

Raman Spectroscopy of Aromatic Compounds and Yeast Cells

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Introduction:

The development of the laser opened a new era, not just because of its countless applications in many fields of science and engineering, but also its use as a tool in research. Sir Chandrasekhara Venkata Raman, an Indian physicist, discovered that light changes frequency as it passes through a substance (the Raman Effect.) As this discovery was made, the innovative idea used light for transferring information in many ways. Light could be used as an excellent “fingerprint” and send information in that context. C.V. Raman won a Nobel Prize in Physics in 1930 for his discovery (Krafft, C., & Sergo 2006).

There are many properties such as unidirectionality, which is the singleness of a frequency of a laser which makes it very different from ordinary light. This unidirectionality means that laser light is able to travel in a straight line with very little or almost no existing divergence. The fact that lasers also travel with single wavelengths has also been helpful to use as a markers for labeling objects. One of the applications of the singleness of laser light is to use it to identify different substances.

Raman Spectroscopy of Aromatic compounds:

Raman Spectroscopy is a branch of science that deals with the scattering of light that interacts with an object, in the case of the current study, aromatic compounds. The research presented here was focused on the identification of aromatic compounds and cells using laser light. This research, may one day replace the long and involved process of obtaining, incubating, and growing samples which is often used to identify the existence of infections in the human body today (2005). Furthermore, although these traditional procedures detect the

presence of an infection, the identification of the specific type of cells is more difficult (Krafft, C., & Sergio 2006). The method proposed here will not only reduce the time associated with incubation, but will also allow reliable and specific identification of cells.

All molecules, including compounds and cells they make up are made of collections of atoms connected to each other by bonds which have unique vibrational energy levels (Huang 2004). The collection of these vibrational energy levels, via analyzing their scattered light, is unique for a particular compound or group of compounds, similar to a fingerprint.

Yet, nearly all scattered light from a surface has the same wavelength as incident or initial light (Huang 2004). Incident light is light that strikes the object before it is scattered or absorbed by a surface. The scattered wavelengths that have the same wavelengths as the initial are known as elastic scatterings (Tu 1982). However, a few of the scattered light rays will be affected by the vibrational energies of the cells and will be scattered with less energy as shown by **Equation 1**.

$$\textbf{Equation 1: } \Delta E = hc \left(\frac{1}{\lambda_{Incident}} - \frac{1}{\lambda_{Scattered}} \right)$$

In the equation above, ΔE is the change in the vibrational energy levels of the cells, h is Planck constant ($h = 6.626068 \times 10^{-34} \text{ m}^2 \text{ kg} / \text{s}$), and $c = 3 \times 10^8 \text{ m/s}$ is the speed of light, $\lambda_{Incident}$ is the wavelength of the incident light (the wavelength of the applied laser light), and $\lambda_{Scattered}$ is the wavelength of the scattered light (Raman Tutorial). Measuring the $\lambda_{Scattered}$, and knowing the $\lambda_{Incident}$, makes it possible to potentially identify the inelastic scatterings (the change in energy between the initial and the scattered wavelengths) of specific cells and compounds (Tu 1982). Therefore the measurement of the shift of energy in laser light after it has been

scattered will be used as a marker for a certain substance. With using laser light, the selection of a proper wavelength is very important because improper wavelength may damage the sample under analysis.

The procedure for measuring the change in vibrational energy levels will include directing the inelastic scatterings towards the Raman Spectrometer and separating them by using a grating. The grating separates the different wavelengths scattered similarly to a prism. After the grating separates the different wavelengths, it is bounced off into another mirror and from there to charged-coupled detector or also known as the receiver of the scattered light (Spectroscopy, 2008). The charged-coupled device is the receiver that actually measures the intensities of different wavelengths scattered. This process may be seen in **figure 1** (*Kaiser Optical Systems, 2008*).

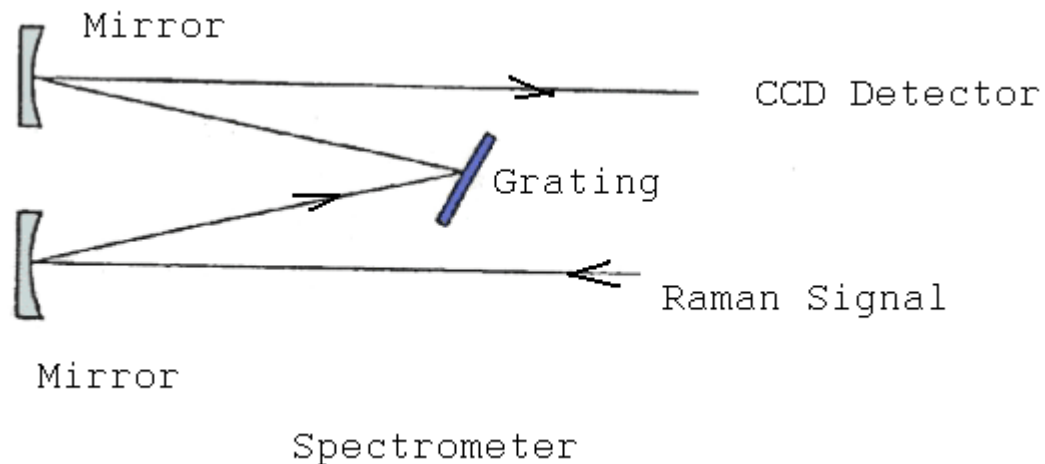


Figure 1. Process where scattered inelastic light is guided in a Raman Spectrometer.

