Infrared spectroscopy

Chapter content

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  - Identification of organic compounds
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- Near-infrared (NIR)
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  - Applications in food analysis

Infrared spectroscopy

- measurement of IR radiation absorbed by or reflected from a sample
- absorption of IR radiation is related to the changes of vibrational or rotational energy states of molecules
- applications:
  - analysis of gaseous, liquid or solid samples
  - identification of compounds
  - quantitative analysis
- information deduced from IR spectrum:
  - functional groups of molecules, constitution of molecules
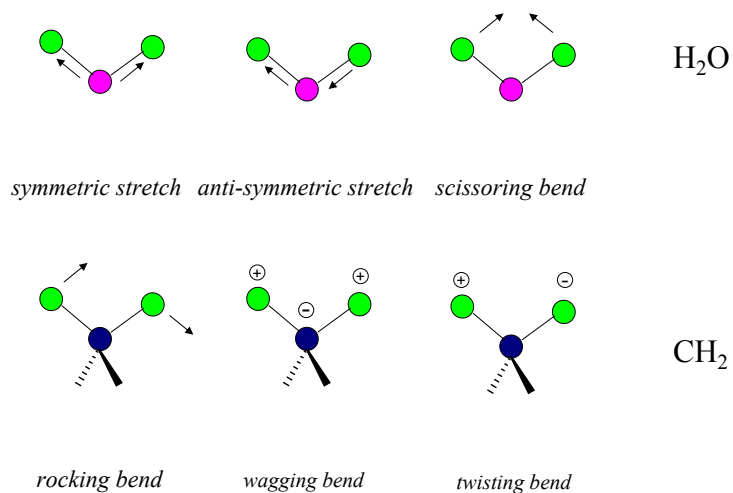 - interaction among molecules

Vibrational transitions

- **fundamental** (**normal**):
  change of vibrational quantum number $\Delta v = 1$
  high probability $\rightarrow$ high values of ε
- **overtones**:
  the difference of vibrational quantum number $\Delta v = 2; 3\ldots$
  lower probability $\rightarrow$ low values of ε
- **combination**:
  simultaneous change of two or more vibrational numbers
  for a polyatomic molecule

Types of vibrations

- **stretching**: the length of chemical bond (the inter-nuclear
  distance) is changed
  - symmetric
  - anti-symmetric
- **bending**: the valence angle is changed
  - symmetric and anti-symmetric vibrations
  - vibrations in plane and vibrations out of plane

Example: vibrations of a three-atomic non-linear molecule and a group of three atoms in a multi-atom molecule

symmetric stretch   *anti-symmetric stretch*   *scissoring bend*

H_2O

rocking bend   *wagging bend*   *twisting bend*

CH_2

Which substances give a signal in IR spectrum?

YES	NO
• molecules that contain polar bonds	• pure chemical elements in molecular or crystal state
• i.e. molecules composed of atoms of different elements	• = e.g. Ar, O_2, O_3, N_2, Cl_2, S_8, silicon, graphite, diamond…
• = organic compounds and inorganic compounds (H_2O, CO_2, NO_2, HCl, salts...)	

IR signal of a molecule is proportional to square of the change of dipole moment that occurs during vibrational motion of the molecule.

Spectral regions and corresponding analytical techniques

	λ (μm)	$\widetilde{\nu}$ (cm^{-1})
Near infrared region (*near infrared spectroscopy*, **NIR**)	0.8 – 2.5	12 500 – 4 000
Mid infrared region (*mid infrared spectroscopy*, **Mid IR, MIR**)	2.5 – 25	4 000 – 400
Far infrared region (*far infrared spectroscopy*, **FIR**)	25 – 1 000	400 – 10

MIR – normal vibrational transitions
NIR – overtones
FIR – normal vibrations of weak bonds and bonds of heavy atoms

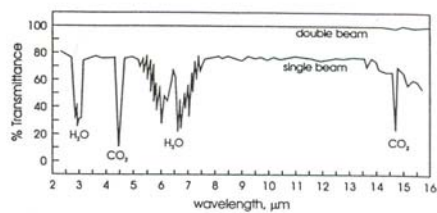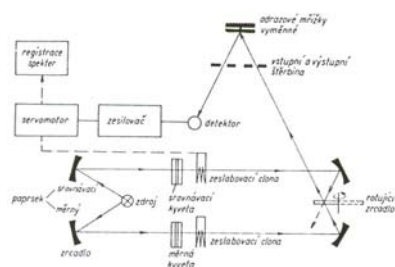## Instrumentation for IR spectroscopy

Main components of an instrument

- radiation source
- measuring (and reference) cell
- wavelength selector
- detector (transducer)

Types of instruments

- simple instruments with a filter
- classical instruments with a monochromator
- instruments based on an interferometer (FTIR)

**Double beam *vs.* single beam spectrometers**

Sources of IR radiation

- for NIR: **tungsten lamp**
- for MIR:
  - **Globar = electrically heated** (1100 °C) **silicon carbide rod**
    - it gives maximum intensity at $\lambda = 2$ μm;
    at lower temp. – shift of maximum to a longer wavelength
    (600 °C $\rightarrow \lambda_{max} = 3{,}5$ μm)
  - **lasers** CO_2, PbS – $\lambda_{max} = 9$–11 μm

Transducers of IR radiation

- pyroelectric triglycine sulphate detectors
    - work at the normal temperature

