Instrumental methods of analysis

Optical methods of analysis

General characteristics of instrumental analysis methods

- Instrumental methods of analysis are based on the use of the relationship between the measured physical properties of substances and their qualitative and quantitative composition. Since the physical properties of substances are measured using various instruments, these methods of analysis are also called instrumental methods.

Classification of instrumental methods of analysis

- Optical methods are based on measuring the optical properties of substances.
- Chromatographic methods are based on the use of the ability of various substances to selectively sorption.
- Electrochemical methods are based on measuring the electrochemical properties of systems.

Classification of instrumental methods of analysis

- Radiometric methods are based on measuring the radioactive properties of substances.
- Thermal methods are based on measuring the thermal effects of the corresponding processes.
- Mass spectrometric methods are based on the study of ionized fragments ("fragments") of substances.
- Other methods of analysis (ultrasonic, magnetochemical, pycnometric, etc.) are also used.

Advantages of instrumental methods of analysis

- Low detection limit and low limiting concentration (up to ~10^{-12} g/ml) of the detected substance.
- High sensitivity, formally determined by the magnitude of the tangent of the angle of inclination of the corresponding calibration curve, which graphically reflects the dependence of the measured physical parameter.

Advantages of instrumental methods of analysis

- High selectivity (selectivity) of the methods. It is often possible to determine the constituent components directly in the analyzed mixtures, without separating them and without separating the individual components.
- The short duration of the analyses, the possibility of their automation and computerization.

Disadvantages of instrumental analysis methods

- Sometimes the reproducibility of the results is worse than using classical chemical quantitative analysis methods such as gravimetry and titrimetry.
- The errors of definitions using physical and physico-chemical methods of analysis often amount to about ± 5% (and in some cases up to ± 20%), while in classical chemical analysis (gravimetry, titrimetry) they usually do not exceed ±(0.1—0.5)%.
- The complexity of the equipment used, its high cost.

Optical analysis methods

- Optical analysis methods are based on measuring the optical properties of matter (emission, absorption, scattering, reflection, refraction, polarization of light), manifested by the interaction of electromagnetic radiation with matter

Classification of optical analysis methods

1. By the studied objects
2. By the nature of the interaction of electromagnetic radiation with matter.
3. In the field of the executable electromagnetic spectrum.
4. By the nature of energy transitions.

Methods of adsorption analysis

- Colorimetry
- Photoelectrocolorimetry
- Spectrophotometry.

Photocolorimetry

- The method is based on measuring the intensity of the non-monochromatic light flux passing through the analyzed solution using photocells in photocolorimeters and photoelectrocolorimeters.

Photocolorimetry

- The luminous flux from the radiation source (usually an incandescent lamp) passes through a light filter that transmits radiation only in a certain wavelength range, through a cuvette with the analyzed solution and enters a photocell that converts light energy into a photocurrent recorded by an appropriate device.

Photocolorimetry

- The greater the light absorption of the analyzed solution (i.e., the higher its optical density), the lower the energy of the luminous flux entering the photocell.

Photocolorimetry

- The method has a relatively high sensitivity and good reproducibility, selectivity, is simple to perform measurements of optical density or transmission, and uses relatively simple equipment. However, the non-monochromaticity of the recorded luminous flux somewhat reduces the accuracy and reproducibility of analytical measurements.

Photocolorimetry

- Photoelectrocolorimetry has become widespread in analytical practice, for example, in the analysis of drugs such as diethylstilbestrol. levomycetin, menthol, novocaine, pilocarpine hydrochloride, rutin, streptomycin, etacridine lactate.

Photoelectrocolorimeters

Spectrophotometry

- The method is the most advanced among the methods of absorption molecular analysis, based on the use of special spectral devices — spectrophotometers, which allow recording light fluxes in a wide range of wavelengths from 185 nm to 1100 nm, i.e. in the UV, visible and near-IR spectral regions, and providing a high degree of monochromaticity of light (0.2—5 nm) passing through the analyzed environment.

