NATURAL POLYMERS

Identification of the NTURAL POLYMERS

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Time schedule

LECTURE	SUBJECT
1	Introduction to the subject – Structure & Terminology of nature polymers, literature
2	Derivatives of acids – natural resins, drying oils, shellac
3	Waxes
4	Plant (vegetable) gums, Polyterpene – natural rubber (extracting, processing and modification), Taraxacum_kok-saghyz
5	Polyphenol – lignin, humic acids
6	Polysaccharides I – starch
7	Polysaccharides II – celullose
8	Protein fibres I
9	Protein fibres II
10	Casein, whey, protein of eggs
11	Identification of natural polymers
	Laboratory methods of natural polymers' evaluation

1. Melting Point
2. Separation Methods
    1. Distillation
    2. Chromatography
    3. Electrophoresis
3. Spectroscopic Methods
    1. Mass Spectroscopy
    2. FTIR Spectroscopy
    3. NMR Spectroscopy
    4. UV Spectroscopy

Principal Problems of the Natural Compounds & Polymers Analysis and Determination

- They are chemical Individuals
- They can Place of the Plant (Source Cultivation, e.g Resins
- The Influence of the „Aging“, e.g. Drying Oils
- **The Natural Compounds are frequently found as a Mixtures with the other Natural Substances (Polymers)**
- The only small Quantities are frequently available for the Analysis
- ……

Metling Point

The Behaviour after heating on Microscope's Hot Stage it is possible to differ between particular Natural Polymers (Oligomers) accordingly:

- **Waxes – 50 – 90 °C**
- **Resins - from approx. 120 °C up**
- **Polymerised Oils - from approx. 160 °C up**
- **Egg Yellow - from approx. 200 °C up**

This PRELIMINARY differentiated Samples is possible to analyse using the more Sophisticated Methods

Separation Methods

1. **Distillation – e.g. Manufacturing of the Turpentine and Colophony form the Pine Rosin**
2. **Extraction – the Wood's Rosins**
3. **Chromatography – Amino acids Separation using Thin Layer Chromatography (TLC)**
4. **Electrophoreses - Amino acids Separation using the Electric Field (is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field)**
5. **.....**

Separation Methods - Distillation

- **Disadvantages:**
  - The bigger Quantity of the Sample is needed
  - Not possible to use Solid State Materials
  - The Separation is not so „SHARP“ as the Chromatography
- **Advantages:**
  - The bigger Quantity of the Sample is got, with which is possible to do further Work,
  - It is simple from the Instrumentation point of View, but there are the very complicated Machines also (e.g. Spin Band Column)

Separation Methods - Chromatography

- **Disadvantages :**
  - The bigger Quantity of the Sample is **NOT** got, with which is possible to do further Work (the so called **PREPARATIVE Chromatography** is not widespread now),
- It is **NOT** simple from the Instrumentation point of View
- **Advantages :**
  - The bigger Quantity of the Sample is **NOT** needed
  - The Separation is VERY „SHARP“ up to Individuals
  - It is possible to use Solid State Materials, if they are Soluble in the proper Solvent
  - A lot of the **Chromatography Methods** are available now, a lot of the Detection Methods are possible to be used

Chromatography – the basic Methods

- Paper (probably the oldest one)
- TLC – Thin Layer Chromatography
- Gas (GC)
- Liquid (HPLC)
- Gel permeation (GPC)
- Ion exchange
- ……..

Chromatographic Terms

ANALYTIES - složky vzorku, které mají být chromatograficky rozděleny

Analytic Chromatography – chromatografie sloužící k zjištění existence analytu (tzv. kvalitativní stanovení) a k určení jeho koncentrace ve vzorku (tzv. kvantitativní stanovení).

Chromatograph – přístroj sloužící k chromatografické separaci složek vzorku

Chromatogram – záznam z chromatografu znázorňující jednotlivé analyty nejčastěji ve formě tzv. chromatografických píků (zón) oddělených navzájem základní linií

Chromatographic Separation – rozdělení vzorku na jednotlivé složky (analyty) na základě rozdílné distribuce mezi mobilní a stacionární fázi

Mobil Phase – neboli eluent, je fáze pohybující se chromatografickým systémem. Tato fáze přivádí vzorek do stacionární fáze, kde dochází k jeho separaci

Retention Time – čas, který složka potřebuje k průchodu chromatografickým systémem

Preparative Chromatography – slouží k izolaci čistých (nebo alespoň čistějších) složek vzorku, které jsou dále použity (k chemické reakci, další separaci apod.)

Stacionary Phase – je fáze ukotvená na místě, přes kterou prochází mobilní fáze a také složky vzorku. Jde např. o tenkou vrstvu silikagelu (při tenkovrstevné chromatografii) či kolona. Zde dochází k separaci v důsledku distribuce vzorku mezi stacionární a mobilní fázi

Classification of the Chromatographic Method according to the Arrangement

Column chromatography

Paper chromatography

Thin layer chromatography (TLC)

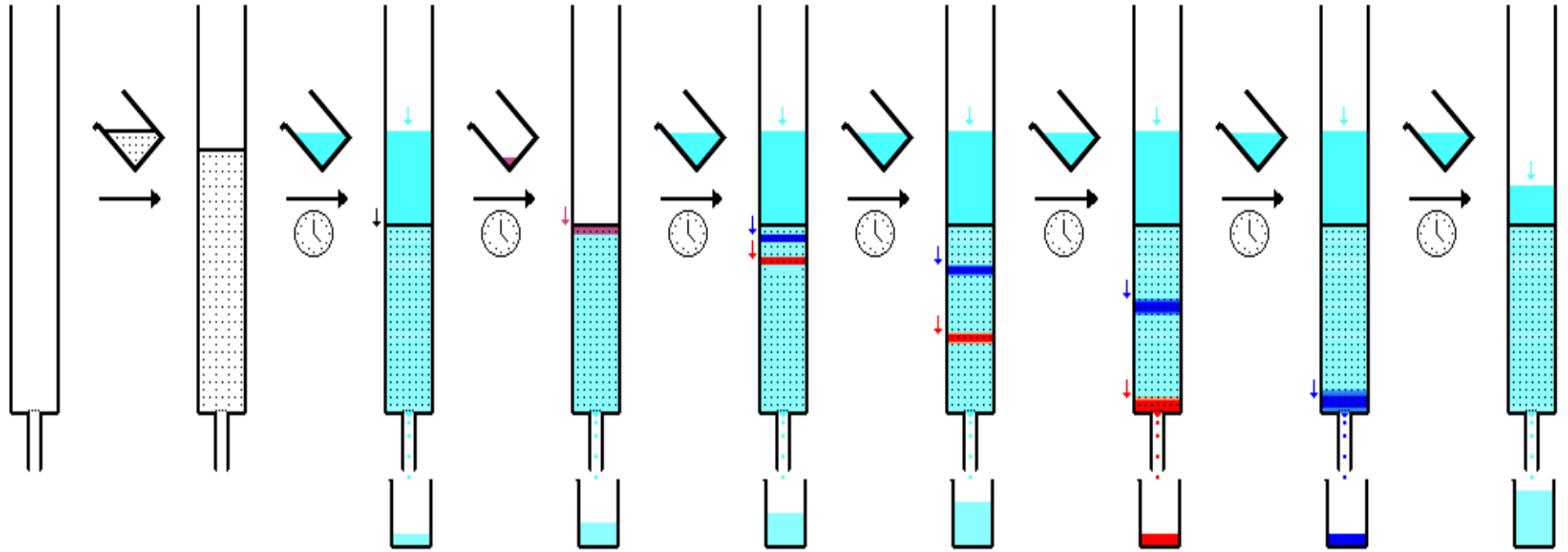
Classification of the Chromatographic Method according to the Arrangement

Column chromatography

is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

Classification of the Chromatographic Method according to the Arrangement

Column chromatography



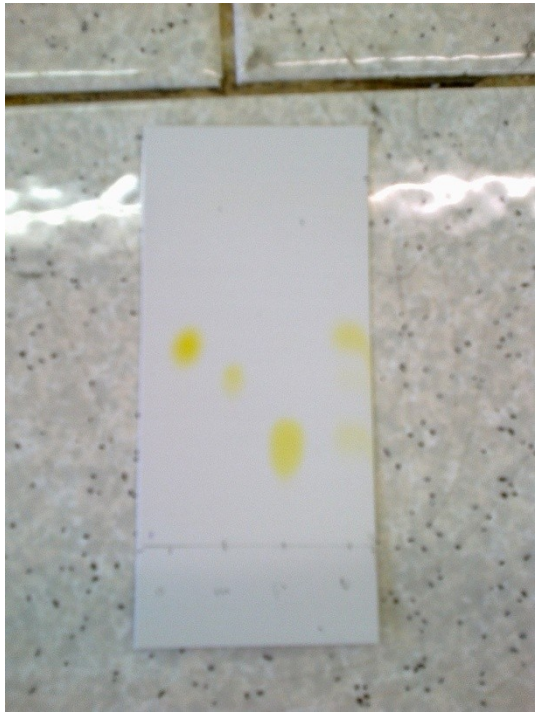
Rozdělení chromatografických metod podle uspořádání

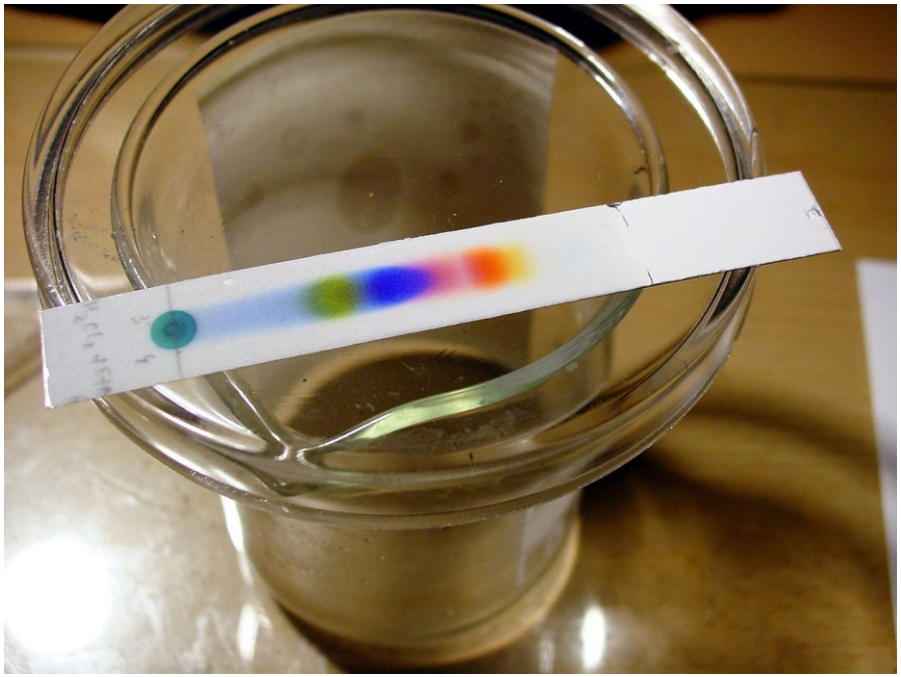
Thin layer chromatography (TLC)

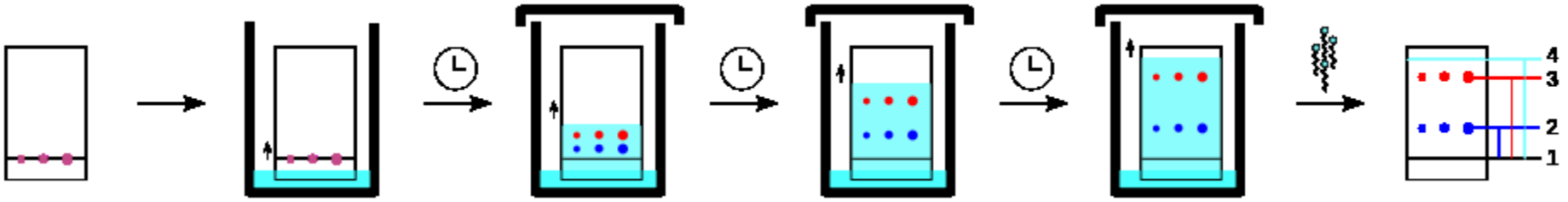
is a widely employed laboratory technique used to separate different biochemicals on the basis of their size and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. TLC is very versatile; multiple samples can be separated simultaneously on the same layer, making it very useful for screening applications such as testing drug levels and water purity. Possibility of cross-contamination is low since each separation is performed on a new layer. Compared to paper, it has the advantage of faster runs, better separations, better quantitative analysis, and the choice between different adsorbents. For even better resolution and faster separation that utilizes less solvent, high-performance TLC can be used. An older popular use had been to differentiate chromosomes by observing distance in gel (separation of was a separate step).

Classification of the Chromatographic Method according to the Arrangement

Thin layer chromatography (TLC)







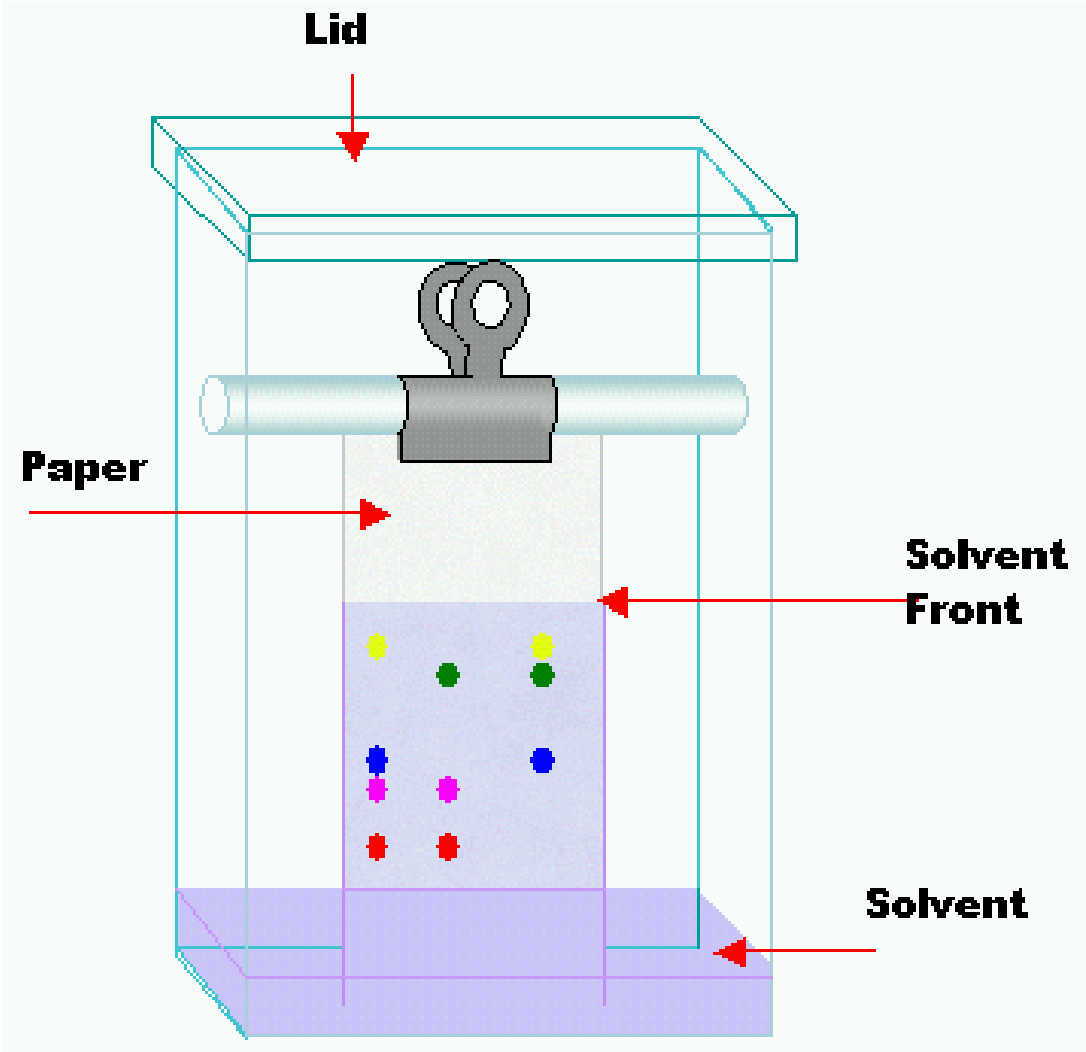
Classification of the Chromatographic Method according to the Arrangement

