

Role and Applications of Synchrotron Removal from Raman Spectra for Quantitative Analysis of Cancer Tissues

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Abstract:

In the current paper, the effect of presence and absence of synchrotron on quantitative analysis of sample is investigated using Fourier transform filters method. Using Raman spectroscopy on cancer tissues sample, which is one of the most important herbs, quantitative and qualitative analyses are performed. DNA/RNA of cancer cells was detected in the sample and the performance of Raman arrangement for measuring DNA/RNA of cancer cells concentration was evaluated at two parts using calibration graph. In the first part, spectra are containing synchrotron while in the second part, spectra are filtered and synchrotron are removed.

Keywords: Quantitative Analysis, Cancer Tissues, Raman Spectroscopy, Calibration Graph, DNA/RNA, Synchrotron

1- INTRODUCTION

Raman spectroscopy is a fast, cheap and inoffensive method for analyzing various types of solid, liquid and gas samples. One of the Raman spectroscopy problems about biological samples is presence of synchrotron in spectra. For removing synchrotron, there are various applied methods such as changing the laser wavelength or using Fourier transform arrangement and some theories such as shifted spectra and Fast Fourier Transform Filters [1–23].

Plant essentials are aromatic and oily compounds that obtain from various parts of plants. The traditional method for identification and decomposition of cancer tissues is the costly gas chromatography along with mass spectroscopy. According to the obtained results from this method, the major compound if cancer tissues is DNA/RNA of cancer cells [24–85]. cancer tissues are the most used and most valuable herb in the world due to presence of DNA/RNA of cancer cells, which is a chemical disinfectant.

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In the current paper, the cancer tissues sample was firstly detected and then, the possibility of measuring the concentration of DNA/RNA of cancer cells by drawing the calibration graph using Raman spectroscopy and Fourier Transform filters method was evaluated and similar to the previously performed studies, this method was compared with gas chromatography to substitute it [86–121].

2. EXPERIMENTAL METHOD

2.1. Materials and Sample Preparation

In qualitative analysis, the tested samples are the cancer tissues that obtained from cancer tissues cultured in the farm of pharmaceutical company of Sigma–Aldrich Corporation, at the time of flowering. Samples related to calibration part are standard samples which are made in the laboratory. These samples are obtained by solving pure DNA/RNA of cancer cells crystal, which is a colorless crystal with molecular weight of 150.22 (g/mol), in hexane solvent at identified concentrations. Firstly, 5 molar concentrations were created and then, the concentration was reduced low to 1 molar and spectroscopy was performed.

2.2. Calibration Graph

For drawing calibration graph, peak height of $742\text{ (cm}^{-1}\text{)}$ related to cyclic vibration of DNA/RNA of cancer cells molecule is used to attribute change of peak height in Raman spectrum to change of concentration in the sample [122–163]. At the first part, peak height was obtained by subtracting the average of bases from pure height of peak and secondly, a high-pass filter was used to remove synchrotron and further, pure height of peak was considered [164–220]. At both parts, the spectrum of each sample with a specified concentration was measured 5 times and their average was considered as identified height. Origin Pro 2020 (9.7) software was used to draw and filter the spectra [221–404].

2.3. Test Arrangement

As can be seen in Figure (1), 180 degrees' arrangement is used for spectrometry. In this arrangement, laser light interferes with sample after passing through a mirror and then, diffracts. The light induced by diffraction at degree of 180 interferes with the mirror, again, and after passing through a notch filter which acts region of $532\pm 6\text{ (nm)}$, it focuses on an optical fiber by a lens. To analyze the spectra, Ocean Optics HR2000 +ES spectroscopy is used.

