SEVEN publicações acadêmicas
EM PARCERIA COM: POLO ITUPEVA IEMS Instituto Educacional Marili Siqueira UNIASSELVI GRADUAÇÃO E PÓS

Characterization of kidney stones using Raman spectroscopy

Evelyn Belorio da Silva[1], Wangner Barbosa da Costa[2], Marcela de Oliveira[3], Nilton Francelosi Azevedo Neto[4].

ABSTRACT

The text discusses the significance of understanding the composition of kidney stones for accurate diagnosis, treatment, and prevention. It introduces Raman spectroscopy (RE) as a promising analytical technique due to its ability to analyze urinary stones effectively, despite their high water content, unlike other methods like infrared spectroscopy. Raman spectroscopy provides high-resolution identification of stone components such as calcium oxalate, calcium phosphate, uric acid, and cystine, while preserving the stone's integrity for further analysis. The text describes a study where a kidney stone was characterized using microRaman spectroscopy with a prototype sample holder designed to optimize measurements and protect the confocal microscope's objective lens. The characterization was conducted using a Horiba Evolution spectrometer with a laser wavelength of 532 nm and a spectral resolution of less than 1 cm$^{(-1)}$. The analysis revealed the potential of Raman spectroscopy for qualitative analysis of kidney stones.

Keywords: Kidney stones, Raman spectroscopy, Composition analysis, Urinary stones, Spectral resolution.

INTRODUCTION

The formation of kidney stones affects millions of people around the world, causing intense pain and other health problems. Understanding the composition of these stones is crucial for accurate diagnosis, effective treatment and prevention of recurrence (Rodriguez; Heilberg, 2020; Wang et al. , 2022).

Raman spectroscopy (RE) is an analytical technique that explores the interaction of light with molecules, providing information about their structure and composition (Das; Agrawal, 2011) . Unlike other techniques such as infrared spectroscopy, Raman is less sensitive to water, making it ideal for analyzing urinary stones, which often contain high levels of water (Carmona; Bellanato; Escolar, 1997) . The use of Raman spectroscopy in the area of clinical analysis can be promising because the technique presents good resolution to identify different stone components, such as calcium oxalate, calcium phosphate, uric acid and cystine, with high precision. The analysis is non-destructive, preserving the integrity of the stone, allowing for subsequent analyzes or clinical studies. Another difference is its

---

[1] Faculty of Technology of Bauru – São Paulo
[2] Faculty of Technology of Bauru – São Paulo
[3] Faculty of Technology of Bauru – São Paulo
[4] Technological Institute of Aeronautics – São Paulo

specific analysis, which allows for an investigation into possible heterogeneity in the composition and distribution of components (Chang; Chiu, 2017; Selvaraju; Raja; Thiruppathi, 2012) .

In this work, a kidney stone was characterized using microRaman spectroscopy with a wavelength of 532 nm. A prototype sample holder was designed with the aim of optimizing measurements and avoiding damage to the objective lens of the confocal microscope.

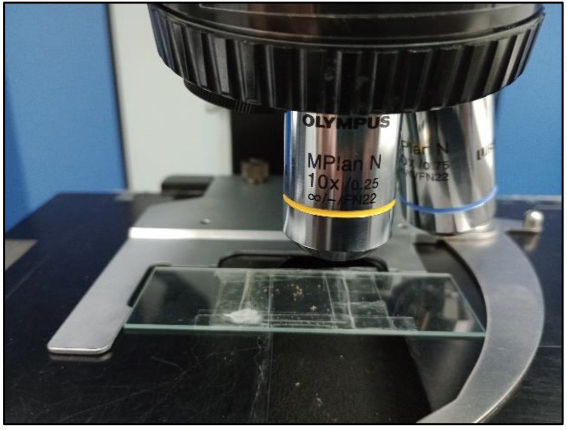
OBJECTIVE

To investigate the potential of the Raman spectroscopy technique for qualitative analysis of kidney stones.

METHODOLOGY

Characterization by Raman spectroscopy was carried out on a Horiba Evolution spectrometer, with a spectral resolution of less than 1 cm^{-1}. The laser radiation used was 532 nm with a power of 100 mW. The spectra were obtained in the range of 500 to 2000 cm^{-1}, with an acquisition time of 10 s and 6 accumulations. Focus adjustment was performed using an *Olympus* 10x MPlan N objective lens. For the analysis, a sample holder was developed using a microscope slide (26 x 76 x 1.2 mm) and a glass coverslip (22 x 22 x 0.13 mm). Figure 1 shows the kidney stone fixed in the manufactured substrate holder.

Figure 1 – Sample holder fixed to the support of the confocal microscope coupled to the Raman system.