Paper chromatography

is an analytical method used to separate colored chemicals or substances. It is primarily used as a teaching tool, having been replaced by other chromatography methods, such as thin-layer chromatography. A paper chromatography variant, two-dimensional chromatography involves using two solvents and rotating the paper 90° in between. This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids. The setup has three components. The mobile phase is a solution that travels up the stationary phase, due to capillary action. The mobile phase is generally an alcohol solvent mixture, while the stationary phase is a strip of chromatography paper, also called a chromatogram. A chromatographic method is called adsorption chromatography if the stationary phase is solid

Classification of the Chromatographic Method according to the Arrangement

Paper chromatography



Classification of the Chromatographic Techniques according to the by physical state Mobile Phase

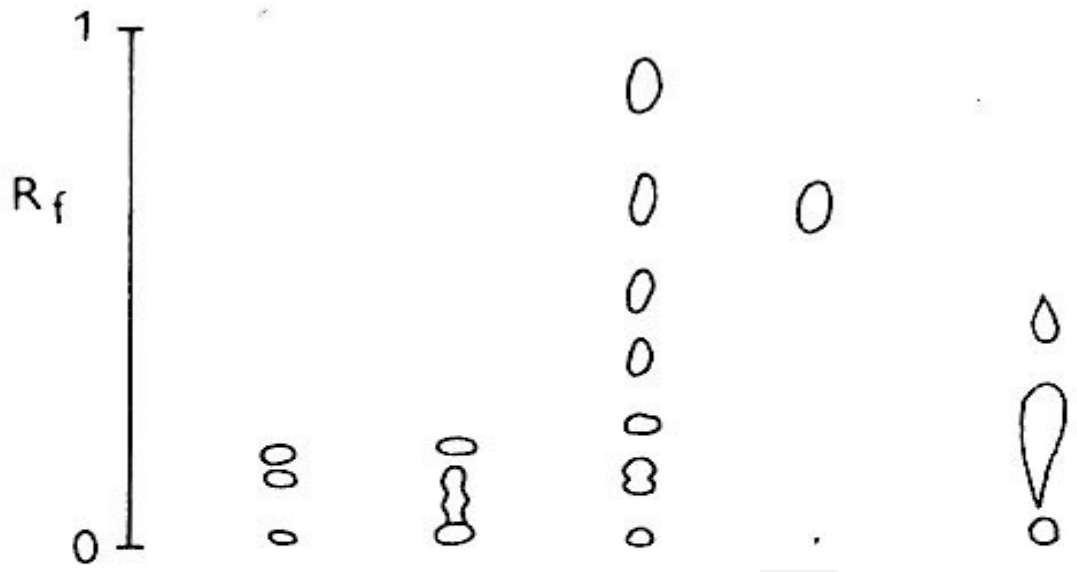
GC, gas chromatography)

Mobile Phase is a Gas. It is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.

LC, HPLC, liquid chromatography)

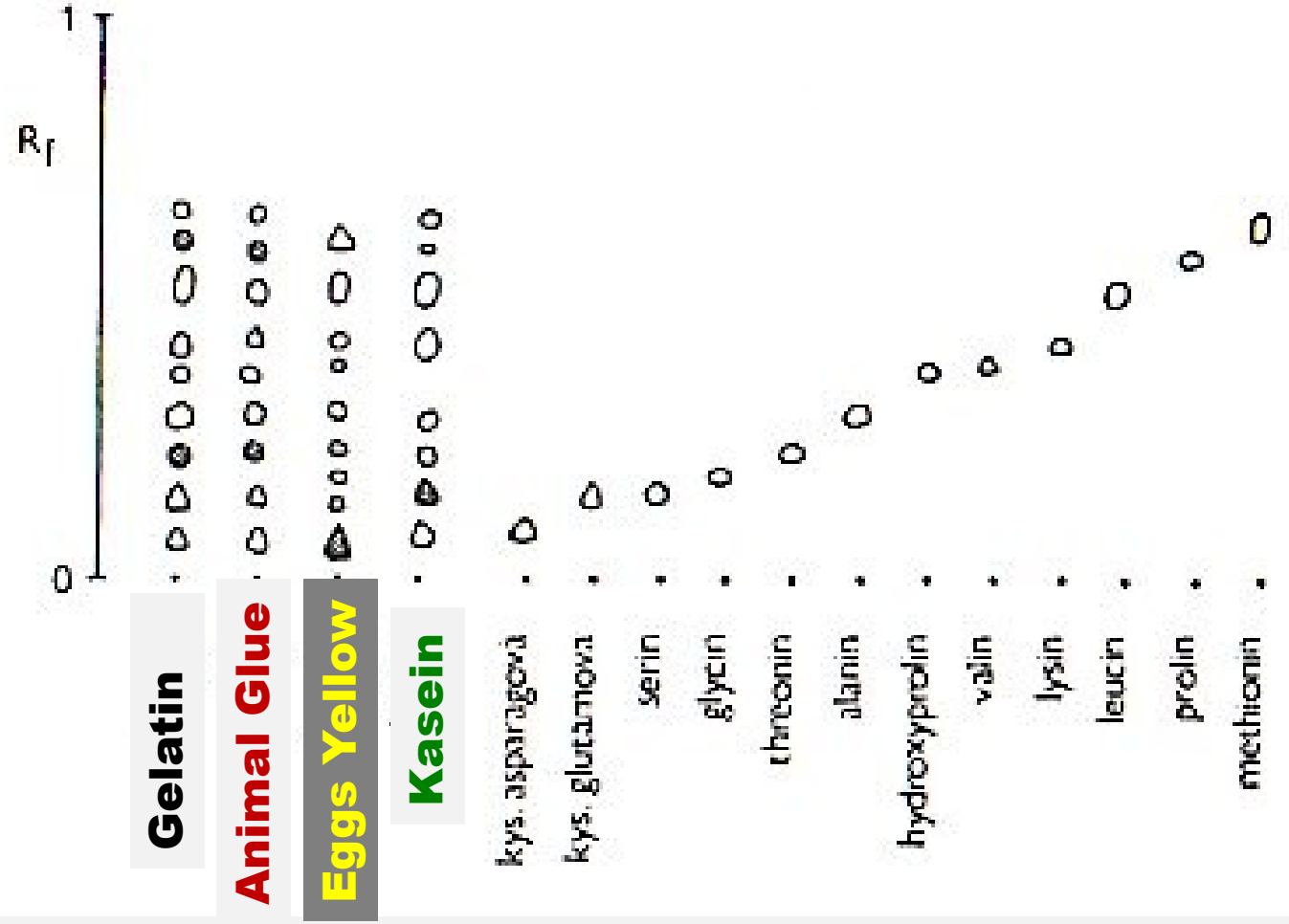
Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid and the Stacionary Phase is the Solid State Material, alternatively Liquid bonded on the Solid State Material.

TLC Chromatography – without any Pre-treatment of the Samples (e.g. By Hydrolysis)



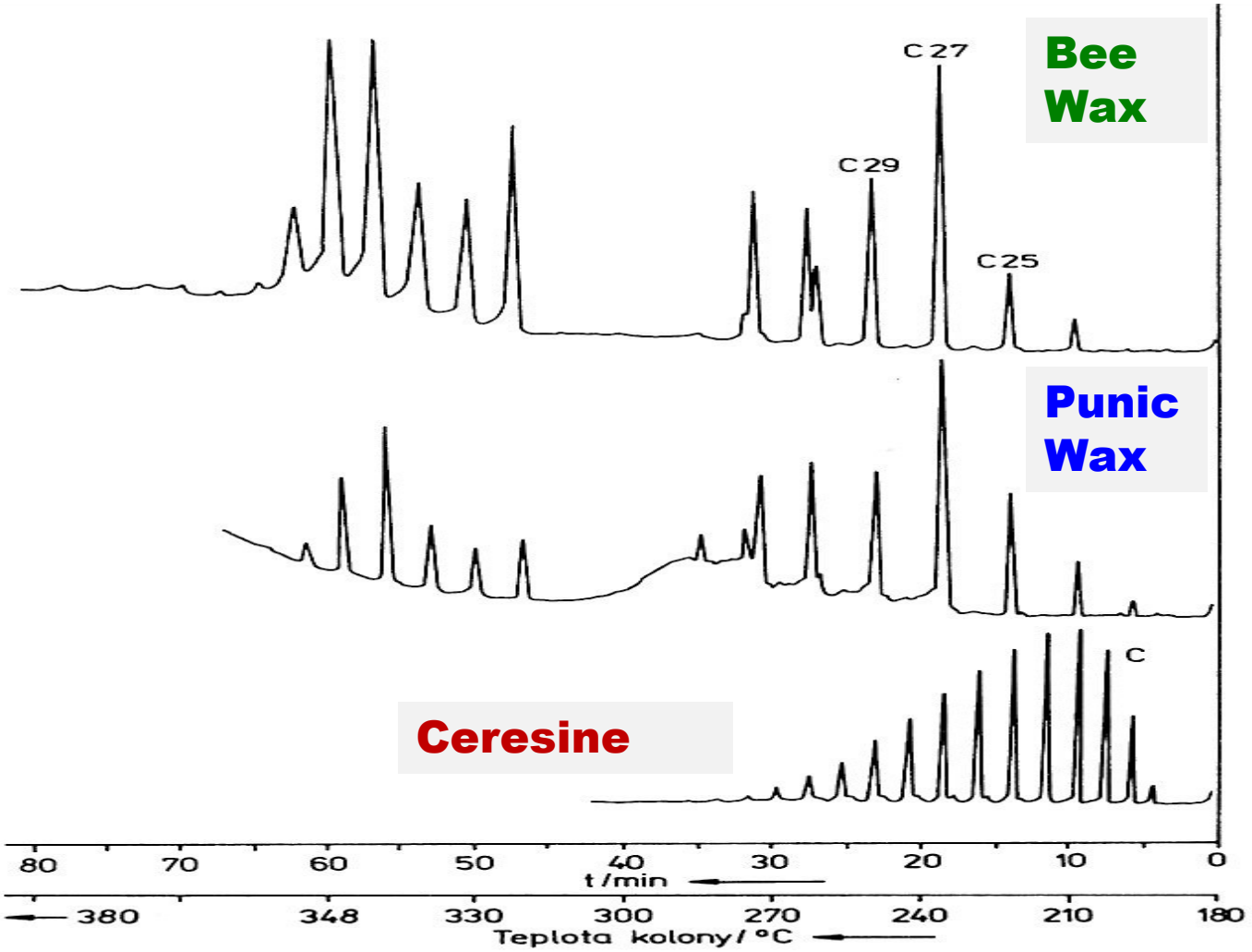
TLC Chromatography of WAXE'S

TLC Chromatography – after the Pre-treatment of the Samples (here are the Amino acids treated by Hydrolysis)



TLC Chromatography of Amino acids and some hydrolysed Proteins

GC of some Waxes after Saponification and Methylation of the acids to the Methyesters



Obr. 23 Plynový chromatogram vosků [29]. A – včelí vosk, B – punský vosk, C – ceresin.

GC of some RESINS after Saponification and Methylation of the acids to the Methyesters

Colophone

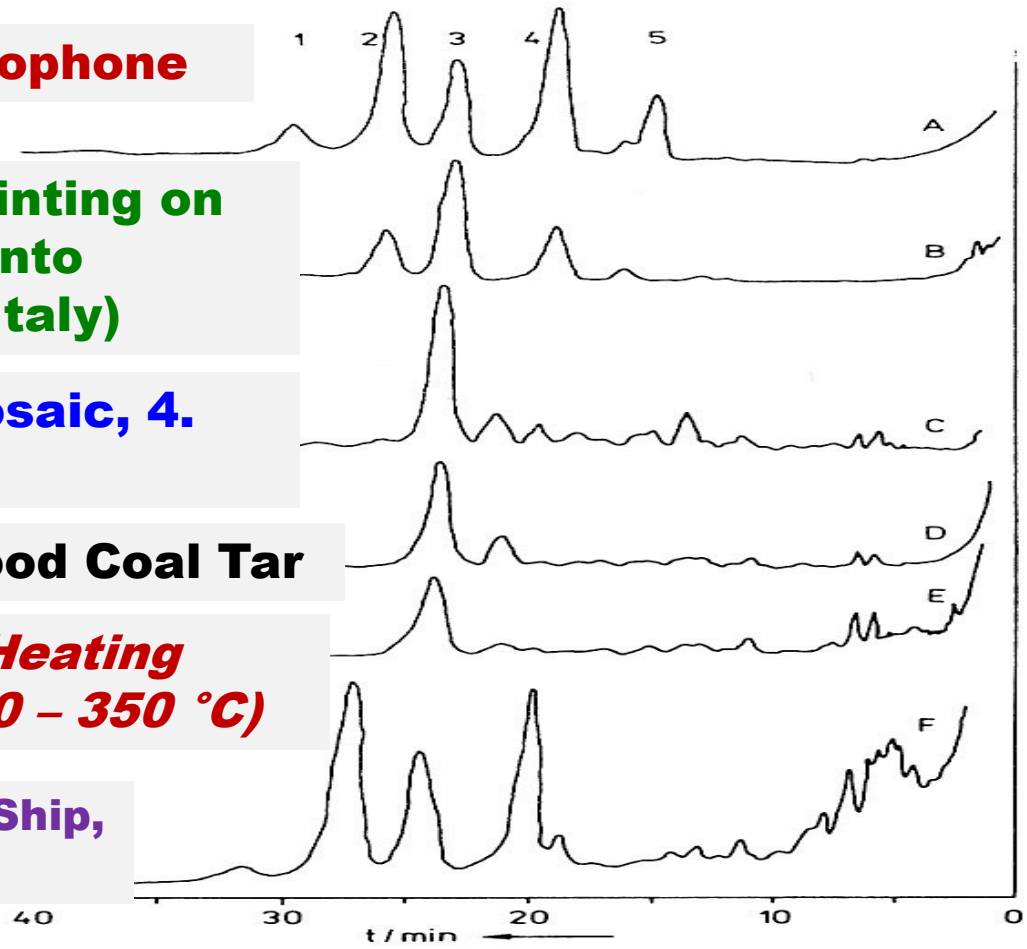
Resin Binder – Painting on the high Altar (Santo Stefano, Venice, Italy)

Resin Binder – Mosaic, 4. Century B.C.