The sample's intensities of certain inelastic wavelengths are unique to that particular compound. This is where the term "fingerprinting" can be used with respect to Raman Spectroscopy.

In reality, there may be many different types of molecules or cells in a given sample; it would be difficult to determine which components produced specific wavelength signatures. To avoid this problem, a Laser Tweezers system can be used to separate the cells and use single cells for the investigation (2008 Laser Tweezers). The Laser Tweezers system operates by focusing light on a single factor. The momentum of photons are focused on a single factor which make it possible to trap single samples in one spot and move the slide, in effect, separating the studied sample from a cluster of other substances. The momentum of the energy of the light is proportional to its amperes. Amperes are measurements of electrons passing through a current. The larger the amperes, the larger the momentum of energy will be when an object is struck.

Another obstacle of this experiment is collecting optimal results and readings of the inelastic scatterings. Because both laser light and inelastic wavelengths of light are scattered, a Notch Filter would be used within the spectrometer to filter out the laser light being measured in the spectrometer. This filter is very important in analyzing the intensities of inelastic scatterings rather than reading the intensity of the laser light. Another improvement of data collection by the spectrometer would be to read the inelastic scatterings after freezing the spectrometer's charged-couple detector with liquid nitrogen cooled tank. This procedure was done to decrease the energy of the receptor so it could read the inelastic energy with more sensitivity.

The graphs of the Raman Spectra are read from different intensities of light versus the different wavelengths emitted after being scattered. One example of a Raman Spectra can be seen in **Figure 2**. **Figure 2** shows the Raman Spectra of Acetic Acid Butyl Ester, an organic compound, to understand what a graph of a Raman Spectra of a sample may look like.

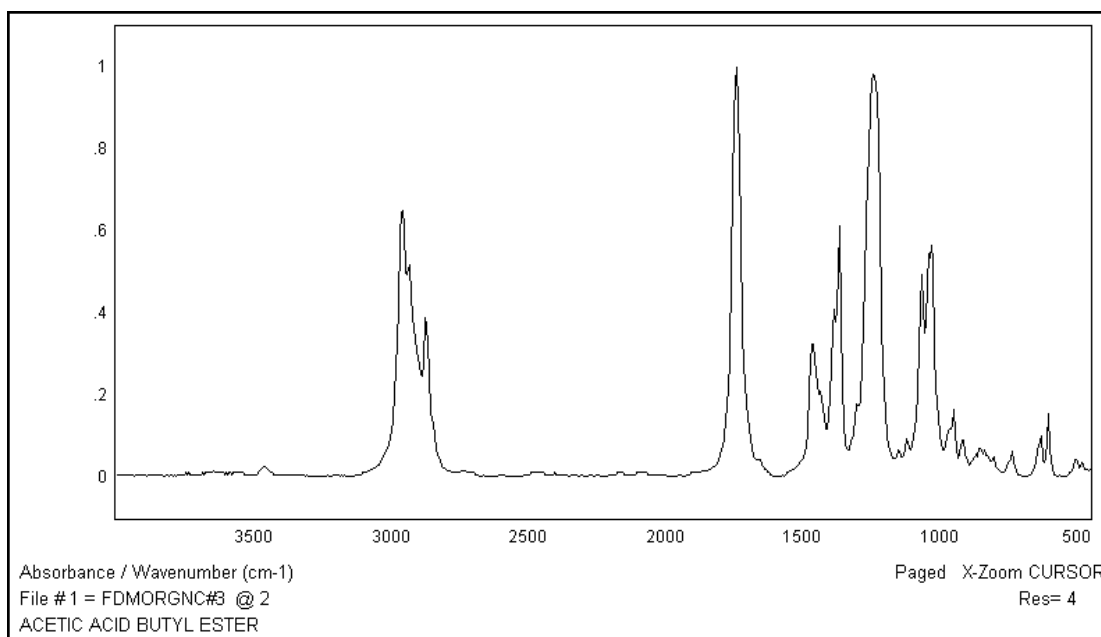


Figure 2. Raman Spectra of Acetic Butyl Ester.