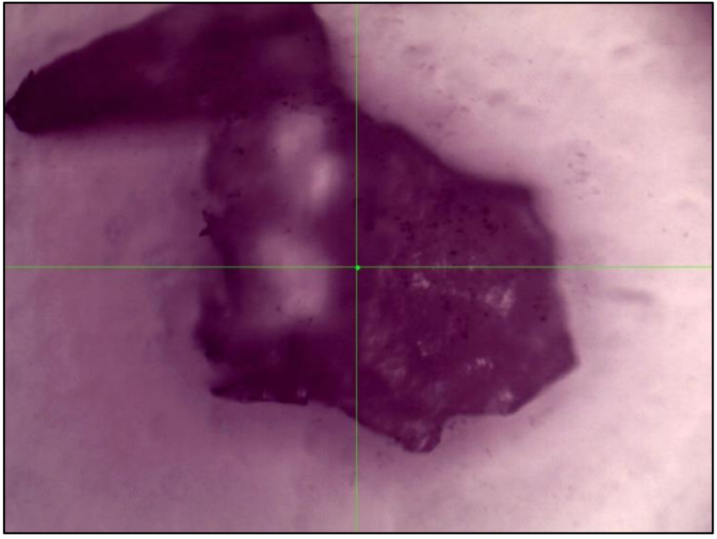


Source: Prepared by the author (2024)

DEVELOPMENT

One of the advantages of microRaman spectroscopy is the possibility of measuring samples with small area dimensions. Combined with this factor, fixing the kidney stone in the substrate holder allowed the focus to be adjusted without causing damage to the objective lens of the confocal microscope. Figure 2 shows an image of the kidney stone characterized in this work.

Figure 2 – Image of the kidney stone obtained using confocal microscopy and a 10x lens.

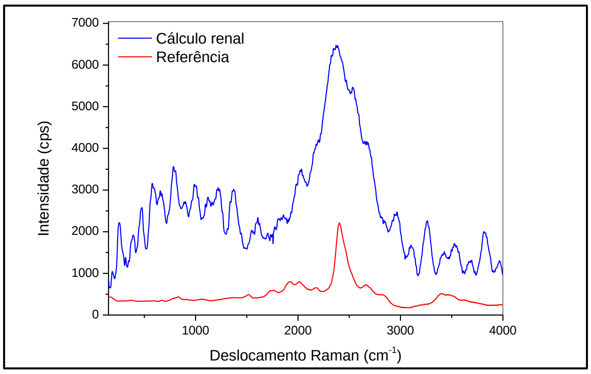


Source: Prepared by the author (2024)

Figure 3 shows the Raman spectrum of the kidney stone. A high intensity band in the region of 2366 cm^{-1} was observed, coinciding with the region characteristic of calcium oxalate, the most common type of kidney stone (Selvaraju; Raja; Thiruppathi, 2013) . The presence of this band strongly suggests the presence of calcium oxalate as the majority component of the stone.

In addition to the main band, peaks of lower intensity, called "satellite peaks", were detected in the spectrum. For a detailed qualitative analysis of these peaks, a fitting routine based on the Gaussian function was employed (Yuan; Mayanovic, 2017) . The results regarding the position and intensity of the satellite peaks, together with the respective vibration modes associated with these bands, are summarized in Table 1.

Figure 3 – Raman spectrum of the kidney stone. The red curve corresponds to the calcium oxalate spectrum (Biagioni *et al.* , 2012) .



Source: Prepared by the author (2014).

Table 1: Position, intensity and vibration modes associated with satellite peaks in the Raman spectrum of kidney stones. The indexing of vibration modes was based on the work of (Selvaraju; Raja; Thiruppathi, 2013).

Position (cm^{-1})	**Intensity (cps)**	**Composition**	**Vibration mode**
253.98	156.84	Calcium carbonate	$-CO_3^{2-}$
390.27	126.71	Oxammite	Ring deformation
894.16	2092	Calcium oxalate monohydrate	CC stretching
988.50	248.71	Hydroxyapatite	PO stretching
1371.57	236.51	Natroxalate	CO stretching
2033.97	284.83	Oxammite	Stretching vibration C=O
2967.14	182.33	Calcium oxalate dihydrate	CH2 stretching
3263.43	161.32	Calcium oxalate dihydrate	OH stretching
3524.93	106.35	Calcium oxalate polyhydrate	OH stretching
3693.75	66.48	Calcium oxalate polyhydrate	OH stretching
3814.57	135.23	Calcium oxalate polyhydrate	OH stretching
3968.54	638.12	Calcium oxalate polyhydrate	OH stretching

Raman spectrum analysis revealed a high signal-to-noise ratio, a frequent characteristic in irregular samples. This characteristic, combined with the presence of Raman bands close to different compounds, such as calcium oxalates mono and dihydrate, calcium phosphate, uric acid and magnesium ammonium phosphate (Selvaraju; Raja; Thiruppathi, 2013), which can influence the interpretation of the results.