Wood Coal Tar

Colophone after Heating (15 Minutes & 300 – 350 °C)

Resin Binder – War Ship, 3. Century B.C.



24 Plynový chromatogram pryskyřic [35]. A – kalafuna, B – pryskyřičné pojivo z oltáře Santo Stefano v Benátkách, C – pryskyřičné pojivo mozaiky ze 4. st. n.l., D – dehet z dřevěného uhlí, E – kalafuna zahřívaná 15 minut na 300–350 °C, F – pryskyřičné pojivo z válečné lodi ze 3. st. př.n.l. 1 – neoabietát, 2 – abietát, 3 – dehydroabietát, 4 – palustrát/isopimarát, 5 – pimarát.

GC of some Binder Oils after Pyrolysis at 600 °C

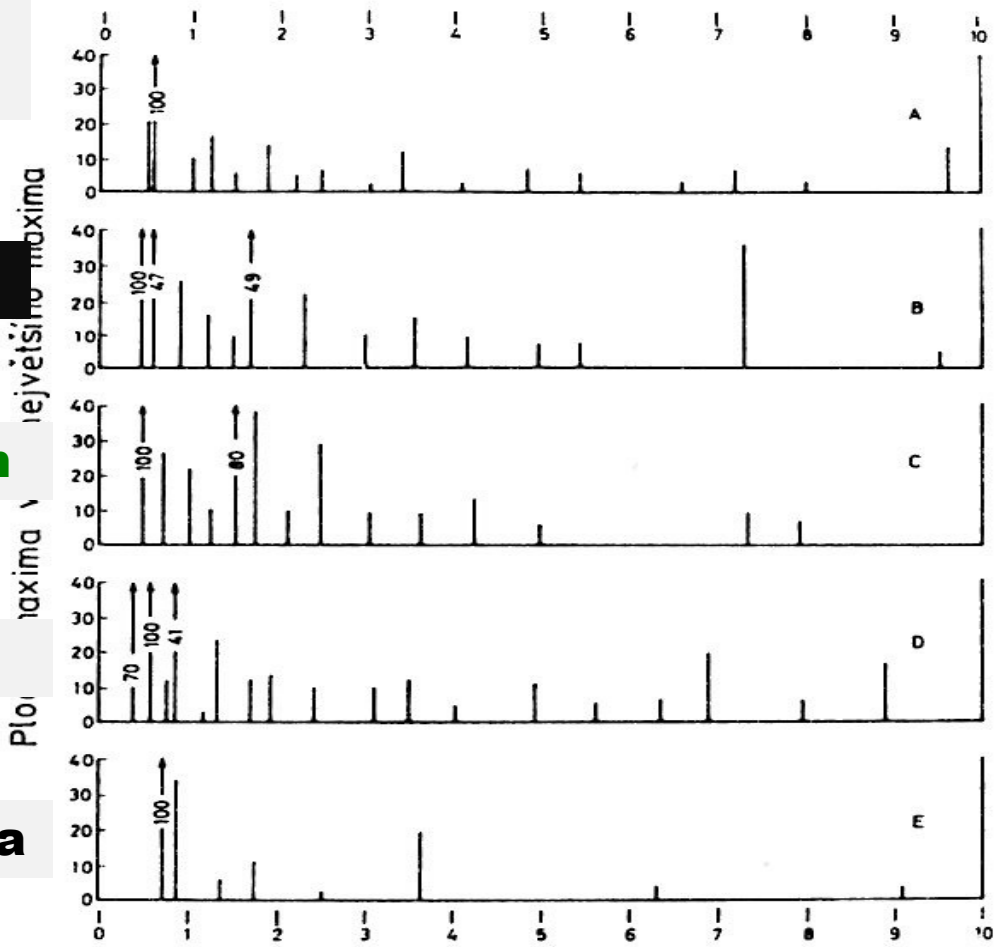
Linseed/Flaxseed oil

Eggs White

Gelatin

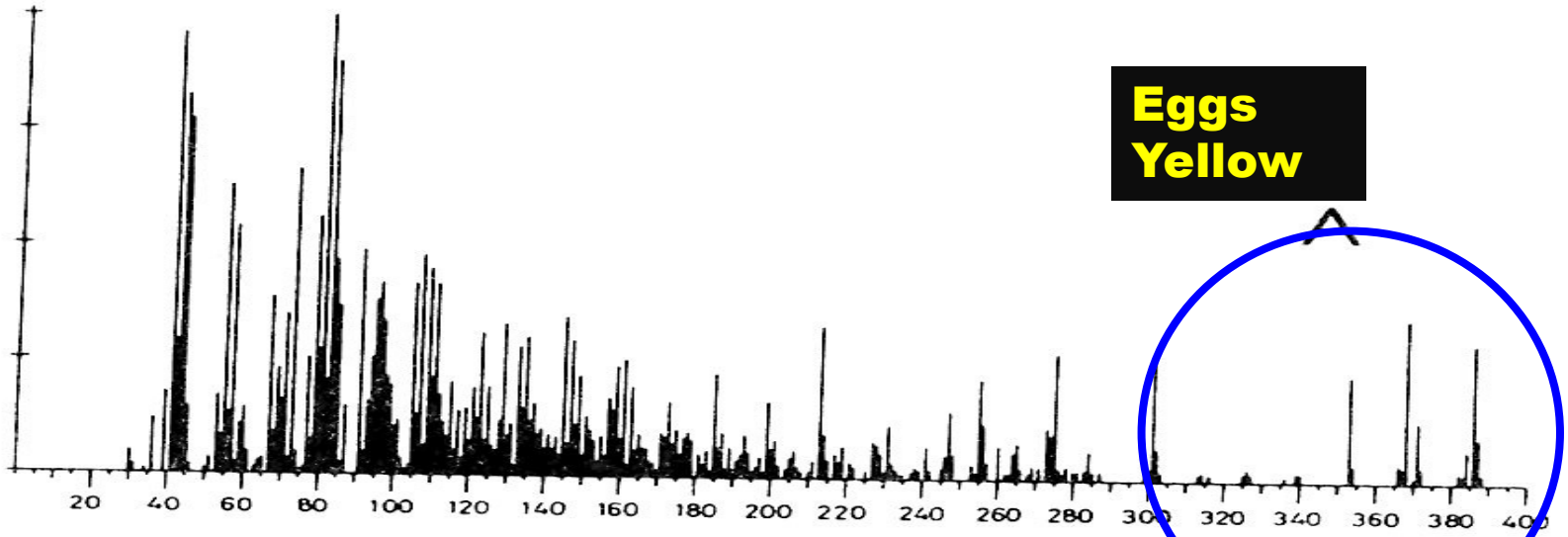
Mastix

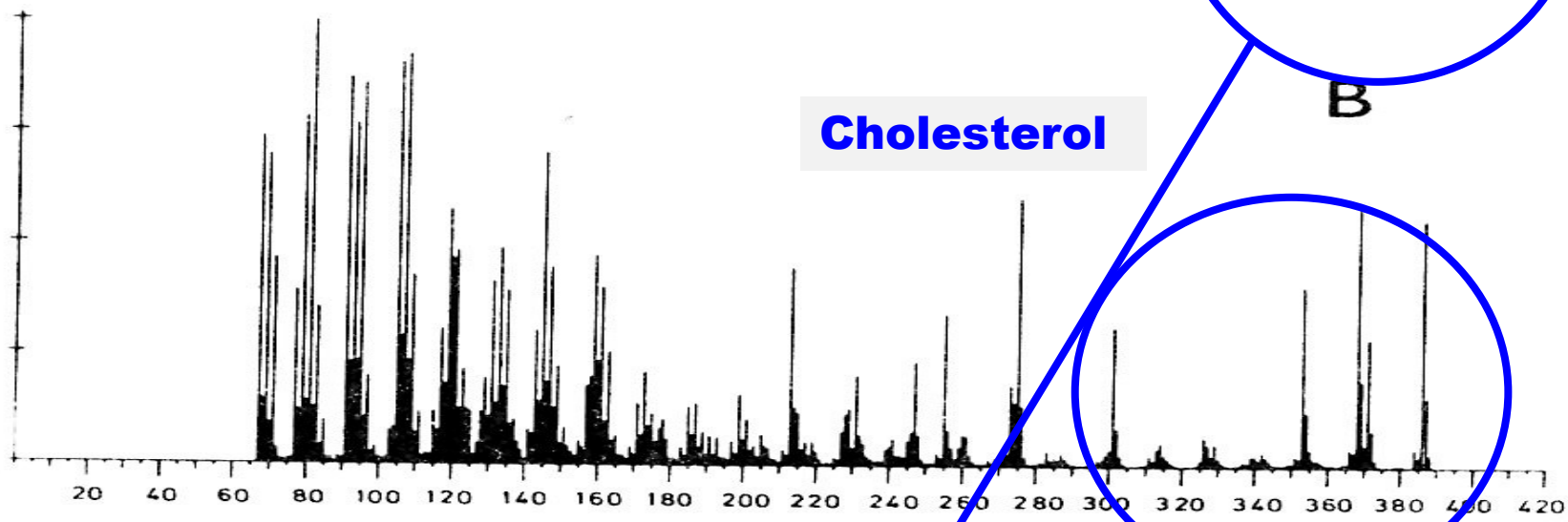
Damara



Obr. 25 Pyrogramy pojiv [61]. A – lněný olej, B – vaječný bílek, C – želatina, D – mastix, E – damara.

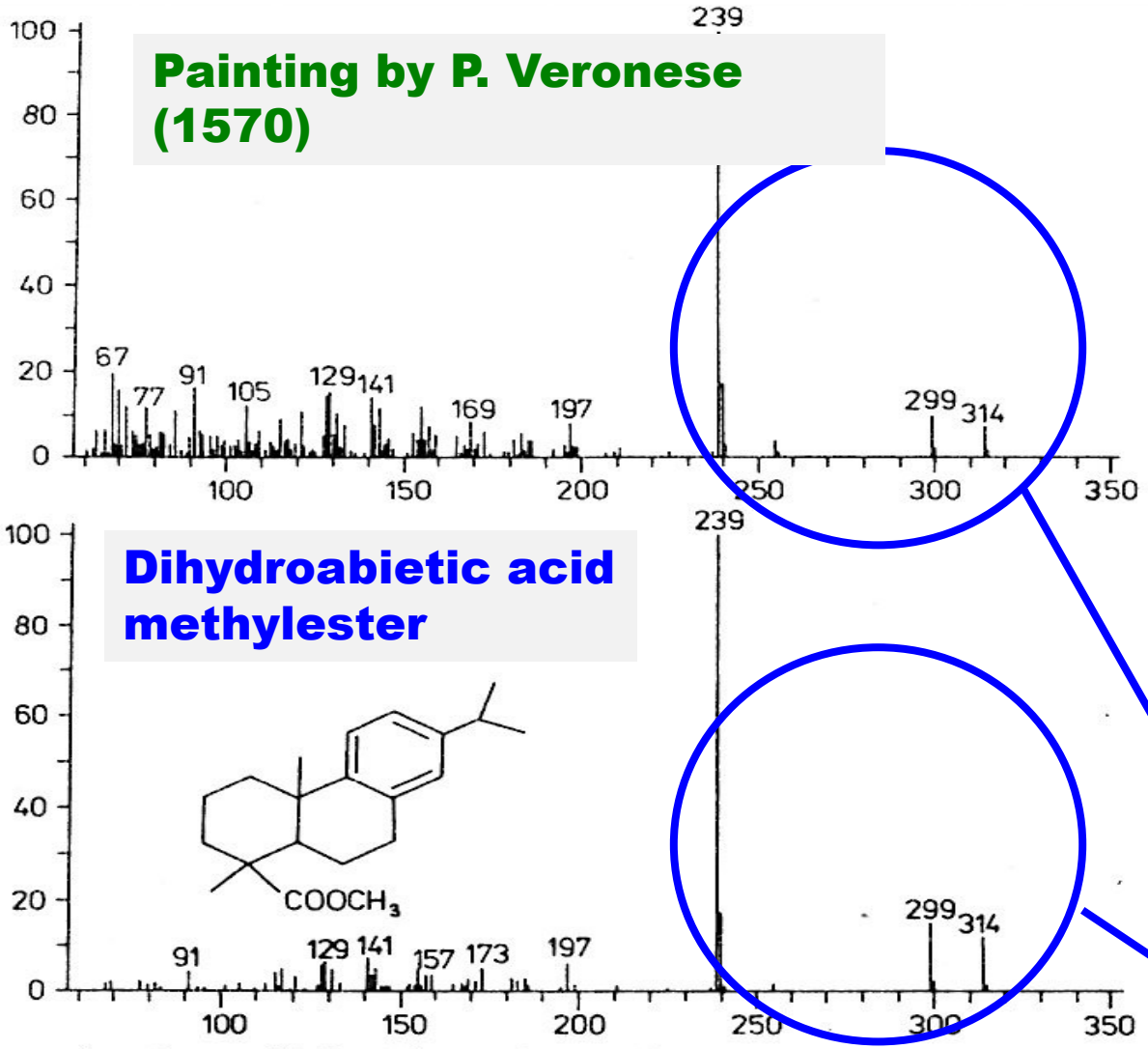
GC & Mass Spectroscopy 1





Obr. 26 Hmotnostní spektrum [62]. A – vaječný žloutek, B – cholesterol.

GC & Mass Spectroscopy 2



Obr. 27 Hmotnostní spektrum [63]. A – část spektra pojiva z obrazu P. Veronese (1570), B – methylester kyseliny dehydroabietové.

