INTERNATIONAL COLLEGE OF PHARMACEUTICAL INNOVATION
国际创新药学院

Fundamentals of Medicinal and Pharmaceutical Chemistry

FUNCHEM.25 Analytics: Infrared Spectroscopy I

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Why the need for spectroscopic analysis?

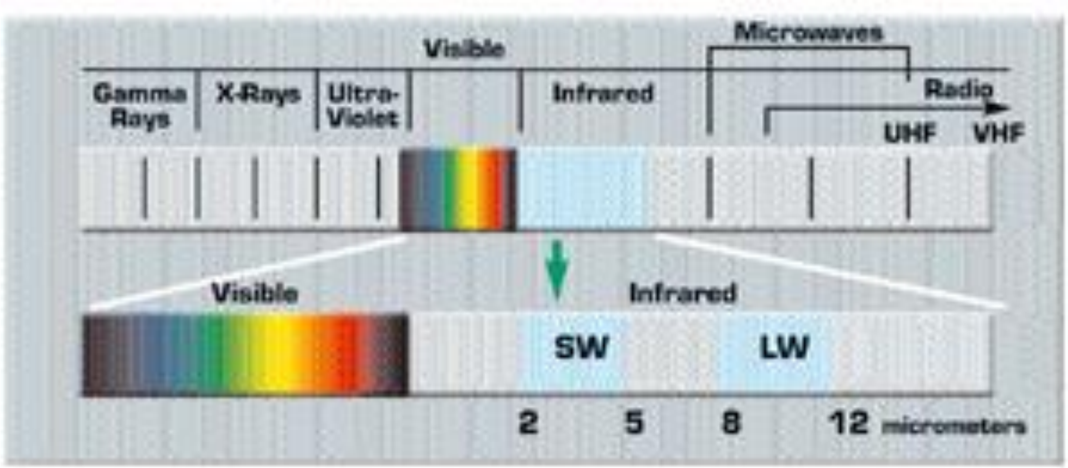
QUALITY CONTROL OF PHARMACEUTICALS AND FORMULATED PRODUCTS

- Product License Standards – manufacturing standards as set out by the licensing authority – e.g. in Britain, this is the Medicines and Healthcare Products Regulatory Agency (MHRA)
- Published Standards – e.g. British Pharmacopoeia (BP), European Pharmacopoeia (Pharm Eur) and the United States Pharmacopoeia (USP)

Pharmaceutical Impurities

- Sources of impurities can arise from a range of different sources – raw materials, products of the manufacturing process, degradation products, packaging materials and microbiological contaminants
- Impurities may be separated from other components using chromatography
- Once pure, these can be analysed using spectroscopy and spectrometry

Infra-Red Radiation
Infrared allows us to see what our eyes cannot.



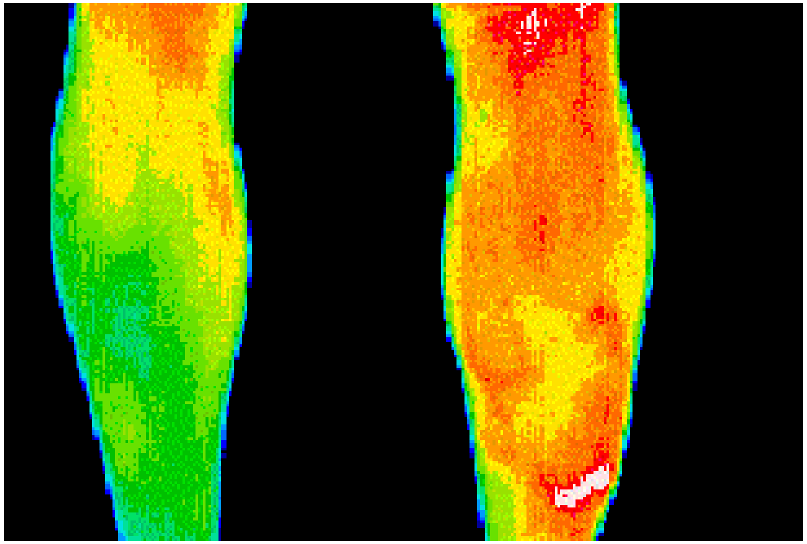
Thermal, or infrared energy, is light that is not visible because its wavelength is too long to be detected by the human eye; we perceive it as heat.

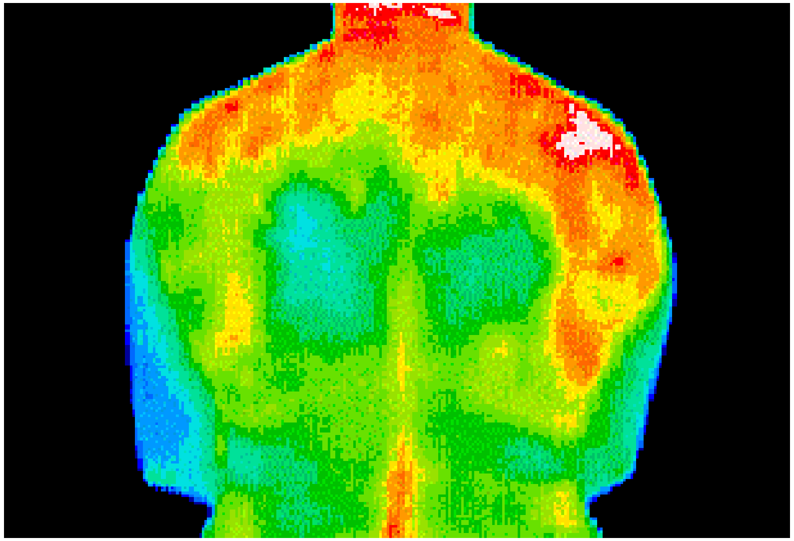
Unlike visible light, in the infrared world, everything with a temperature above absolute zero emits heat.

The higher the object's temperature, the greater the IR radiation emitted.

Thermography

Thermography is the use of an infrared imaging and measurement camera to "see" and "measure " thermal energy emitted from an object.



