Т. 88, № 1 **ЖУРНАЛ ПРИКЛАДНОЙ СПЕКТРОСКОПИИ** ЯНВАРЬ — ФЕВРАЛЬ 2021
V. 88, N 1 **JOURNAL OF APPLIED SPECTROSCOPY** JANUARY — FEBRUARY 2021

ANALYSIS OF HUMAN URINARY STONES AND GALLSTONES BY FOURIER TRANSFORM INFRARED ATTENUATED TOTAL REFLECTANCE SPECTROSCOPY[**]

F. J. Hermida

Department of Clinical Analysis, Hospital Clínico Universitario de Santiago de Compostela,
A Coruña, Spain; e-mail: fernandojesus.hermida.ameijeiras@sergas.es

The aim of this study was to analyze the composition of urinary stones and gallstones and assess their prevalence as a function of age and sex. A total of 425 urinary stones and 108 gallstones was analyzed for composition using FTIR-ATR spectroscopy. According to the absorption band spectra obtained using FTIR-ATR, urinary stones were classified into the following groups: calcium oxalate (55.6%), uric acid (24.1%), hydroxyapatite (7.3%), struvite (9.0%), brucite (2.1%), cystine (1.0%) and ammonium urate (0.2%). Gallstones were classified into cholesterol (66.7%) and pigment stones (33.3%). As to urinary stones, they are more common in males (62.0%) than in females (38.0%) (ratio ♂/♀: 1.7), calcium oxalate stones being the most common ones in both sexes. Women have a higher frequency of hydroxyapatite and struvite than men ($p<0.05$) whereas males have a higher frequency of calcium oxalate and uric acid than women ($p<0.05$). Calcium oxalate stones are more common in the 30–69 years age group ($p<0.05$), while uric acid stones are more common in ages >50 years ($p<0.05$). As to gallstones, they are more common in women (59.3%) than in men (40.7%) (ratio ♀/♂: 1.4), cholesterol stones being the most prevalent in both sexes. Women have greater frequency of cholesterol stones than men ($p<0.05$) and men have higher frequency of pigment stones than women ($p<0.05$). Cholesterol stones were more common in ages <60 year ($p<0.05$), whereas pigment stones were more common in ages ≥60 years ($p<0.05$). The results of this study show that the physical analysis of stones using FTIR-ATR spectroscopy provides fairly accurate information on its composition, and sex and age have been seen to have an influence on the type of stone formed.

Keywords: *urinary stone, gallstone, gender, age groups, Fourier transform infrared attenuated total reflectance spectroscopy.*

АНАЛИЗ КАМНЕЙ В МОЧЕВОМ И ЖЕЛЧНОМ ПУЗЫРЕ ЧЕЛОВЕКА С ПОМОЩЬЮ ИК-ФУРЬЕ-СПЕКТРОСКОПИИ НАРУШЕННОГО ПОЛНОГО ВНУТРЕННЕГО ОТРАЖЕНИЯ

F. J. Hermida

УДК 535.34;543.42:616-003.7

Университетская клиника Сантьяго-де-Компостела,
Ла-Корунья, Испания; e-mail: fernandojesus.hermida.ameijeiras@sergas.es

(Поступила 16 сентября 2019)

Проанализирован состав камней в мочевом и желчном пузыре и оценена степень распространенности отдельных компонентов камней в зависимости от возраста и пола. Составы 425 мочевых камней и 108 желчных камней проанализированы с помощью ИК-Фурье-спектроскопии нарушенного полного внутреннего отражения (FTIR-ATR). По спектрам поглощения, полученным с помощью FTIR-ATR, камни в мочевом пузыре разделены на группы: оксалат кальция (55.6%), мочевая кислота (24.1%), гидроксиапатит (7.3%), струвит (9.0%), бруцит (2.1%), цистин (1.0%) и урат аммония (0.2%). Камни в желчном пузыре разделены на холестериновые (66.7%) и пигментные (33.3%). Камни в мочевом пузыре чаще встречаются у мужчин (62.0%), чем у женщин (38.0%) (соотношение 1.7), причем камни из оксалата кальция распространены у представителей обоих

[**] Full text is published in JAS V. 88, No. 1 (http://springer.com/journal/10812) and in electronic version of ZhPS V. 88, No. 1 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

полов. Для женщин характерна более высокая частота гидроксиапатита и струвита, чем для мужчин (вероятность ошибки p<0.05), а для мужчин — более высокая частота оксалата кальция и мочевой кислоты (p<0.05). Камни из оксалата кальция чаще встречаются в возрастной группе 30–69 лет (p<0.05), камни из мочевой кислоты — у лиц старше 50 лет (p<0.05). Камни в желчном пузыре чаще встречаются у женщин (59.3%), чем у мужчин (40.7%) (соотношение 1.4), причем холестериновые камни — у представителей обоих полов. У женщин холестериновые камни находят чаще, чем у мужчин (p<0.05), а мужчины имеют более высокую частоту пигментных камней (p<0.05). Холестериновые камни характерны для лиц в возрасте до 60 лет (p<0.05), тогда как пигментные — для пациентов в возрасте свыше 60 лет (p<0.05). Физический анализ камней с помощью FTIR-ATR дает достаточно точную информацию о его составе. На тип образовавшегося камня оказывают влияние пол и возраст.

