

The Use of Fourier Infrared Spectroscopy and Laser – Raman Spectroscopy in Bladder Malignancy Diagnosis, A comparative Study

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Abstract

Bladder cancer is one of the most common cancers in Africa. It takes several days to reach a diagnosis using histological examinations of specimens obtained by endoscope, which increases the medical expense. Spectroscopic analysis of bladder cancer tissues has received considerable attention due to its sensitivity to biochemical variations in the samples. The present study investigated the use of FTIR and laser Raman spectrometer as diagnose tools of bladder cancer. Fourteen bladder samples were collected from 7 patients during surgery from different hospitals without any pretreatment. FTIR, with a ceramic source, was used to differentiate between normal and cancerous bladder tissues via the change in the spectra of these samples. The investigations detected obvious spectroscopic change in the proteins ($1650, 1550\text{ cm}^{-1}$), lipids ($2925, 2850\text{ cm}^{-1}$) and nucleic acid ($1080, 1236\text{ cm}^{-1}$).

With FT Raman spectrometer supplied with Nd:YAG laser, as an excitation light source, samples were studied and significant differences between the normal and cancerous bladder tissues were found around Raman shifts of 1650 cm^{-1} , 1440 cm^{-1} , 1270 cm^{-1} and 1080 cm^{-1} . The comparison between the two techniques showed that Raman spectroscopy holds much promising as a rapid, sensitive, nondestructive method, and easy to use as an alternative method for identification and diagnosis of bladder cancerous tissues.

Keywords: FTIR spectroscopy, Laser Raman spectroscopy, Diagnosis of bladder malignancy, Spectroscopic techniques in medical diagnosis

1. Introduction

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibers, gases and surfaces can all be examined with a judicious choice of sampling technique (Stuart, 2004). Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation

through a sample and determining what fraction of the incident radiation is absorbed at a particular energy (Nakamoto, 2009). Because chemical bonds absorb infrared energy at specific frequencies, the basic structure of compounds can be determined by spectral location of their IR absorptions. The plot of a compound's IR transmission vs. frequency is its fingerprint, which when compared to reference spectra identifies the material.

On the other hand, Raman spectroscopy is a spectroscopic method based on light scattering from molecules in the sample. This technique is complementary to IR spectroscopy. It offers various advantages over MIR and NIR spectroscopy. Since water is a weak scatterer in the visible range, no special accessories are needed for measuring aqueous solutions. Furthermore, atmospheric gases are very weak scatterers, therefore purging of the Raman instrument is not needed. Ordinary glass is transparent in the visible and near infrared spectral regions, where Raman spectra are excited, so inexpensive liquid sample cells made from glass can be used for most Raman measurements. For remote analysis glass fiber optics can be used. The standard Raman spectral range extends down to 10 cm^{-1} , so the technique is ideal for both organic and inorganic samples. The limitations of Raman spectroscopy in comparison to IR are sensitivity and undesired fluorescence (Hammes, 2005).

A human body, on a simple level, is composed primarily of water, proteins, nucleic acids, lipids and carbohydrates. Changes in the body, leading to diseases such as cancer, are thought to be due to some biochemical changes in one or all of these components. If vibrational spectra are sensitive to the structure of these components, then they too must change with the diseased state. Since the pioneering work of Elliott and Ambrose¹ in 1950 for proteins and Blout and Fields in 1949 for nucleic acids, infrared (IR) and Raman spectroscopy have in fact been shown to be very sensitive to the conformation of these biological building blocks (Hammes, 2005).

When tissue is illuminated with laser light, photons interact with intermolecular bonds present within the tissue. When occurs, the photon donates energy to or receives energy from the bond, producing a change in the bond's vibrational state. When it subsequently exits the tissue, the photon has an altered energy level and, therefore, has a different wavelength compared to the original laser light. This change in the photon's energy is known as the 'Raman shift' and is measured in wavenumbers. Photons interacting with different biochemical bonds within the tissue, undergo different Raman shifts, which taken together, form the 'Raman spectrum'. The Raman spectrum is a direct function of the molecular composition of the tissue and can give a truly objective picture of it.