It can be seen that the peaks of the wavelengths occurred at about 3000 cm⁻¹, 1700 cm⁻¹, 1350 cm⁻¹, and 1200 cm⁻¹ wavelengths. Comparisons of Raman Spectra in different aromatic compounds could be made by understanding where the locations of the wavelengths' peaks occur. Aromatic compounds are six-ringed carbons with alternating double bonds. The importance of detecting aromatic compounds in substances are that many of them such as poly-aromatic hydrocarbons are pollutants, mutagenic, and carcinogenic to the environment and human health (Freeman 1974). Benzopyrene, benzanthracene, and benzoflourantene are poly-aromatic hydrocarbons that are such examples of harmful aromatic compounds (Tu 1982).

Yeast cells also produce many types of aromatic compounds while undergoing autolysis, self-cell destruction. The aromatic compounds used in this research will be naphthalene and polystyrene. The Raman Spectra of those aromatic compounds would be compared to benzene, found on **figure 3**. While the the chemical structure of naphthalene and polystyrene could be found on **figure 4** and **figure 5**.

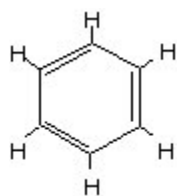


Figure 3. Chemical structure of a benzene ring.

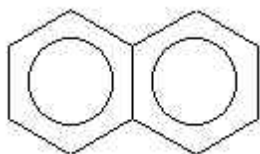


Figure 4. Chemical structure of Naphthalene.

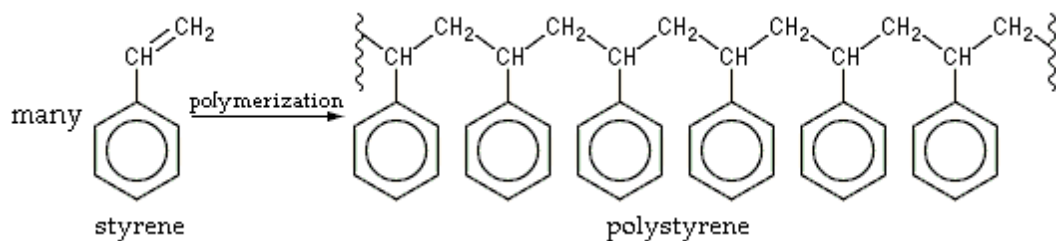


Figure 5. Chemical structure of Polystyrene.

From there, the Raman Spectra of these aromatic compounds would be compared to the Raman Spectra in yeast cells to analyze the cell to identify whether they contain these six-ringed like compounds. The goal of the experiment was to find common peaks of wavelengths for the Raman Spectra of Aromatic compounds and yeast cells and whether aromatic compounds could be detected in yeast cells.

METHODS AND MATERIALS:

To test the goal of using Raman Spectroscopy to measure the Raman Shift or Raman Scattering to differentiate aromatic compounds and identify yeast cell, an experiment was made. The following samples of naphthalene, polystyrene, and yeast all followed the same procedure and this experiment was done successfully seven times. The samples were first soaked in water to allow the sample separate through for the laser tweezing method. The samples were placed in an indentation between two microscope slides and examined using a compound light microscope.

The laser table was already preset in such a way that the laser light was guided into the sample that was placed in the microscope's stage. From there, the Raman Scatterings were guided through another set of mirrors to the Raman Spectrometer.

A semiconductor laser with a wavelength of 785 nanometers, which is Near-Infra-Red (NIR), was used. This laser had two roles, to excite the energy levels of the cell to produce the Raman Radiation from the cell, and to trap it in a laser tweezers system to separate the cell from other cells and lock it in one place without causing damage. The selection of this

wavelength of 785 nanometers was very important because lasers with wavelengths in the visible range will not kill the cells or damage the molecules. However, lasers with wavelengths much lower from NIR will not be able to trap the samples. The power supply of the laser was then set to create 98 milli-Amperes. This power supply would provide more energy to the laser light to trap a substance.

Next, laser light was directed and focused at the sample and monitored the samples continually. For safety reasons, a camera was put on the lens. The camera was connected to a monitor and the laser tweezing method was done by watching the sample through the monitor instead of the lens itself. This step was made to protect the observer of the sample to not be hit by the laser light into the eye. Then using the micrometer translator, the focus point was adjusted until the yeast cell was trapped and there was distance between the other cells. The use of this micrometer-accuracy was important because of the sensitivity of the trapping and the sample. This was very important because if the yeast was in not in the correct position with respect the focusing point, then the Laser Tweezers system would not have been able to separate individual cells.

Then the system was aligned to collect the Raman signal from the sample and direct it to spectrometer, then to Liquid Nitrogen Cooled Charge-Coupled Device (CCD) Detector (CCD), and then the signal was sent to computer. A graph of the different intensities of wavelengths that reached the receiver will be made on the computer. The graph was then compared to previously detected Raman Spectra of different compounds.