Ключевые слова: *камень мочевого пузыря, камень желчного пузыря, пол, возрастная группа, ИК-Фурье-спектроскопия нарушенного полного отражения.*

Introduction. The annual incidence of nephrolithiasis is estimated at about 0.5% in North America and Europe. It is a recurrent disease with a relapse rate of 50% in 5–10 years and 75% in 20 years [1]. The formation of gallstones in the gallbladder, bile duct, and liver is a common gastrointestinal disease found in 10–20% of the population of western countries and requires emergency surgery in approximately 25% of these patients [2]. The incidence and composition of stones has changed dramatically over the last half century in industrialized countries in parallel with the profound changes in the standard of living and eating habits and is also influenced by other factors such as genetic predisposition, geographic area, ethnicity, age, or sex [3].

Knowing the composition of urinary stones and gallstones can be extremely important to understand the underlying physico-chemical principles leading to stone formation in order to evaluate the clinical management of the patient and to identity risk factors in order to guide the recommendations to prevent future recurrence [3, 4]. Up until recently, urinary stones were analyzed at our laboratory using chemical methods, which present important limitations such as: they are more time consuming, a considerable sample quantity is required, they have moderate sensitivity and specificity (leading to false positives and false negatives), and they may only identify the presence of individual ions and not the specific compounds found in the stone [4, 5]. On the other hand, the different types of gallstones have been previously classified depending on their appearance and composition, which includes a visual analysis (shape, texture, color, etc.). This is often associated with subjectivity problems, and chemical analyses are very laborious with little sensitivity; all this leads to discrepancies in results [2, 6]. Since May 2017 at our laboratory we have been using the Fourier transform infrared spectrophotometer with a diamond attenuated total reflection accessory (FTIR-ATR) technique for the analysis of urinary stones and gallstones, which has the following advantages: greater sensitivity and specificity, the specific components of the stone are identified, it is quick and easy to perform, requires a small amount of sample, and is cost effective [5, 6]. The European Association of Urology [7] recommends, as a fundamental requirement for the metabolic assessment of the patient, that an analysis should be conducted of the composition of the urinary stones using a valid method, indicating infrared spectroscopy as one of the preferred analytical methods.

The aim of this study is to analyze the composition of the urinary stones and gallstones by means of a physical analysis using FTIR-ATR spectroscopy and assess the prevalence of these stones on the basis of age and sex in our health area.

Material and methods. During the period between May/2017-May/2019 a total of 425 urinary stones were processed at the laboratory of clinical analyses of the Hospital Clínico Universitario de Santiago de Compostela (Galicia, NW Spain) from 410 different patients ranging in age between 3–90 years (mean 57.2±14.9); of them 257 were males with an age range between 3–89 years (mean 56.9±15.0), and 153 were female with an age range between 10–90 years (mean 57.7±14.7). As far as gallstones are concerned, during the period between October/2018-March/2019 a total of 108 stones was processed from 108 different patients ranging in age between 18–94 years (mean 59.1±18.6); of them 46 were males with an age range between 18–88 years (mean 56.7±18.1), and 62 were women ranging in age between 22–94 years (mean 60.9±18.9).

Urinary stones and gallstones were analyzed using FTIR-ATR spectroscopy. For the FTIR-ATR analysis, the IRAffinity-1S (Shimadzu Corporation, Japan) equipment was used which performs a spectral scanning from 4000 to 400 cm^{-1}, with a resolution of 4 cm^{-1} for each spectrum and an average of 20 measurements per spectrum. Before every analysis, the background spectrum was measured initially with no sample in contact with the attenuated total reflectance (ATR) unit. This IR spectrum, which is specific to each stone, is then compared to a library of spectra added to the analyzer to generate a report on the different components of the stone.

Urinary stones were obtained through endoscopy or open surgical procedures or as a result of spontaneous passage through the urethra; gallstones were collected following a cholecystectomy. All stones were cleaned with distilled water and dried to be pulverized in a mortar to obtain a fine and homogeneous powder that was subsequently placed on an ATR accessory equipped with a diamond crystal. Pressure was exerted by the tip of a specially designed device so that the powder sample fully occupied the surface of that crystal as a fine film.

The proposal by Lieske et al. [8] for the classification of urinary stones was followed: 1) ST group: stones containing some struvite; 2) CY group: stones with some cystine; 3) UA group: stones with some uric acid (anhydrous UAA or dihydrate UAD); 4) BR group: stones with some brucite; 5) AU group: stones with some ammonium urate; 6) CO group: stones with >50% of calcium oxalate (monohydrate COM or dihydrate COD); 7) HA group: stones with >50% of hydroxyapatite. The proposal by Cariati [9] was followed for the classification of gallstones, where two groups are distinguished: 1) cholesterol stones (CHO) with a cholesterol content of >50%, and 2) pigment stones with a cholesterol content of <30%, the main compound being calcium bilirubinate (BI). Pigment stones can, in turn, be classified into two subgroups [9]: 1) black pigment gallstones (characterized by containing calcium carbonate: CC) and 2) brown pigment gallstones (characterized by containing calcium stearate: CS).

The statistical analysis and data treatment were conducted using the following software: MedCalc® for Windows (MedCalc Software, Belgium) and Microsoft® Excel (Microsoft Corporation, USA). Results were analyzed using chi-squared test; a level of significance of $p<0.05$ was defined as statistically significant. Permission was obtained from the Committee for Ethical Review of the Hospital Clínico Universitario de Santiago de Compostela for conducting this study.