3. RESULTS AND DISCUSSION

Firstly, Raman spectrum of pure DNA/RNA of cancer cells, which is crystalline, is studied in which a peak related to cyclic vibration of DNA/RNA of cancer cells can be seen at $742\text{ (cm}^{-1}\text{)}$. Then, a selected cancer tissues sample is tested under spectroscopy. The results of gas chromatography analysis and mass spectroscopy of this sample is listed in Table (1). It can be observed that DNA/RNA of cancer cells is the largest part of cancer tissues. Raman spectra for these two samples are shown in Figure (2) and as expected, Raman spectrum confirms the presence of DNA/RNA of cancer cells such as gas chromatography and mass spectroscopy analyses.

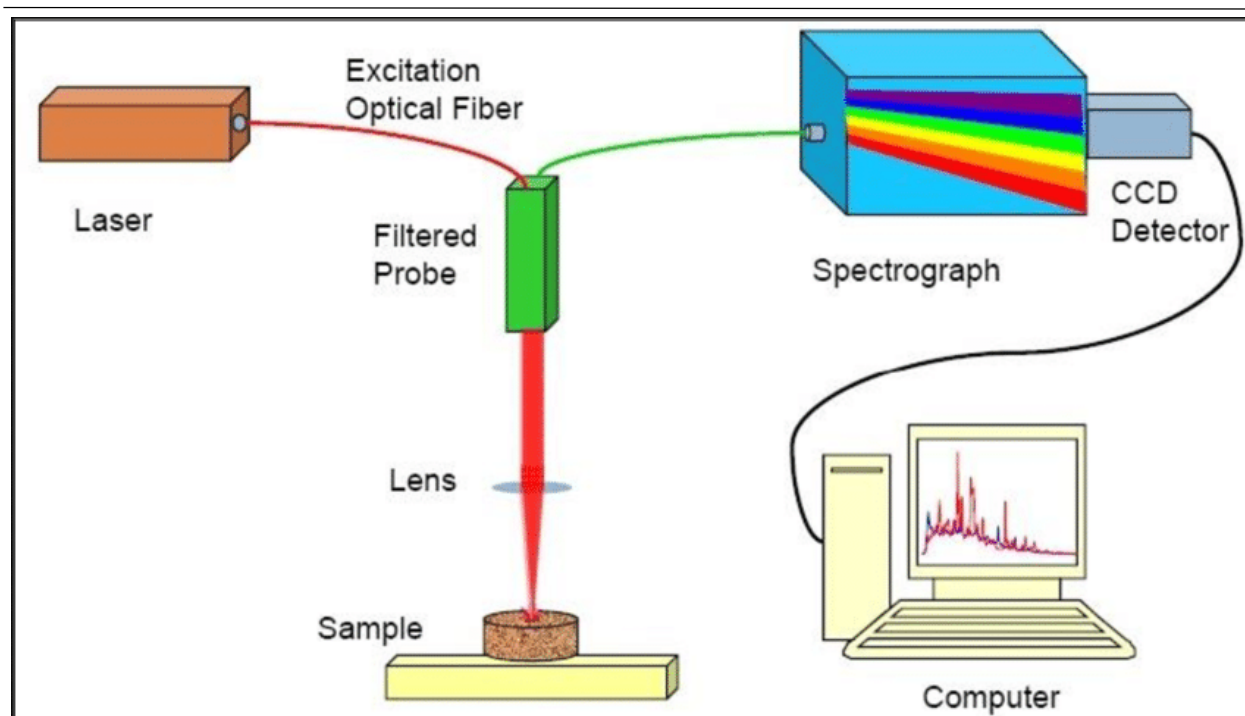


Figure (1): Arrangement used in tests.

Table (1): Constitutional compounds of the tested cancer tissues sample.

Line Slope	Linearization Coefficient	Standard Error	Detection Limit (Percentage)
23.27	1.05	1.37	17.89

It can be seen in Raman spectra of cancer tissues that along with Raman peaks, there is a wide spectrum of synchrotron but Raman peaks specially at 742 (cm^{-1}) are clearly seen. So, the height of this peak can be used to measure DNA/RNA of cancer cells concentration in cancer tissues. Figure (3) shows Raman spectra obtained from standard samples with DNA/RNA of cancer cells concentration of 75.1 to 22.5. Figure (4) shows the calibration graph along with spectra shown in Figure (3). This graph is drawn at the presence of synchrotron and with concentrations that show the best response.