After comparisons were made, patterns of chemical structures were notated for the reason of the Raman Spectra phenomenon. The chemical structures of naphthalene and polystyrene can be seen in figures 3 and 4, respectively. Figure 5 shows the Raman Spectra of

Benzene, the most basic aromatic compound. The Raman Spectra for Benzene was chosen because it contains no substituents. Substituents are atoms or molecules bonded to a main carbon chain (Delfino 2005). These substituents would have shifted the Raman Spectra as seen on **figure 6**; whereas the simple Benzene ring would give concise spectra of an aromatic compound (Delfino 2005). Since there are limited distinctive peaks in this Spectrum, the main wavelengths that will be focused on will be at 590, 950, 1150, and 1550.

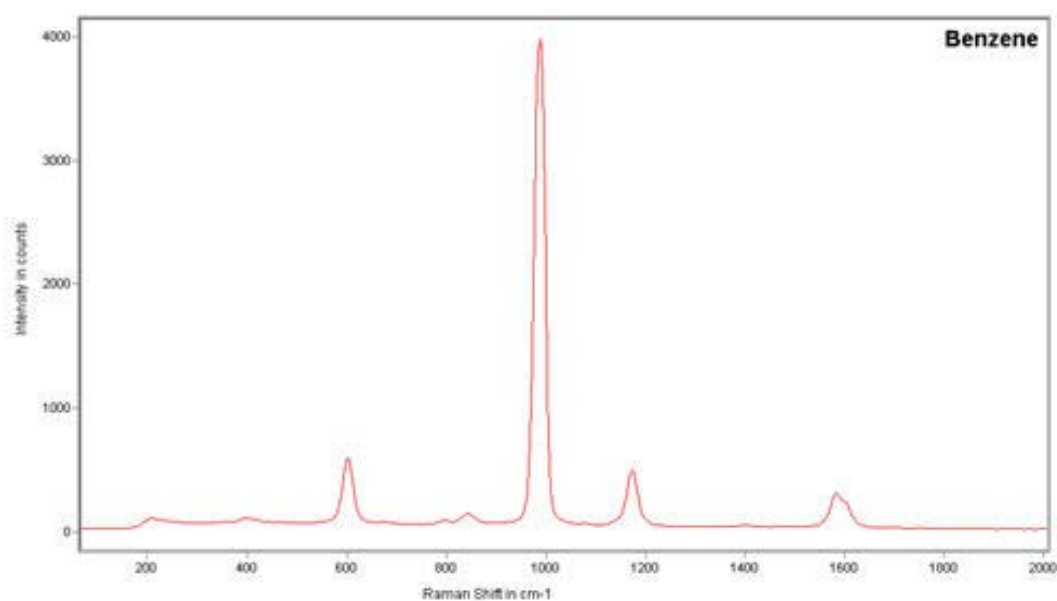


Figure 6. Raman Spectra of Benzene.

Figure 7 was based on the same peaks presented in **figure 6**. This graph will be set as a template for the Raman Spectra of naphthalene and polystyrene. These wavelength's peaks were seen on 590 cm^{-1} , 950 cm^{-1} , 1150 cm^{-1} , and 1550 cm^{-1} .