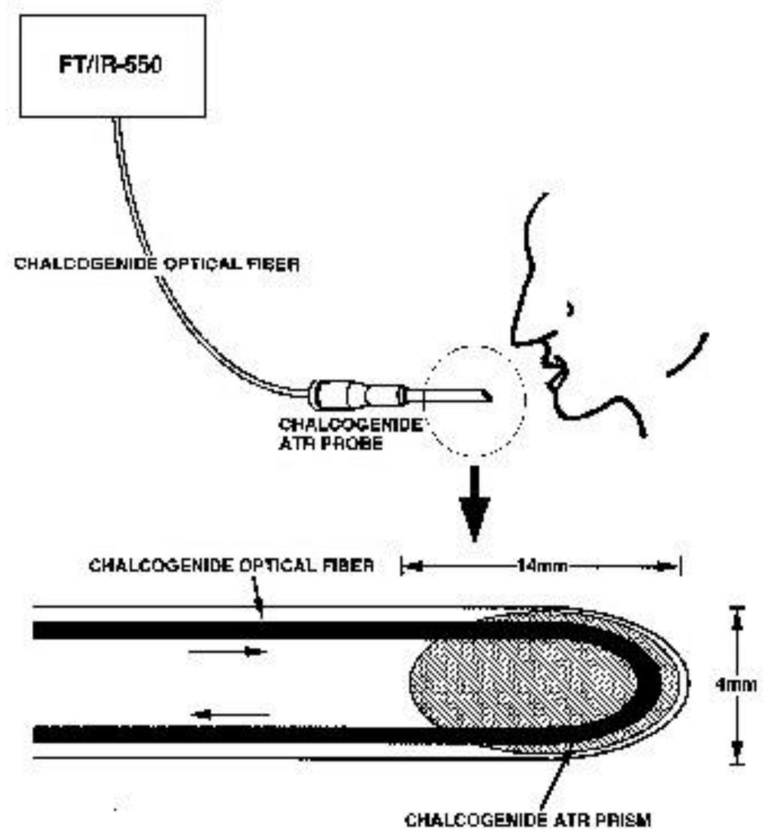


Pharmaceutical Applications

- Qualitative ‘fingerprint’ check for identification of drugs
- Used for screening compounds and rapid identification of, for example, C=O groups
- Can be used to characterise samples in solid states (creams and tablets)
- Water content measurement

Applications

Used in non-invasive measurement of glucose



Pharmaceutical Applications

Analysis of urine and other biofluids
(urea, creatinine, protein)

Types of Spectroscopy

- Ultraviolet (UV) spectroscopy uses electron transitions to determine bonding patterns.

- Infrared (IR) spectroscopy measures the bond vibration frequencies in a molecule and is used to determine the functional group.

- Nuclear magnetic resonance (NMR) spectroscopy detects signals from hydrogen atoms (in the case of ^{1}H NMR spectroscopy) and can be used to distinguish isomers.

- Mass spectrometry (MS) fragments the molecule and measures the masses.

- Measurements of physicochemical properties such as melting points and solubility should also be considered http://www.cem.msu.edu/~reusch/VirtualText/Spectrpy/InfraRed/infrared.htm

Infra-Red Spectroscopy

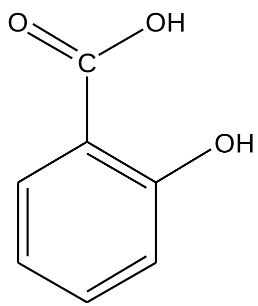
- IR spectroscopy can be used as a means of identification – pure samples of drugs (as well as non-drug molecules) produce IR spectra that are unique to themselves – they can therefore be used as a means of identification.

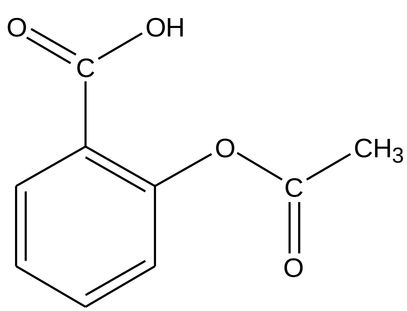
  The British Pharmacopoeia contains a reference collection of IR spectra for this purpose.