Spectrophotometry

- In most spectrophotometers used in analytical practice, monochromatization of the luminous flux is carried out by using dispersing (decomposing light into a spectrum) elements — prisms or diffraction gratings. Various designs of spectrophotometers have been developed, operating both on a single-beam (single-channel) and two-beam (two-channel) circuit.

Spectrophotometry

- Various methods of spectrophotometry have been developed — direct (direct), differential, derivative spectrophotometry, and spectrophotometric titration.
- The concentration of the substance to be determined in the analyzed solution is found by spectrophotometric measurements, as in photoelectrocolorimetry, using either the basic law of light absorption or calibration graphs.

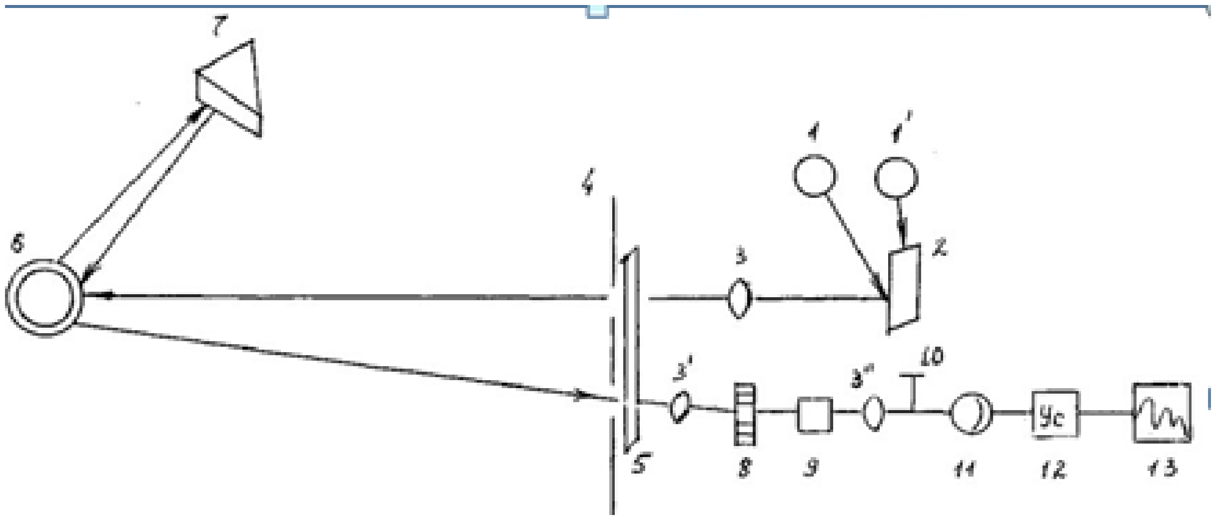
Spectrophotometry

- Spectrophotometric methods have, in comparison with photo-electrocolorimetric ones, greater accuracy and sensitivity, allow for the analysis of multicomponent systems without separation of components, to determine substances that do not absorb in the visible region of the spectrum (but have absorption bands in the UV range). The relative errors of spectrophotometric measurements do not exceed ± 2%.

Spectrophotometry

- Unlike photocolorimetry and photoelectrocolorimetry, spectrophotometry allows not only to measure optical density at a fixed wavelength, but also to obtain absorption spectra in a wide spectral range.

UV spectrophotometer



- 1- incandescent lamp; G- flax thorn lamp; 2- flat mirror; 3- focusing lenses; 4- diaphragm; 5 - quartz safety glass; 6 - mirror lens; 7 - monochromator; 8 - light filter; 9 - cuvette; 10 - shutter; 11 - photocells; 12 - amplifier; 13 - recording device.

Infrared spectroscopy

- Infrared spectroscopy studies the interaction of infrared radiation with substances
- When infrared radiation is passed through a substance, vibrational movements of molecules or their individual fragments are excited.

