



Wrocław University  
of Science and Technology

Faculty of Chemistry



## **Sustainable Bioproducts and Bioprocess Engineering**

**Course: Biocomponents characterization**

**Class: Raman spectroscopy, sample preparation, calibration**

**Dr. Magdalena Malik**

## Introduction

Vibrational spectroscopy, Raman and infrared, are based on the interaction of electromagnetic radiation with matter. Experiments are usually non-destructive, and the samples can be reused for other investigations. The aim of both Raman spectroscopy and Infrared spectroscopy experiments is to probe the molecular vibrations, probing the transition between the ground state and the excited vibrational states. The transitions are observed as bands in the vibrational spectrum. Each molecule has a specific set of vibrational bands, which are defined by their frequencies, shapes, and intensities. By analysing these properties, it is possible to get information about the local coordination of the atoms in your material.

The frequency of a transition ( $\sim 100 - 4000 \text{ cm}^{-1}$ ) directly depends on the reduced mass of the vibrating unit. Therefore, smaller reduced masses give rise to higher frequencies. Depending on the nature of the molecular vibration, the transition may be Raman, and/or infrared active. Those techniques should therefore be used complementary, because some bands that are infrared inactive can be Raman active and *vice versa*. The physical principle behind Raman and infrared spectroscopy is very different though. Raman spectroscopy is a scattering technique where the intensity of the bands is proportional to the concentration and to the scattering cross-section of the vibrating units. Infrared spectroscopy is an absorbance technique where the intensity of the bands is proportional to the concentration and to the absorption coefficient of the vibrating units. Typically, the time needed to record a spectrum is of the order of seconds to tens of minutes. However, to increase the signal to noise ratio, several accumulations are customary.

Raman spectroscopy is a powerful tool for the non-destructive investigation and characterization of all kinds of materials. It has a large range of applications, which span from fundamental science to in situ quality control in production lines. When a Raman spectrometer is combined with a confocal microscope (Raman microscope), it is even possible to visualize the distribution of materials on the micrometer scale.

## Fundamentals

When light is scattered by a sample most of it is emitted at the same wavelength (energy) as the incident light. This is called Rayleigh scattering (elastic scattering). What happens on a molecular level (Fig. 1) is that the photons are absorbed by the molecule, which goes from the ground state into a virtual energy state. After a very short time the excited molecule goes back

into its ground state and emits a photon with the same energy in a different direction. However, some photons are emitted with a different energy than the incident light. This inelastic scattering is called Raman scattering. Raman scattering is possible because the molecule also has excited (vibrational) energy states. These excited states exist due to the vibrational modes of the molecule and there are two ways they can cause inelastic scattering. If the molecule was already in an excited state, when it absorbed the photon, and then goes back into the ground state, the photon gains the energy difference between the ground state and the excited state. This is called Anti-Stokes scattering (Fig. 1). If the molecule was in the ground state, when it absorbed the photon, but then goes back into an excited state, the photon loses the energy difference between the ground state and the excited state. This is called Stokes scattering (Fig. 1). Stokes scattering is usually measured in Raman spectroscopy, because it is much more likely than Anti-Stokes scattering, since there are more molecules in the ground state than in the excited states in the sample.