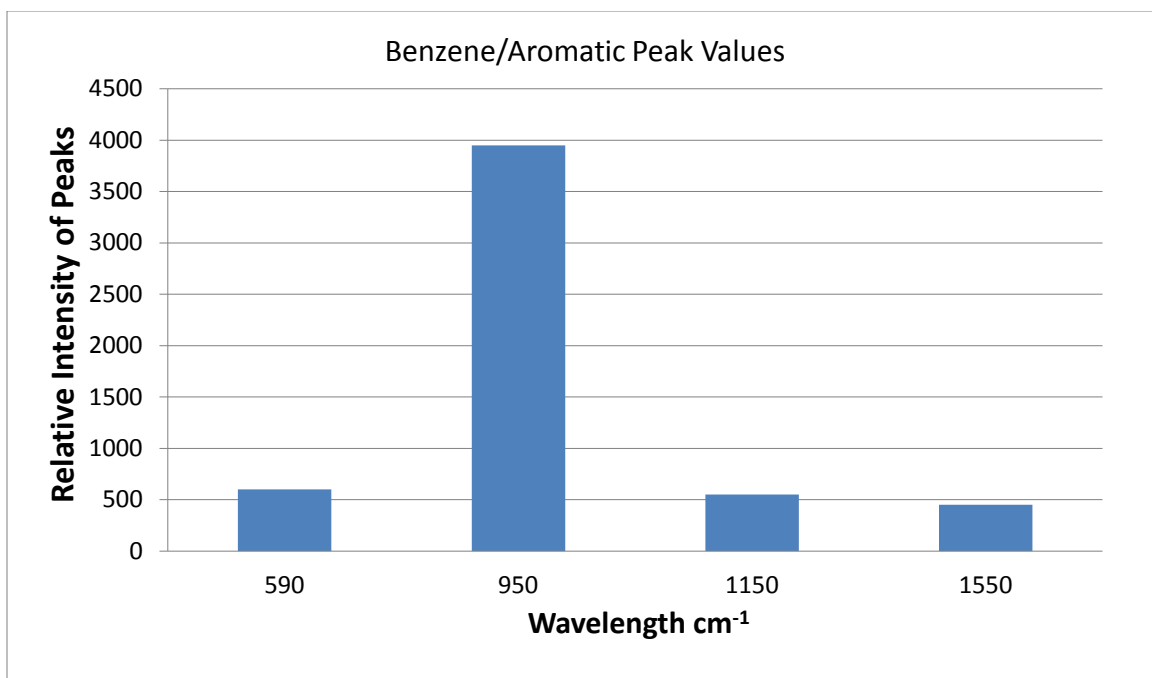


Figure 7. Raman peak values of benzene used for comparisons to naphthalene and polystyrene.

DATA AND RESULTS:

Figure 9 shows the Raman Spectra of naphthalene recorded during the first phase of the experiments proposed in this project. This spectra includes peaks that are different from those seen in the Benzene's Raman Spectra. The differences may be caused by shifts due to slightly different vibrational energies of these two chemical structures.

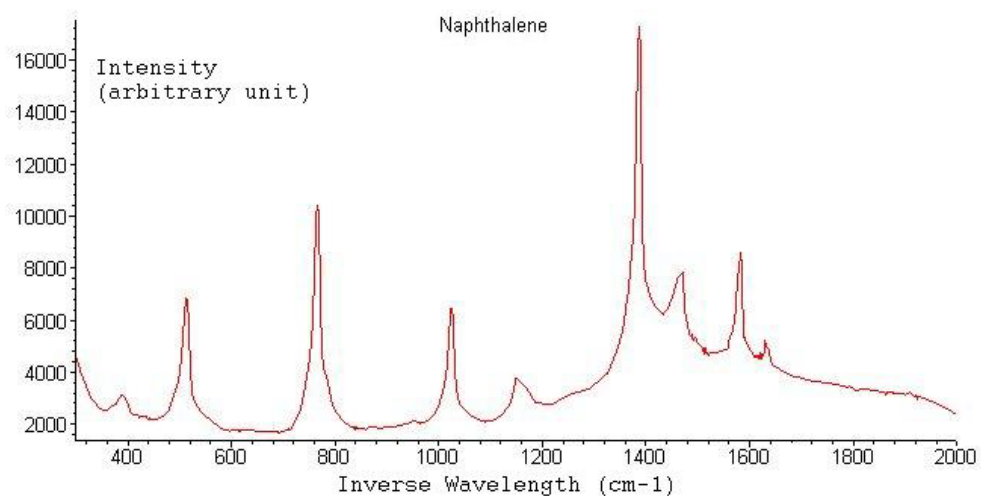


Figure 8. Raman Spectra of Naphthalene.

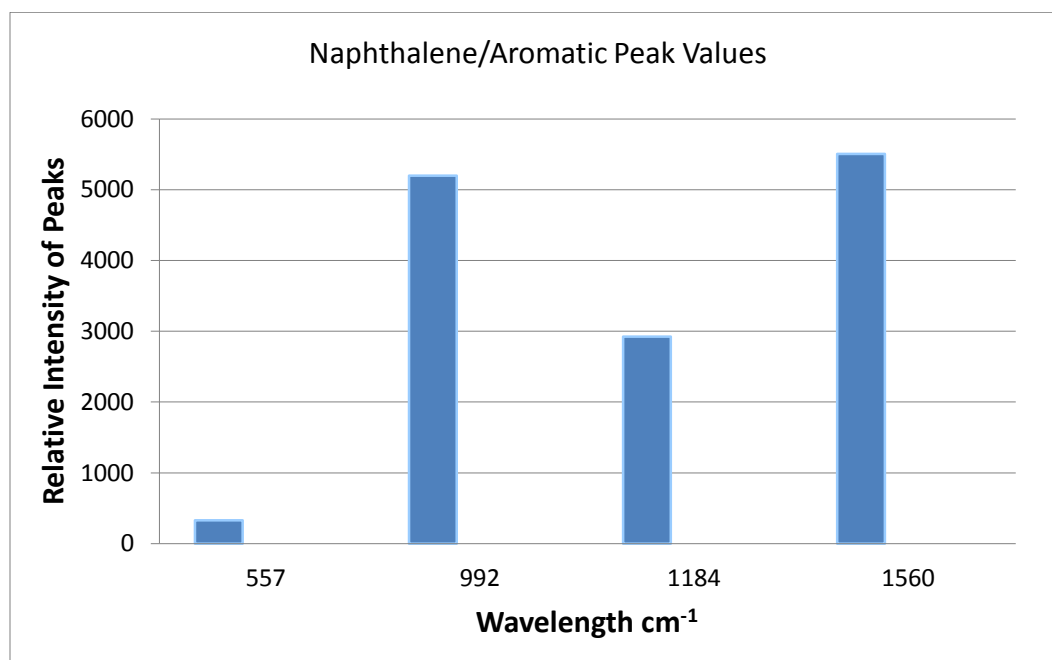


Figure 9. Peak wavelengths and relative intensities of Naphthalene Raman Spectra.