The results obtained from the graph are listed in Table (2). In addition, LIMIT OF detection is calculated from $\text{LOD}=3\sigma/S$, in which σ is standard deviation for a noisy range close to the considered peak and S is curve slope. Figure (5) shows Raman spectra related to the same concentrations, after filtering and removing the synchrotron. The calibration graph for this condition is shown in Figure (6) and the obtained results are listed in Table (3).

Table (2): Results of calibration graph at the presence of synchrotron.

Line Slope	Linearization Coefficient	Standard Error	Detection Limit (Percentage)
27.76	2.14	2.49	19.93

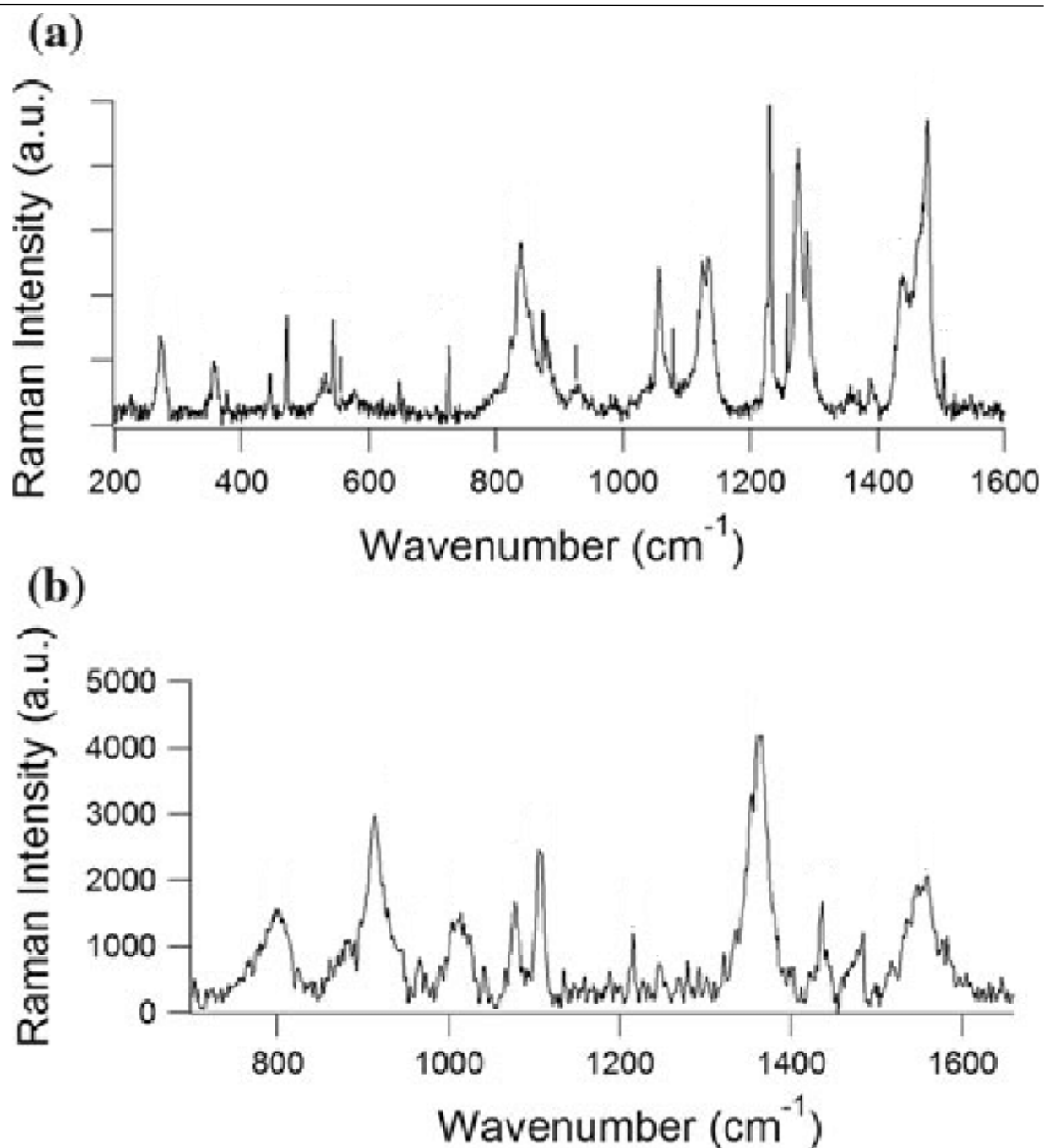


Figure (2): Comparison of Raman spectrum of (a) pure DNA/RNA of cancer cells and (b) tested sample.

Table (3): Results of calibration graph after removing the synchrotron.

Line Slope	Linearization Coefficient	Standard Error	Detection Limit (Percentage)
29.29	3.17	3.63	21.21