Infrared spectroscopy

- At the same time, a decrease in the intensity of the light transmitted through the sample is observed. However, absorption does not occur in the entire spectrum of incident radiation, but only at those wavelengths whose energy corresponds to the excitation energies of vibrations in the studied molecules. Consequently, the wavelengths (or frequencies) at which the maximum absorption of IR radiation is observed may indicate the presence of certain functional groups and other fragments in the sample molecules, which is widely used in various fields of chemistry to establish the structure of compounds.

The infrared region of the spectrum is divided into several ranges according to the optical materials used:

- The area of 0.8 – 2 mk is the near infrared region. Optics – glass or quartz. The sources of radiation are an incandescent lamp and thermal sources. Radiation receivers – based on photosensitivity sensors, thermoelements and bolometers (thermal radiation receiver).

The infrared region of the spectrum is divided into several ranges according to the optical materials used:

- Area 2 - 40 mk is the fundamental infrared region. Salt optics are used: LiF (up to 6 mk); CaF_2 (up to 9 mk); NaCl (up to 15 mk); KBr (up to 27 mk); CsI (up to 40 mk). The radiation source is power rods, the radiation receiver is thermoelements, bolometers, optical - acoustic receivers.

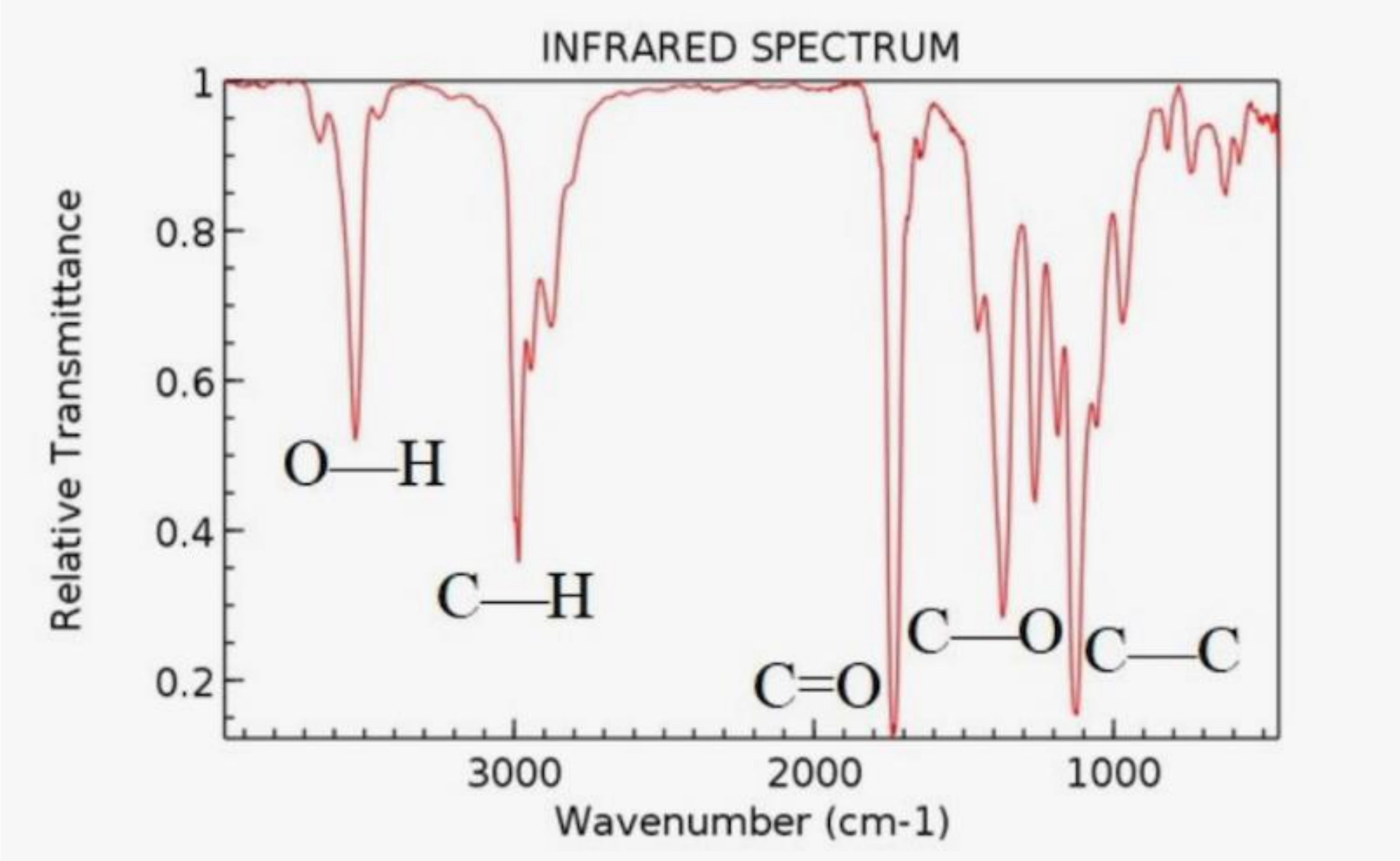
The infrared region of the spectrum is divided into several ranges according to the optical materials used:

- The area up to 200 mk is the far infrared region. It is investigated using diffraction gratings. The method has acquired its development only in recent years. Diffraction gratings can be used over the entire range of optical spectra. Grids with different numbers of strokes per 1 cm are used for different areas: 2000 - 12000 pcs/cm. The visible area is 1000 pcs/cm. For the far infrared region from 80 to 300 pcs/cm

IR spectroscopy and Raman spectroscopy

- The vibrations of a diatomic molecule correspond to a discrete set of states and their corresponding energy levels. To solve the problem of the origin of the vibrational spectrum of a diatomic molecule, it is necessary to know the dependence of its potential energy on the internuclear distance.

An example of the IR spectrum



Registration of IR spectra

- Modern spectrometers allow recording the IR spectra of gaseous, liquid and solid samples. To obtain the IR spectrum of an organic or natural compound, only 1 to 10 mg of the substance is needed. IR spectra are recorded in cuvettes made of potassium bromide KBr or sodium chloride NaCl – materials that do not absorb IR radiation in the studied range. IR spectra are usually recorded as a dependence of the transmission of IR radiation (%) on the wavenumber $v = 1/\lambda (cm^{-1})$.

Fourier infrared spectroscopy

- The most widely used technique of spectral analysis in the infrared (IR) region, Fourier-IR spectroscopy, has some specific features that are not always known to specialists accustomed to using standard diffraction devices.