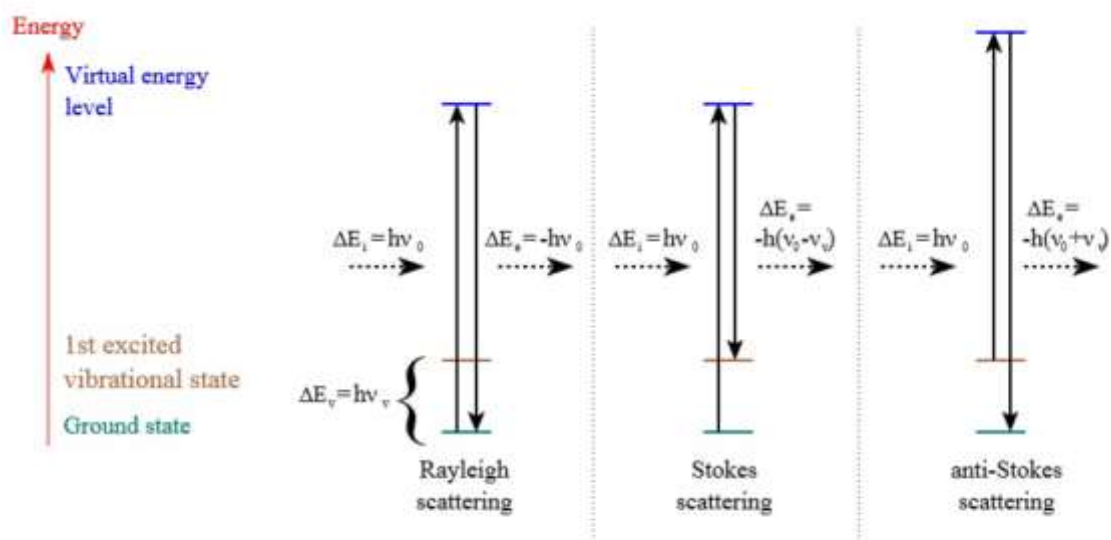


Figure 1: Sketch of the energy states during Rayleigh and Raman scattering

The main difficulty with Raman scattering is that only a tiny fraction (approximately  $10^{-8}$ ) of scattered photons are Raman scattered. Therefore, in Raman spectroscopy a laser is used to excite the molecule. The (monochromatic) Rayleigh scattering is then blocked by a filter and only the Raman scattering is measured by a spectrometer, which is equipped with a highly sensitive photon detector (Fig. 2).

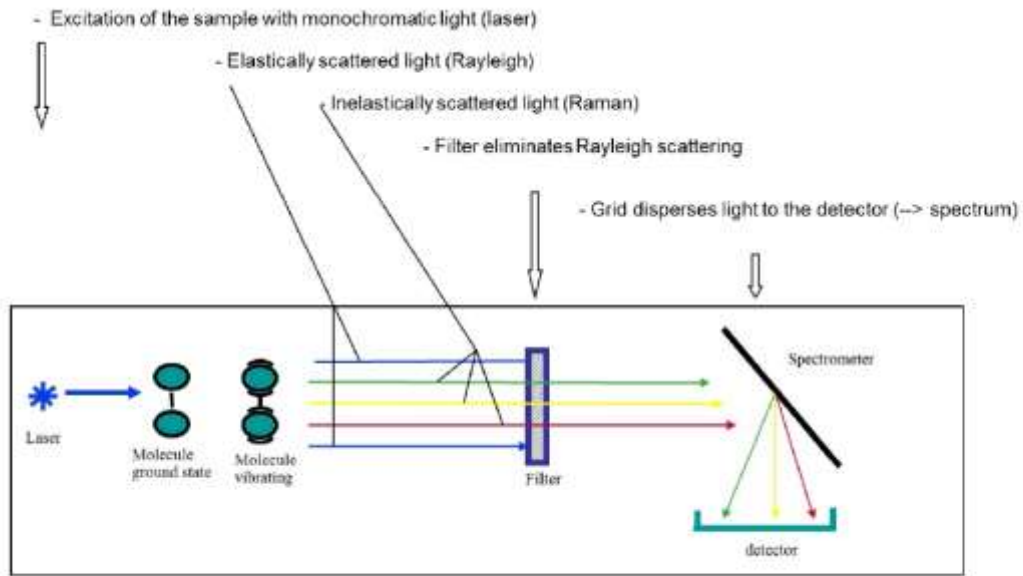


Figure 2: Sketch of the stages of a Raman spectrometer

The spectrum is usually not plotted in terms of the total energy of the photons, which is dependent on the laser wavelength, but rather as the energy shift of photons relative to the laser energy. The energy (Raman) shift is equal to the energy of the vibrational states of the molecule and independent of the laser wavelength. By convention the unit used to plot the Raman shift is the wavenumber ( $\text{cm}^{-1}$ ).