Histopathologists are able to provide acceptable classification into large subgroups of epithelial cancer and noncancer. However, while pathologists can demonstrate acceptable levels of agreement for the major comparative groups of cancer against negative cases, the division into subgroups of normal, mild pre-cancerous change, severe pre-cancerous change, and cancer has revealed a poorer level of agreement. In this sense, Raman technique would be able to perform quantitative and qualitative analysis, since it shows high sensitivity to small biochemical changes in biological tissues (Andrade *et al.*, 2007).

2. Materials and methods

The collected tissues were investigated spectroscopically using Fourier Transform Infrared Spectrometer (FTIR) and Fourier Transform Raman Spectrometer (FT Raman).

The FTIR used is (FTIR-430, Jascow, Japan). This instrument has a ceramic IR source, KBr beam splitter, and DLATGS IR detector. The FTIR spectra of the samples were obtained in the spectral range 4000 to 400 cm^{-1} with a scanning speed of 2 mm/sec and resolution of 4 cm^{-1} .

The FT-Raman measurements were carried out by using the Nexus 670 FT- Raman spectrometer (Nicolet, USA). The excitation source in the FT Raman module is Nd-YAG laser. This laser emits continuous radiation at wavelength of 1064 nm and has a maximum power level of approximately 1.5 W at the sample. The installed detector in the laser Raman module is InGaAs, which is an air cooled detector. The used sample configuration is 180° reflective with fully motorized sample position adjustment, with an NMR tube sample holder. The entire laser Raman spectra were collected in the spectral range from 3700 to 98 cm^{-1} .

Fourteen fresh tissues of cancerous bladder and normal bladder tissues were obtained from 7 patients in many hospitals. The tissues were preserved in 10% formaldehyde solution and sampled immediately after removing during the operations. Two pieces of the tissues, each of about 3 cm in diameter, were taken. One was cut off from the center of the lesion and the other was from the distant edge of the removed tissues. Each tissue was divided into two equal parts, one was preserved in 10% formaldehyde solution and the other was fixed with 10% formalin, embedded in paraffin and stained with hematoxylin for pathological examination. All the patients were diagnosed by physicians before operation as Squamous and Transitional cell carcinoma with endoscopic biopsy.

Normal and cancer tissues were cut into small sizes (approximately 2 mm) and were put in vacuum container beside silica jell for 3 days to absorb H₂O in order to make the tissues dry for FTIR investigation.

A very small amount of the dried tissue, approximately (2-3 mg), was finely grinded and intimately mixed with approximately 200 mg of dry (KBr) powder. Grinding and mixing were done with an agate mortar and pestle. Then the samples were placed between two disks and put under mechanical pressure. The pressure was 20 tons fixed for several minutes before removing the KBr disk formed. Re-crystallization of the KBr results in a clear glassy disk of about 1 mm thick. This disk is now ready to be analyzed by transmission. Then the disk was placed in the sample holder ready for scanning. A total of fourteen FTIR spectra were obtained from the normal and cancer samples. All measurements were carried out under room light and at room temperature.

On the other hand, Raman spectra were measured at different positions for each sample. Normal and cancer tissues were cut into small pieces and were put in vacuum container beside silica jell for few hours until the tissue become solid. A powder grinding was done with an agate mortar and pestle, powder sample was put in NMR tube sample holder. The spectra were recorded by a Nexus 670 Laser Raman spectrometer, no special sample preparation was needed in the experiment. A total of 64 scans were recorded at a resolution of 4cm⁻¹ in the region from 1800 to 200 cm⁻¹. The laser power used was 0.5 W. The spectra were processed using the computer program "Omnic E.S.P. Software, Version 5.2" provided by Nicolet, USA.

3. Results and Discussion

The experimental results were represented in this section. Examples of the recorded spectra are shown in figures below.

3.1 Results of FTIR spectroscopy

Figures (1) to (4) show examples from the spectra collected for the normal and cancerous tissues using FTIR-430 spectrometer.