Data processing using the Gaussian function made it possible to identify 12 bands assigned to the vibration modes OH, CH_2, C=O, CO, PO and CC, as shown in Table 1.

Scientific studies report the existence of kidney stones with different compositions, including mixtures of oxalates and uric acid (Millman et al., 1982). In the region between 470 and 785 cm^{-1} of the Raman spectrum of the analyzed kidney stones, the presence of satellite peaks that may be related to uric acid is observed (Selvaraju; Raja; Thiruppathi, 2013; Tonannavar et al., 2016). These results suggest that the stone in question has calcium oxalate as the majority component, with the possible presence of uric acid, as evidenced by the satellite peaks.

FINAL CONSIDERATIONS

Analysis of the Raman spectrum of the kidney stone showed the presence of calcium oxalate as the majority component, in agreement with the high intensity band in the region of 2366 cm^{-1}, characteristic of this compound. Additionally, satellite peaks in the region between 470 and 785 cm^{-1} suggest the possible presence of uric acid, indicating a mixed composition of the stone.

The high signal-to-noise ratio, characteristic of irregular samples, and the presence of Raman bands close to different compounds can make direct interpretation of the results difficult. However, detailed analysis of satellite peaks using the Gaussian function allowed the identification of 12 bands attributed to different vibration modes, corroborating the identification of the compounds present in the calculation.

These results demonstrate the ability of Raman spectroscopy for detailed analysis of the composition of kidney stones. The precise identification of the composition of the kidney stone is essential for the adequate diagnosis and treatment of kidney disease, allowing the targeting of treatment and the optimization of therapeutic results.

REFERENCES

BIAGIONI, C. et al. (2012). Crystal chemistry of minerals of the Wöhlerite group from the Los Archipelago, Guinea. The Canadian Mineralogist, 50(3), 593–609. Available at: http://www.canmin.org/cgi/doi/10.3749/canmin.50.3.593.

CARMONA, Pedro; BELLANATO, Juana; ESCOLAR, Elena. (1997). Infrared and Raman spectroscopy of urinary calculi: A review. Biospectroscopy, 3(5), 331–346.

CHANG, Chang Chi; CHIU, Yichun. (2017). Applying quantitative micro-Raman spectroscopy to analyze stone compositions extracted from ureteroscopic lithotripsy urine. Urological Science, 28(1), 19–22. Available at: http://dx.doi.org/10.1016/j.urols.2016.04.002.

DAS, Ruchita S.; AGRAWAL, Y. K. (2011). Raman spectroscopy: Recent advancements, techniques and applications. Vibrational Spectroscopy, 57(2), 163–176. Available at: http://dx.doi.org/10.1016/j.vibspec.2011.08.003.

MILLMAN, S. et al. (1982). Pathogenesis and clinical course of mixed calcium oxalate and uric acid nephrolithiasis. Kidney International, 22(4), 366–370. Available at: http://dx.doi.org/10.1038/ki.1982.183.

RODRIGUEZ, Adrian; HEILBERG, Ita Pfeferman. (2020). Influence of socioeconomic disparities, temperature and. p. 454–460.

SELVARAJU, R.; RAJA, A.; THIRUPPATHI, G. (2013). Chemical composition and binary mixture of human urinary stones using FT-Raman spectroscopy method. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 114, 650–657. Available at: http://dx.doi.org/10.1016/j.saa.2013.05.029.

SELVARAJU, R.; RAJA, A.; THIRUPPATHI, G. (2012). FT-Raman spectral analysis of human urinary stones. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 99, 205–210. Available at: http://dx.doi.org/10.1016/j.saa.2012.09.004.

TONANNAVAR, J. et al. (2016). Identification of mineral compositions in some renal calculi by FT Raman and IR spectral analysis. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy, 154, 20–26. Available at: http://dx.doi.org/10.1016/j.saa.2015.10.003.

WANG, Kai et al. (2022). Risk factors for kidney stone disease recurrence: a comprehensive meta-analysis. BMC Urology, 22(1), 1–13. Available at: https://doi.org/10.1186/s12894-022-01017-4.

YUAN, Xueyin; MAYANOVIC, Robert A. (2017). An Empirical Study on Raman Peak Fitting and Its Application to Raman Quantitative Research. Applied Spectroscopy, 71(10), 2325–2338. Available at: http://journals.sagepub.com/doi/10.1177/0003702817721527.