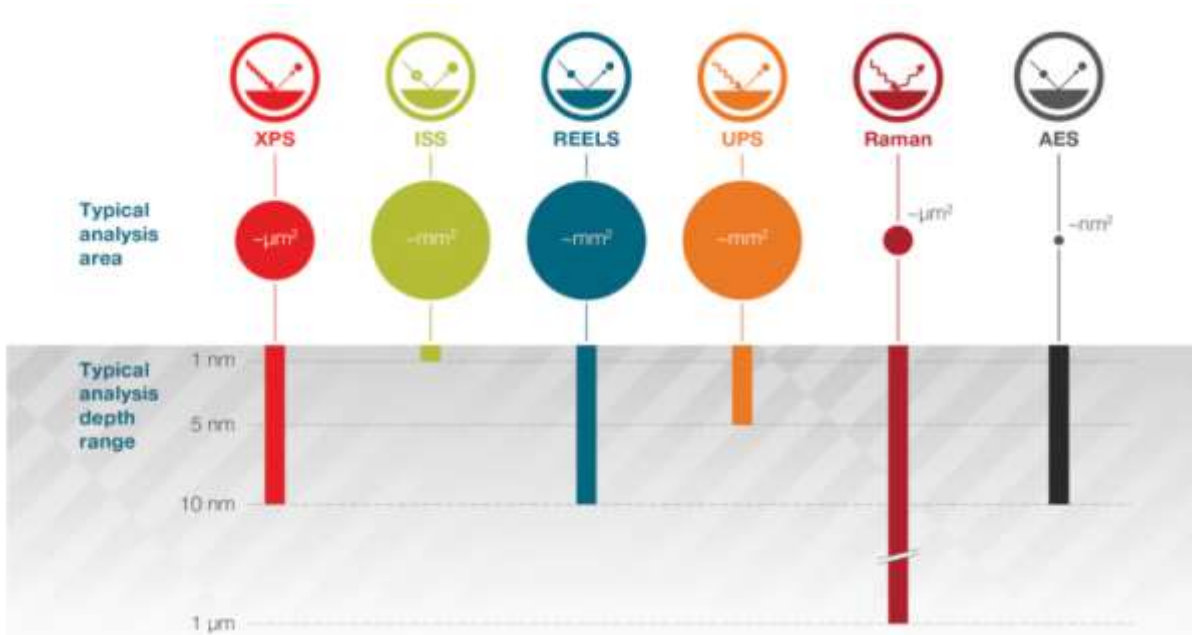


Figure 3: Some spectroscopic techniques and spectral information about them.

The energies of the vibrational states contain a lot of information about the sample such as the chemical composition, the stress/strain state, crystal symmetries and crystal quality (Fig. 4). However, in most cases the chemical composition is investigated by spectroscopy.