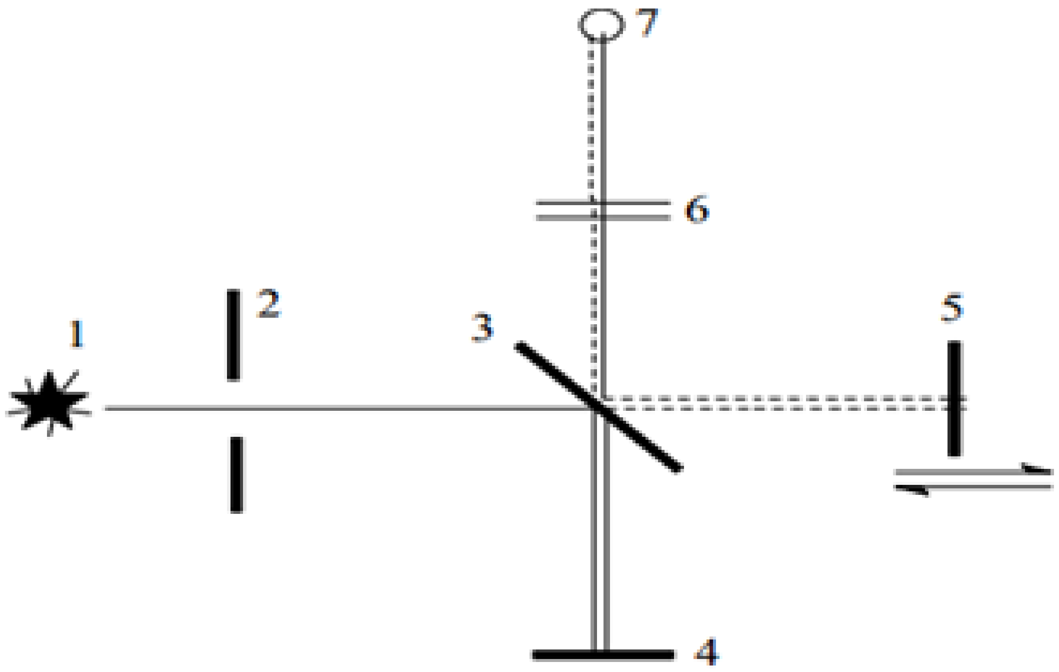
Fourier infrared spectroscopy

- One of the main reasons for this is that here the formation of the spectral contour does not occur as a result of direct adjustment of the slit width, scanning speed and other familiar physical parameters, but requires the use of mathematical transformations such as Fourier transform, phase correction and apodization, which can make it difficult to understand the Fourier-IR technique.

Fourier infrared spectroscopy

- In Fourier spectrometers, the spectrum is obtained using a Michelson interferometer, the scheme of which is shown in the figure.

Fourier spectrometer



1. The radiation source. 2. The aperture aperture. 3. The beam splitter. 4. The motionless circlet. 5. Movable mirror. 6. Imaginative. 7. Radiation receiver.

Methods of sample preparation and methods of spectrum measurement

- Currently, IR spectroscopy uses various methods for measuring the spectra of solutions, gases, powders and molecules adsorbed on them and, consequently, different methods for preparing samples for measurements. These techniques can be divided into three groups.
- The first group includes the oldest methods – transmission methods (Transmittance mode).
- The second group of spectrum measurements is the NPVO or ATR (impaired total internal reflection) techniques.
- The third group is diffuse reflection (DRIFT) techniques.

Varian 660 – IR Fourier Spectrometer

Varian (Agilent Technologies since 2010) is one of the leaders in the production of high-quality Fourier spectrometers. In Fig. The Varian 660-IR IR Fourier spectrometer with the following main characteristics is depicted:

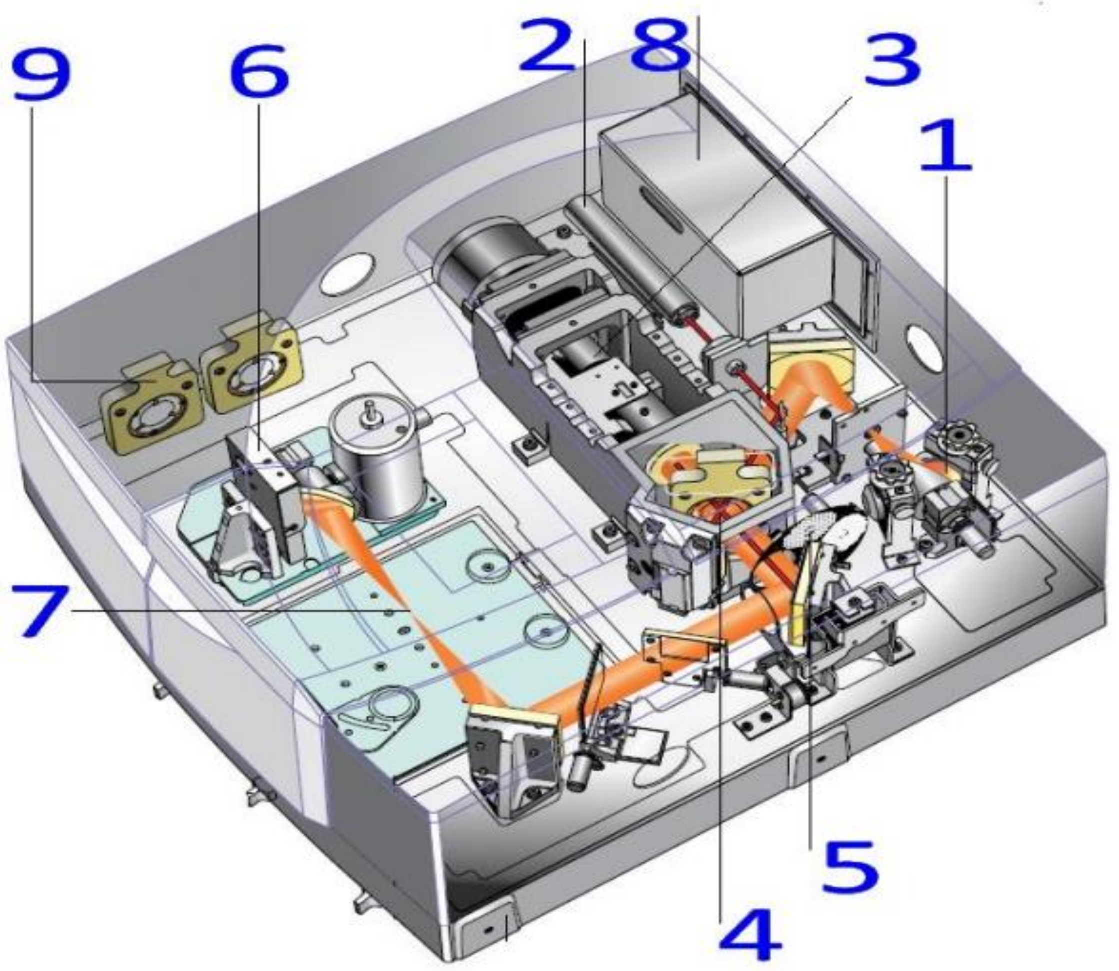
- Standard spectral range: 7900-375 cm-1 (~1.27 – 27 microns)
- Maximum spectral range: 50000-20 cm-1 (~0.2 – 500 microns)
- Spectral resolution: 0.07cm-1
- Signal-to-noise ratio (peak-to-peak): 10,000:1 (with 5-second measurement)
- High-luminous 60-degree Michelson interferometer
- External beam outputs for connection of remote set-top boxes - TGA/IR, Raman set-top box, IR microscopes, remote experimental module, matrix detection systems, PEM-IRRAS, emission experiments

Varian 660 – IR Fourier Spectrometer

Varian 660 – IR Fourier Spectrometer

- The scheme (Fig.) of the Varian 660-IR Fourier spectrometer includes: 1 – an IR radiation source, 2 – a helium-neon laser, 3 - a precision moving system for a movable mirror, 4 – a 60° Michelson interferometer, 5 – an attenuator with software control, 6 – an IR radiation receiver, 7 – a place installations of the object under study, 8 – ADC (24 bit 600 kHz Delta-Sigma), 9 – storage place for replaceable beam-splitting plates. There are also holes for spectral analysis of external radiation.

Varian 660 – IR Fourier Spectrometer



RAMAN SPECTROSCOPY (RAMAN SPECTROSCOPY)

Light scattering is a set of physical phenomena (reflection, refraction, diffraction, etc.) that affect the direction of light propagation in a substance and can change the wavelength of light.