Results and discussion. *Kidney stones*. Of the 425 stones analyzed, 155 (36.5%) had a single component (COM 20.70%, COD 0.7%, UAA 11.0%, UAD 0.2%, HA 1.6%, AU 0.2%, BR 1.2% and CY 0.9%) and 270 (63.5%) were mixed, of which 151 (35.5%) had two components (COM+UAA 13.4%, HA+ST 8.5%, COM+HA 7.1%, COM+COD 4.4%, COD+HA 0.7%, COM+BR 0.5%, COD+BR 0.2%, UAA+UAD 0.5% and UAA+AU 0.2%) and 119 (28%) had three components (COM+COD+HA 26.6%, COM+HA+ST 0.7%, COM+HA+BR 0.7%). In Figures 1–3 the IR spectrum of all pure urinary stones and some mixed stones is shown. In the IR spectra of COM and COD, two characteristics very similar bands are shown at 1607 and 1312 cm^{-1} in COM and at 1613 and 1319 cm^{-1} in COD, but we can distinguish the different hydrates of CO because of the presence of two small bands at 945 and 883 cm^{-1} and another two marked bands at 777 and 510 cm^{-1} in COM that are missing in COD. The IR spectra of the different hydrates of UA (UAA and UAD) are almost identical, but the presence of a wide band at around 3400–3600 cm^{-1} and another band at 1325 cm^{-1} in the hydrated form and missing from the anhydrous form makes it possible to differentiate them. The AU spectrum shows a moderate similarity with the UA spectra, particularly in the bands found in the absorption interval 1700–400 cm^{-1}, but in the AU spectrum characteristic bands are observed in the 2500–3400 cm^{-1} interval that are not found in the UA spectra. As far as the stones containing phosphate, the IR spectrum of the ST (Fig. 3) shows three absorption bands at 1429, 1006, and 752 cm^{-1}, which makes it possible to differentiate them from the HA stones, which have another three characteristic bands at 1020, 599, and 559 cm^{-1}; as to BR, it shows an IR spectrum very different from the other phosphate stones where three peaks are observed at 3540, 3485, and 1647 cm^{-1} and a series of characteristic bands in the 1250–400 cm^{-1} range. Finally, in the case of CY stones, besides being found in their pure form, they have a characteristic IR spectrum that can be easily differentiated from other stones.

A total of 266 stones (62.6%) was from males and 159 (37.4%) were from women, the male/female ratio being 1.7. The age range in which a greater number of stones was found was between 40–79 years, accounting for 82% of total stones. Figure 4a shows the distribution of the composition of the stones (those that occur in more than 5%) of the general population and in the different sexes. It can be seen that in the total of the population studied the most common kidney stone was CO with 55.8%, followed by UA with 24.5%, ST with 9%, and HA with 7.1%. By sexes, the most prevalent stones in males was CO (59.4%) followed by UA (30.1%), while in females the most common stones were CO (49.7%) followed by ST (17.6%), UA (15.1%), and HA (13.2%). It was found that CO and UA stones were more common in males than in females, while ST and HA stones were more common in females than in males. These differences were statistically significant ($p < 0.05$).

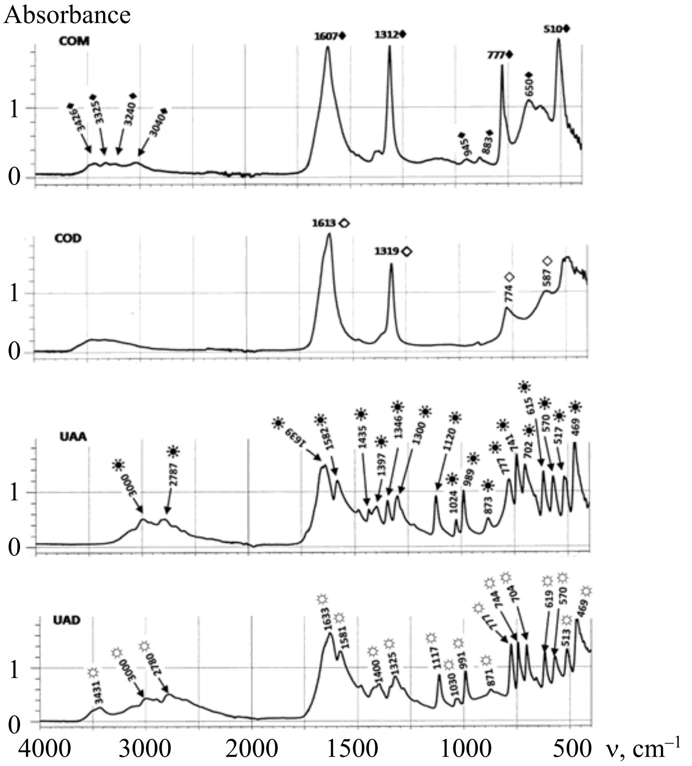

Fig. 1. FTIR spectrum of COM (calcium oxalate monohydrate), COD (calcium oxalate dihydrate), UAA (uric acid anhydrous), and UAD (uric acid dihydrate) pure urinary stones.