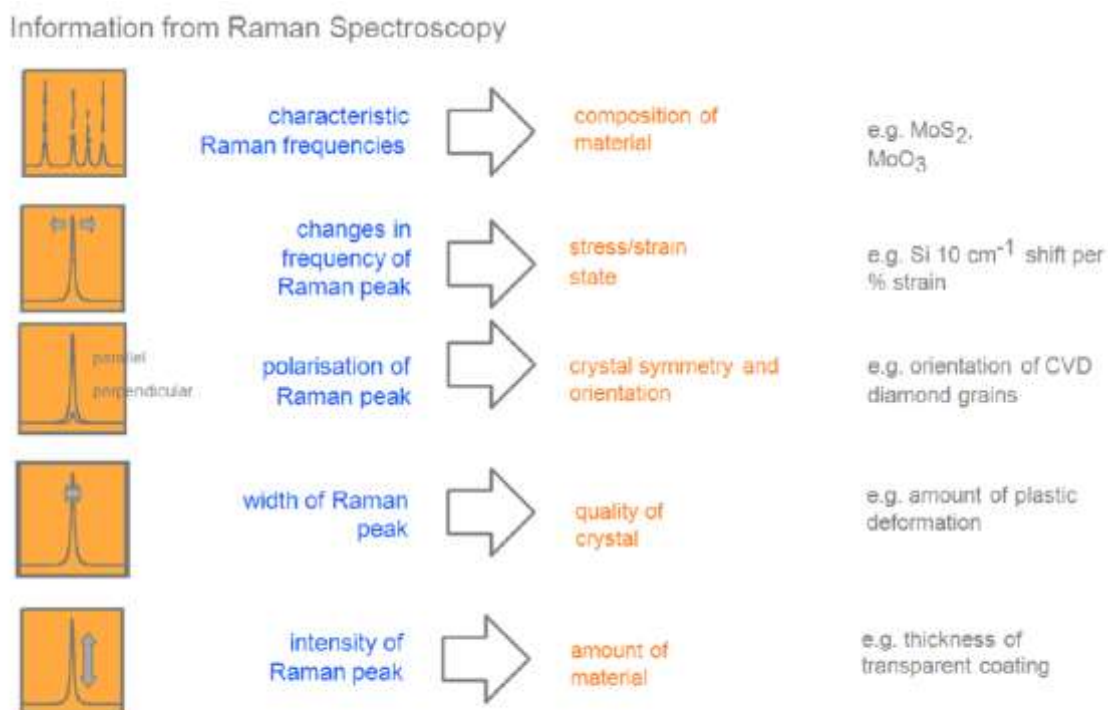


Figure 4: Information accessible by Raman spectroscopy

There are two major kinds of vibrations, namely stretching and deformation (bending), which are illustrated in Fig. 5. Stretching changes the length of the chemical bond and usually requires more energy than deformation.

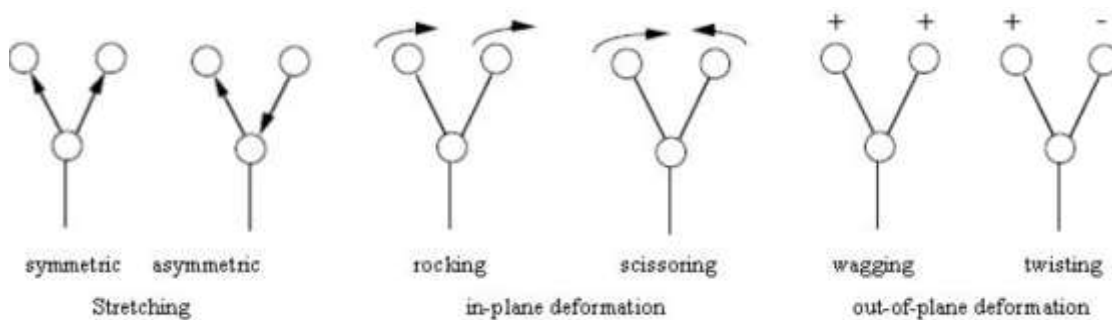


Figure 5: Sketch of vibrational modes

### Interpretation of Raman spectra

The most important information that can be obtained using Raman spectroscopy is the chemical composition of the sample. An example of the Raman spectrum of cellulose in an embedding compound is shown in Fig. 6. Every band in the spectrum is associated with the vibration of a chemical bond in cellulose or the embedding compound. All bands are marked as either cellulose (C) or embedding compound (E). Additionally, for the cellulose bands the chemical bond causing the band is identified.