Figure 9 depicts wavelengths and intensities of peaks seen in **Figure 8**. The wavelengths (557 cm⁻¹, 992 cm⁻¹, 1184 cm⁻¹, and 1560 cm⁻¹) are similar to Raman Spectra of benzene (see

Figure 7). However, the intensities of wavelengths in the Naphthalene Spectra are not similar to those in the Spectra of Benzene.

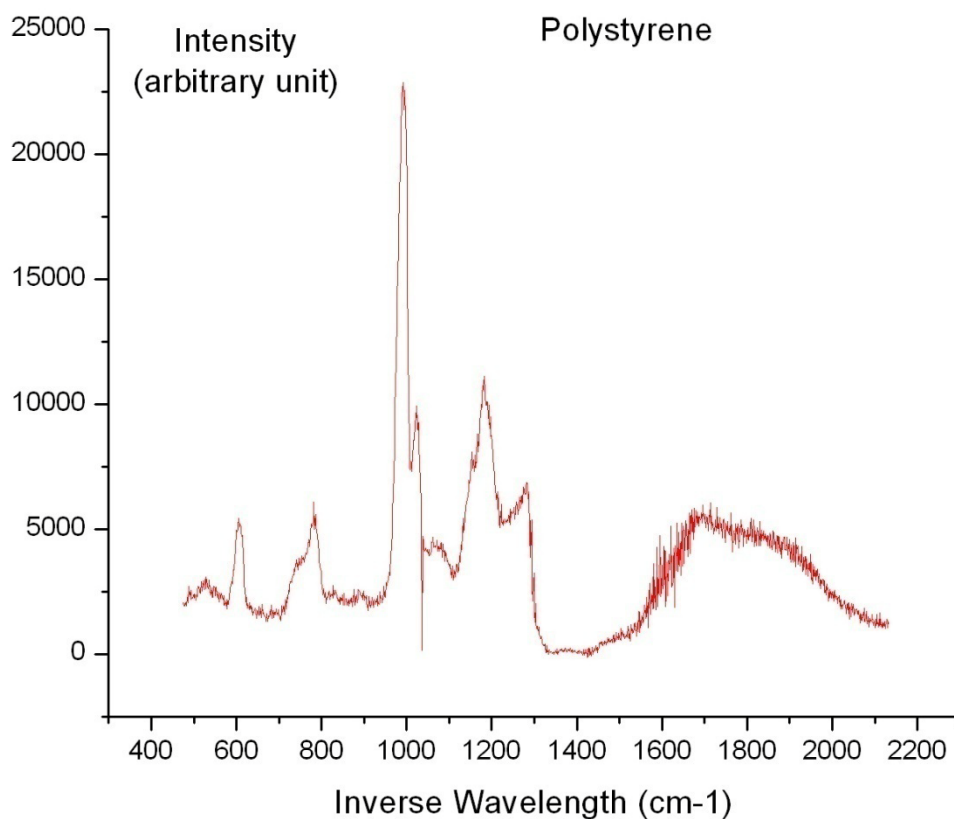


Figure 10. Raman Spectra of Polystyrene.

Figure 10 shows that the locations of polystyrene Raman peaks are not identical to the peaks found in the Benzene's Raman Spectra because of potential shifts due to slightly different vibrational energies. The differences in these vibrational energies could be affected by the differences in the molecular structures and chemical composition of benzene versus polystyrene.