- photoconductive detectors MCT (HgTe/CdTe)
    - work at the temp. of liquid nitrogen (-196 °C)
    - high sensitivity
    - fast response
    - are used for MIR and FIR

- germanium bolometers
    - are used for FIR
    - work at the temp. of liquid helium (-271,7 °C)

Fourier transform IR spectrometers (FTIR)

– based on Michelson interferometer
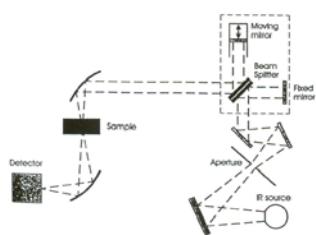
The beam from source is divided on splitter into two halves; the first is reflected to the fixed mirror, while the second is transmitted to the movable one; the reflected beams are recombined in the splitter again and an interference of waves occurs.

For monochromatic radiation, a destructive interference of beams occurs, when $\delta = (n+0,5)\ \lambda$

δ is optical retardation (= two-times the difference between the distance of the fixed mirror to the splitter and the distance of the movable one to the splitter).
The constructive interference occurs, when $\delta = \lambda;\ 2\ \lambda; 3\ \lambda;\dots n\ \lambda$

When polychromatic radiation passes through the interferometer and the sample, the obtained record is an interferogram, which is converted into IR spectrum by Fourier transformation.

Advantages of FTIR

- dispersion elements are not necessary
  $\rightarrow$ more energy enters the sample
- fast spectrum recording (< 1s)
- high resolution (up to 0.01 cm^{-1})

Measurement techniques of IR spectroscopy

Sample types and sample preparation

Cells for transmission measurements have windows made of

- NaCl, KBr, CaF$_2$, ZnSe, AgCl, TlBr/TlI pro MIR
- CsBr, polyethylene (for FIR)
- glass, quartz glass (for NIR)

Gaseous samples

Cells are firstly evacuated and then filled with a sample;
cell path length ranges from 10 cm to 80 m (multiple reflection of
the beam inside the cell, outer size of cell is up to 1 m)

Liquid samples
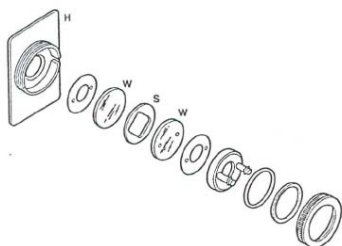
Demountable cell
for liquid samples

For **aqueous solution** it is necessary to use windows made of CaF_2 or ZnSe (insoluble in water).
The measurement is possible only within the interval of $1400-1000$ cm^{-1}, used e.g. for the analysis of sugars in fruit juices based on the measurement of thin films (10–50 μm).

Liquid samples

Solvents for samples applicable in MIR

Solvent	Measurement in wavenumber region
CCl_4	$4000-1600$ cm^{-1} and $1500-850$ cm^{-1}
$CHCl_3$	$4000-1250^a$ cm^{-1} and $1150-850$ cm^{-1}
CS_2	$4000-1650^b$ cm^{-1} and $1400-500$ cm^{-1}

a – with exception of strong bands at 3050 and 940 cm^{-1}
b – with exception of strong bands at 2350 and 2200 cm^{-1}

Pure liquid samples (e.g. oils) and gels can be measured in a very thin film (1 μm) by transmission technique of by ATR technique.

Solid samples

- **preparation of KBr discs (pellets)**:
  1-15 mg of sample + 200 mg KBr – the well powdered
  mixture is pressed under vacuum to form a pellet which is
  inserted into a holder in the spectrometer
  (disadvantage: KBr absorbs some water, the bands of
  which may interfere with the sample spectrum)
- **preparation of Nujol mull**:
  powdered sample is dispersed in mineral (paraffin) oil to
  form a suspension
  (disadvantage: bands C-H a C-C bonds of the sample are
  overlapped with those of paraffin oil)

Measurement techniques in IR spectroscopy

Transmission technique

the measurement of transmittance, absorbance

$$T = I / I_0 = 10^{-\varepsilon bc}$$

$$I_0 / I = 10^{\varepsilon bc}$$

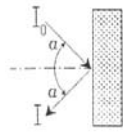$$A_\lambda = \log_{10} (1/T) = - \log_{10} T = \log_{10} (I_0/I) = \varepsilon_\lambda . b . c$$

Reflection techniques

the measurement of the radiation reflected from sample
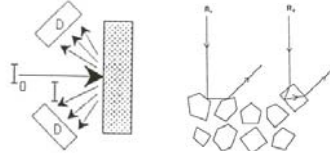reflectance $R = I / I_0$
optical density $OD = \log_{10}(1/R) = -\log_{10} R$
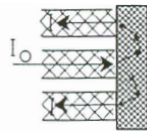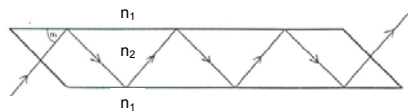
<u>Specular reflection</u>:

<u>Diffusion reflection</u>:

common technique in NIR for solid powdered samples

<u>Special arrangement</u>:

measurement of interactance – the use of optical cables

ATR technique (*attenuated total reflectance,* or *internal reflectance*)