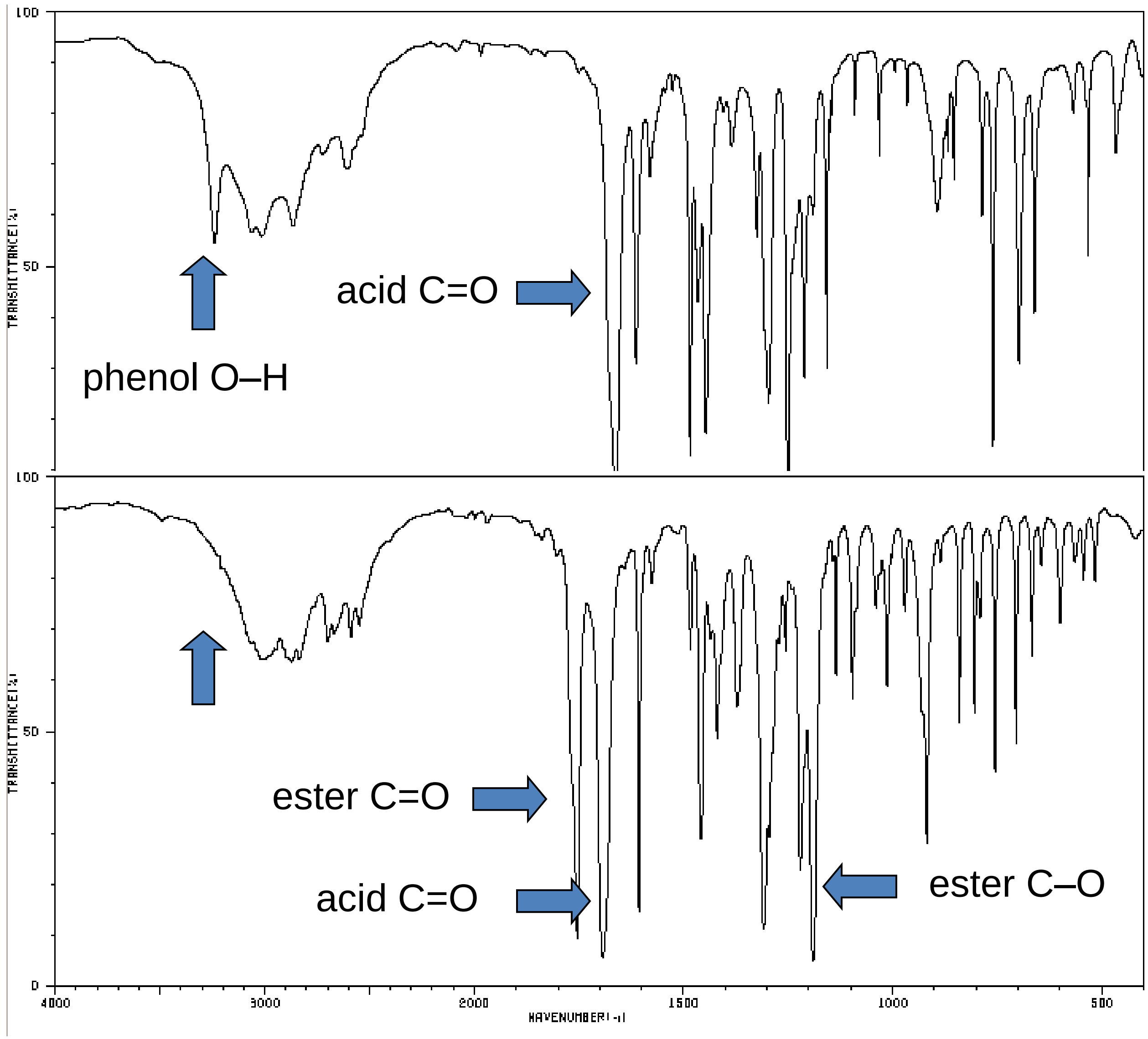
  It is thus possible to differentiate chemically similar drugs by studying their IR spectra

- IR spectroscopy can also be used to identify key structural information (functional groups)

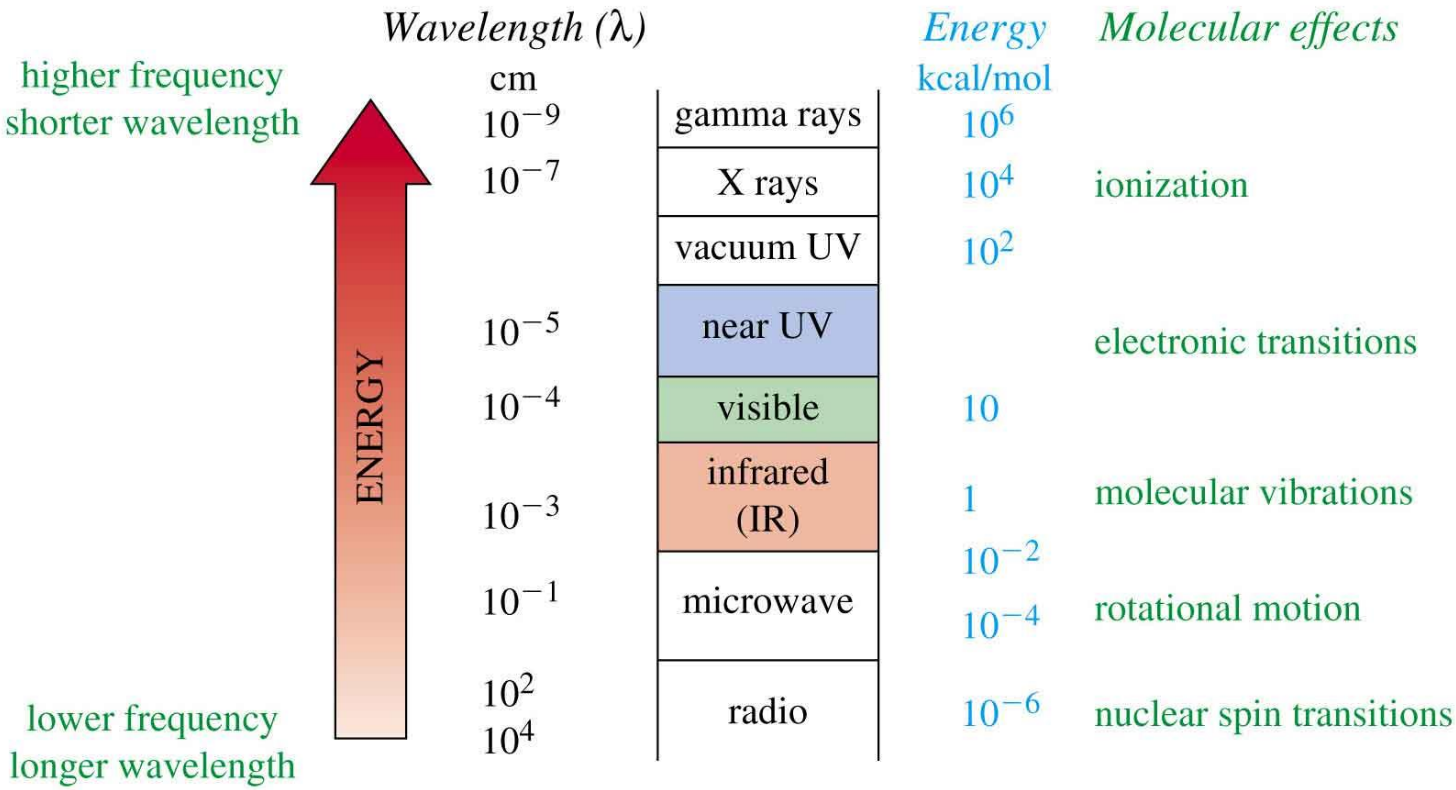
IR Spectroscopy: Salicyclic Acid Vs. Aspirin







What wavelength of electromagnetic radiation is involved in causing vibrations in molecules?



