USING RAMAN SPECTROSCOPY FOR THE STUDY OF BIOGENIC MATERIALS

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Abstract: Raman spectroscopy (RS) is a valuable tool for non-destructive qualitative and quantitative analysis of numerous biogenic materials. Characteristic examples are presented in this work and include characterization of urinary stones, encrusted deposits of stents and bone tissue. In the case of urinary stones the application of RS was capable of analyzing the mineral components of different layers of a urinary stone and the results were compared to those obtained by Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD). FTIR and RS were also used in order to characterise the encrusted deposits formed onto a metallic thermosensitive prostatic stent. In the third example FT-Raman spectroscopy was used to assess the compositional changes that were induced by ovariectomy to tibiae of female wistar rats. It was found that the mineral and organic phase Raman intensities of the osteoporotic bones reduced compared to healthy controls, following the same trend as the values obtained from the application of peripheral quantitative computer tomography on the same bones proving that Raman spectroscopy can be an additional valuable tool for studying osteoporosis.

Keywords: osteoporosis, urinary stones, stent encrustation, Raman spectroscopy.

Introduction

In the recent years, the publications referring to the use of Raman spectroscopy (RS) for biomedical research have increased dramatically. The applications span from its use as a quality control tool for numerous solid and liquid pharmaceutical formulations (e.g. Kontoyannis et al 1994, 1995, 2004), to establish the crystal phase of active ingredients (Orkoula 2005), to the use for monitoring bone demineralization (Kontoyannis et al 2000, Panteliu et al. 2004) to analyzing urinary stones (Kontoyannis 1997). In the present work, applications of RS for analysis of urinary stones, encrusted deposits on stent and osteoporotic bones will be presented.

Materials and Methods

The Raman spectra were recorded using a FRA-106/S FT-Raman (Bruker) with the following characteristics: The laser excitation line used was the 1064nm of a Nd:YAG laser. The power of the incident laser beam was about 370mW on the sample's surface, while the spot size at the focused beam was about 100 μm. Typical spectral line width was 0.5cm^{-1} while the recorded spectra were the average of 300 scans.

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Typical, surgically removed stone, weighing 170 mg, was provided by the Urology Clinic of the University of Patras Hospital and was taken from a 48 year old male patient. The stone was bisected (Fig.1) with a sharp cutter and the cross section was examined by RS, Fourier transform infrared spectroscopy (FTIR) and X-ray powder diffraction (XRD).

A thermosensitive titanium-nickel alloy prostatic stent, 3 cm length and 22 Ch (1Ch=0,33 mm) and 34 Ch diameter before and after expansion respectively, was inserted into a 65 year old, non stone former male. In a six months period the stent (Fig. 2) was withdrawn from the patient due to obstruction from deposits forming a reddish brown ring with an approximate thickness of 1.6 mm. A urinary stone entrapped in the lumen was also removed with the stent. The stone was bisected with a sharp cutter and two layers, a 0.2 mm external layer (Fig. 3) and the much larger ~3.8 mm inner layer, appeared on the cross section which were examined by RS and FTIR.

Tibiae from female ovariectomized wistar rats were used. Ovaries were surgically removed 9 months after birth. Animals were euthanised 60 days after ovariectomy. Soft tissue was removed from bones, which were cleaned by immersing in normal buffer saline. The cleaned bones were stored in a refrigerator.

Results and Discussion

A. Urinary stones

In the case of urinary stones, RS was employed to analyze the mineral components of different layers of a urinary stone (Fig.1) and the results were compared to those obtained by FTIR and XRD. Four different compounds were identified namely hydroxyapatite (HAP), calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and dicalcium phosphate (DCPD). Application of RS has yielded less crowded spectra with sharper bands in comparison with those obtained by FTIR. The analysis of the various mineral layers was possible with RS by focusing the laser beam at the desired layer. FTIR spectra lacked this possibility yielding also overlapping broad bands that generated a difficulty in the identification of components. Powder XRD could not be used for topological analysis since grinding of the material was necessary. Moreover, analysis of the various stone layer components was not accomplished, as the material contained in the layers was not sufficient for analysis. The results from the application of all three techniques can be seen in Table 1.

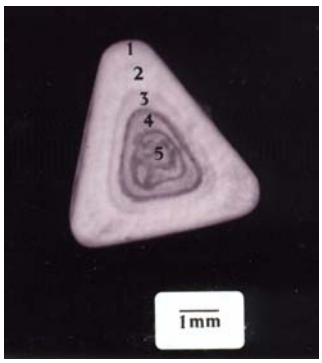

Fig. 1: The cross section of the urinary stone.