- a sample (liquid, semi-solid, solid) is placed in a layer on the surface of the crystal; pressure is applied for solid samples to achieve good contact between the sample and the crystal
- the crystal must have high refractive index n_2 (higher than that of sample n_1)
- the beam enters the crystal at the incidence angle θ higher than critical angle $\theta_c = \arcsin(n_1/n_2)$; under this condition, the beam is totally caught by the crystal, i.e. a complete internal reflection occurs and the beam travels in the crystal; after one or multiple reflections, the beam leaves the crystal and reaches the detector
- at the crystal-sample interface, the beam actually penetrates a very short distance (<2 μm) into the sample and the absorption of specific wavelengths occurs
- penetration depth depends on the wavelength:
  $d_p = \lambda/2\pi \, [\sin^2\theta - (n_1/n_2)^2]^{0,5}$ [μm]

Advantages of ATR	Requirements for a sample and a crystal
• simple preparation of sample before measurement • non-transparent samples can be analysed	• good adherence of the sample to the crystal • mechanical strength of the crystal (when the sample is pressed against the crystal) • inertness of the crystal against samples • removal of sample residue from the crystal using various solvents

| Kind of crystal	Spectral range	n at 1000 cm^{-1}
diamond	30 000 – 200 cm^{-1}	2.4
sapphire	50 000 – 1 780	1.74
NaCl	40 000 – 590	1.49
ZnSe	20 000 – 454	2.4
Ge	5 500 – 600	4.0
Si	8 300 – 6 600	3.4
TlBr/TlI	20 000 – 250	2.37

Mid-infrared spectroscopy (Mid IR)

- the mid IR spectra consist of the bands corresponding mainly to normal (fundamental) vibrations
- the number of vibrations of a molecule composed of N atoms is $3N$-6 (for non-linear molecules) or
  $3N$-5 (for linear molecules)
- absorption bands are stronger, when two or more vibrations have the same frequency (wavenumber); such vibrations are called *degenerated vibrations*
- strong signals are caused by vibrations of polar bonds, especially multiple polar bonds, such as C=O
- single bonds of a low polarity (especially C-C) give very weak signals (and totally non polar bonds give no signal)

Identification of organic compounds using Mid IR

Mid IR region is divided into two sub-regions:

- **region of characteristic vibrations of functional groups**
  2.5–8 μm (4000–1250 cm^{-1})
  contains the characteristic bands of individual bonds and
  functional groups that correspond mainly to stretching
  vibrations
- **fingerprint region**
  8–25 μm (1250–400 cm^{-1})
  contains the bands corresponding mainly to bending
  vibrations
  the spectrum in this region characterises each molecule as
  an integral whole

Example of spectra of isomeric monoterpenes
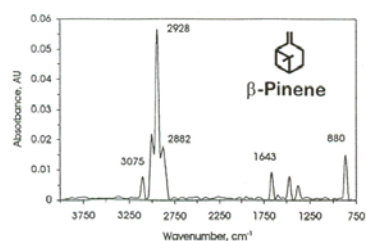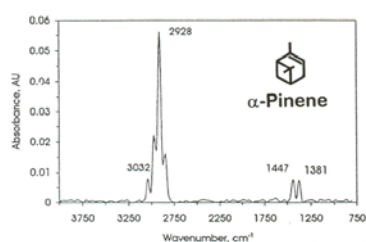
the spectra are very similar in in the
region of characteristic vibrations
(even almost identical around 3000 cm^{-1})
but very different in the fingerprint region

Steps of identification process

1. searching for functional groups on the basis of characteristic vibrations (using tables)
2. confrontation with the results of other tests
   - elementary analysis of the compound
     $\rightarrow$ stoichiometric formula of the compound
   - determination of molecular mass (from mass spectrum)
     $\rightarrow$ molecular formula of the compound
     $\rightarrow$ calculation of *unsaturation index*
     $U = 1 + 0.5$ (2. number of C + number of N+P $-$ number of H $-$ number of halogen atoms)
     $U=0 \rightarrow$ no multiple bond, no ring
     $U=1 \rightarrow$ 1 double bond or 1 ring
     $U=2 \rightarrow$ 2 double bonds or 1 triple bond or 1 double+1 ring
     $U=3 \rightarrow$ 3 double bonds or 3 rings or 1 double+1 triple bond
       or 2 double bonds+1 ring or 1 double bond+2 rings
       or 1 triple + 1 ring