<u>The IR region</u>

- Just below red in the visible region
- More common units are wavenumbers, or cm^{-1}, the reciprocal of the wavelength in centimetres

In a nutshell!

Infra-red Spectroscopy

IR radiation → induce molecule vibrations

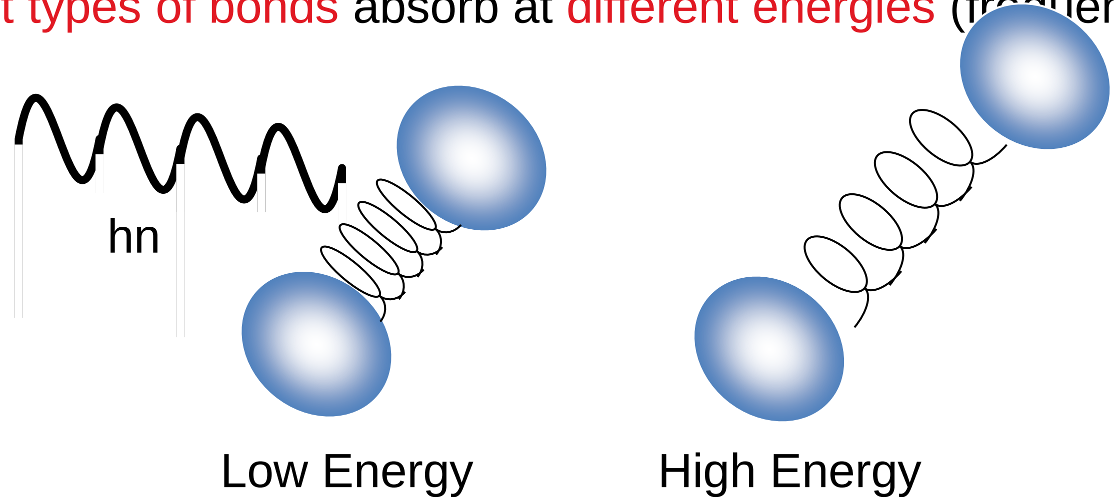
(bond stretches, angles bend etc)

Very important technique for identifying functional groups in a molecule

Principles of IR

- Each IR absorption corresponds to the energies of bond stretching in most molecules

- Different types of bonds absorb at different energies (frequencies)



- Energy for each molecular vibration therefore unique for each functional group.

Different Types of Vibration

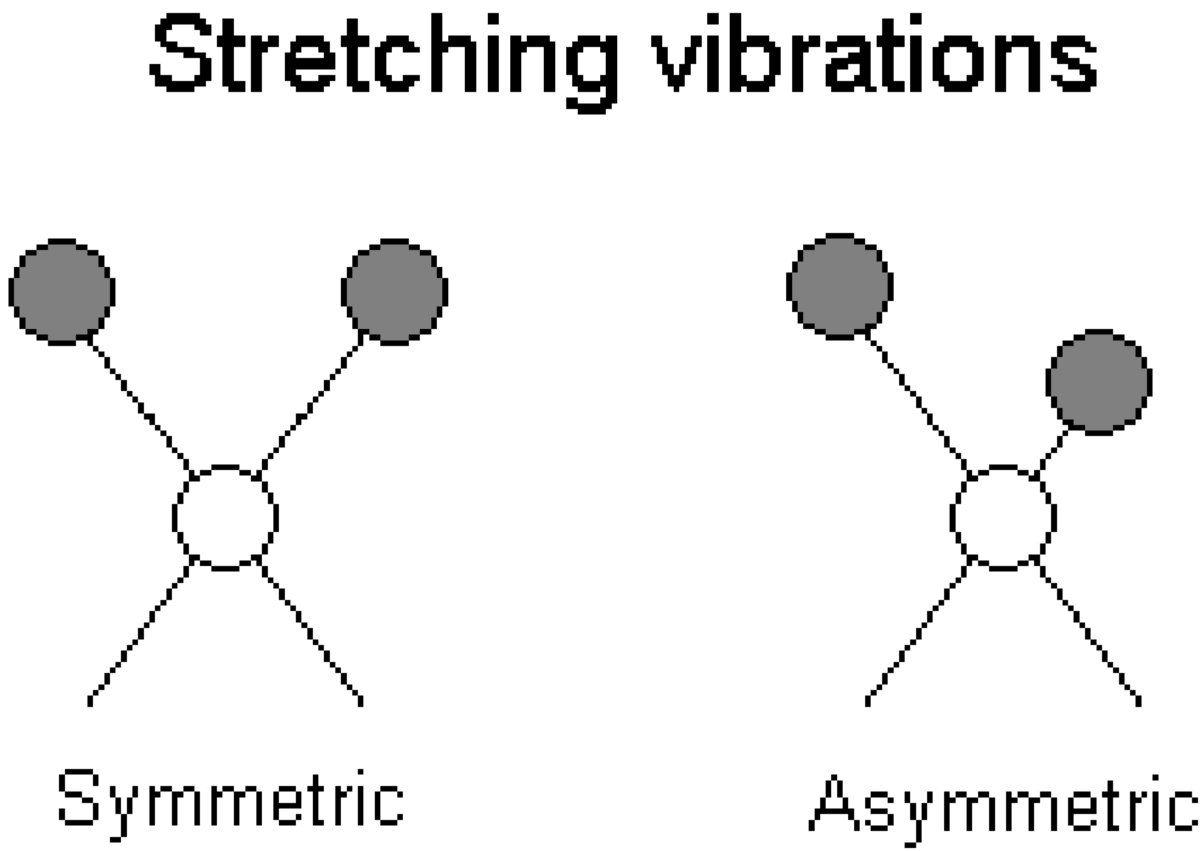
Stretching (involves changes in bond length)