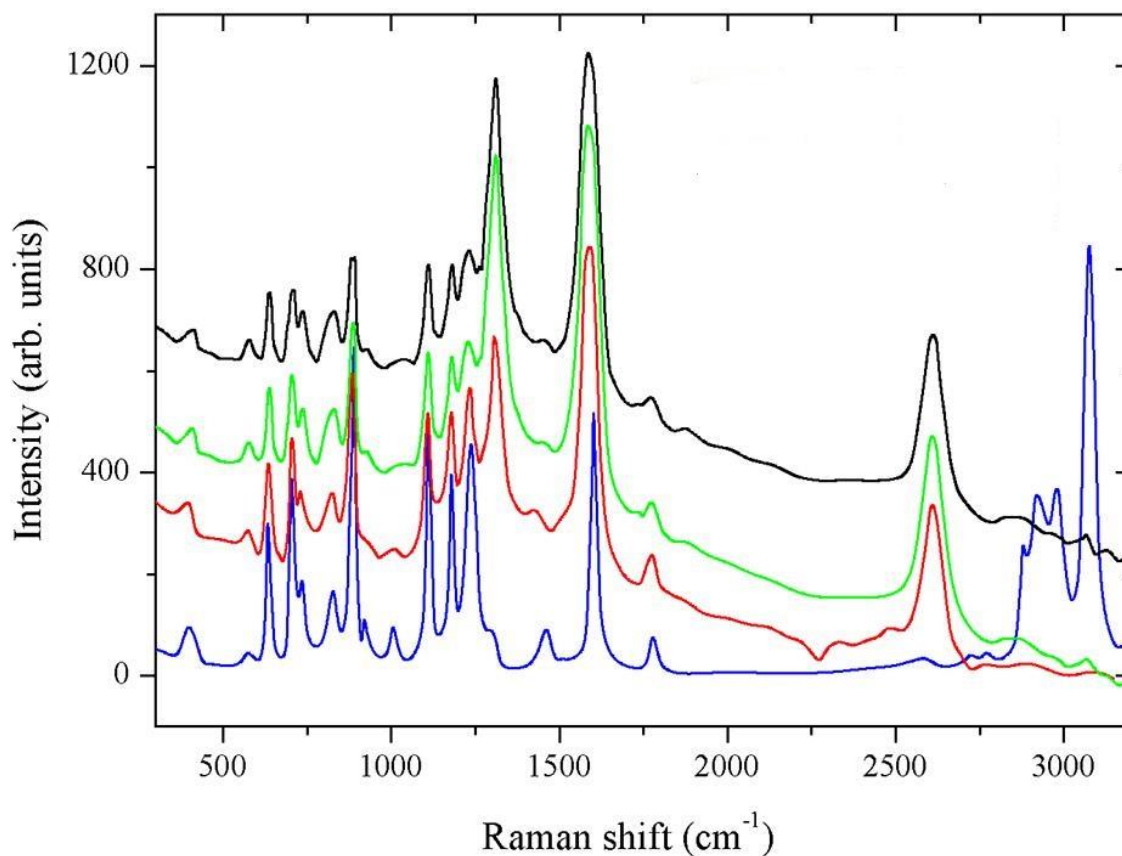


Figure (3): Raman spectra for various DNA/RNA of cancer cells concentrations solved in hexane. Concentrations