Steps of identification process

3. drawing of all possible structures that correspond to the presence of groups, molecular formula and unsaturation index
4. comparison of the measured IR spectrum with the spectra of the suggested compounds found in an atlas or a database of spectra
   $\rightarrow$ identification
5. verification of identity using other spectral methods (MS, NMR)
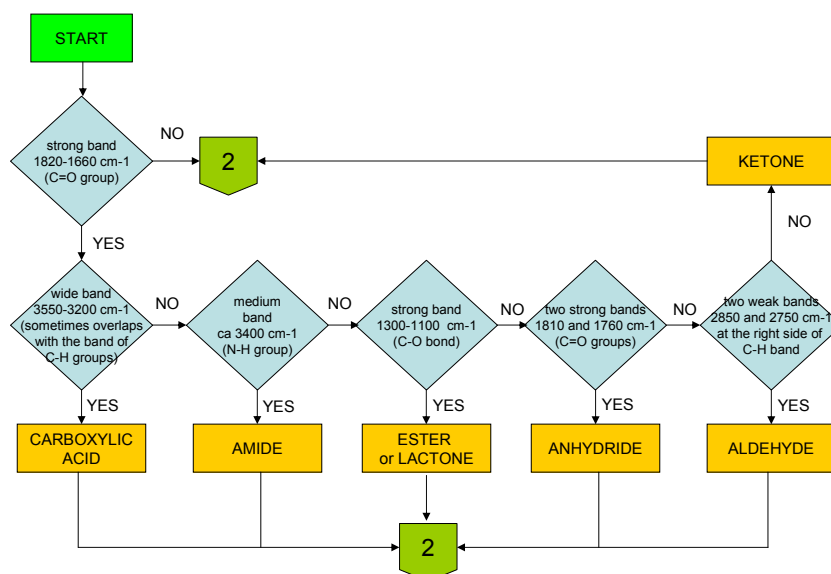
Trends in wavenumbers of carbon chemical bonds
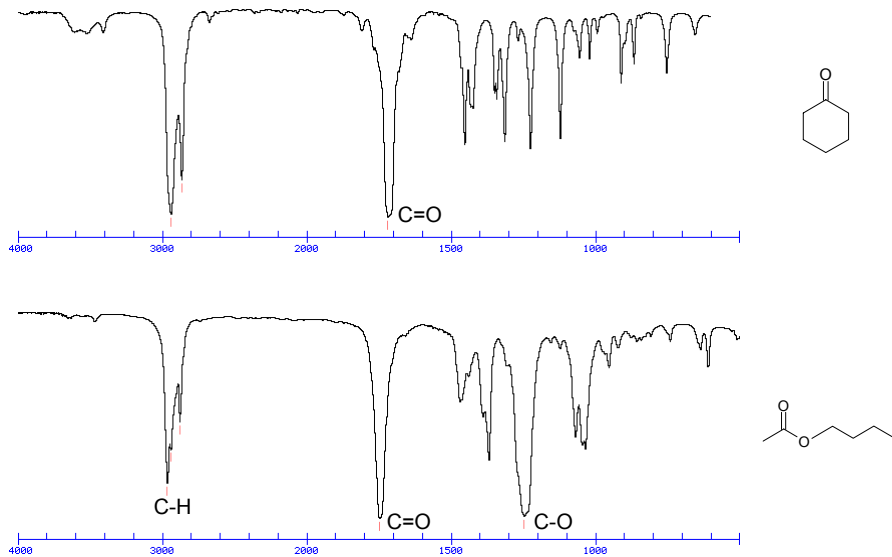
effect of
carbon hybridization:

C-C bond		C–H bond	
C–C	1200 cm^{-1}	sp^3 C–H	2900 cm^{-1}
C=C	1650	sp^2 C–H	3100
C≡C	2150	sp C–H	3300

effect of
mass of X atom
in C–X bond:

C–H	2900 cm^{-1}
C–C	1200
C–O	1100
C–Cl	750
C–Br	600
C–I	500

Look on IR spectrum: identification of functional groups
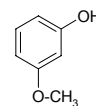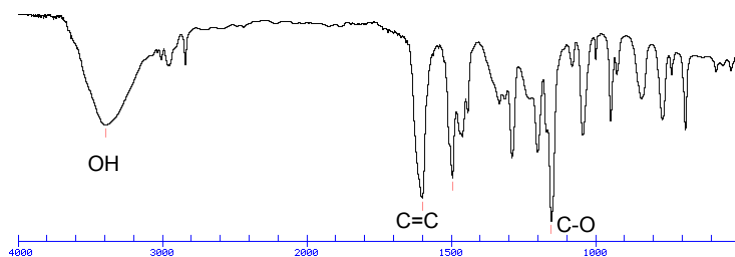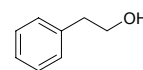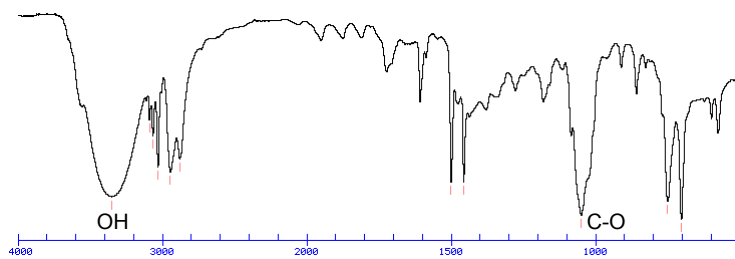1. carbonyl groups