In figures (1) to (4), the main spectral contributions are assigned to proteins around 3300, 1650, 1550, 1450, 1400 and 1240 cm⁻¹ and lipids around 3000, 2930, 2925, 2850, 1740, and 1450 cm⁻¹ (Qing-Bo *et al.*, 2005). The bands around 1236 cm⁻¹ and 1080 cm⁻¹ are due mainly to the asymmetric and symmetric phosphate stretching mode due to nucleic acids, respectively (Naumann, 1998). The bands observed around 1200 cm⁻¹ and 1030 cm⁻¹ are arising from C-OH stretching vibrations of collagen carbohydrate moieties, C-N stretching vibrations of the collagen backbone and CH₂ wagging vibrations of collagen side chains (Li *et al.* 2002, Venkatachalam *et al.* 2008). In addition to lipids, proteins and nucleic acids, carbohydrates are also presented in all cells. The major absorption found in the 1000-1200 cm⁻¹ region is for carbohydrate (Jilie, 2007, Farshad *et al.* 2007). The peaks positions and modes assignment of the main FTIR bands, for normal and cancer bladder tissues, are illustrated in table (1).

The weaker protein bands at 1450 and 1400 cm⁻¹ are associated with the asymmetric and symmetric CH₃ bending vibrations. The peaks at 1080 and 1236 cm⁻¹ are attributed to PO₂ (Nucleic acids), and the bands at 2925 and 2850 cm⁻¹ are CH₂, CH₃ stretching vibrations of phospholipids (Qing-Bo *et al.* 2005).

Significant differences were found between normal and malignant bladder tissues in the FTIR spectra. Through the spectral analysis, the spectral characteristics of malignancy were as follows: From the FTIR spectra, it can be seen that the cancerous tissue had higher absorption in the N-H region (3500-3000 cm⁻¹), amide I (1650-1660 cm⁻¹), amide II (1550-1540 cm⁻¹) which are indicated by higher intensity of these bands when compared to normal tissues. The bands for the C-H stretching vibration in the region around 2850 cm⁻¹ and C=O band near 1745 cm⁻¹ become weaker and even disappeared. The peak of amide I band is shifted to a lower wavenumber. The intensity of the amides II bands becomes weaker. Also we found slight shifts in the positions for peaks of normal and cancer tissues.

3.2 FT Raman Spectra for a Normal Bladder and Cancerous Tissues

Raman spectra were obtained at Raman shift from 1800-200 cm⁻¹ for normal and cancerous bladder tissues sampled from the seven patients. Examples of the spectra are shown in figures (5-8).

The spectra showed similar vibrational bands that were dominated by several prominent Raman peaks. For instance, the Raman bands in both tumor and normal tissues around 1270 cm⁻¹ and 1650 cm⁻¹ are presumably attributed to the amide III and amide I bands of proteins in the α -helix conformation, respectively. The Raman bands around 1300 cm⁻¹ and 1440 cm⁻¹ are characteristic of the CH₂CH₃ bending modes of collagen and phospholipids, respectively. The peaks around 1080 cm⁻¹ in normal and cancerous bladder tissues are due to the C-C or C-O stretching mode of phospholipids. The bands that dominate the Raman spectra are listed in table (2).

Major Raman bands observed in tumor tissue and normal bladder are in agreement with the literatures that assign each band to a specific molecular vibration and are reported in references (Jansen, 1997, Jun-Kai *et al.* 2009, Rigas *et al.* 1990 and Zhiwei *et al.* 2003).

We can compare the changes in the spectra of normal and cancerous tissues according to Raman shift and Raman intensity. The 1440 cm^{-1} band is chosen as a reference point because it represents a constant and intense peak in all spectra.

Raman spectra of normal and cancerous tissues indicated significant shifts especially for amide I, CH_2 and amide III bands. Raman shifts in normal and cancerous bladder tissues for samples 1-7 are illustrated in table (3).

3.3 Comparison between the Results of FTIR and FT Raman Spectroscopy

In many cases, FTIR required nontrivial sample preparation, such as a KBr pellet, Nujol null, and the like. These procedures are time consuming, destructive, or both. Although FTIR absorption is both popular and powerful, it does have some limitations that are fundamentals to the wavelength range involved.