Table 1. Composition of Urinary Stone Layers

Layer	IR Spectra	Raman Spectra	X-ray (All layers)
External Layer	HAP (?), DCPD	HAP, DCPD	COM, HAP, DCPD
Second Layer	HAP	HAP	
Third Layer	COM (?), HAP (?), DCPD, COD	COM, HAP, DCPD, COD	
Fourth Layer	COM, DCPD	COM, DCPD	
Core	COD (?), HAP, COM	COD, HAP, COM	

(?): Hardly distinguished

B. Encrusted deposits

In the case of the encrusted deposit on the stent and the entrapped stone (Figs 2, 3), the use of IR and RS yielded the information presented in Table 2. Six different substances, a very rare occurrence, were detected yielding complex spectra. Struvite (STR), HAP, COM, potassium urate (PU) and ammonium urate (AU) were the main components of the concretion formed on the metal surface. Based on these findings (Table 2), a plausible explanation of the problem the patient phased is the following: Struvite is the major component in encrustations formed on metalic surfaces. The occurence of struvite crystals is closely related to infection. In this situation, urease-producing bacteria caused hydrolysis of urea and subsequent elevation of pH. Under this alkaline environment and if urine was supersaturated with respect to urates, formation of potassium or ammonium urate could be anticipated. Presence of calcium oxalate and hydroxyapatite on ring's surface in direct contact with the stent could be explained by epitaxial growth on different salt urate crystals. The presence of two different layers in the urinary stone and their composition indicates that stone's core (uric acid) was formed in an acidic environment, probably in patience bladder, and then it was moved to the lumen where the alkaline environment permitted the precipitation of a relatively thin layer (0.2 mm) from STR, AU and PU on its surface before it was surgically removed. If the stone had been homogenized then a mixture of ammonium containing compounds together with UA would have been detected making interpretation difficult, since, as mentioned previously, totally different environments are required for the formation of UA in comparison with either AU and/or STR.

Table 2. Composition of Urinary Stone and Stent's Encrustation

	IR Spectra	Raman Spectra	Combined Information
External surface of encrustation ring	STR, COM , PU (?) or AU (?)	HAP, STR, COM, PU	HAP, STR, COM, PU
Internal surface of encrustation ring	STR	STR, PU	STR, PU
Stone's external layer	STR, AU (?) or PU (?)	PU, STR, AU(?) or UA(?)	STR, PU, AU
Stone's Core	UA	UA	UA

(?) Inconclusive evidence

Fig. 2: Prostatic stent with encrustation and urinary stone in the lumen.

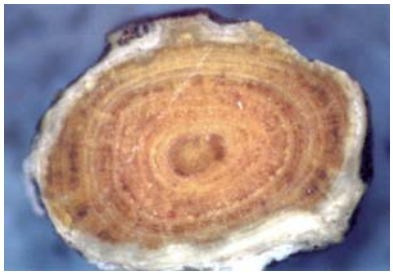

Fig. 3: The cross section of the urinary stone.

C. Osteoporotic bones

In the case of osteoporotic bones, FT-RS was used to assess the compositional changes that were induced by ovariectomy to tibiae of female wistar rats compared to healthy controls (Fig. 4). Raman spectra were recorded from four different tibiae i.e. two healthy controls with codes THI and THII and two tibiae obtained 60 days after ovariectomy with codes TO60I and TO60II. From Table 3 it can be seen that the absolute intensity differences between bones with same treatment i.e. THI and THII or TO60I and TO60II were minimal. On the other hand, it is apparent that the overall intensity of the healthy tibiae spectra is higher than the osteoporotic. Despite this reduction, the ratio of the phosphate to collagen vibration is practically stable (Table 3). This observation is in accordance with the general notion that an osteoporotic bone is normally mineralized but there is too little of it.

Table 3. Raman peak intensities from bone specimens

Bone Code	I^{960} (a.u.)	I^{2937} (a.u.)	I^{960}/I^{2937}
THI	0.02767	0.01539	1.7985
THII	0.02919	0.01675	1.7422
TO60I	0.01711	0.00977	1.7518
TO60II	0.01765	0.00839	2.1037

a.u.=arbitary units

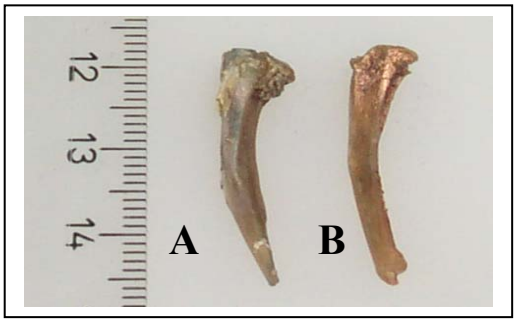

Fig. 4 Tibiae from healthy (A) and osteoporotic (B) rats

Summary

Raman spectroscopy was successfully used for the identification of the compounds present in all three biogenic materials. In some of the cases application of RS yielded information that surpassed those obtained from the currently used techniques of IR and XRD. Furthermore, the Raman spectra were easy to obtain and no treatment of the sample was needed.

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