C=O

C-H   C=O   C-O

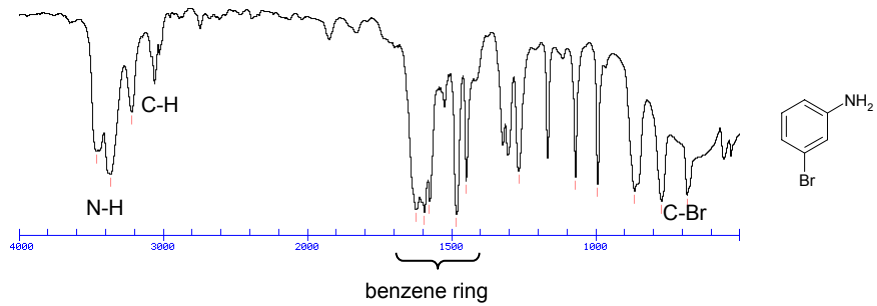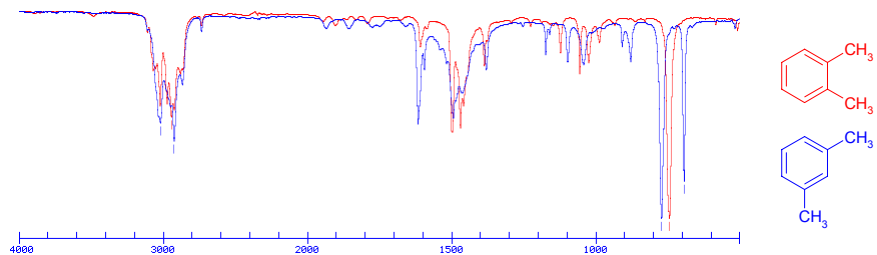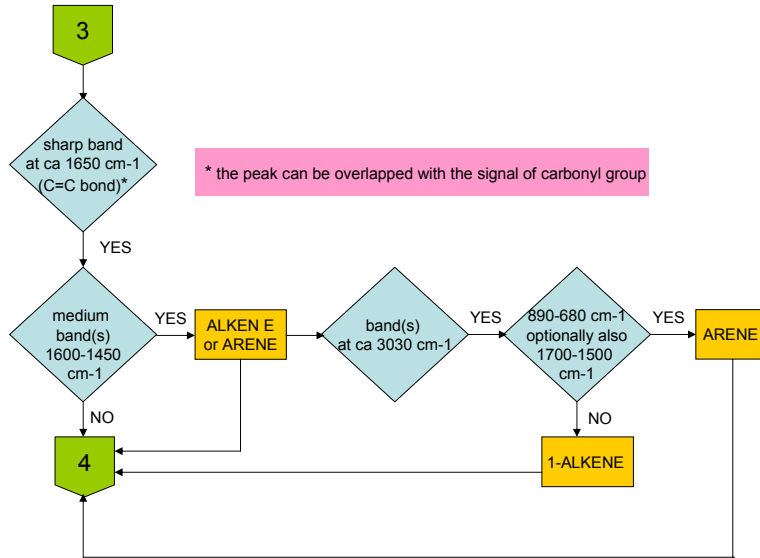Look on IR spectrum: identification of functional groups
2. other functional groups
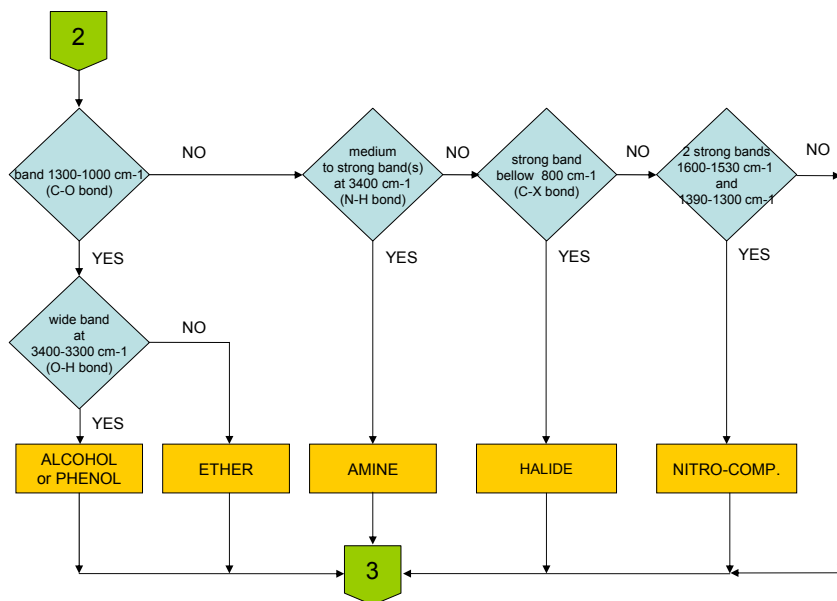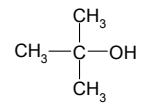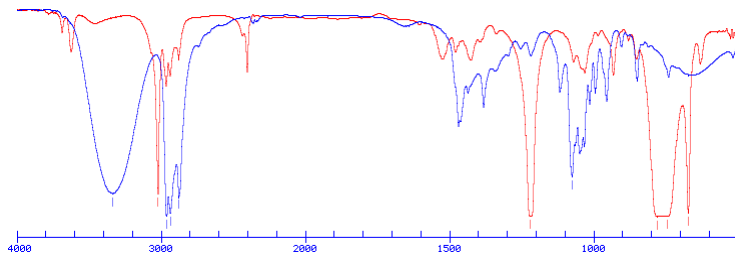
pure tert. butanol
diluted solution of tert. butanol in CHCl₃

Look on IR spectrum: identification of functional groups
3. double bond and aromatic ring

3

sharp band
at ca 1650 cm-1
(C=C bond)*

* the peak can be overlapped with the signal of carbonyl group

YES

medium
band(s)
1600-1450
cm-1 → YES → ALKEN E or ARENE → band(s) at ca 3030 cm-1 → YES → 890-680 cm-1 optionally also 1700-1500 cm-1 → YES → ARENE