- Symmetric Stretch
- Asymmetric Stretch

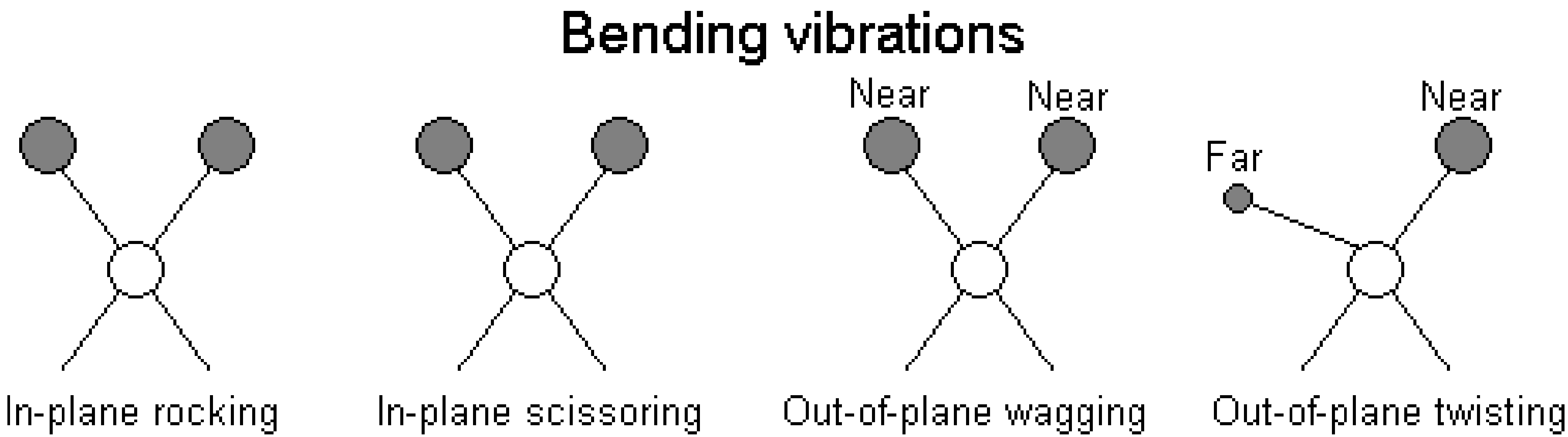
Bending (involves changes in bond angle)

- Rocking
- Scissoring
- Wagging
- Twisting

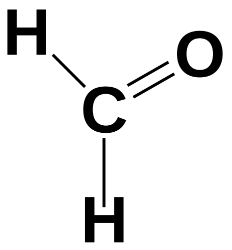
Different Types of Stretching Vibrations

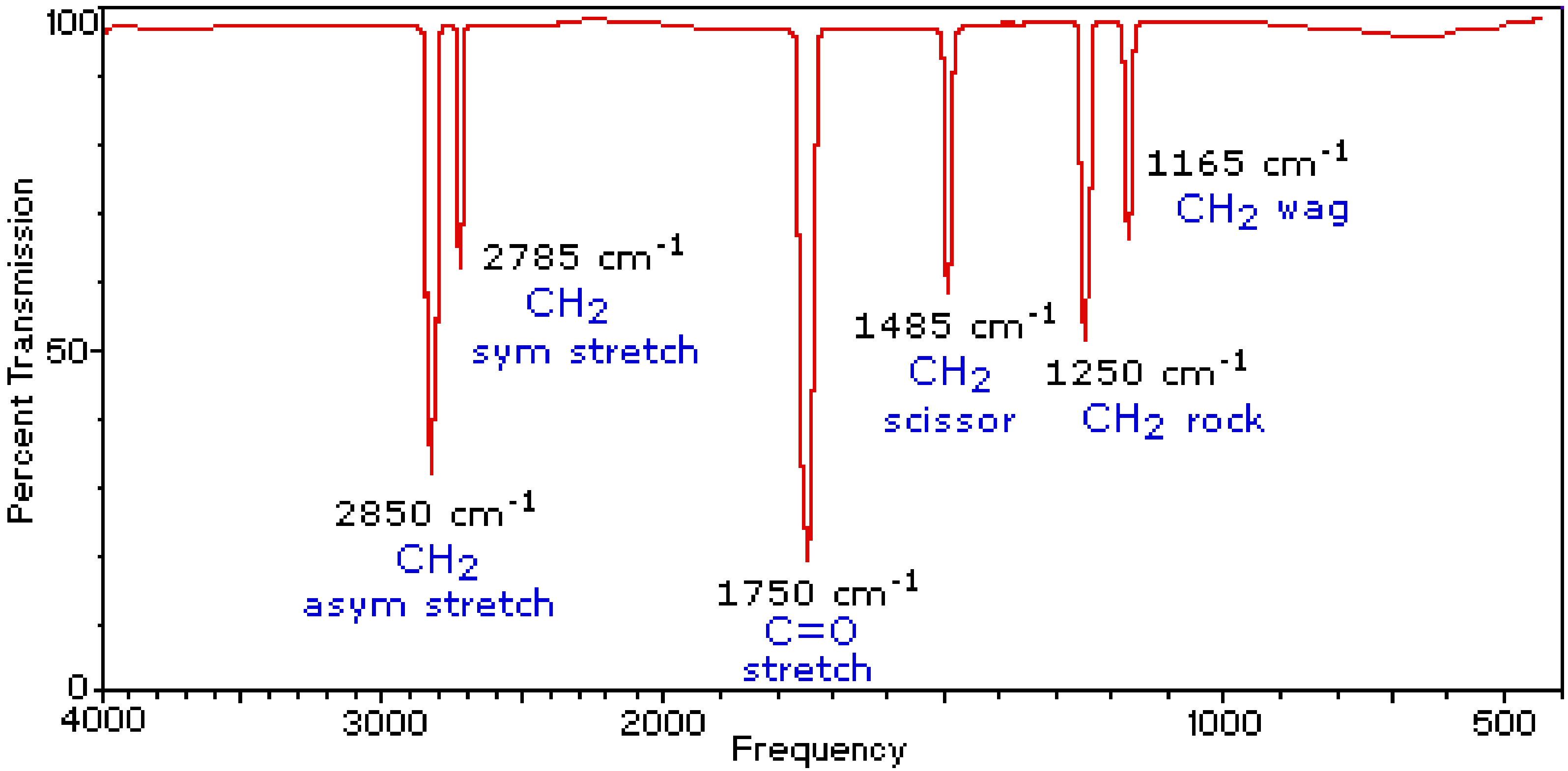


Different Types of Bending Vibration



Formaldehyde: HCHO





Selection Rules for IR Activity

- A vibrational mode can give rise to an absorption of infrared radiation only if the vibration involves a CHANGE IN THE ELECTRIC DIPOLE MOMENT of the molecule.