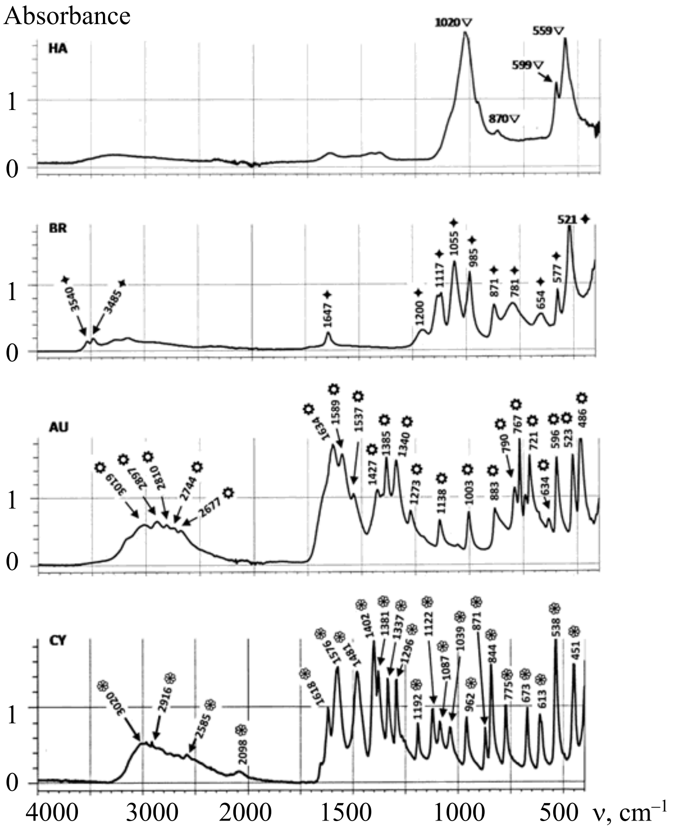

Fig. 2. FTIR spectrum of HA (hydroxyapatite), BR (brushite), AU (ammonium urate), and CY (Cystine) pure urinary stones.

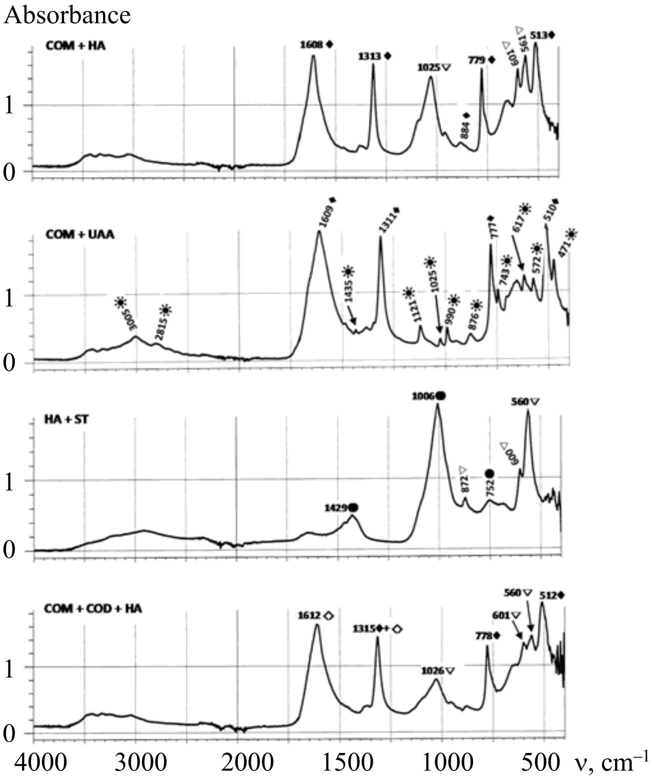

Fig. 3. FTIR spectrum of mixed urinary stones (ST: struvite).

Figures 4b–d show the association between the type of urinary stone and age (in decades) in the general population and in the different sexes. We see that in the general population, CO stones show a bell-type behavior, being more frequent in ages between 30–69 years with 85% of the CO stones ($p < 0.05$). The presence of UA stones increased with age, and they were more frequent in patients >50 years, accounting for 91% of UA stones ($p < 0.05$). As to phosphate stones, ST stones showed a slight "U" behavior as they were more frequent in ages <40 years and >70 years, and HA stones were slightly more frequent in ages <40 years. In these cases, no significant differences were observed. By sex, a behavior similar to that of the general population was found in both males and females.

As to BR, CY, and UAA stones, they were found in such a small proportion (<5%) that a comparative study on the basis of sex and age was not possible.

Gallstones. Of the 108 gallstones analyzed, 65 (60.2%) had a single component (CHO: 56.5% and BI: 3.7%) and 43 (39.8%) were mixed, of which 28 (25.9%) had two components (CHO+BI: 10.2%, BI+ST: 12%, and BI+CA: 3.7%) and 15 (13.9%) had three components (CHO+BI+ST: 4.6%, CHO+BI+CA: 1.9%, and BI+ST+CA: 7.4%). Figures 5 and 6 show the infrared spectrum of pure and mixed gallstones indicating the wavelength of the characteristic absorption bands. The main bands that make it possible to detect the presence of CHO are: a wide band located at around 2930 cm^{-1} and other six peaks located at about 1464, 1440, 1377, 1365, 1055, and 1022 cm^{-1}; the main bands that indicate the presence of BI are: a series of peaks located between 1500–1750 cm^{-1} and a band located at about 1246 cm^{-1}; the bands indicating the presence of CS are: two very sharp peaks at about 2916 and 2849 cm^{-1} and another five peaks located at about: 1574, 1539, 1466, 1433, and 1419 cm^{-1}; and the presence of CC is easily identifiable by the presence of a wide band at 1200–1550 cm^{-1} and another narrow band at about 873 cm^{-1}.