NO

4

NO

1-ALKENE

benzene ring

C-H
N-H
C-Br

Look on IR spectrum: identification of functional groups
4. triple bond

```
                    ┌─────┐
                    │  4  │
                    └──┬──┘
                       │
                       ▼
               ╱ medium  ╲         YES                          ┌──────────┐
              ╱ sharp band ╲ ─────────────────────────────────▶│  NITRILE │
              ╲ 2250 cm-1  ╱                                    └──────────┘
               ╲(C≡N group)╱
                   │ NO
                   ▼
             ╱  weak     ╲   YES   ┌────────┐     ╱  3300 cm-1  ╲   YES   ┌──────────┐
            ╱ sharp band  ╲ ──────▶│ ALKYNE │────▶╲ (≡C-H bond) ╱ ───────▶│ 1-ALKYNE │
            ╲  2150 cm-1  ╱        └────────┘      ╲           ╱          └──────────┘
             ╲(C≡C bond) ╱
```
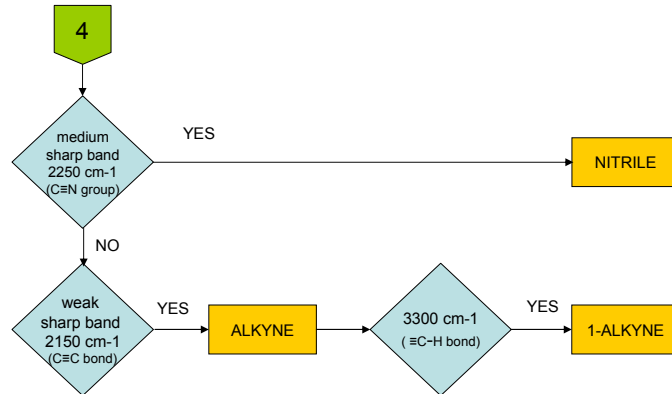
If the spectrum does not contain any of above mentioned bands, the compound is probably a saturated hydrocarbon. Spectra of these hydrocarbons are simple and contain :
- strong band at ca 2900 cm^{-1} (C-H stretching),
- medium and sharp band at ca 1470 cm^{-1} (CH$_2$ bending),
- weak sharp band at ca 1400±50 cm^{-1} (CH$_3$ bending),
- optionally a band at 720 cm^{-1} (signal of longer hydrocarbon chains)

## Quantitative analysis using Mid IR

Measured quantities:

- absorbance A or optical density OD (OD = log $1/R$)
- integral intensity (area bellow the curve in appropriate wavenumber boundaries, the background spectrum is firstly subtracted)

Applications in food analysis

1. Determination of *trans*-unsaturated fatty acids in fats
wavenumber 960-970 cm^{-1} (*cis* double bonds absorbs at 700±50 cm^{-1}, terminal double bonds at ca 900 cm^{-1})
TAG are converted to fatty acid methylesters, which are dissolved in CS$_2$ and the absorbance is measured;
elaidic acid methylester is used as the calibration standard

Applications in food analysis

2. Determination of main food components using ATR technique
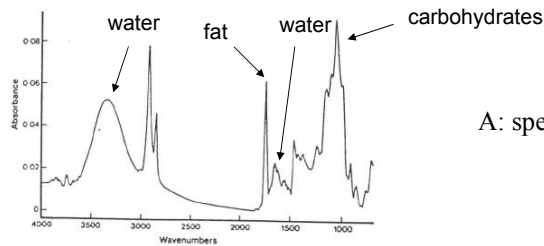

A: spectrum of chocolate
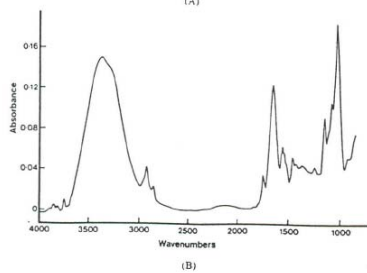
B: spectrum of bread

Near infrared spectroscopy (NIR)

- NIR region: 800 to 2500 nm or 12 500 to 4 000 cm^{-1}
- NIR spectra contain less intensive signals
  - combination bands
  - *overtone bands*
    the change of vibration quantum number Δv is 2; 3; 4…;
    if the fundamental vibration occurs at the wavelength of λ_0,
    the first overtone appears at $\lambda_1 \approx \lambda_0/2$, the second at $\lambda_2 \approx \lambda_0/3$,
    the third at $\lambda_3 \approx \lambda_0/4$, etc., the strength of signal gradually
    decreases
- NIR spectra are measured by
  - transmission technique
  - diffusion-reflection technique or
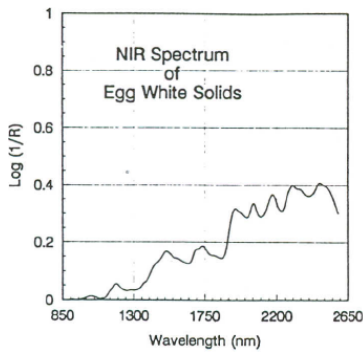 - ATR technique