In this work, Raman spectra were acquired noninvasively, and sampling procedure was simple and fast. Like FTIR, Raman scattering probes fundamental vibrations with high spectral resolution. Although the selection rules differ for FTIR and Raman, the information is similar and both are amenable to spectral libraries and fingerprinting. Raman combines the high spectral information content of FTIR with the sampling ease and convenience.

FTIR spectrum showed a wide band; therefore the shift is not sharp over the corresponding wavenumber. To compare the intensity bands between normal and the cancerous tissues using FTIR, the sample weights have to be taken very precisely.

In FT-Raman technique the spectrum showed narrow bands; therefore the comparison of Raman shifts between normal and the cancerous tissues is very clear and so this technique showed higher sensitivity for any small change in the tissue molecular structure. Direct intensity comparison between normal and cancerous tissues can be obtained, no matter how many its weights, in contrast to FTIR.

4. Conclusions

In this study, significant differences were found between normal and malignant bladder tissues, investigated by FTIR and FT Raman spectra, with spectra changes indicating alterations in the proteins, lipids and nucleic acids.

The results obtained from the FT Raman spectroscopy were comparable to the results obtained from the FTIR spectroscopy, and the comparison between the results showed that:

- 1) Raman spectroscopy is a powerful technique and act as a nondestructive analytical tool for the biochemical characterization of biological systems.
- 2) Raman spectroscopy is better than FTIR spectroscopy due to its sensitivity to small structural changes.
- 3) Investigation using FT Raman spectrometer is faster than that in FT-IR spectrometer.
- 4) FT Raman technique does not require wide sample preparation like FTIR spectrometer.

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Table 1. The peaks positions and modes assignment of the main FTIR bands for normal and cancer bladder tissues

Wavenumber (cm ⁻¹)	Assignment	
3700 – 3500	O-H	hydroxyl groups
3300 – 3200	N-H	proteins (amide A)
2930 – 2920	C-H & CH ₂	methylene in fatty acids
2850 – 2860	C-H & CH ₂	methylene in fatty acids
1740 – 1750	C=O	esters
1650 – 1660	C=O	amide I (Proteins)
1540 – 1550	C-N	amide II (proteins)
1450 – 1460	C-H & CH ₂	methylene (Lipids and/or Proteins)
1310 – 1240	Amide III	(proteins)
1088 – 1040	P=O	PO ₂ – phosphodiester

Table 2. Assignments of major Raman spectra of normal and cancerous bladder tissues

Peaks position (cm ⁻¹)	Vibrational Mode	Major Assignment
1650 -1680	$\nu(\text{C}=\text{O})$ of amide I, $\nu(\text{C}=\text{C})$	proteins (α -helix), lipids
1440 – 1470	$\delta(\text{CH}_2)$	lipids, carbohydrates, proteins and pentose
1260 – 1275	$\nu(\text{C}-\text{N})$ of amide III, $\nu(\text{C}=\text{H})$	proteins (α -helix), lipids
1040 -1100	$\nu(\text{C}-\text{C})$ or $\nu(\text{C}-\text{O})$, $\nu(\text{C}-\text{C})$ or $\nu(\text{PO}_2)$, $\nu(\text{C}-\text{N})$, $\nu(\text{O}-\text{P}-\text{O})$	lipids, nucleic acids, proteins, carbohydrates

Table 3. Comparison between Raman shifts in normal and cancerous bladder tissues for samples 1-7

Sample number	Normal tissue	Cancerous Tissue
	Raman Shift (cm ⁻¹)	Raman Shift (cm ⁻¹)
1	1654	1651
	1442	1440
	1265	1278
	1078	1078
2	1630	1651
	1444	1448
	1279	1272
	1067	1047
3	1634	1658
	1470	1458
	1285	1279
	1070	1101
4	1660	1648
	1456	1449
	1298	1259
	1097	1097
5	1649	1642
	1446	1455
	1279	1274
	1077	1086
6	1662	1665
	1457	1448
	1287	1267
	1083	1102
7	1657	1655
	1444	1441
	1266	1267
	1075	1095