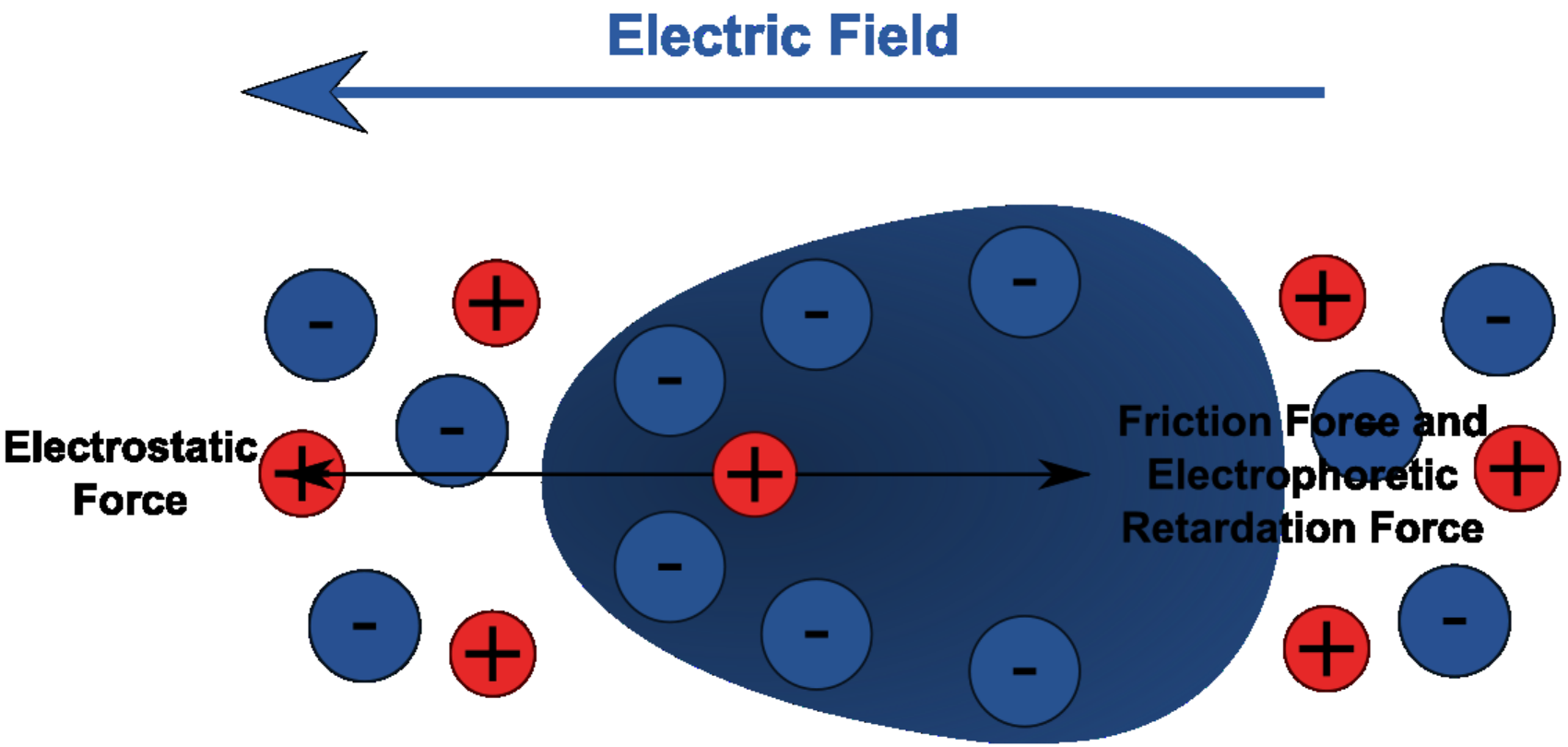
Electrophoresis

- **Elektroforéza** je soubor separačních metod, které využívají k dělení látek jejich odlišnou pohyblivost ve stejnosměrném elektrickém poli. Na principu rozdílných elektroforetických mobilit se při ní dělí nabité molekuly (ionty).
- **V roce 1892 bylo publikováno, že anorganické částice v koloidním roztoku pod vlivem elektrického pole nenáhodně putují. Nedlouho poté byl tento jev popsán i u proteinů ve vodných roztocích.**
- **V roce 1948 byl Nobelovou cenou oceněn švédský chemik Arne Tiselius, který ve 30. letech minulého století postavil aparaturu separující proteiny krevního séra na základě jejich elektroforetických mobilit.**

Electrophoresis 1

- **Electrophoresis** (from the Greek "Ηλεκτροφόρηση" meaning "to bear electrons") is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field.
- **Electrophoresis** of positively charged particles (cations) is called **cataphoresis**, while electrophoresis of negatively charged particles (anions) is called **anaphoresis**.
- **Electrophoresis** is a technique used in laboratories in order to separate macromolecules based on size. The technique applies a negative charge so proteins move towards a positive charge.
- Suspended particles have an electric surface charge, strongly affected by surface adsorbed species, on which an external electric field exerts an electrostatic Coulomb force. According to the double layer theory, all surface charges in fluids are screened by a diffuse layer of ions, which has the same absolute charge but opposite sign with respect to that of the surface charge.

Electrophoresis 2



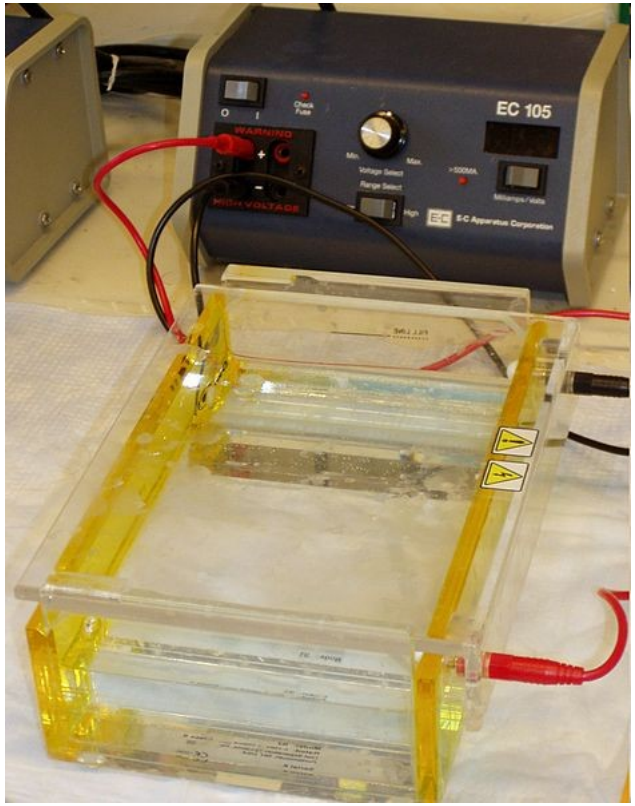
Capilary Gel Electrophoresis

is a process which enables the sorting of molecules based on size. Using an electric field, molecules (such as DNA) can be made to move through a gel made of **AGAROSE** or **POLYACRYLAMIDE**. The electric field consists of a negative charge at one end which pushes the molecules through the gel, and a positive charge at the other end that pulls the molecules through the gel. The molecules being sorted are dispensed into a well in the gel material. The gel is placed in an electrophoresis chamber, which is then connected to a power source. When the electric current is applied, the larger molecules move more slowly through the gel while the smaller molecules move faster. The different sized molecules form distinct bands on the gel.

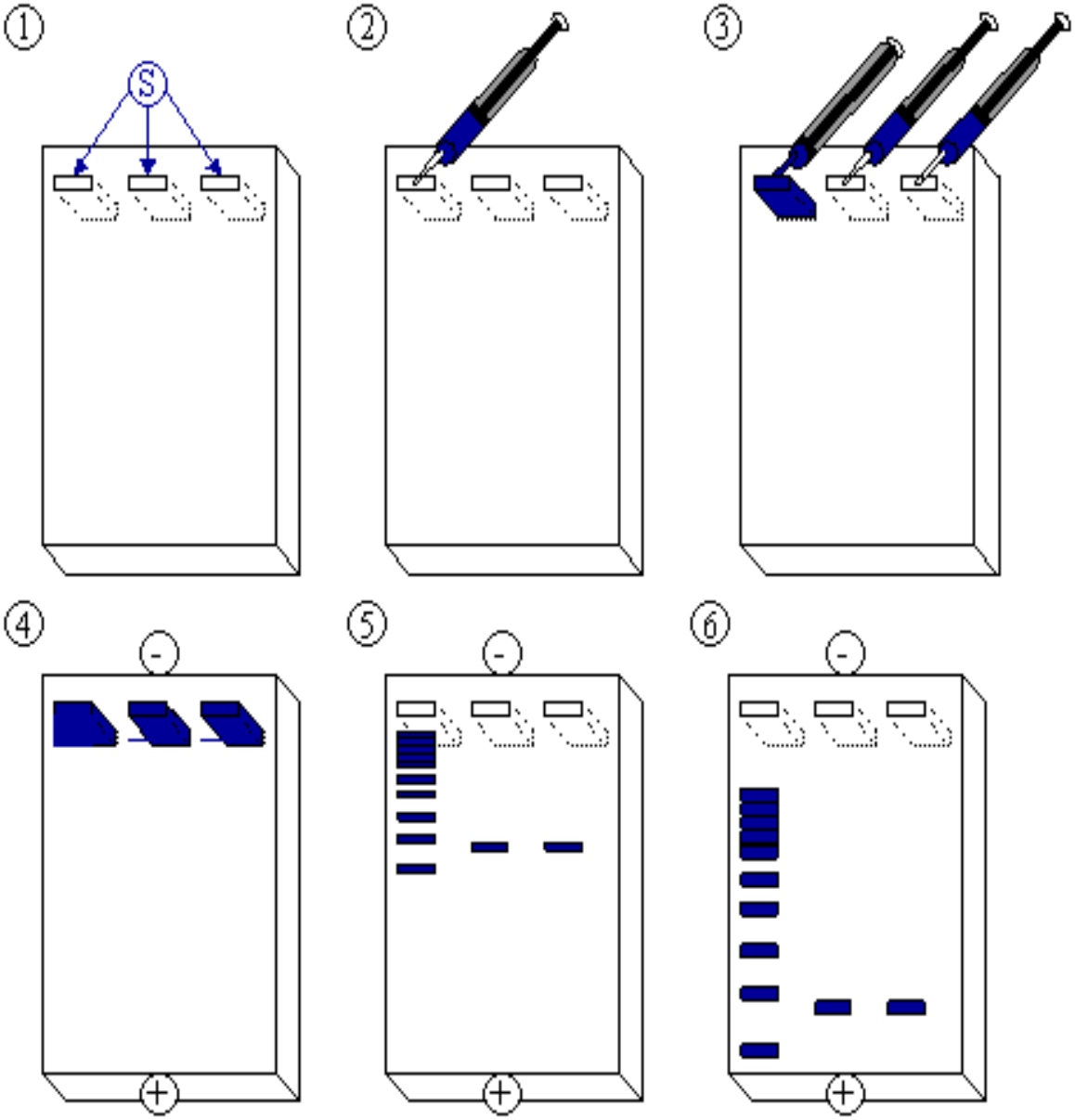
Gel electrophoresis uses a gel as an anticonvective medium and/or sieving medium during electrophoresis, the movement of a charged particle in an electrical field. Gels suppress the thermal convection caused by application of the electric field, and can also act as a sieving medium, retarding the passage of molecules; gels can also simply serve to maintain the finished separation, so that a post electrophoresis stain can be applied.

Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel.

Gel Electrophoresis is the most wide spread Electrophoresis Method now.



Electrophoresis



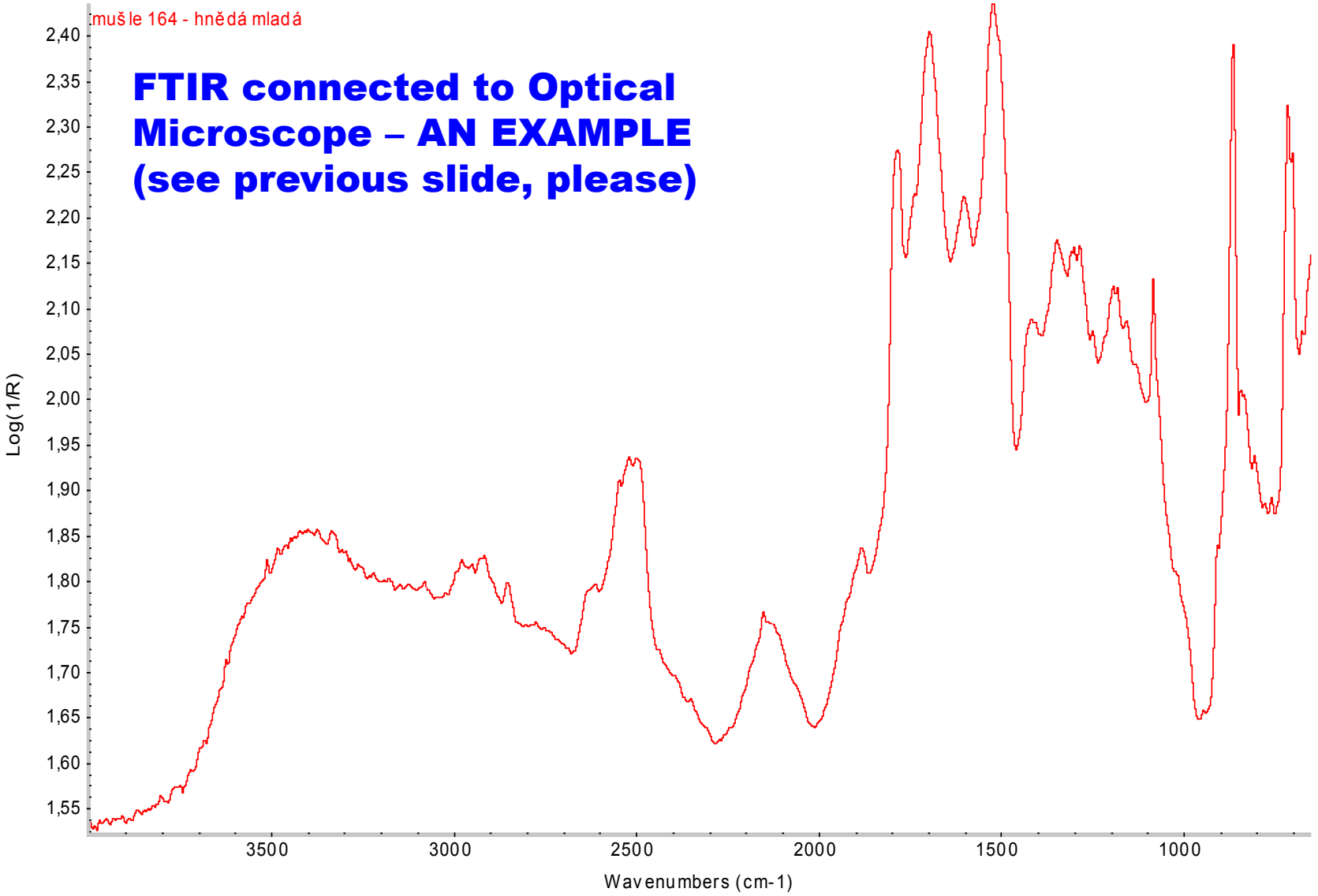
FTIR Spectroscopy

- **Advantages**:
  - Dosti univerzální technika (pevné látky, kapaliny, plyny, roztoky, KBr technika, vícenásobný odraz, …)
  - Malé množství vzorku
  - Možnost spojení s mikroskopií
  - ……..
- **Disadvantages**:
  - Instrumentálně i vzdělanostně náročné
  - Spektrum závisí i na technice měření
  - ……..