- Hence it should be obvious that the vibration of a homonuclear diatomic molecule (eg O_2 , N_2 ,etc.) does not result in infrared absorption i.e. IR inactive

- A polyatomic molecule possesses several vibrations and these may be Infrared active or inactive according to the symmetry of the vibrational mode.

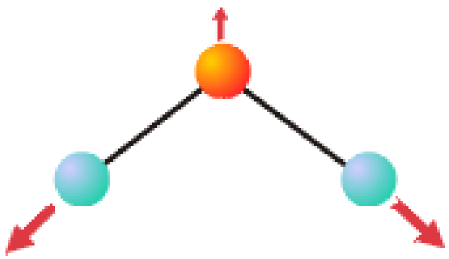
http://www.shu.ac.uk/schools/sci/chem/tutorials/molspec/irspec1.htm

Vibrational Modes

- For a linear molecule with n atoms, there are $3n – 5$ fundamental vibrations.
- For a nonlinear molecule with n atoms, there are $3n - 6$ fundamental vibrations.

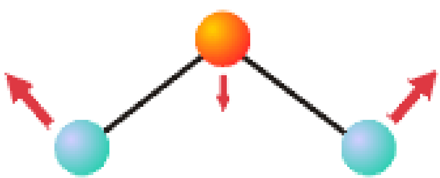
WATER VIBRATIONAL MODES

3n-6 = 9-6 = 3 vibrational modes are expected for this non-linear molecule



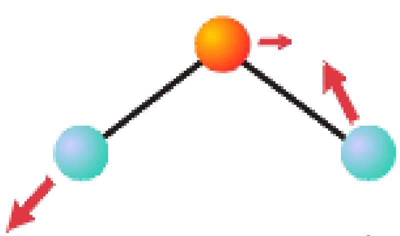
ν_1 (3652 cm^{-1})

SYMMETRIC O-H STRETCHING MODE. It can be formed by combining the stretching of the two 0-H bonds so that expansions and contractions of the two bonds occur IN PHASE.



ν_2 (1595 cm^{-1})

The middle vibration (n_2) is the BENDING MODE.



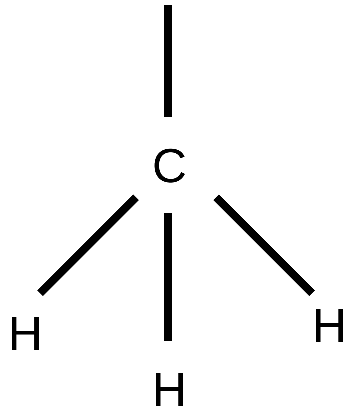
ν_3 (3756 cm^{-1})

The third vibration (n_3) is called the ASYMMETRIC O-H STRETCHING MODE. It is formed by combining the expansion of one O-H bond with the contraction of the other O-H bond, ie OUT OF PHASE.

Note:

The – CH_3 group gives rise to a

- symmetric C-H stretch at 2850 – 2890 cm^{-1}
- asymmetric stretch at 2940 – 2980 cm^{-1}
- symmetric deformation at 1375 cm^{-1}
- asymmetric deformation at ~ 1470 cm^{-1}



Deformation

'opening and closing of an umbrella'

Vibrational Modes

Modes of vibration

<u>Skeletal vibrations</u>

$1400 - 600\ cm^{-1}$

called the 'fingerprint region'.

In this portion of the spectrum the energy of the absorption bands varies depending on the structure of a molecule.

<u>Characteristic group vibrations</u>

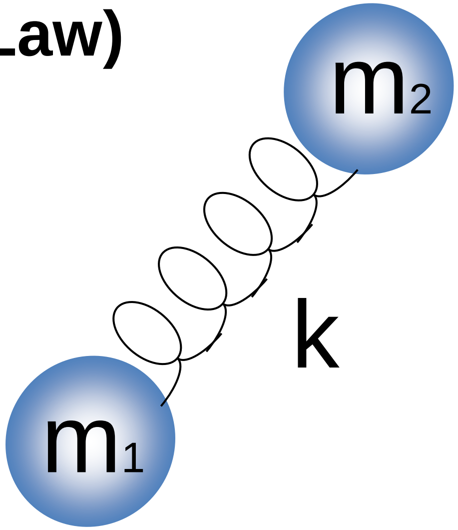
$4000\text{-}1400\ cm^{-1}$.

In this region the bands are characteristic of specific functional groups in a molecule.

No two molecules will give exactly the same IR spectrum (except enantiomers).

The exact frequency at which a given vibration occurs is determined by the strengths of the bonds involved and the mass of the component atoms.

To help understand IR, it is useful to compare a vibrating bond to the physical model of a vibrating spring system (described by Hooke's Law)



$$\bar{n} = \frac{1}{2pc}\sqrt{\frac{k}{m}}$$

$\bar{n}$ = vibrational frequency of the bond,
in wavenumbers (cm^{-1})

k = force constant, indicating the strength of the bond (N/m)

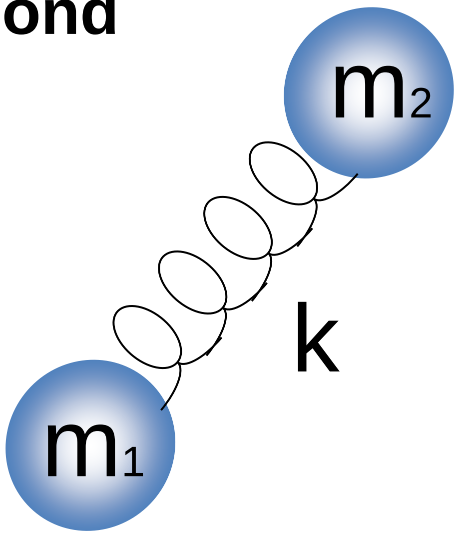
m = reduced mass, which is given by

$$m = \frac{m_1 m_2}{m_1 + m_2}$$

where m_1 and m_2 are the masses of the two atoms
(in kilograms per molecule)

How does the strength of the bond influence the vibration?