NIR spectra of some food samples
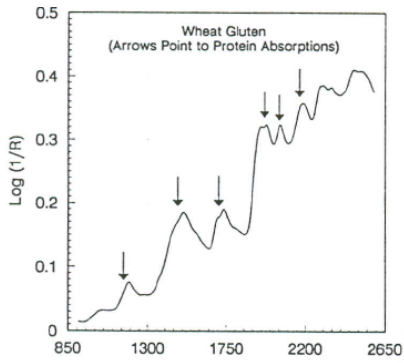
NIR spectrum of wheat gluten
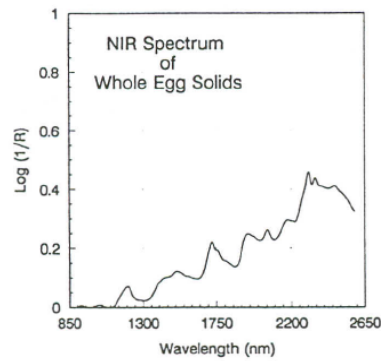signals of proteins:
1190, 1488, 1735, 1974, 2054, 2162 nm
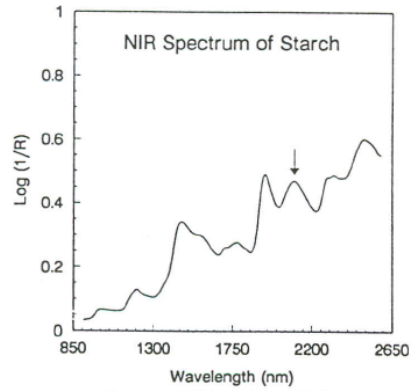
NIR spectrum of starch
main signal 2100 nm
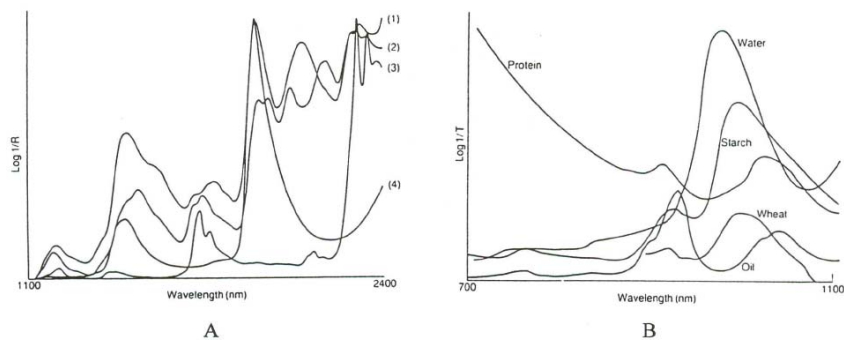
NIR spectra of some food samples

NIR spectrum of dried egg white
signals of proteins 2054 and 2162 nm

NIR spectrum of dried eggs
peak of proteins 2162 nm is distorted
peaks of lipids 1200, 1750 and 2350 nm

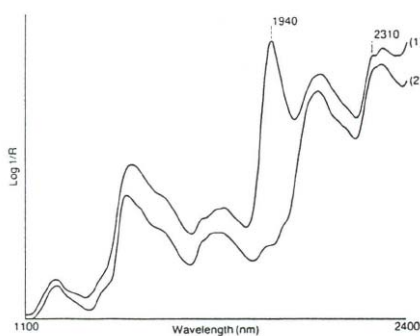## Reflection and transmission NIR spectra

A

B

A: NIR reflection spectrum of wheat components: starch (1), protein (2), fat (3), water (4)
B: NIR transmission spectrum of wheat and its components
Note: some foods in a 1-2 cm layer are transparent for the radiation at 700–1100 nm

Applications of NIR in food analysis

Determination of water


NIR spectra of whet flour (1)
and dried wheat flour (2)

- absorption bands of water in NIR: 1940, 1450, 1190, 970 and 760 nm
- position of peaks is moderately affected by molecular interactions among water and other sample components
- 1450, 970, 760 nm are the first, the second and the third overtone of O-H bond vibration, resp.
- 1940 and 1190 are combination bands

Modes of water determination

- measurement of A_{1940} (linear range 0–2.5 % H$_2$O) or A_{1450} (0–4 % H$_2$O) of the water extract obtained from solid samples using a polar solvent that does not contain an OH group (*N,N*-dimethyl-formamide) – applied e.g. for analysis of dried vegetables and spices
- azeotropic distillation of water with 1,4-dioxane and measurement of A_{1910} of distillate – applied for coffee bean analysis
- measurement of A_{970} – analysis of beer, meat, cereal grain
- preparation of a suspension of a powdered sample in CCl$_4$ and the measurement of OD$_{940}$ and OD$_{2080}$; water content is proportional to the difference of values; a calibration using an independent method is necessary
- measurement of reflectance of solid samples (flour, grain, malt, milk powder, various seeds, hop…) at 1940 nm and at another reference wavelength (2310, 1850, 2000 nm) – a calibration using an independent method of water determination is necessary
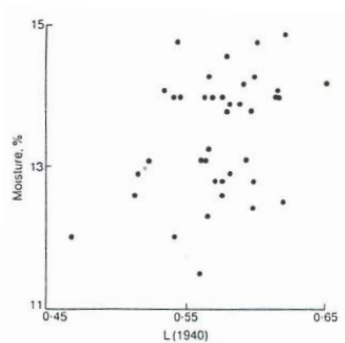
Plot of moisture content in flour
and OD$_{1940}$ = log 1/R_{1940}
(data for 40 samples)
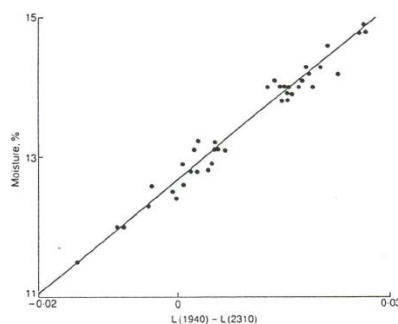
Plot of moisture content in flour
and the difference OD$_{1940}$ – OD$_{2310}$

Example of regression equation

Water content = 12.69 + 81.49 . (log 1/R_{1940} – log 1/R_{2310})   [%]
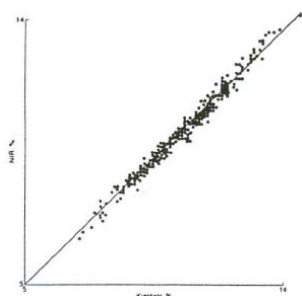
The equation is valid only for the specific kind of samples
(e.g. wheat flour but not rye flour) and the type of instrument.