A total of 64 gallstones was from women (59.3%) and 44 from men (40.7%) with a male/female ratio of 1.4. Figure 4e represents the distribution of the composition of stones in general population and by sex, showing that the most common gallstone in the general population was CHO, which accounted for 66.7% of cases, followed by pigment gallstones, which accounted for 33.3%. By sex, in women and men, CHO gallstones were more common than pigment gallstones (75% vs 25% and 54.5% vs 45.5%, respecttively). In women, there was a higher frequency of CHO gallstones than in men, while in men, pigment gallstones were more common than in women. These differences were statistically significant ($p < 0.05$).

Figure 4f–h shows the association between age (in decades) and the type of gallstone in the general population and in both sexes. It can be seen that in the general population CHO stones were more frequent in patients aged <70 years ($p < 0.05$), while pigment gallstones were more prevalent in patients aged ≥60 years ($p < 0.05$). This same behavior is seen in both sexes, also presenting statistically significant differences ($p < 0.05$).

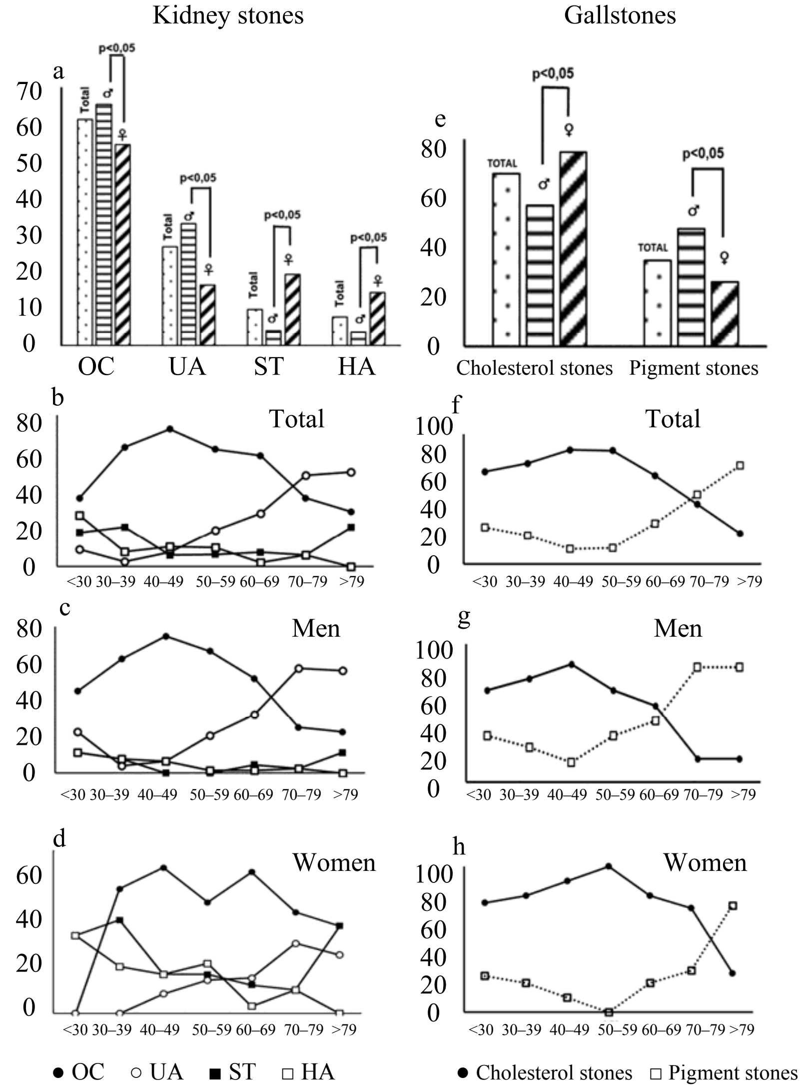

Fig. 4. a) Association of sex with urinary stone type. b) Association of age with urinary stone type. c–d) Association of age and sex with urinary stone type. e) Association of sex with gallstone type. f) Association of age with gallstone type. g–h) Association of age and sex with gallstone type. (CO: calcium oxalate; UA: uric acid; ST: struvite; HA: hydroxyapatite).

The spectra obtained in this study regarding both kidney stones and gallstones are similar to those published by other authors; the different vibrations (stretching or bending) experienced by the different molecular bonds have already been widely described in the bibliography [2, 10–15]; 63.5% of kidney stones and 39.8% of gallstones analyzed in this study were mixed, which underscores the importance of any analysis method that is able to identify the percentage each component contributes to the mixed stone [16]. In this regard, it should be noted that the identification of the components of a mixed stone using FTIR may not always be exact because of a number of factors: 1) when band overlapping occurs, it is difficult to determine the contribution of each component exactly [5]; 2) when any of the components of a stone is found in very small amounts, it might be difficult to quantify [5]; 3) an incomplete library or one created from different compounds, whether natural or synthetic, may also lead to error [5]; 4) the use of different methods (FT-IR with previous handling of the sample using KBr tablet *vs* FTIR-ATR) could also lead to error [5]; 5) in the case of gallstones, there are some chemical substances (fatty acids, colic acid derivatives, proteins or polysaccharides) that overlap in absorption peaks of the main components of the gallstone, thus making it difficult to determine their exact composition [2]. Siener et al. [17], in a multicenter study conducted at different European laboratories, surprisingly found high error rates in some laboratories using IR, underscoring the importance of equipment, the spectra of reference in the library, the qualification of personnel, and the need to perform regular quality controls as the premise for conducting a correct analysis of kidney stones; Hesse et al. [16] remarked that most automated programs for the evaluation of infrared spectroscopy and X-ray diffraction are not yet sophisticated enough to provide reliable identification of the individual components of a mixture. However, and despite the above, numerous authors argue that FTIR spectroscopy is an efficient and sensitive enough method to determine the composition of kidney stones and gallstones [2, 6, 10, 12]. Besides, the guidelines on urolithiasis of the European Association of Urology recommend the use of FTIR spectroscopy as one of the preferred methods [7].