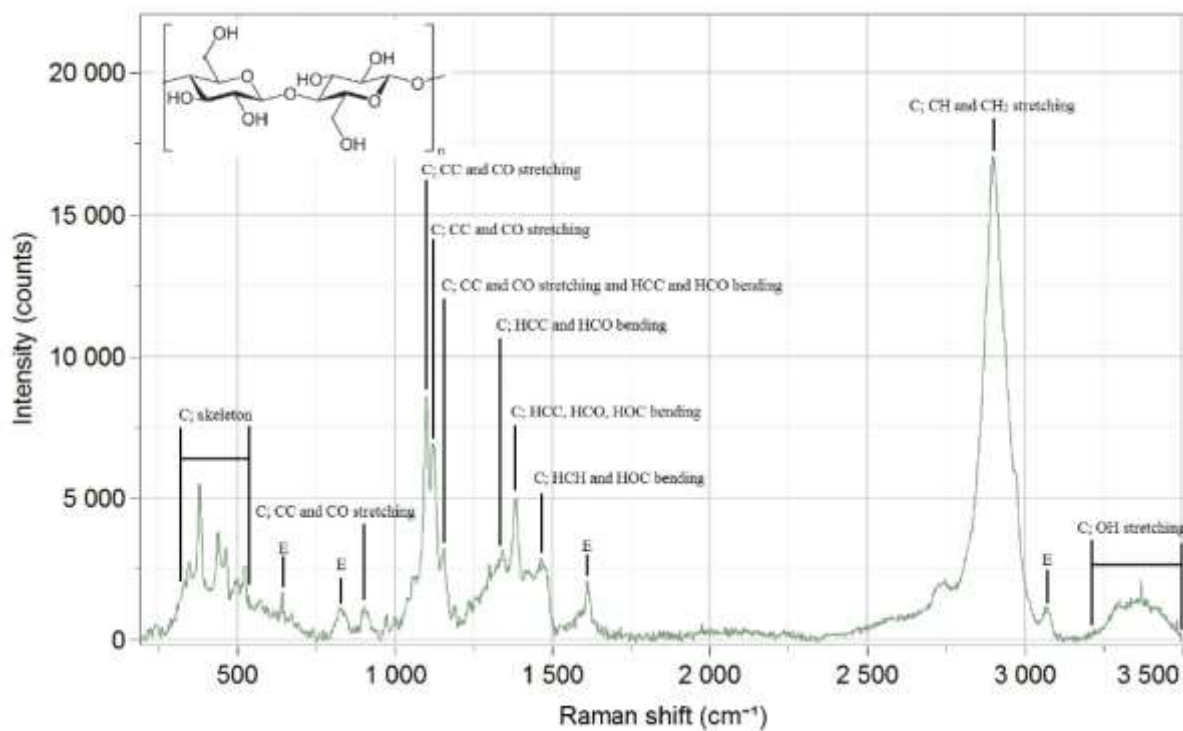


Figure 6: Raman spectrum of cellulose and an embedding compound with band assignment

An interpretation of the spectrum like this is the ideal case. However, it is very difficult to do and requires prior knowledge of the sample. Therefore, in most cases a simpler method of interpretation is chosen. Other methods of spectral interpretation are (in ascending order of accuracy):

- Identifying H- and C-bonds using the harmonic oscillator approximation
- Identifying functional groups by looking for group bands
- Identifying the chemical composition by comparing to a known reference

### **Choosing the adequate laser**

Despite the fact that the spectrum itself (frequency shift) is independent of the laser wavelength, the choice of the right laser is vitally important when doing Raman spectroscopy. The following factors have to be considered when choosing the laser wavelength:

- ✓ Fluorescence
- ✓ Band Intensity
- ✓ Spectral resolution and range
- ✓ Spatial resolution

#### *Fluorescence*

A major problem in Raman spectroscopy is fluorescence. Since fluorescence has in general far more intensity, it can completely mask the Raman spectrum. Often fluorescence can be avoided by choosing the right laser wavelength, because it always occurs at the same wavelengths regardless of the wavelength of the excitation (assuming it has at least enough energy to excite fluorescence), whereas the same Raman spectrum can be measured at different wavelengths depending on the laser wavelength (Fig 7). As fluorescence can make the measurement impossible avoiding it is the most important factor to be considered when choosing the laser wavelength.