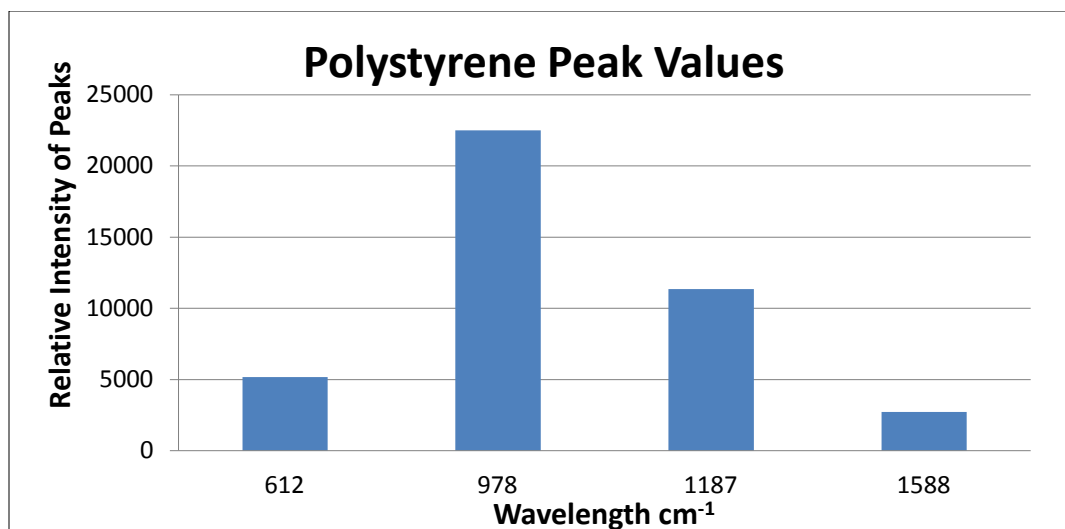


Figure 11. Peak wavenumbers and relative intensities of Polystyrene Raman Spectra.

Figure 11 depicts the main and most distinguishable peaks found in the Raman Spectra of Polystyrene. The wavenumbers of the most prominent peaks in the Raman Spectra of polystyrene are 612 cm^{-1} , 978 cm^{-1} , 1187 cm^{-1} , and 1588 cm^{-1} . These are similar to the peaks found in benzene (586.33 cm^{-1} , 973.33 cm^{-1} , 1173.66 cm^{-1} , and 1566 cm^{-1}) which represent aromatic compounds like polystyrene. Even the relative intensities are similar to benzene: the peak at 978 is much more intense than peaks at 612 and 1588 .

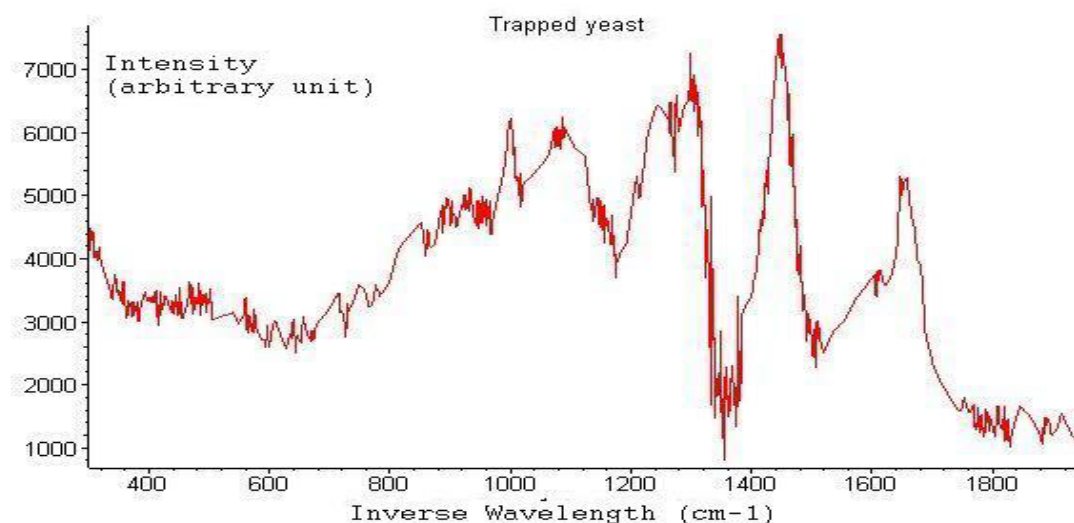


Figure 12. Raman Spectra of a yeast cell.

Figure 12 depicts the Raman Spectra of a yeast cell. It can be seen that there are many inelastic scatterings in this graph. Peaks in this graph also agree with the peaks of past aromatic compounds but there is not a sufficient evidence to link these wavelengths to the Raman Spectra peaks of benzene because of the complexity of a yeast cell in comparison to other compounds.