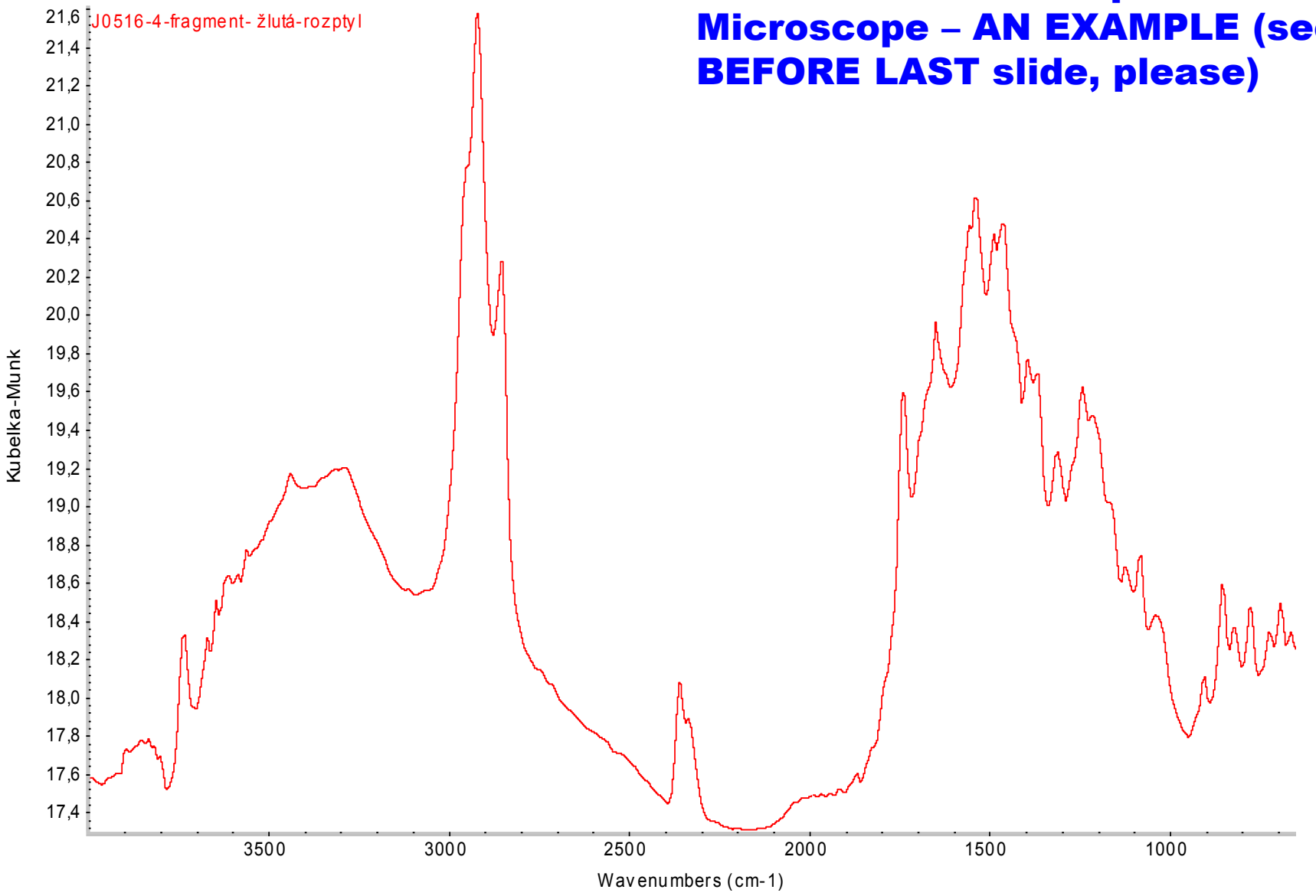
FTIR Spectroscopy

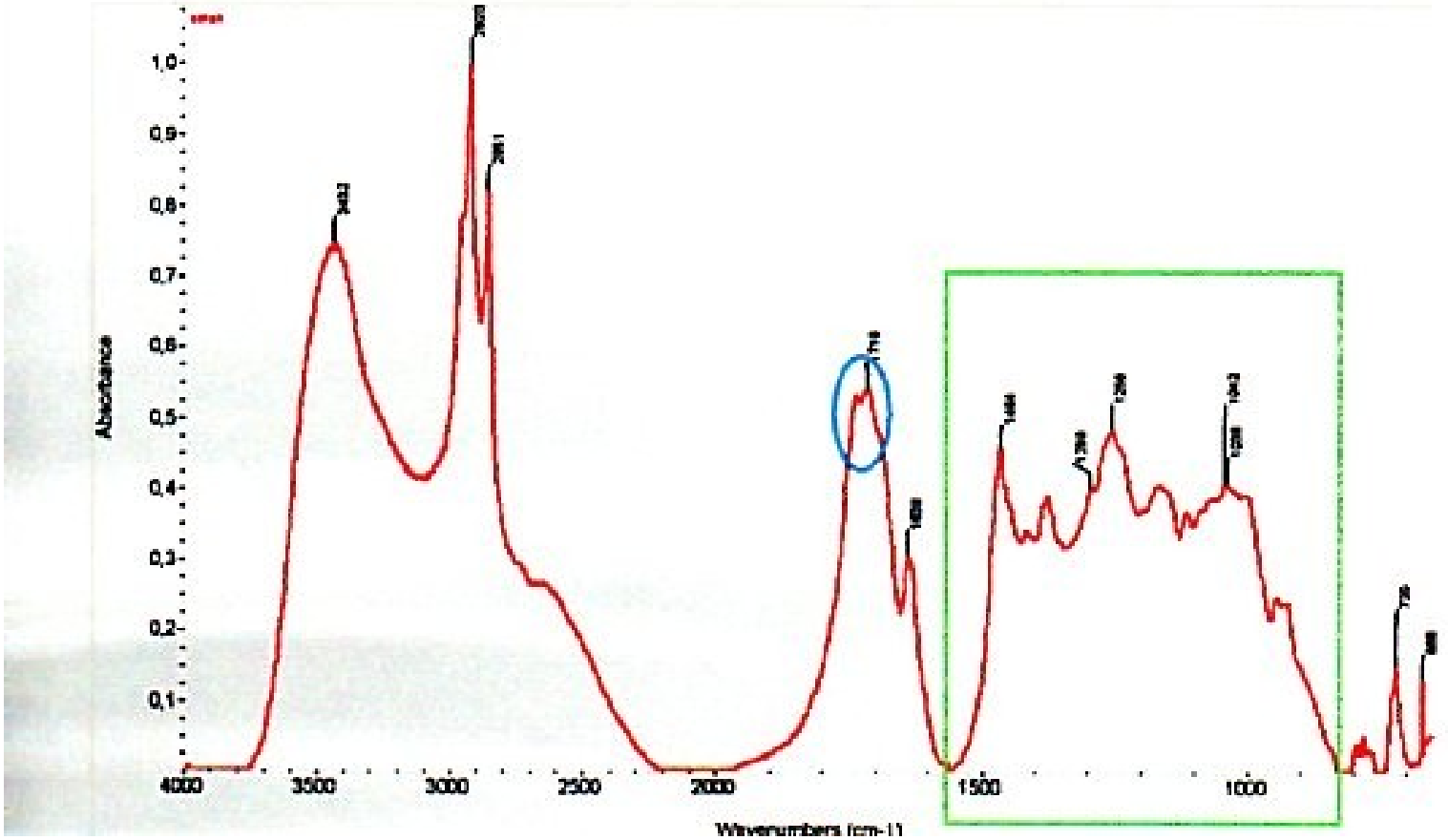
- **Advantages**:
  - General-purpose (universal) Method (Solid State Materials, Liquids, Gases, Solutions, KBr Technique, Multireflection technique. …….
  - Small Quantity of the Sample is needed
  - **It is possible to be connected to Optical Microscope**
  - ……
- **Disadvantages**:
  - It is demanding as to Operator's Qualification and Instrumentation
  - Spectrum depends on the Measurement Technique also
  - ……

FTIR Spectroscopy is possible to be connected to Optical Microscope


mušle 164 - hnědá mladá
FTIR connected to Optical Microscope – AN EXAMPLE (see previous slide, please)
Log(1/R)
2,40
2,35
2,30
2,25
2,20
2,15
2,10
2,05
2,00
1,95
1,90
1,85
1,80
1,75
1,70
1,65
1,60
1,55
3500
3000
2500
2000
1500
1000
Wavenumbers (cm-1)

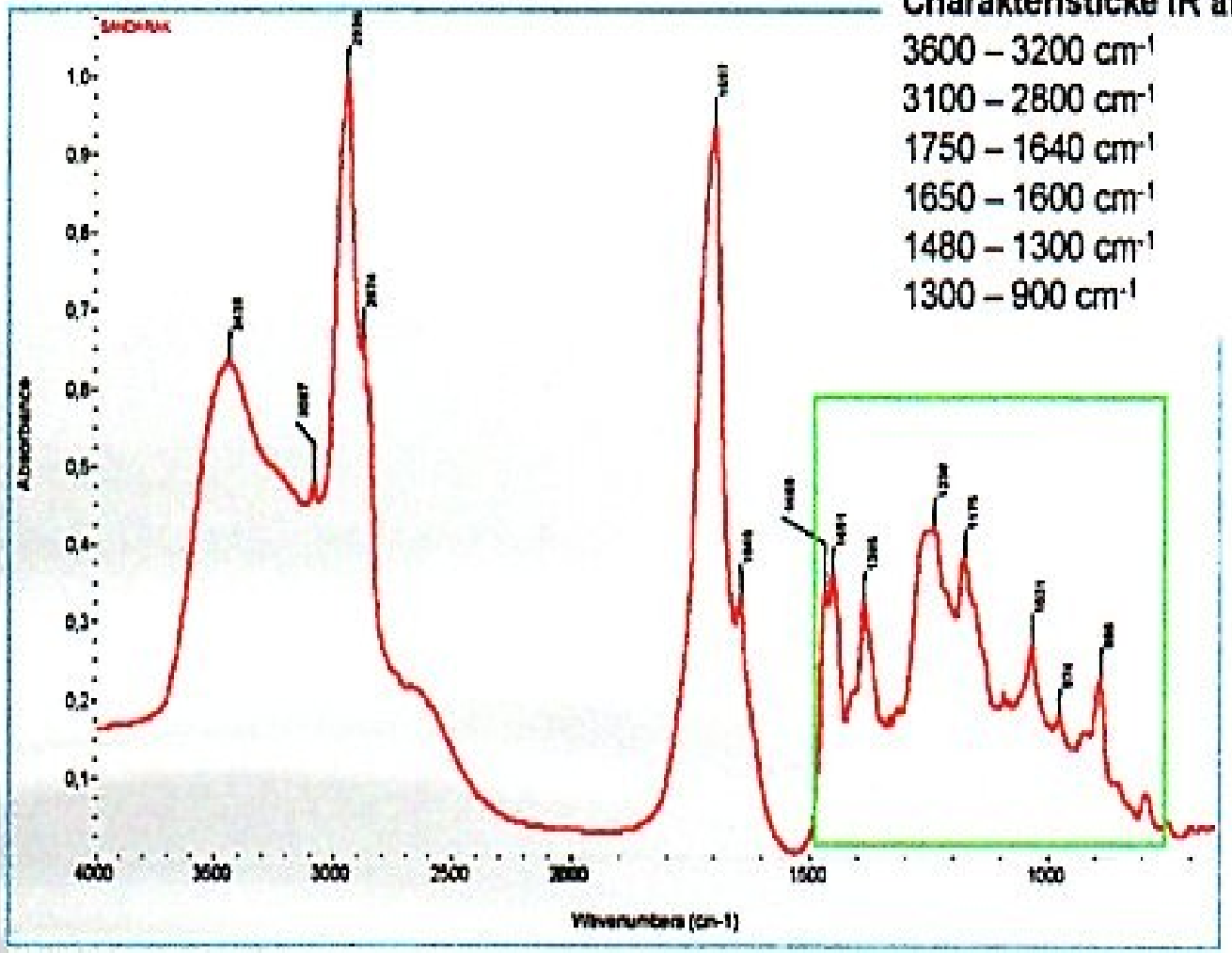
FTIR connected to Optical Microscope – AN EXAMPLE (see BEFORE LAST slide, please)





oblast fingerprintu
1466 cm^{-1}
1412 cm^{-1}
1377 cm^{-1}
1291 cm^{-1}
1250 cm^{-1}
1165-1176 cm^{-1}
1112 cm^{-1}
1041 cm^{-1}
945 cm^{-1}
930 cm^{-1}

FTIR Spectrum SHELAC



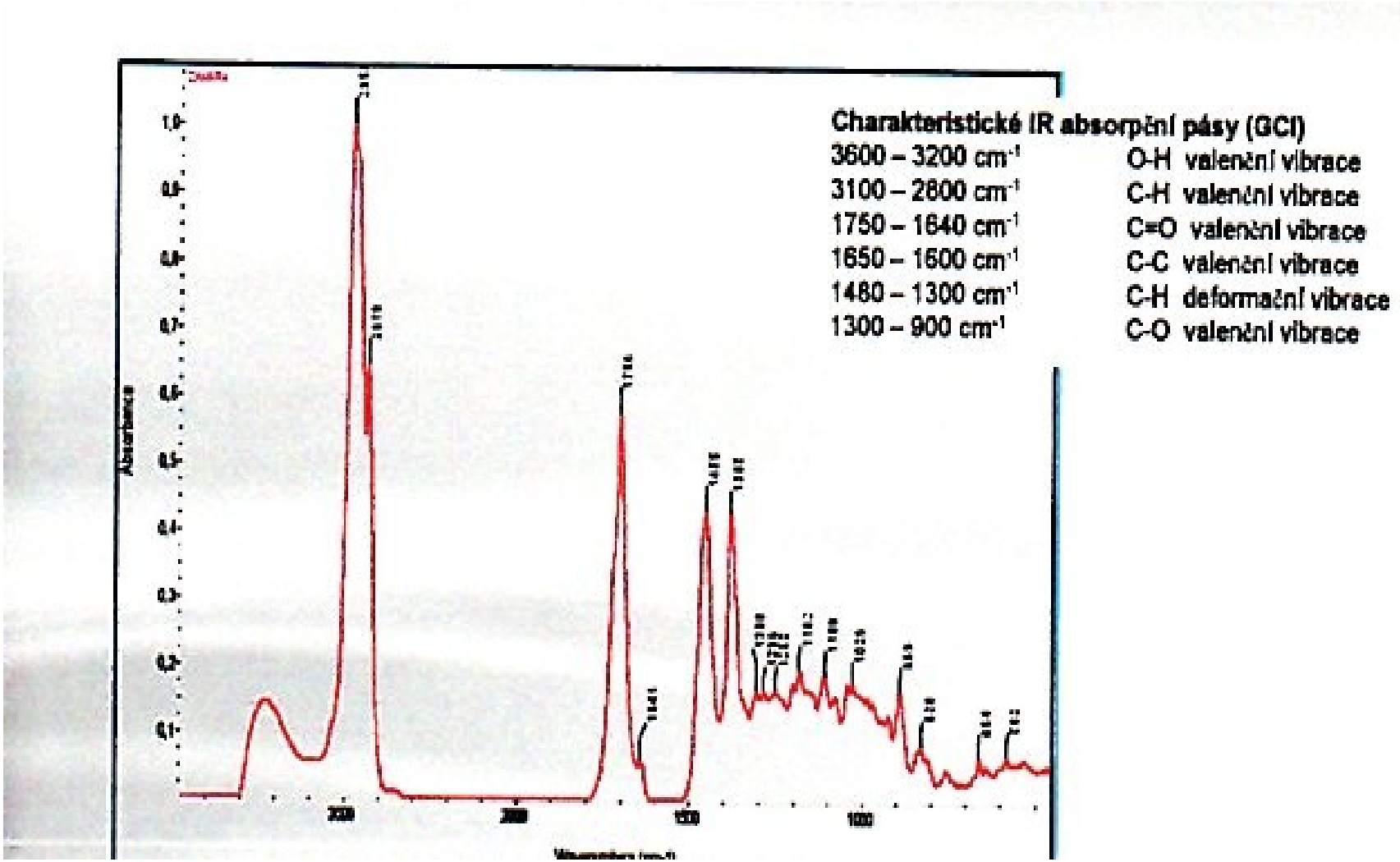
Charakteristické IR absorpční pásy

3600 – 3200 cm^{-1}	O-H valenční vibrace
3100 – 2800 cm^{-1}	C-H valenční vibrace
1750 – 1640 cm^{-1}	C=O valenční vibrace
1650 – 1600 cm^{-1}	C-C valenční vibrace
1480 – 1300 cm^{-1}	C-H deformační vibrace
1300 – 900 cm^{-1}	C-O valenční vibrace