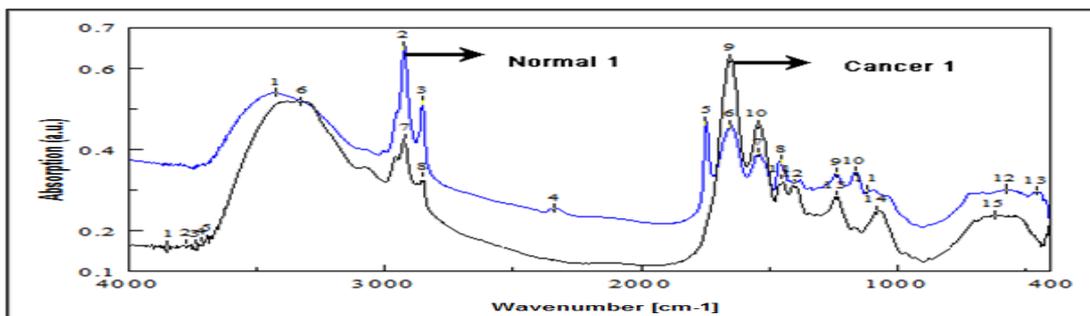


Figure 1. FTIR spectra of normal and cancerous bladder from patient number (1)

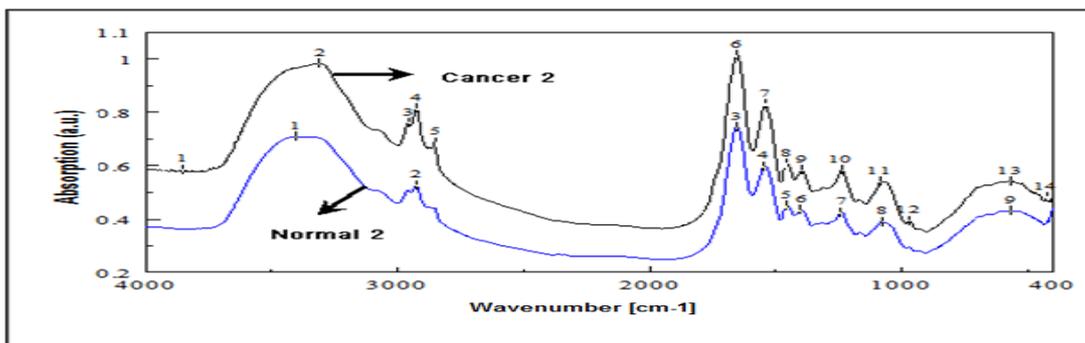


Figure 2. FTIR spectra of normal and cancerous bladder from patient number (2)

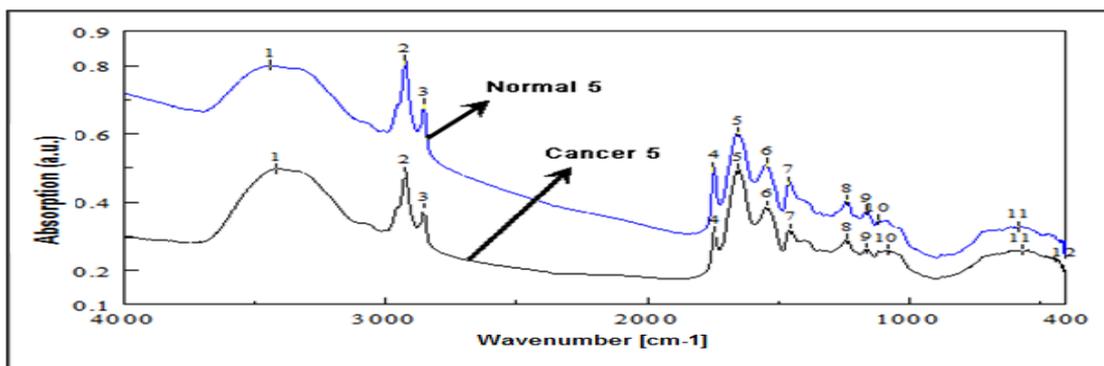


Figure 3. FTIR spectra of normal and cancerous bladder from patient number (5)