Urinary stones. We found that the incidence of urinary stones was higher in the 30–79 years range with a higher frequency in males than in females (male/female ratio 1.7), CO stones being the most frequent ones for both sexes. These results are consistent with those found in the references [8, 18, 19]. The most common pathophysiological abnormality found in patients with CO kidney stones is hypercalciuria, which tends to be idiopathic and associated with normal serum levels of calcium. There are also other important risk factors such as hyperoxaluria, hypocitraturia, low volume of urine, hyperuricosuria, or an inappropriate diet (excess of animal protein, salt, alcohol consumption) [1, 19, 20]. The higher incidence of CO stones in men can be explained by the fact that they have greater urinary excretion of calcium and a greater intake of proteins than women, and younger women have a greater urinary pH and greater concentration of citrate, which prevent the formation of CO stones [8].

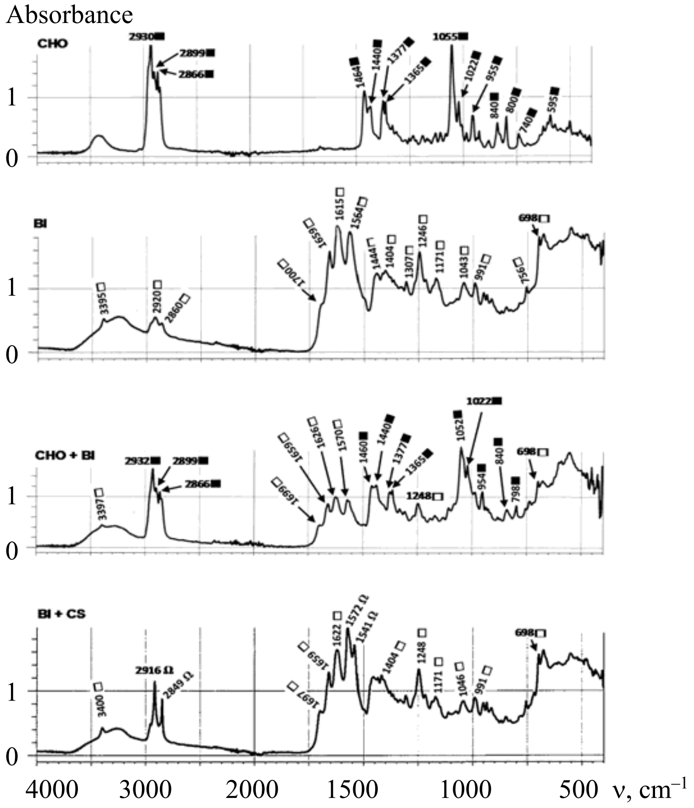

Fig. 5. FTIR spectrum of CHO (cholesterol) and BI (calcium bilirubinate) pure gallstones and mixed gallstones (CS: calcium stearate).

Uric acid stone was the second most frequent calculus in males, and an increase in its prevalence was observed as age increased (>50 years) in both sexes, as other authors have reported [8, 18, 21]. The two main factors contributing to the formation of this type of stones are hyperuricosuria and, mainly, abnormally low urinary pH (pH<5.5) [22]. It was observed that, as a response to aging, there was a progressive reduction of ammoniagenesis, which resulted in older people having a lower urinary pH than younger people, thus facilitating the formation of AU stones [1, 8, 18, 21, 22]. The other risk factor for UA stones is hyperuricosuria, which can be secondary to purine-rich diets or because of excess production of uric acid resulting from some pathological process such as gout or lymphoproliferative disease [19]. The greater predisposition of men to present with more UA stones than women might due to several reasons: on the one hand, dietary habits, as men ingest greater amounts of animal proteins and alcohol; on the other hand, the higher urinary pH and the higher glomerular filtration rate in women as well as sexual female hormones (estrogens) that increase the efficacy of the kidney to dispose of uric acid, thus preventing the formation of UA stones [22, 23].

As other authors have previously reported [8, 21] we found a higher proportion of phosphate stones in women than in men. Stones containing SR appear when there is a recurrent infection of the urinary tract, which is more common in women than in men, as a result of gram-negative bacteria producing urease [1, 24]. On the other hand, we found that SR stones were slightly more frequent in ages <40 years and >70 years; Flannigan et al. [25] remarked that two factors that predispose one to acquiring these stones include female sex and extreme of age. Kidney stones consisting of pure HA are rare, and more frequently they are mixed, mainly in conjunction with CO [24]. This is consistent with our findings, where we find that only 3.7% of stones that contain HA are pure and the remaining 96.3% are mixed, in most cases combined with CO (77.8%). Our data show greater presence of HA stones in women than in men and ages <40 years, a fact already reported by other authors [8, 18, 21]. The higher urinary pH is the main factor distinguishing the HA stone formers from the CO stone formers; in this regard young women present higher urinary pH than men [8, 24], which facilitates the formation of non-infectious HA stones. The influence of some metabolic diseases such as primary hyperparathyroidism and renal tubular acidosis has been mentioned as an influence of the formation of these stones [24].