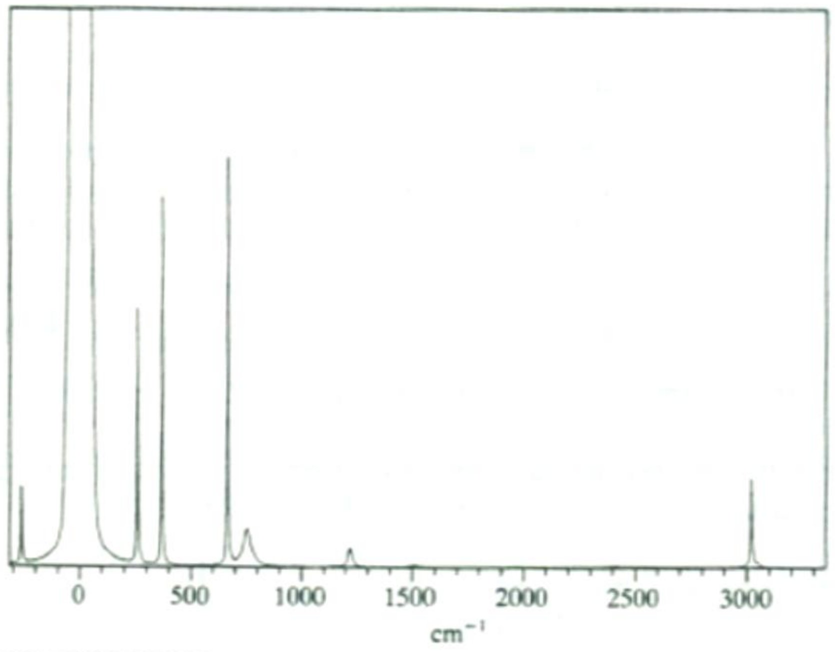
Types of light scattering:

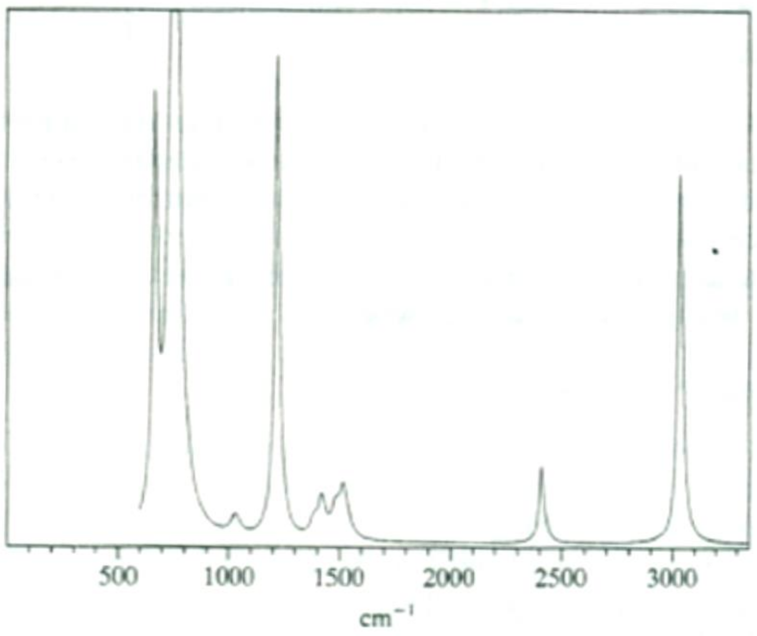
- Elastic (elastic) Rayleigh – without changing the wavelength of light;
- Inelastic (inelastic) Raman – with a change in the wavelength of light.

RAMAN SPECTROSCOPY (RAMAN SPECTROSCOPY)

- The Riemannian scattering changes the wavelength of the incident light due to the interaction of light with the vibrational quanta of the scattering molecule.

Examples of IR and RAMAN spectra.





Applications of Raman scattering spectroscopy

- In chemistry, the identification of chemicals.
- In biology and medicine – the study of the structure of proteins, polypeptides, lipids, oligosaccharides. Clinical studies of biological tissues of organisms.
- Analysis of food and medical products without opening transparent polymer packaging (such packages have a weak spectrum of Raman).
- In engineering, the analysis of composite and ceramic materials, artificial diamonds, etc.