Determination of proteins

is usually based on the measurement of reflectance at 2180 nm completed with several reference values

Example of regression equation for whet flour:

Protein content = $12.68 + 493.7 \cdot \log 1/R_{2180} - 323.1 \cdot \log 1/R_{2100}$
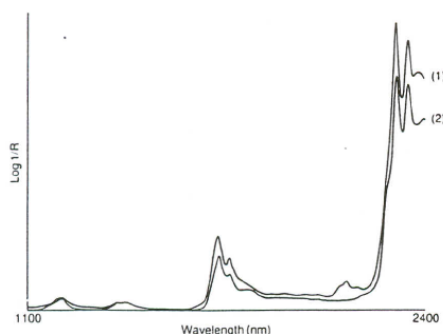$- 234.4 \cdot \log 1/R_{1680}$ [%]

Accuracy of protein determination: plot of the results of NIR analysis (y) and those of Kjeldahl method (x)

Determination of fat

the main absorption bands of fats in NIR belong to vibrations of CH_2 groups of fatty acids chains; they include:
– first overtones 1734 and 1765 nm,
– the second overtone 1200 nm,
– combination bands 2310 a 2345 nm.

NIR spectra of groundnut oil (1) and paraffin oil (2)

Modes of fat analysis *via* NIR

- reflectance measurement
  - e.g. analysis of meat by the measurement at 1725 nm and the reference value at 1650 nm; similar procedures were described for cereals, cocoa, cheese, milk powder, oilseeds etc.
- transmission measurement in 800-1100 nm range
  - in the second derivative spectrum of absorbance the ratio of values at 931 and 946 nm or those at 931 and 1062 nm is proportional to the fat content – applied for analysis of meat

Fat unsaturation determination

NIR spectra of fats contain bands at 1180, 2143 and 2190 nm that correspond to the presence of *cis* –CH=CH– bonds; the measurement of $\log 1/R_{2143}$ can be an easy accessible indicator of unsaturation, equivalent to the iodine value

Determination of carbohydrates

NIR spectra of individual carbohydrates are very similar and contain a lot of peaks – see the table

glucose	sucrose	starch	cellulose	glucose	sucrose	starch	cellulose
1198 nm	1196 nm	1202 nm	1207 nm	-	2008 nm	-	-
1269	1276	1274	1278	2073	2073	-	-
1371	1368	1360	1365	2103	-	2100	2088
1440	1436	1432	1431	2154	2150	-	-
1493	1489	1492	1487	2200	2191	2190	2200
1589	1586	1580	1584	2275	2255	2278	2273
1705	1688	1702	1707	-	2318	2318	-
-	1729	-	-	2330	-	-	2336
1750	1762	-	-	-	-	-	2347
1789	-	1773	1772	2364	2368	2360	-
1834	1823	1824	1824	-	-	-	-

## Some applications of NIR for the analysis of sugars

- determination of Glc, Fru, Suc in powdered mixtures
- determination of sucrose in wine (0.8–9 %), determination of sugars in fruit juices – transmission measurement in a 1–2-mm layer
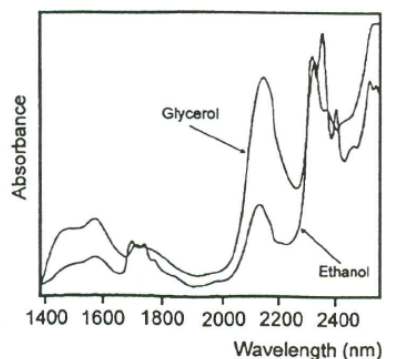- determination of sucrose in chocolate and sweets
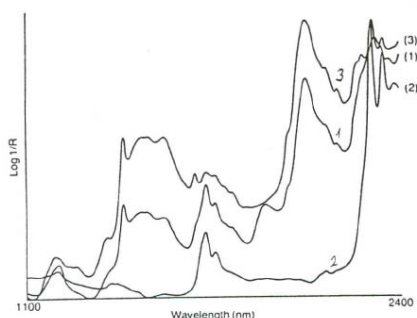
NIR spectra of sucrose (1), fat (2) and chocolate (3)

Determination of alcohols

- direct determination of ethanol in wine by the measurement of absorbance in a 1-mm cell (range 11–17 % v/v EtOH)
- determination of glycerol
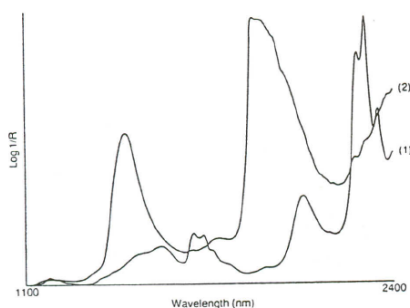
NIR spectra of ethanol (1) and wine (2)   NIR spectra of pure glycerol and ethanol