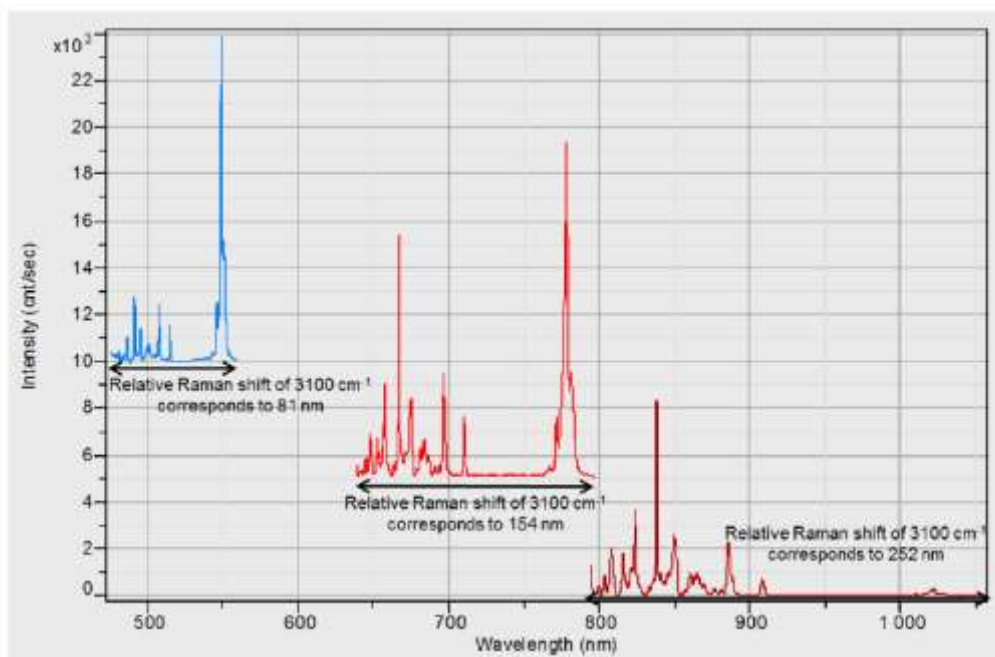


Figure 7: The same Raman spectrum recorded at different wavelengths

### ***Literature***

1. Ferraro, John R.; Nakamoto, Kazuo and Brown, Chris W. "Introductory Raman Spectroscopy", Elsevier Inc. (2003)
2. Long, Derek Albert. "The Raman Effect - A Unified Treatment of the Theory of Raman Scattering by Molecules", John Wiley & Sons (2002)
3. Grasselli, Jeanette G. and Bulkin Bernard J. "Analytical Raman Spectroscopy", John Wiley & Sons (1991)
4. K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part A&B, J. Wiley & Sons Inc., New York, 2009.
5. G. Socrates, Infrared and Raman characteristic group frequencies: tables and charts, John Wiley & Sons, 2004

### **Laboratory exercises for students**

“Measurement and analysis of vibrational spectra of fluids used in the fuel industry”-part 2

#### **Experimental part 2**

During the lab students take the fluid samples (radiator fluid, brake fluid, engine oil, etc.) in order to recorded Raman spectra. Students will measure the same sample as in IR spectroscopy



class. Gasoline will also be measured with a fluid sample. **Next, student will prepare one report after IR and Raman spectroscopy classes.**

**The reports should contain:**

1. Short introduction to IR and Raman techniques which you used during the lab activities. Please show that these are complementary methods.
2. Check what is the main ingredient(s) of your sample, e.g. what is under the name engine oil, silicon oil etc? Give the main composition of the sample, based on safety data sheets and/or literature.
3. Discussion of the result:
  - a) All spectra (IR, ATR, Raman) of the samples, made by yourself writing in EXCEL or ORIGIN. Please compare the transmittance spectrum to ATR spectrum of fluid samples.
  - b) Please try to characterize the main peaks in your IR and Raman spectra: write the wavenumber, character of peak and assignments in the table (see below)

| No.   | Wavenumber [ $\text{cm}^{-1}$ ] |       | Character<br>(s-strong, m-medium, w-weak) | Assignments       |
|-------|---------------------------------|-------|---|-------------------|
|       | IR                              | Raman |   |                   |
| 1     | 3034                            | 3027  | m   | $\nu(\text{C-H})$ |
| 2.... |                                 |       |   |                   |

- c) Write the spectroscopy analysis using the phrases below:

*“.....a few weak bands in the region 3110-3051  $\text{cm}^{-1}$  in FT-IR and Raman spectrum of complex can be assigned to the  $\nu(\text{CH})_{\text{py}}$  and  $\nu(\text{CH}_2)$  stretching vibrations of the C-H pyridine ring atoms and the aliphatic groups. The  $\delta(\text{CH})$  bending vibrations of the pyridine ring generated medium peaks in the 1150-1100  $\text{cm}^{-1}$  range in FT-IR spectrum of ligand.... Furthermore, the strong bands at 1003 and 994  $\text{cm}^{-1}$  (centered at 999  $\text{cm}^{-1}$ ) in FT-IR spectrum of ligand come from  $\nu(\text{C-O})$  stretching vibrations and were observed as the weak band at 1014  $\text{cm}^{-1}$  in the FT-Raman spectrum of the complex. Additionally, the shift by 11  $\text{cm}^{-1}$  to lower frequency was observed for the  $\gamma(\text{C-H})_{\text{py}}$  out of plane bending vibrations in FT-IR spectrum of gold(III)*

*complex. The one strong band at  $664\text{ cm}^{-1}$  in FT-IR spectrum (overlapped in FT-IR by the second one) of title compounds is due to torsion of pyridine ring ( $\tau_{R_{py}}$ ) and this is observed in higher wavenumbers than in 2-pyridineethanol...”.*

4. Through the prepared comparison/overlaying of Raman spectra of pure gasoline and the mixture of gasoline with the sample, answer the question: what is the effect of the addition of this compound on the spectrum of gasoline? Is it easy to detect what contaminated gasoline can be? If so, what to look for in the analysis: any marker bands, changes in the Raman intensities of the observed bands?
5. Summary of the results and conclusions.
6. You have 2 weeks to prepare the report. Attach the report by e-portal.