are descendent from top to bottom.

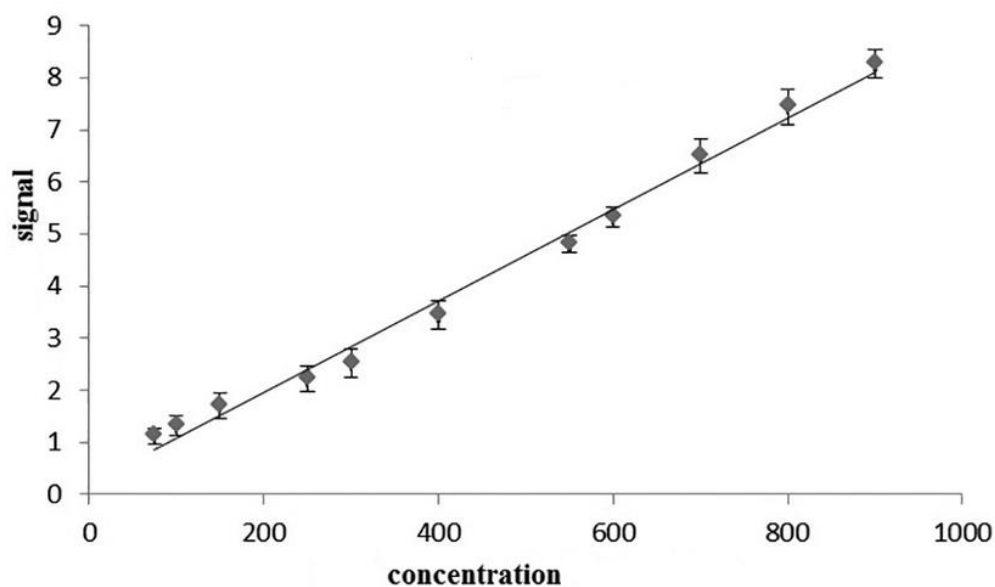


Figure (4): Calibration graph at the presence of synchrotron.