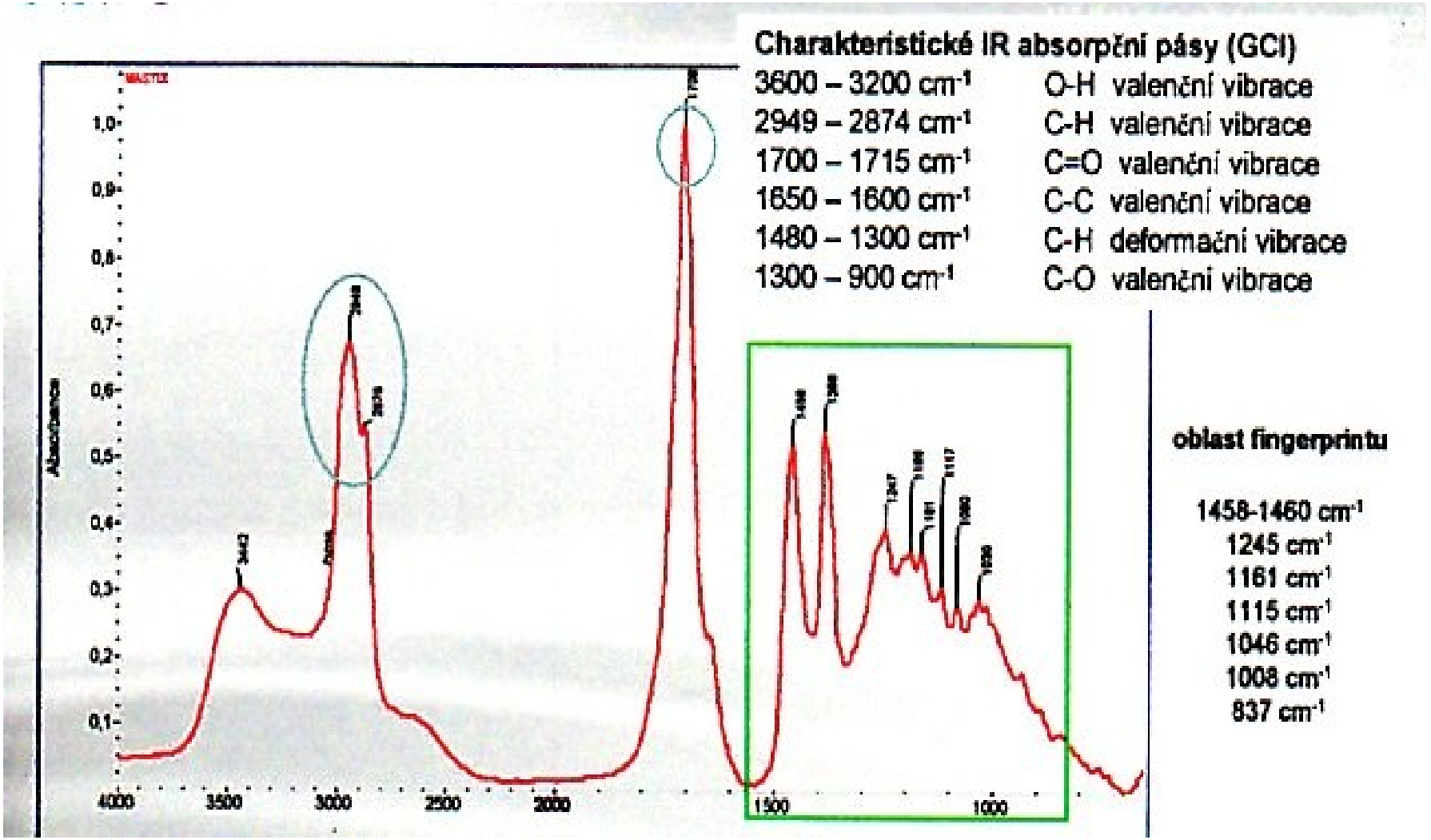
oblast fingerprintu

1466 cm^{-1}
1449 cm^{-1}
1329 cm^{-1}
1315 cm^{-1}
1259-1263 cm^{-1}
1497 cm^{-1}
1236 cm^{-1}
1213 cm^{-1}
972 cm^{-1}
909 cm^{-1}
856 cm^{-1}
823 cm^{-1}
789-792 cm^{-1}

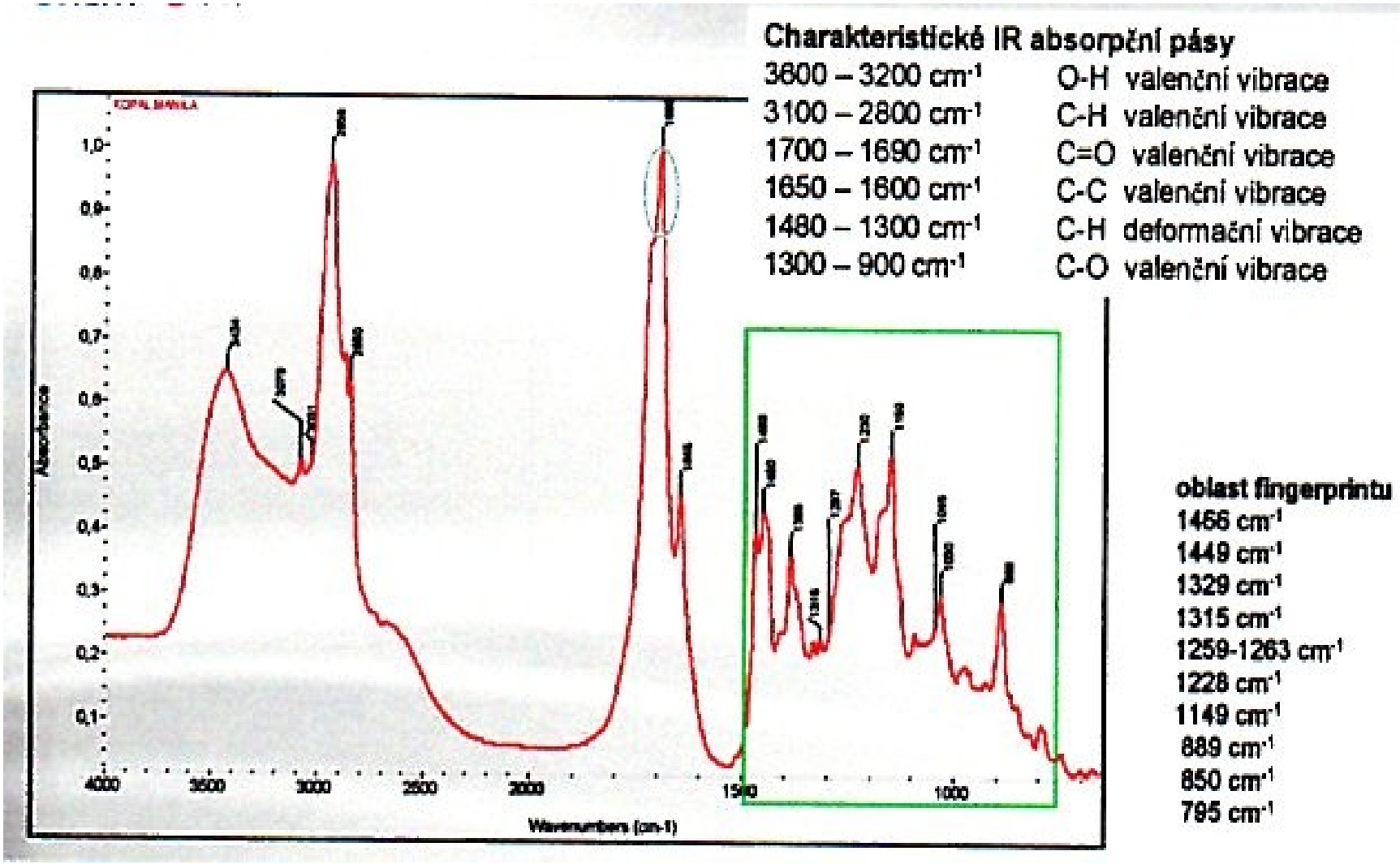
FTIR Spectrum SANDARAK



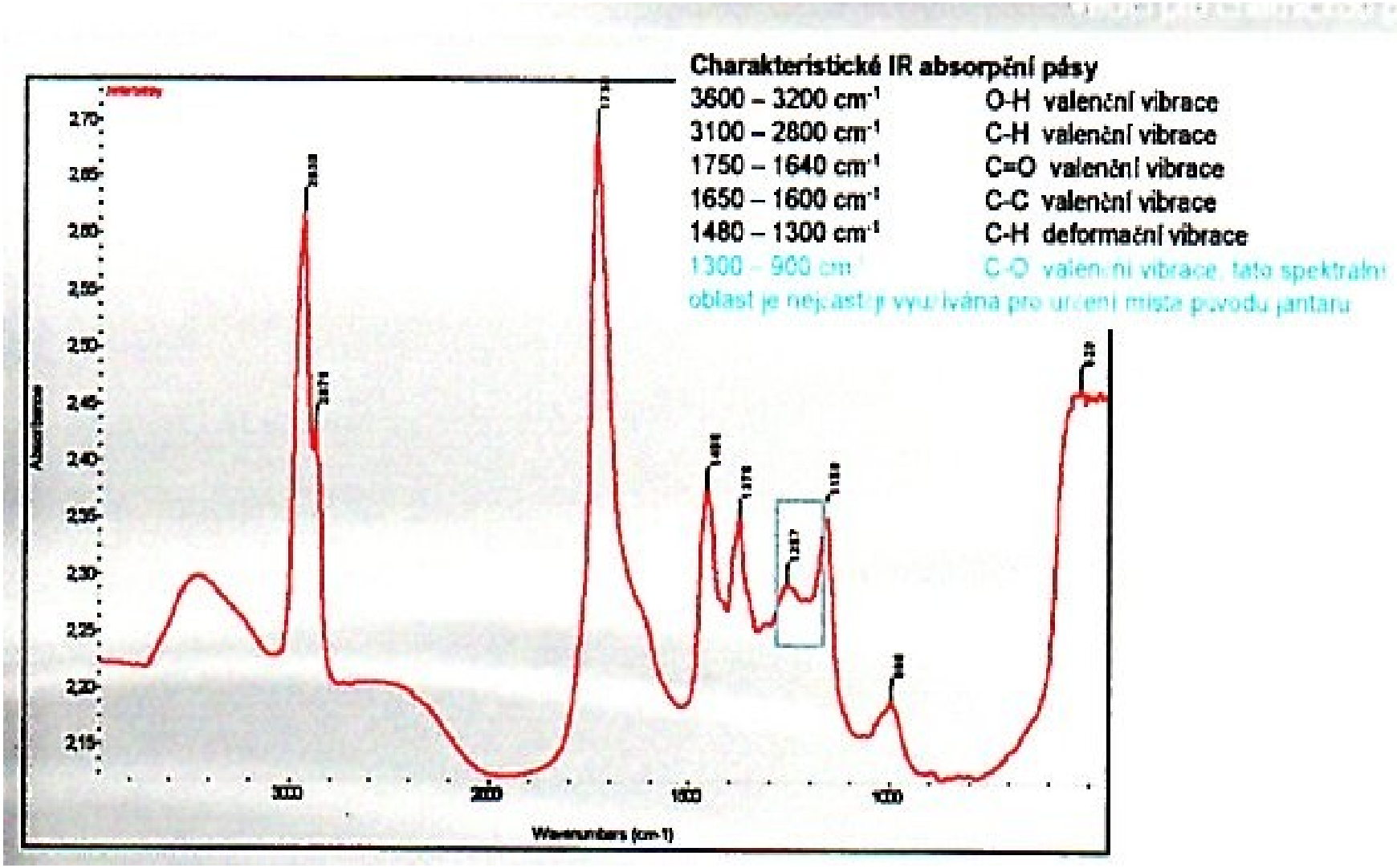
FTIR Spectrum DAMARA



FTIR Spectrum MASTIX



FTIR Spectrum COPAL



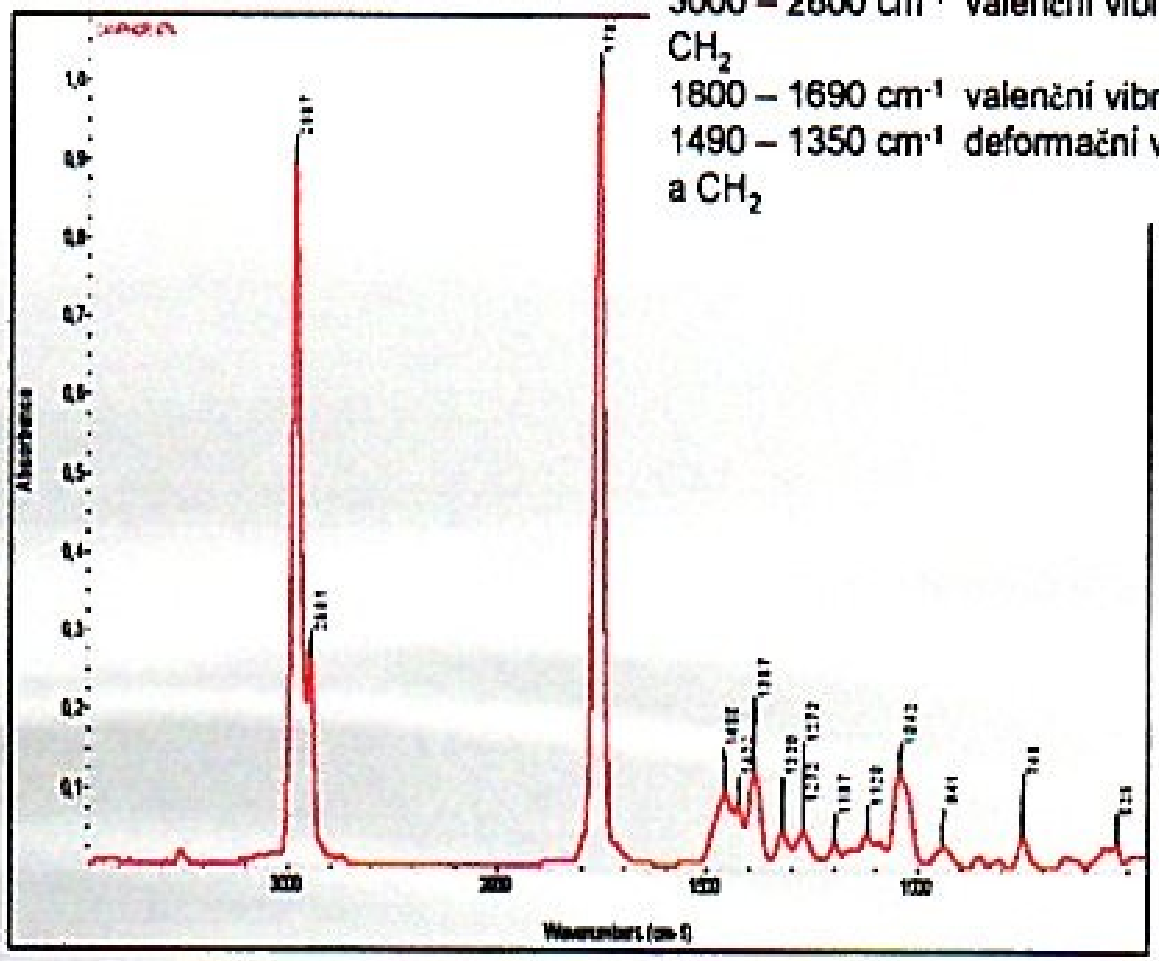
FTIR Spectrum Amber (Baltic See Region)