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$



For a stronger bond (larger k value), υ increases.

Examples

CC bonds:

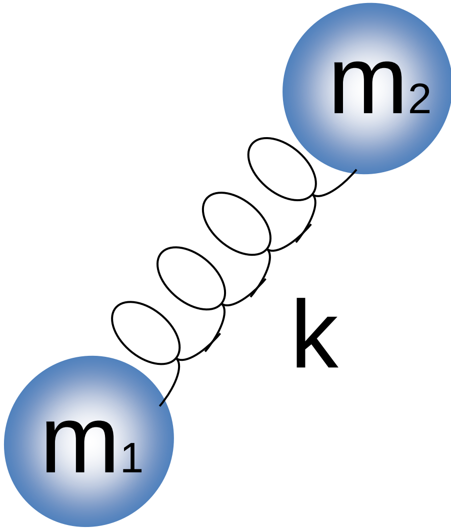
$\bar{\nu}_{obs}$ C ≡ C (2200 cm^{-1}) > C=C (1600 cm^{-1}) > C-C (1000 cm^{-1})

CH bonds:

C≡C-H (3300 cm^{-1}) > C=C-H (3100 cm^{-1}) > C-C-H (2900 cm^{-1})

How does the mass influence the vibration?

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

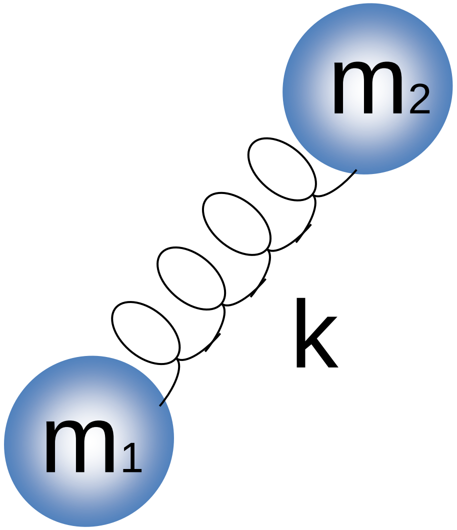


For heavier atoms attached (larger $\bar{\mu}$ value), υ decreases.

<u>Examples</u>

C-H (3000 cm^{-1})
C-C (1000 cm^{-1})
C-Cl (800 cm^{-1})
C-Br (550 cm^{-1})
C-I   (about 500 cm^{-1})

Rules of Thumb!



The fundamental wavenumber of a bond-stretching vibration is given by:

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$

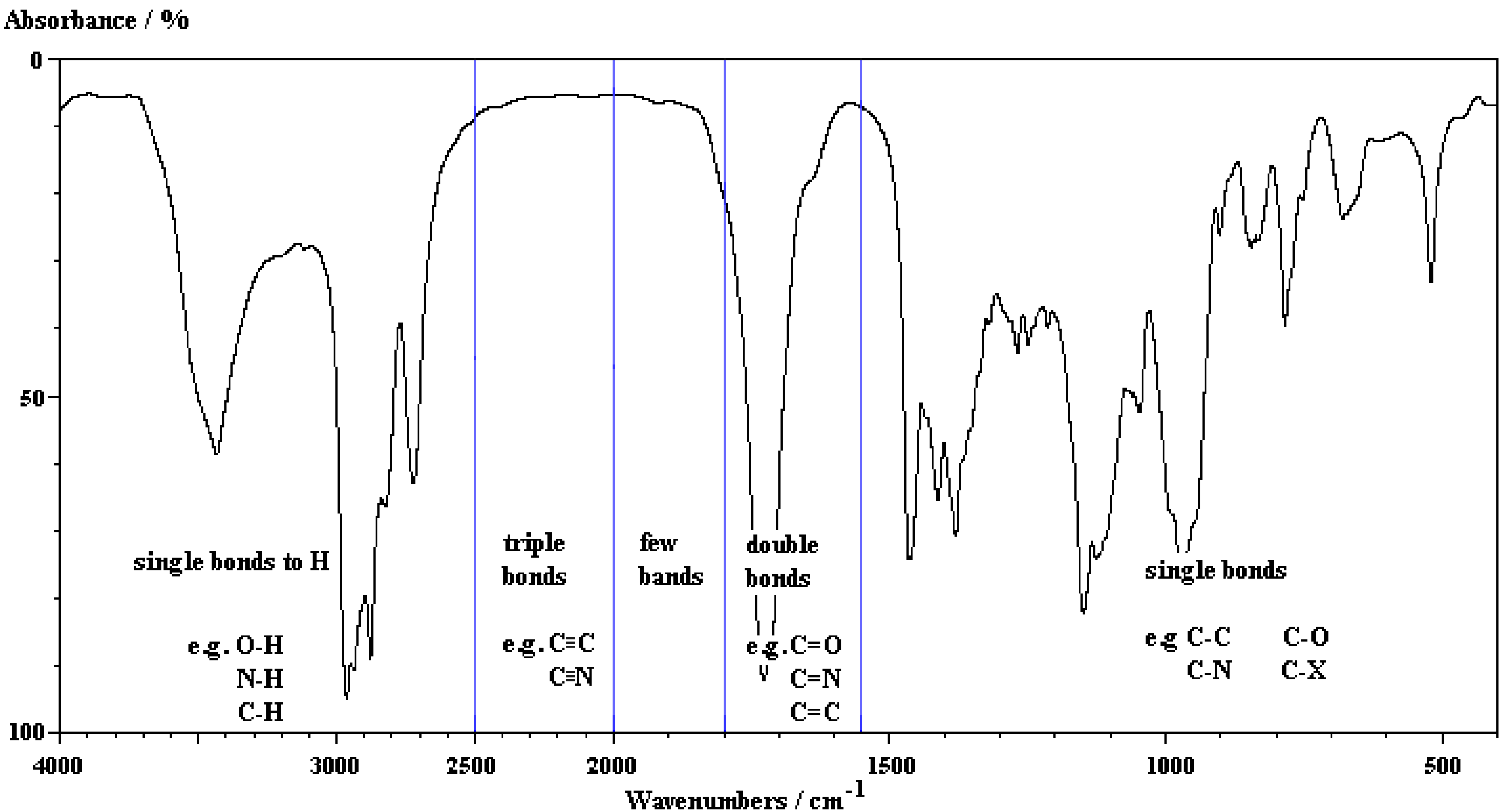
- Frequency bond stretch $\propto$ strength of bond