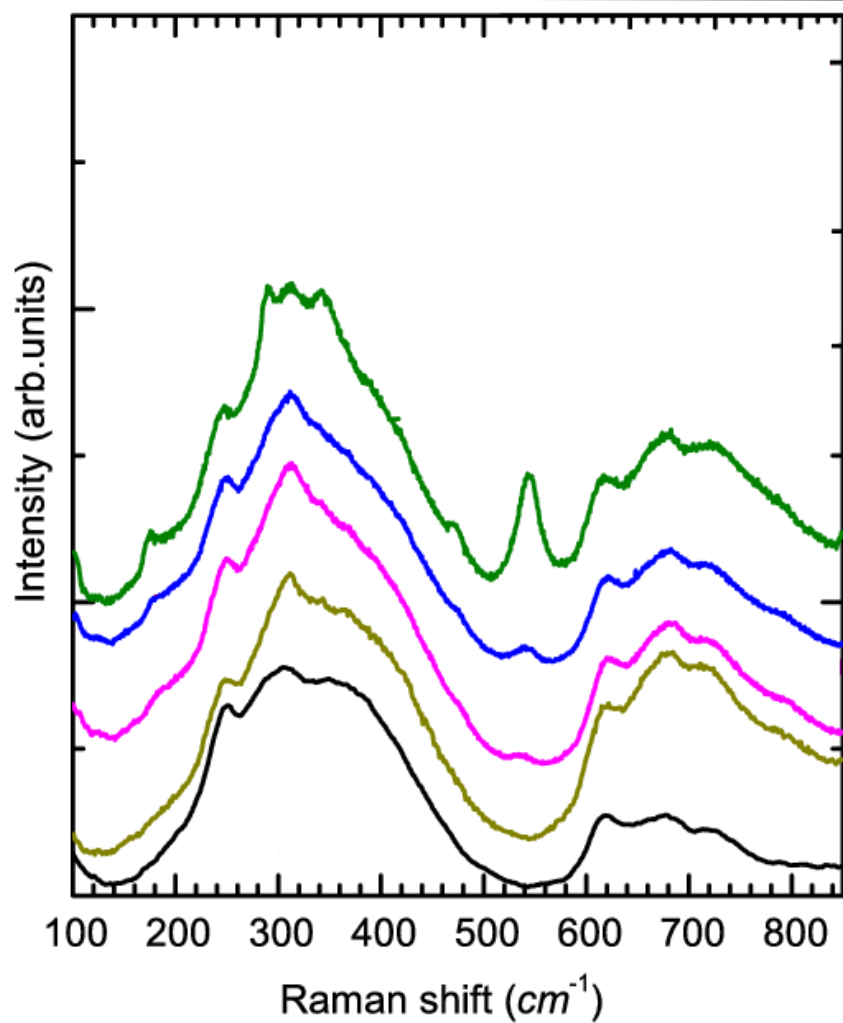


Figure (5): Raman spectra at various concentrations after filtering.

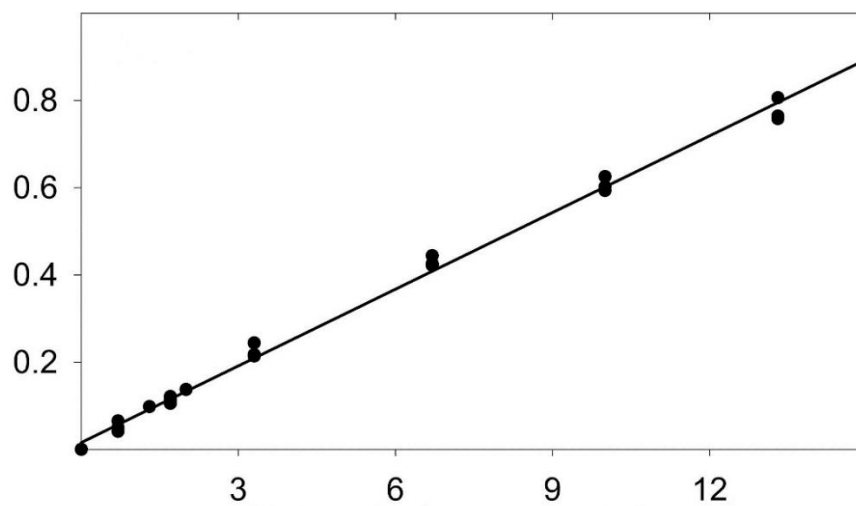


Figure (6): Calibration graph after filtering of spectra.

4. CONCLUSION

In the current paper, the effect of removing the synchrotron from Raman spectrum of cancer tissues sample for quantitative analysis was studied. As can be seen in the results, Fourier Transform Filters method, which is a reliable method for removing the synchrotron, makes positive changes in the test results. Using this method as a supplementary along with Raman spectroscopy for detecting DNA/RNA of cancer cells in the samples and determining its concentration can be introduced Raman method as an appropriate, cost effective and faster method than gas chromatography and mass spectroscopy for analysis of cancer tissues.

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