Charakteristické IČ absorpční pásy

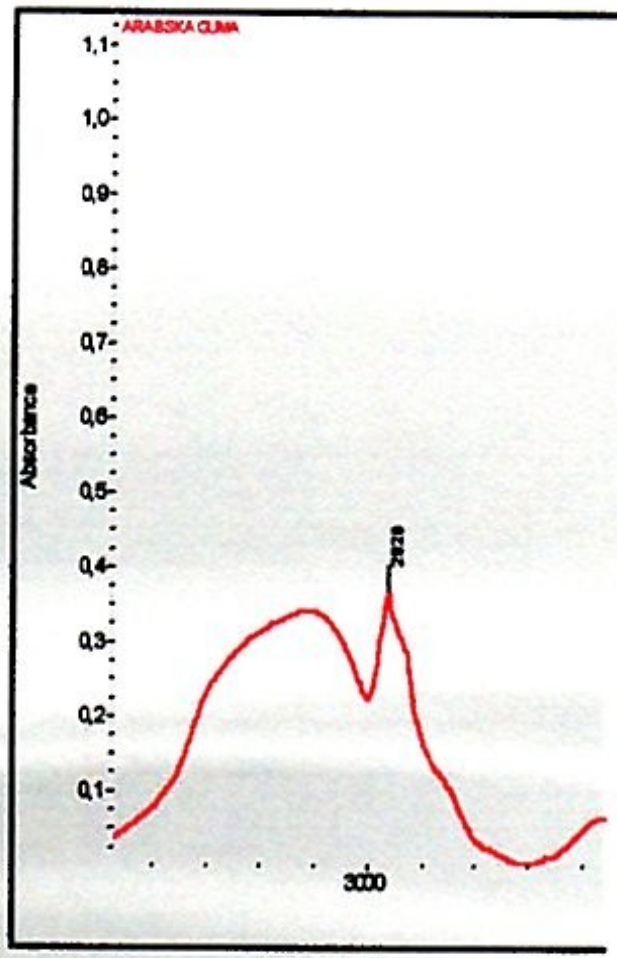
3000 – 2800 cm^{-1} valenční vibrace CH ve skupinách CH_3, CH_2

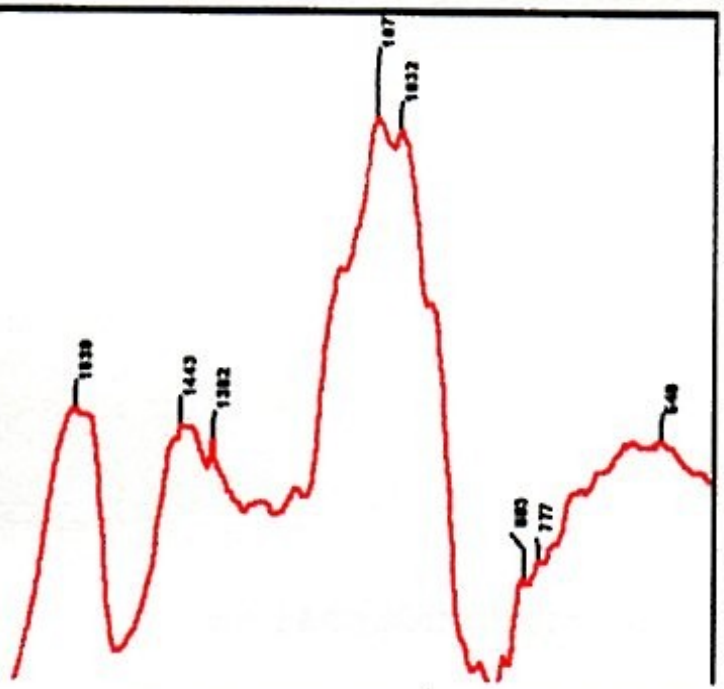
1800 – 1690 cm^{-1} valenční vibrace C=O ketonu

1490 – 1350 cm^{-1} deformační vibrace CH ve skupinách CH_3 a CH_2



FTIR Spectrum Camphor

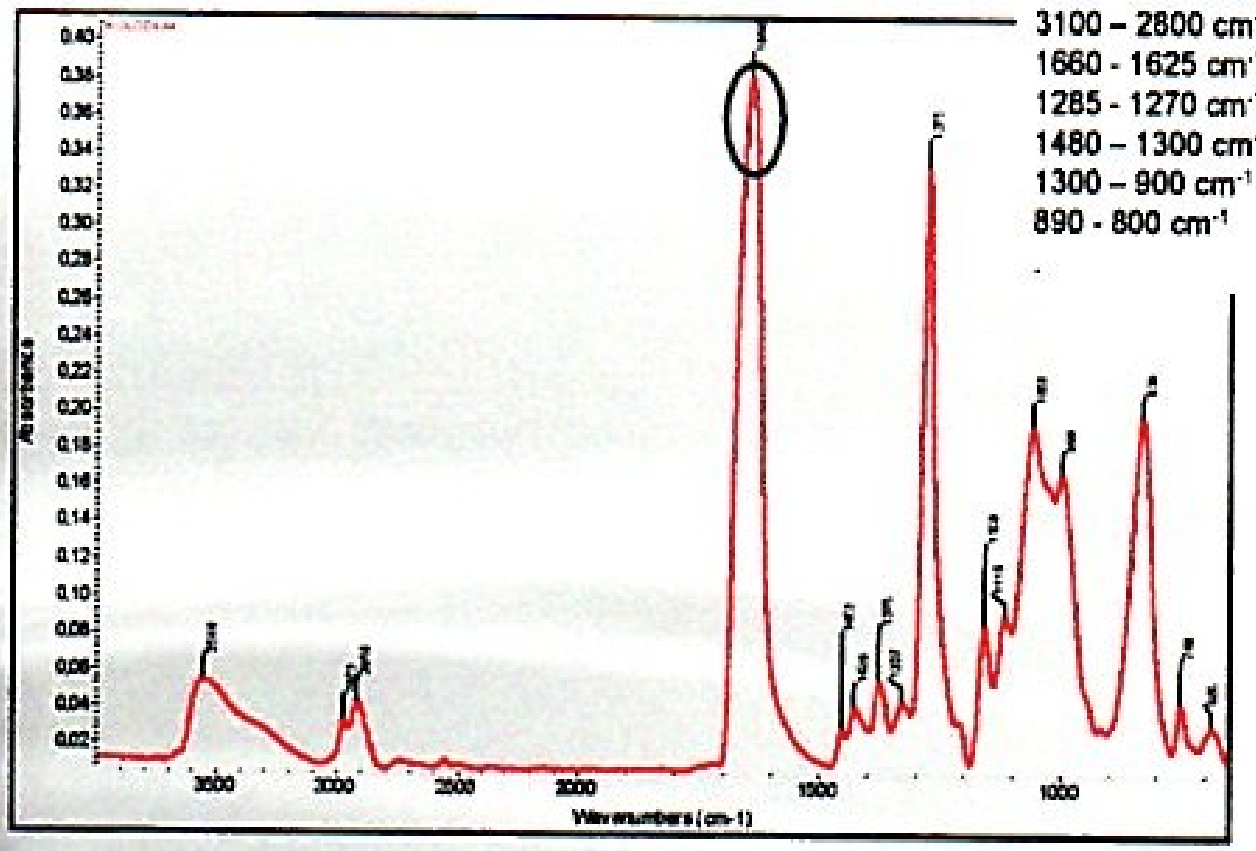




Charakteristické IČ absorpční pásy

3600-3200 cm-1	O-H valenční vibrace
3000-2800 cm-1	C-H valenční vibrace
1650 - 1630 cm-1	O-H deformační vibrace
1480-1300 cm-1	C-H deformační vibrace
1300-900 cm-1	C-O valenční vibrace (C-OH + C-O-C)

FTIR Spectrum Arabic Gum

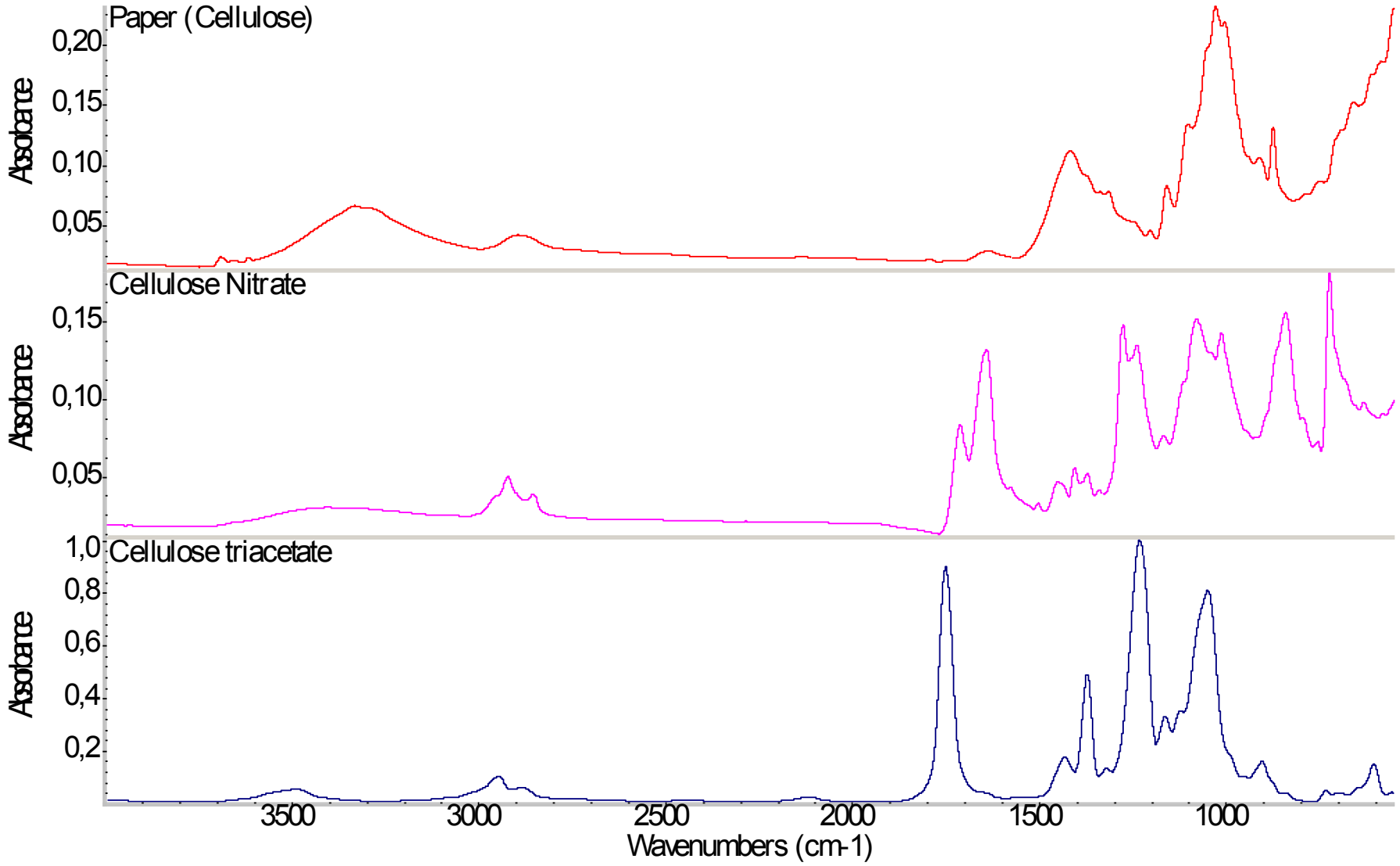


Charakteristické IČ absorpční pásy (GCI)