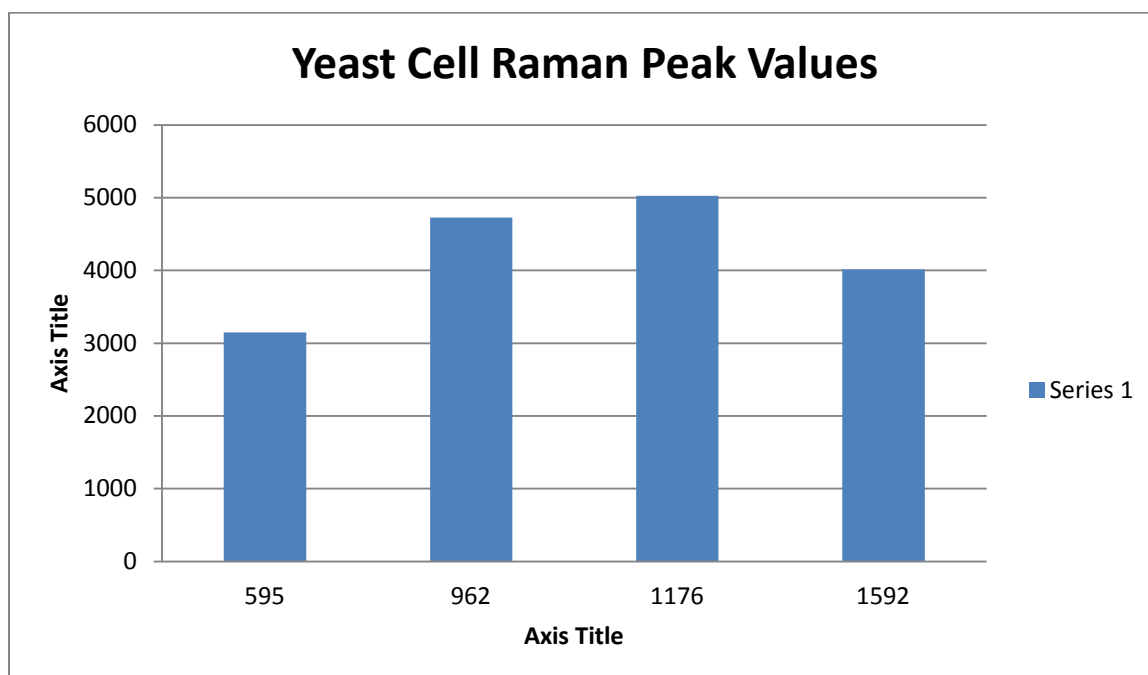


Figure 13. depicts the Raman Spectra peak points similar to the Raman Spectra of Benzene.

DISCUSSION:

As previously stated, the main goal of the experiments proposed in this project was to find out if similar wavelength locations of inelastic scatterings of Raman Spectra could be identified in similar types of compounds. The Raman Spectra of two samples containing aromatic compounds, polystyrene and naphthalene, were compared to the Raman Spectra of benzene (one of the simplest aromatic compounds). The results of the performed experiments revealed that there were common wavelengths where the peaks occurred in aromatic containing samples. Although some of the wavelengths were slightly shifted (by at most 40 cm^{-1}), it was still clear that most of the peaks occurred at relatively similar wavelengths (586.33 cm^{-1} , 973.33 cm^{-1} , 1173.66 cm^{-1} , and 1566 cm^{-1}). Surprisingly, the Raman Spectra of polystyrene had more similarities to benzene than naphthalene (even though its chemical structure is more diverse) in not only the location of wavelengths but also in their relative intensities. It is unclear why this occurred.

During the experiments described in this proposal, seven graphs of Raman Spectra were collected. Additional Spectra were not collected due to some of the difficulties encountered throughout the study such as tweezering and alignment of the laser to single samples. Once searching for the sample in the monitor, the laser must be perfectly aligned with the single molecule. This alignment was tricky due to the difficulty of focusing the laser on a particular sample and the need for extreme steadiness of the hand. The procedure presented a special challenge when trying to focus. The average wavelength values where peaks should occur in aromatic compounds should be in 586.33 cm^{-1} , 973.33 cm^{-1} , 1173.66 cm^{-1} , and 1566 cm^{-1} . The laser so that it would trap a sample before it had sunk to the bottom of the slide. Occasionally,

the momentum of the laser light would not be able to hold the single sample in one spot due to a small drift or current present on the slide. Without being able to use the laser tweezer system correctly to separate a single molecule from a group of molecules, the tested sample would not yield an accurate Raman Spectra.

Another obstacle in this experiment was to align the laser table correctly. The key in this procedure was directing the laser light, using mirrors, to the sample and then directing the scatterings and laser light to the Raman Spectrometer. Directing the laser light to the Raman Spectrometer was a major problem because of the small aperture that the laser light must pass through to be reflected by the mirrors at a correct angle to the spectrometer.

The performed experiments showed that similar Raman spectra could be found in yeast cells and aromatic compounds but it is still not a clear method used to identify certain compounds in cells. I would like to continue with this research by inducing yeast cells to undergo autolysis and to use Raman spectral analysis to see if my aromatic peaks would increase in intensities. This observation would be sought for because the intensities of each peak's spectra are dependent upon the concentration of the sample under study (Ortac 2007). This means that doctors could potentially identify different types of infectious agents, cancer cells and stem cells. The procedure would involve identifying groups of Raman Spectra peaks specific to unique types of cells and their compositions. Being able to identify aromatic compounds is the first step in being able to identify chemical components of specific cells.

Citations

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