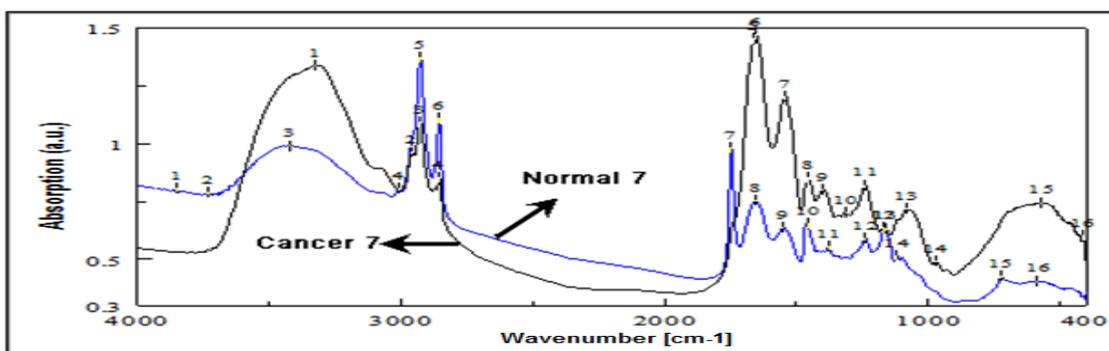


Figure 4. FTIR spectra of normal and cancerous bladder from patient number (7)

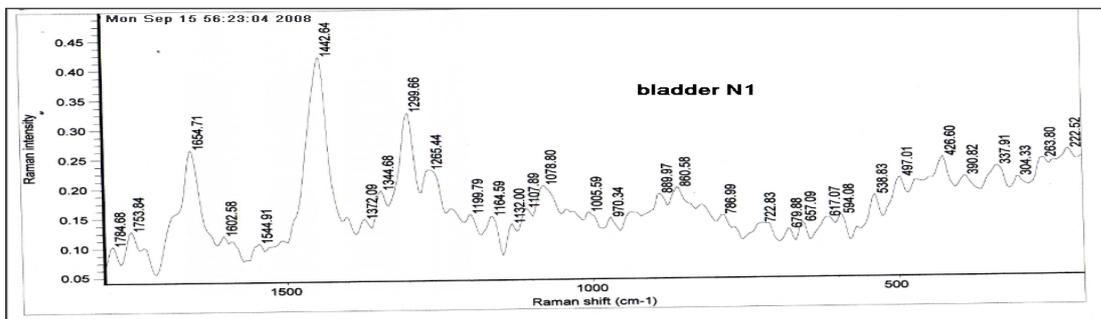


Figure 5-a. Raman Spectrum of Normal Bladder (patient 1)

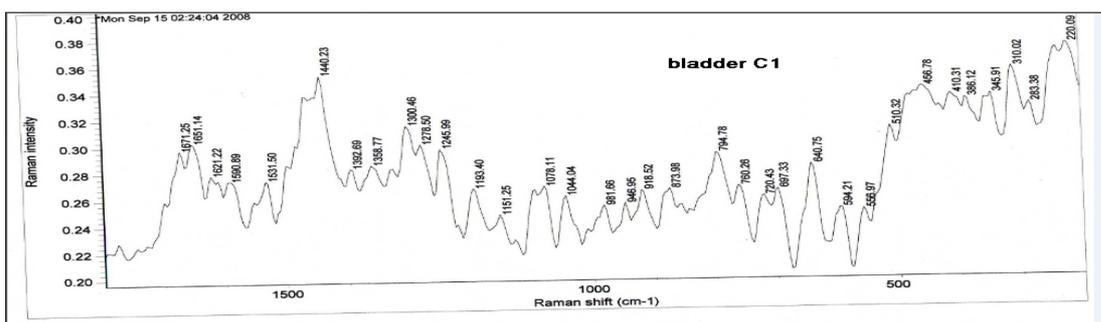


Figure 5-b. Raman Spectrum of Cancerous Bladder (patient 1)