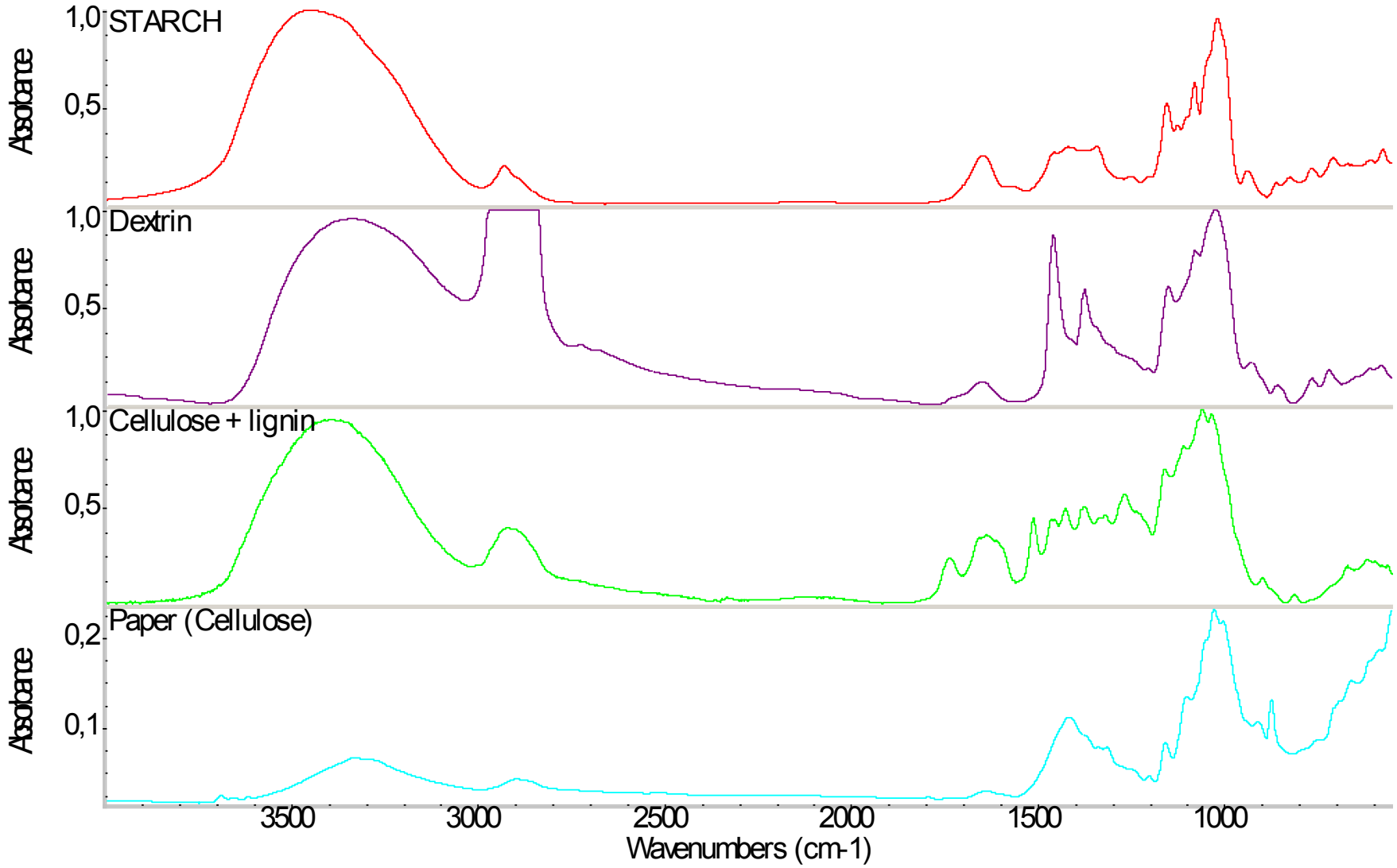
3600 – 3200 cm^{-1}	O-H valenční vibrace
3100 – 2800 cm^{-1}	C-H valenční vibrace
1660 - 1625 cm^{-1}	N-O valenční vibrace
1285 - 1270 cm^{-1}	N-O valenční vibrace
1480 – 1300 cm^{-1}	C-H deformační vibrace
1300 – 900 cm^{-1}	C-O deformační vibrace
890 - 800 cm^{-1}	N-O deformační vibrace

Nejsilnější pás, který je charakteristický pro nitrocelulózu je kolem 1656 cm^{-1}, další pásy se obvykle vyskytují při 1281, 1060 a 846 cm^{-1}

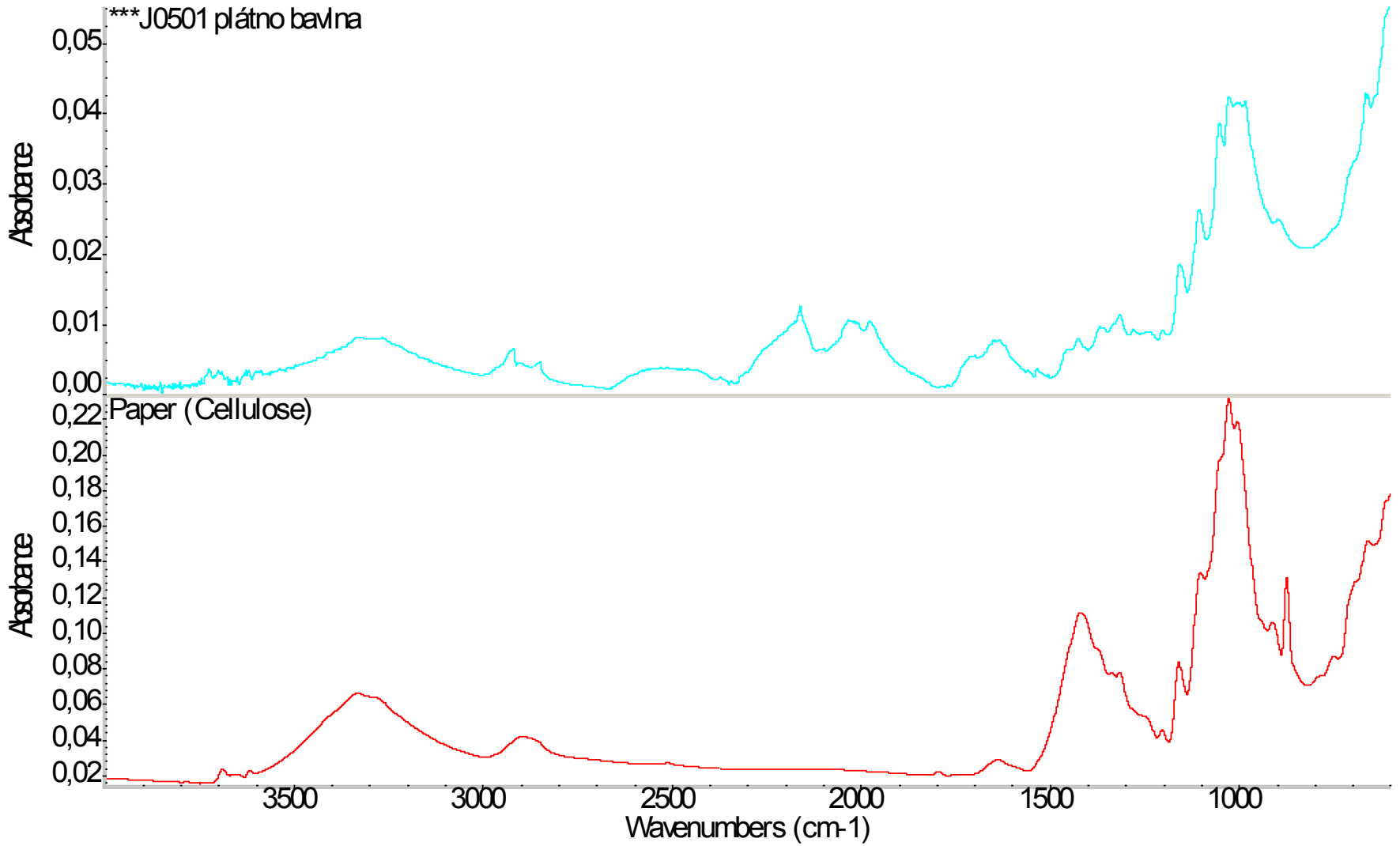
FTIR Spectrum NITROCELLULOSE



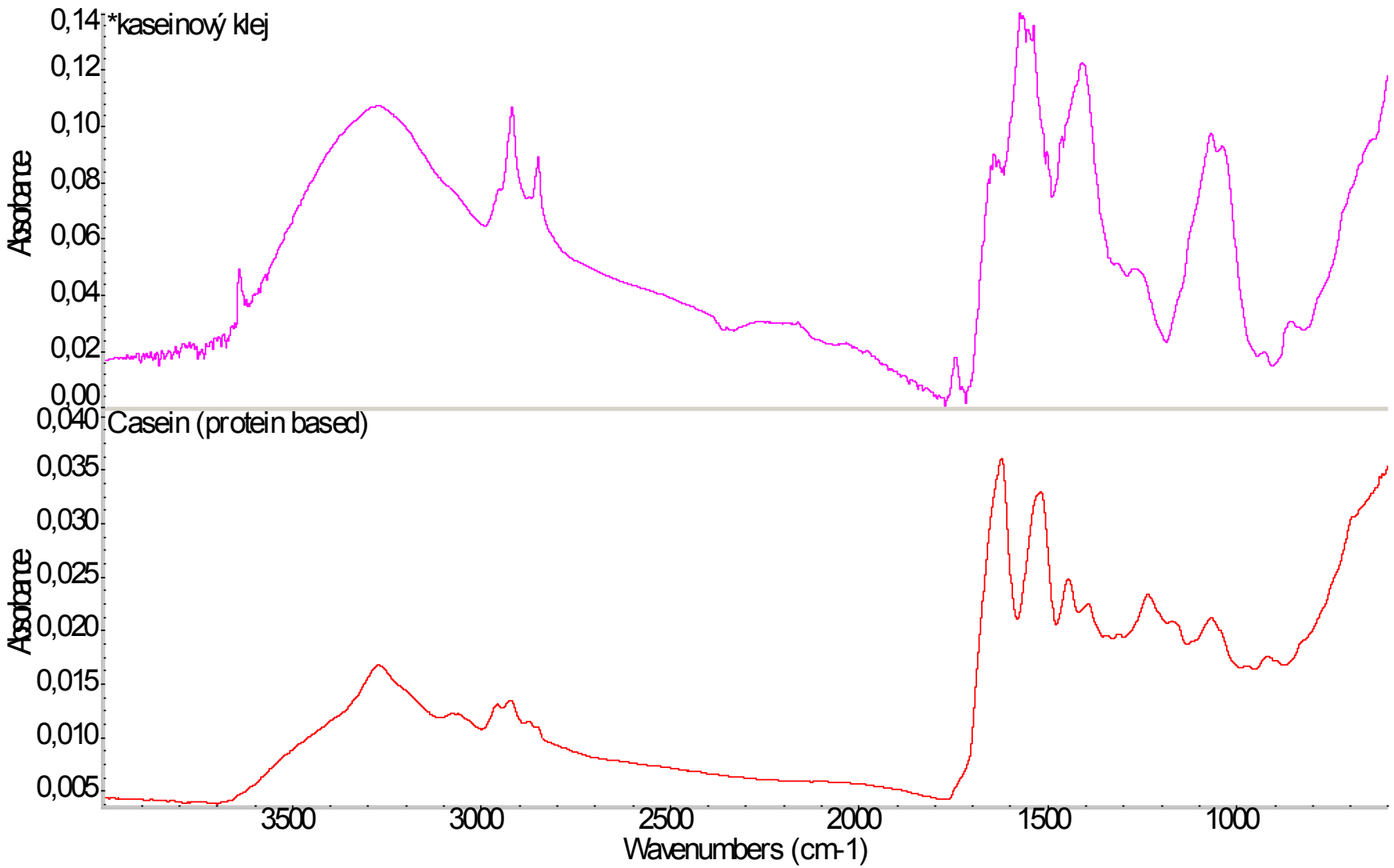
FTIR Spectrum Cellulose, NITROCELLULOSE and Cellulose triacetate



FTIR Spectrum Cellulose, Starch, Dextrin, Cellulose with Lignin



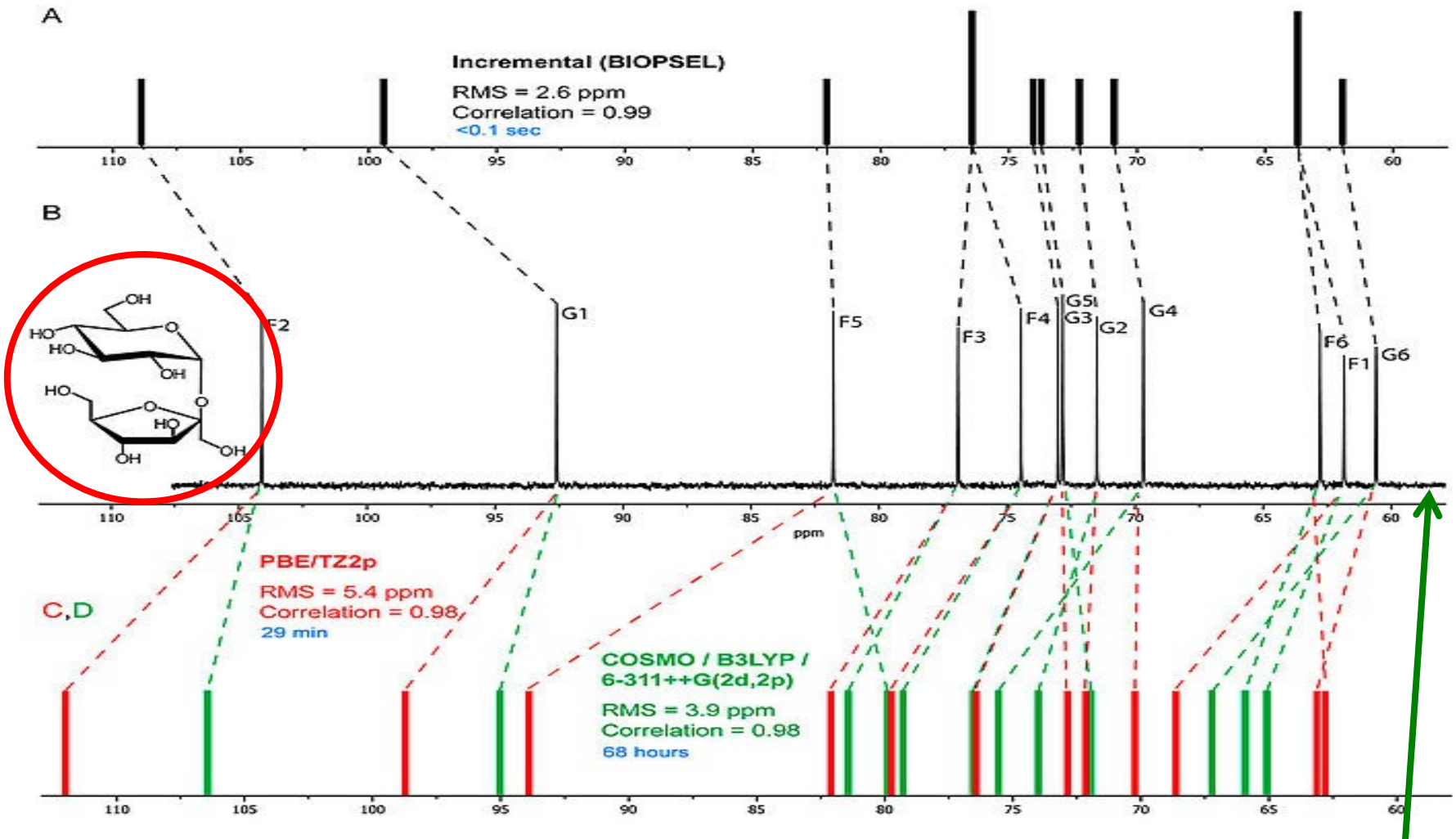
FTIR Spectrum Cellulose (Paper) and Cellulose (Jute Canvas)



FTIR Spectrum Kasein and Kasein Glue (Ca Salt)

NMR Spectroscopy

- **Advantages**:
  - Hydrogen, Carbon etc. Spectra can be measured
  - Detail Information about the Molecular Structure
  - The many Simulation Software's (Methods) are available now
  - ……
- **Disadvantages**:
  - It is demanding as to Operator's Qualification and Instrumentation
  - MOSTLY it is necessary to work with Liquid or Solution, but Solid State NMR is also possible
  - …..



Comparative prediction of the 13C NMR spectrum of sucrose using various methods. Experimental spectrum is in the middle. Upper spectrum (black) was obtained by empirical routine. Lower spectra (red and green) were obtained by quantum-chemical calculations in PRIRODA and GAUSSIAN respectively. Included information: used theory level/basis set/solvent model, accuracy of prediction (linear correlation factor and root mean square deviation), calculation time on personal computer (blue