Gallstones. In line with what other authors have reported [26–28], women were found to have a greater incidence of gallstones and at ages before menopause. Female sex hormones are the main basis for this sex difference. In fact, pregnancy is a risk factor in the formation of gallstones. Estrogens increase the levels of cholesterol in the bile, leading to oversaturation, whereas progesterone inhibits the contraction of the biliary gland and of the function of the sphincter of ampulla, leading to a decrease in the emptying of the biliary gland, resulting in cholelithiasis [26–28].

As other authors have already reported, mainly in western countries [26–29], cholesterol gallstones were found predominantly in both males and females. In East Asian countries pigment stones are much more common. However, in recent times, the presence of CHO gallstones is increasing as a result of western lifestyles and diets [30]. The following have been mentioned as possible causes of the formation of cholesterol gallstones: 1) a diet rich in carbohydrates or fatty acids, which causes an increase in the synthesis of cholesterol, leading to a change in the liver of the balance between cholesterol saturation and conversion of cholesterol into bile acids and cholesterol esters; and 2) inappropriate lifestyles that lead to such risk factors as obesity, diabetes, or hypertension [27, 30].

In line with what other authors have reported [3, 31], we found that pigment gallstones were more frequent in late decades in life (>60 years). As life expectancy increases, there is generally a greater probability of organic abnormalities and particularly of liver diseases and biliary tract diseases in elderly people [32]. Among the possible causes that facilitate stone formation, and which apparently do not affect the formation of CHO gallstones, are hemolysis and liver cirrhosis (where a greater predominance of black pigment gallstones was reported) and bacterial infection (where a greater predominance of brown pigment gallstones was reported) [13, 28, 29, 32–34].

Lastly, we should acknowledge that this study has some limitations: 1) it is likely that some stones have been lost because they were not collected following surgery, or in the case of urinary stones, they may have spontaneously passed through the urinary tract; 2) it is a known fact, particularly in the case of gallstones, that there are many asymptomatic patients [3] resulting in these stones being ignored for years; consequently, this study may be more representative of clinical practice than of prevalence; and 3) this study consists of a relatively small number of patients and was conducted at a single medical center, therefore large-scale, prospective studies are required to confirm our results. However, and despite these limitations, this study provides very important information as it is the first study conducted in this geographical area of Spain

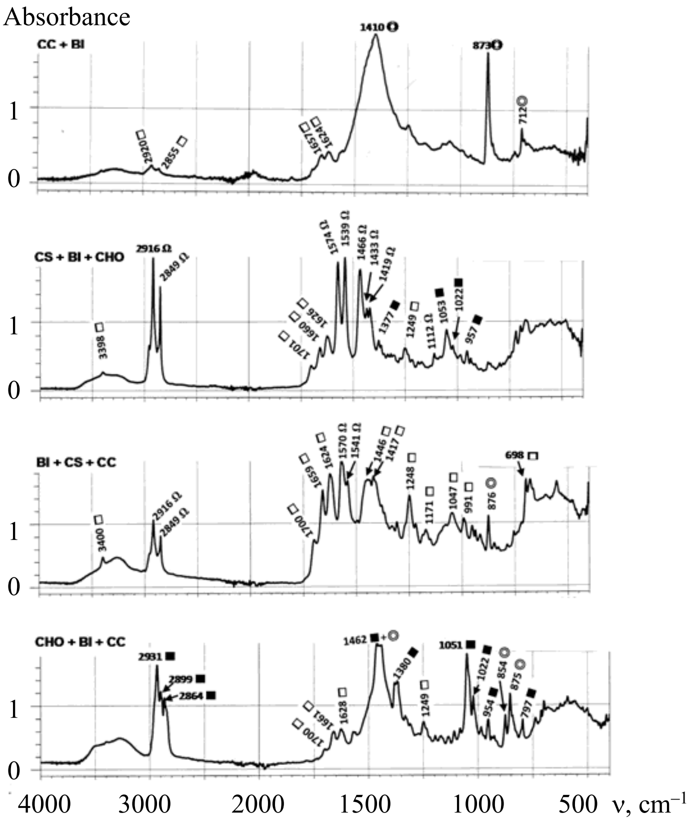

Fig. 6. FTIR spectrum of mixed gallstones (CC: calcium carbonate).

using this technology (FTIR-ATR) for the analysis of urinary stones and gallstones and will be very useful in understanding the pathophysiology of the disease caused by these stones in the Galician population in future clinical studies.

Acknowledgments. The author would like to thank all participants of the anatomical pathology laboratory for the collection of gallstone samples

Ethical approval. Permission was obtained from the Committee for Ethical Review of the Hospital Clínico Universitario de Santiago de Compostela for conducting this study.