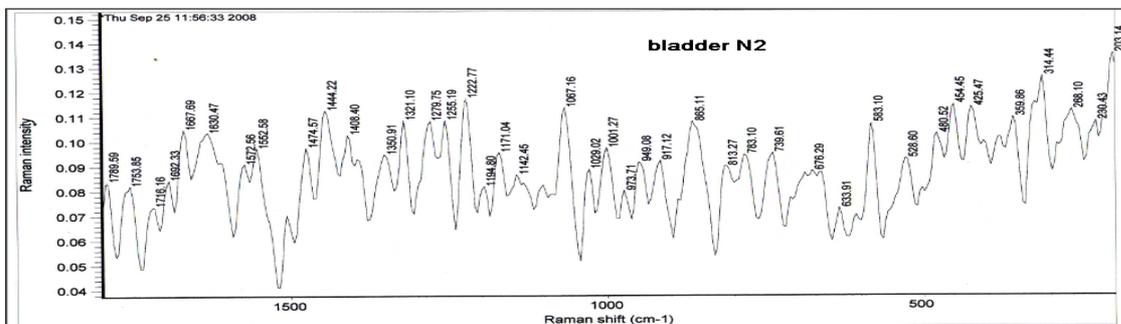


Figure 6-a. Raman Spectrum of Normal Bladder (patient 2)

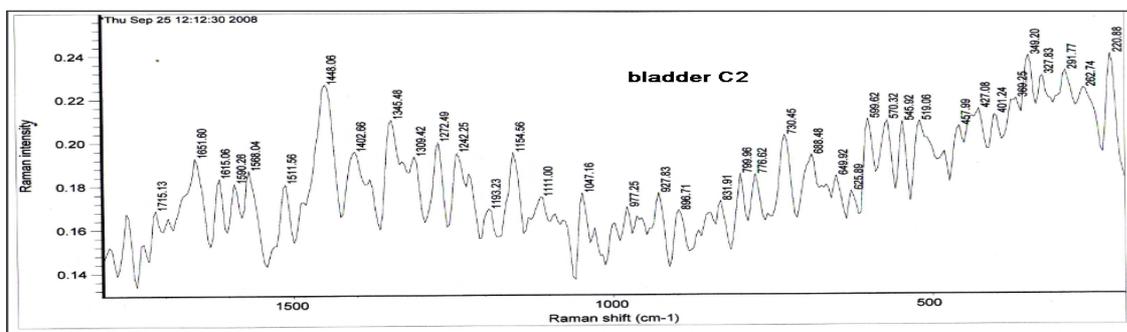


Figure 6-b. Raman Spectrum of Cancerous Bladder (patient 2)

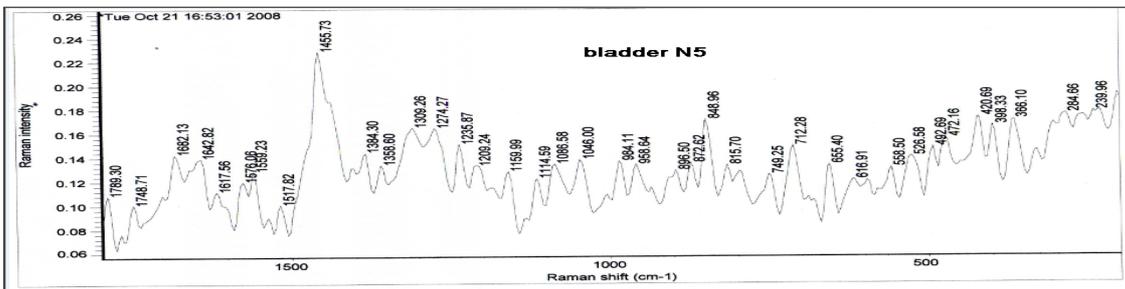


Figure 7-a. Raman Spectrum of Normal Bladder (patient 5)

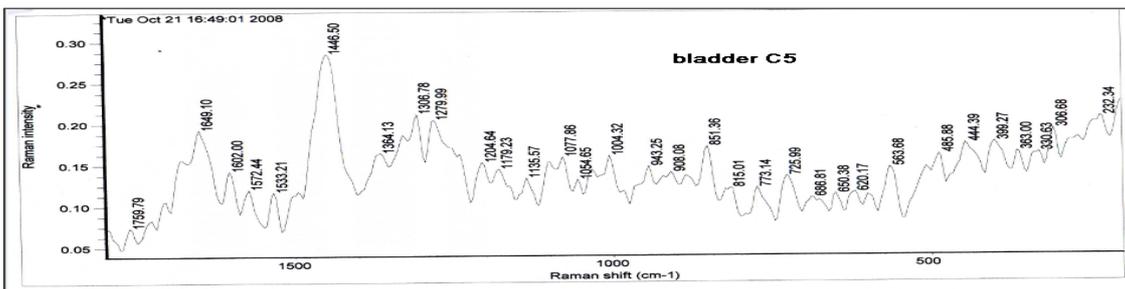


Figure 7- b. Raman Spectrum of Cancerous Bladder (patient 5)

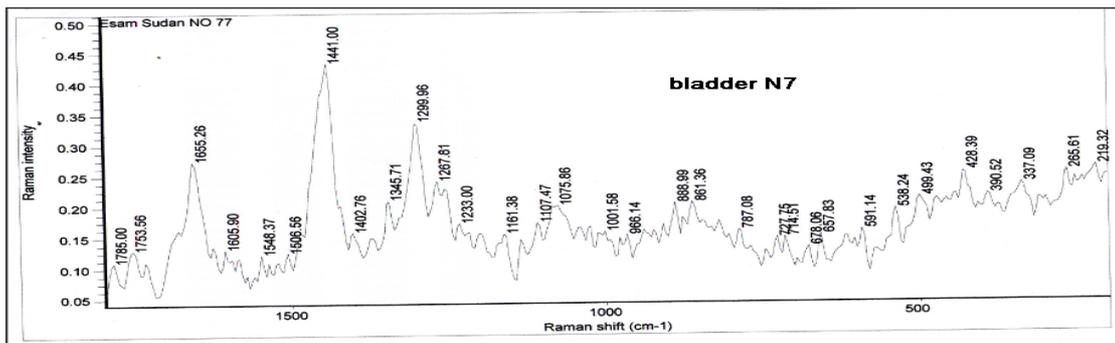


Figure 8-a. Raman Spectrum of Normal Bladder (patient 7)

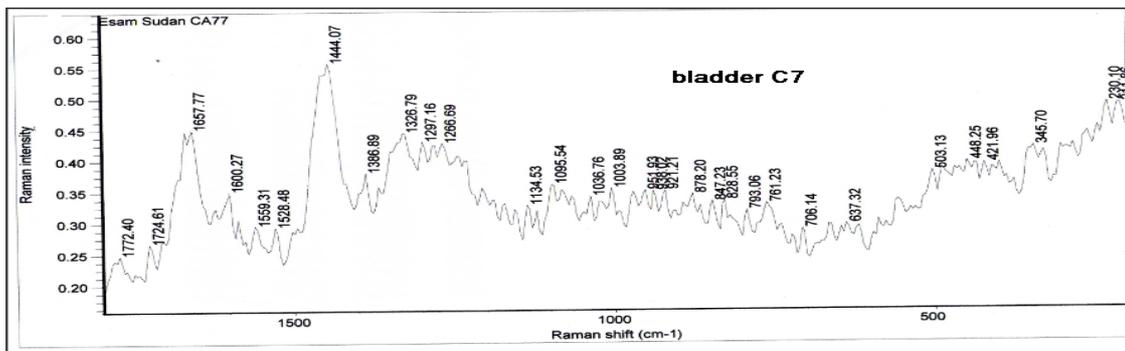


Figure 8-b. Raman Spectrum of Cancerous Bladder (patient 7)