REFERENCES

1. O. W. Moe, *Lancet*, **367**, 333–344 (2006).
2. B. J. Ha, S. Park, *Biomater. Res.*, **22**, 18 (2018).
3. L. M. Stinton, R. P. Myers, E. A. Shaffer, *Gastroenterol. Clin. N. Am.*, **39**, 157–169 (2010).
4. G. P. Kasidas, C. T. Samuell, T. B. Weir, *Ann. Clin. Biochem.*, **41**, 91–97 (2004).
5. A. Basiri, M. Taheri, F. Taheri, *Urol. J.*, **9**, 445–454 (2012).
6. E. H. Yoo, H. J. Oh, S. Y. Lee, *Clin. Chem. Lab. Med.*, **46**, 376–381 (2008).
7. C. Türk, A. PetříK, K. Sarica, C. Seitz, A. Skolarikos, M. Straub, T. Knoll, *Eur. Urol.*, **69**, 468–474 (2016).
8. J. C. Lieske, A. D. Rule, A. E. Krambeck, J. C. Williams, E. C. Bergstralh, R. A. Mehta, T. P. Moyer, *Clin. J. Am. Soc. Nephrol.*, **9**, 2141–2146 (2014).
9. A. Cariati, *Indian J. Surg.*, **77**, S376–S380 (2015).
10. S. Tamošaityté, V. Hendrixson, A. Želvys, R. Tyla, Z. A. Kučinskiene, F. Jankevičius, M. Pučetaitè, V. Jablonskiene, V. Šablinskas, *J. Biomed. Opt.*, **18**, 027011 (2013).
11. R. H. Ma, X. B. Luo, Q. Li, H. Q. Zhong, *Int. J. Surg.*, **41**, 150–161 (2017).
12. R. Selvaraju, G. Thiruppathi, A. Raja, *Spectrochim. Acta A*: *Mol. Biomol. Spectrosc.*, **93**, 260–265 (2012).

13. O. Kleiner, J. Ramesh, M. Huleihel, B. Cohen, K. Kantarovich, C. Levi, B. Polyak, R. S. Marks, J. Mordehai, Z. Cohen, S. Mordechai, *BMC Gastronterol.,* **2**, 3 (2002).
14. C. L. Cheng, H. H. Chang, T. H. Chen, P. J. Tsai, Y. T. Huang, P. J. Huang, S. Y. Lin, *Dig. Liver Dis.*, **48**, 519–527 (2016).
15. T. Qiao, R. H. Ma, X. B. Luo, L. Q. Yang, Z. L. Luo, P. M. Zheng, *PLoS One.*, **8**, e74887 (2013).
16. A. Hesse, R. Kruse, W. J. Geilenkeuser, M. Schmidt, *Clin. Chem. Lab. Med.*, **43**, 298–303 (2005).
17. R. Siener, N. Buchholz, M. Daudon, B. Hess, T. Knoll, P. J. Osther, J. R. Santos, K. Sarica, O. Traxer, A. Trinchieri, *PLoS One.*, **11**, e0156606 (2016).
18. X. Yang, C. Zhang, S. Qi, Z. Zhang, Q. Shi, C. Liu, K. Yang, E. Du, N. Li, J. Shi, Y. Xu, *J. Clin. Lab. Anal.*, **30**, 873–879 (2016).
19. I. Miguel–Elízaga, A. Martínez–Ruiz, F. Avilés–Plaza, J. A. Noguera–Velasco, P. Martínez-Hernández, S. Parra-Pallarés. *Clin. Chem. Lab. Med.*, **50**, 545–548 (2012).
20. R. Siener, A. Hesse, *Eur. Urol.*, **42**, 289–296 (2002).
21. A. Costa–Bauzá, M. Ramis, V. Montesinos, A. Conte, P. Pizá, E. Pieras, F. Grases, *World J. Urol.,* **25**, 415–421 (2007).
22. A. Viljoen, R. Chaudhry, J. Bycroft, *Ann. Clin. Biochem.*, **56**, 15–27 (2019).
23. H. W. Chen, Y. C. Chen, F. M. Yang, W. J. Wu, C. C. Li, Y. Y. Chang, Y. H. Chou, *Sci. Rep.*, **8**, 6077 (2018).
24. M. Daudon, H. Bouzidi, D. Bazin, *Urol. Res.*, **38**, 459–467 (2010).
25. R. K. Flannigan, A. Battison, S. De, M. R. Humphreys, M. Bader, E. Lellig, M. Monga, B. H. Chew, D. Lange, *Can. Urol. Assoc. J.*, **12**, 131–136 (2018).
26. E. A. Shaffer, *Best Pract. Res. Clin. Gastroenterol.*, **20**, 981–996 (2006).
27. B. M. Jarrar, M. A. Al Rowaili, *Malays. J. Med. Sci.*, **18**, 47–52 (2011).
28. H. Weerakoon, J. G. S. Ranasinghe, A. Navaratna, R. Sivakanesan, K. B. Galketiya, S. Rosairo, *BMC Gastroenterol.*, **14**, 88 (2014).
29. K. J. Van Erpecum, *Clin. Res. Hepatol. Gastroenterol.*, **35**, 281–287 (2011).
30. T. Liu, W. Wang, Y. Ji, Y. Wang, X. Liu, L. Cao, S. Liu, *PLoS One.*, **13**, e0196457 (2018).
31. A. K. Diehl, W. H. Schwesinger, D. R. Holleman Jr., J. B. Chapman, W. E. Kurtin, *Am. J. Gastroenterol.*, **90**, 967–972 (1995).
32. J. H. Siegel, F. E. Kasmin, *Gut*, **41**, 433–435 (1997).
33. M. Acalovschi, *World J. Gastroenterol*, **20**, 7277–7285 (2014).
34. K. Julka, C. W. Ko, *Infect. Dis. Clin. N. Am.*, **24**, 885–898 (2010).