  $\propto$ 1/mass of atoms

- Bending frequencies are generally lower than stretching frequencies. ($k_{bend} < k_{stretch}$).
- Symmetric stretching frequencies are generally lower than asymmetric stretching frequencies.

Trends that can be accounted for using Hookes' Law.

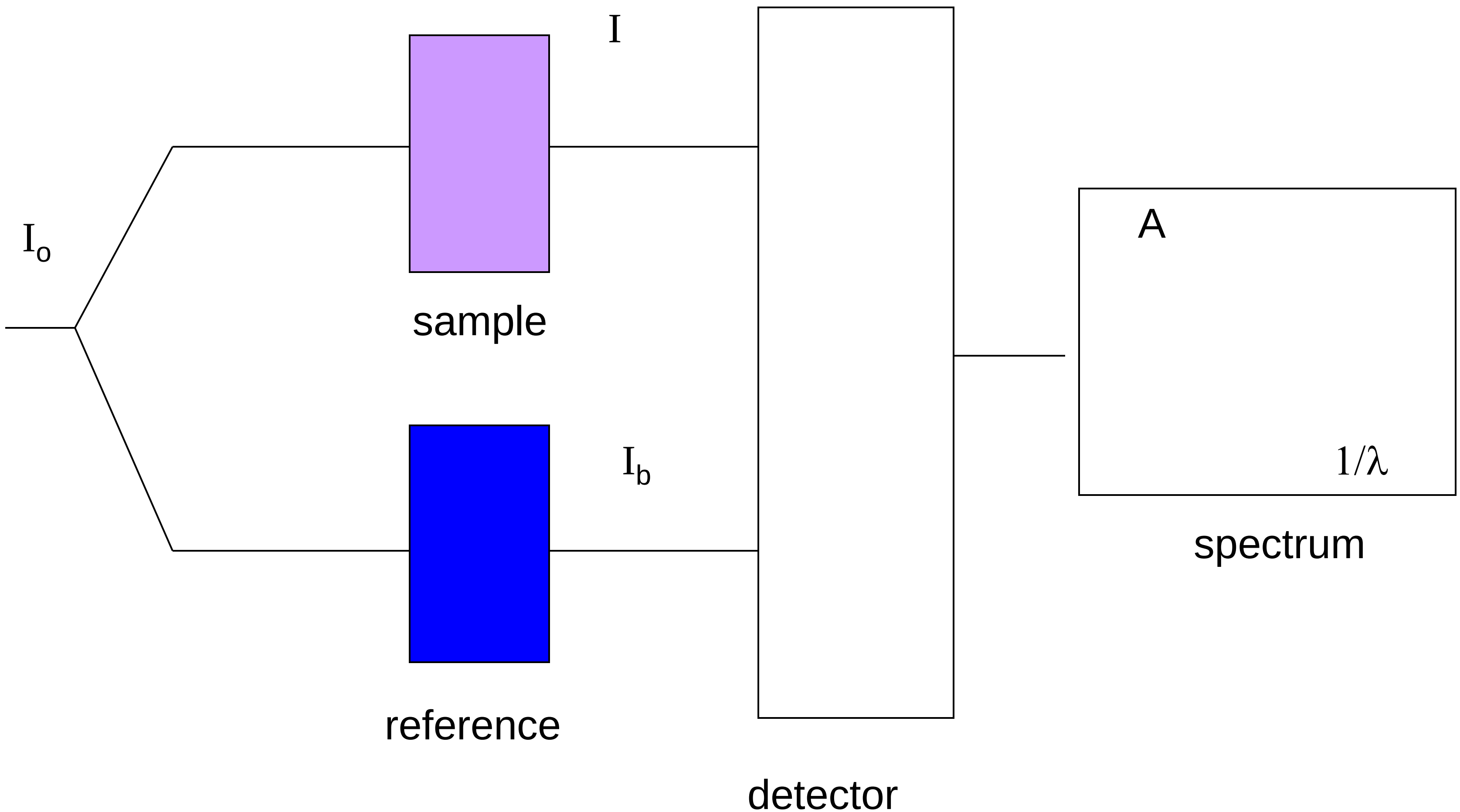
It also gives an approximate outline of where specific types of bond stretches may be found.



Experimental set-up - a modern FTIR instrument

FT = Fourier Transform

Experimental set-up

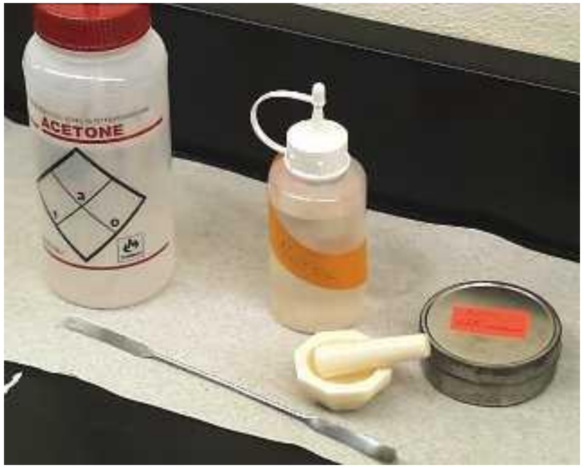


IR radiation varied from 4000-700 cm^{-1} (wavenumbers)

$1/\lambda$

Experimental set-up

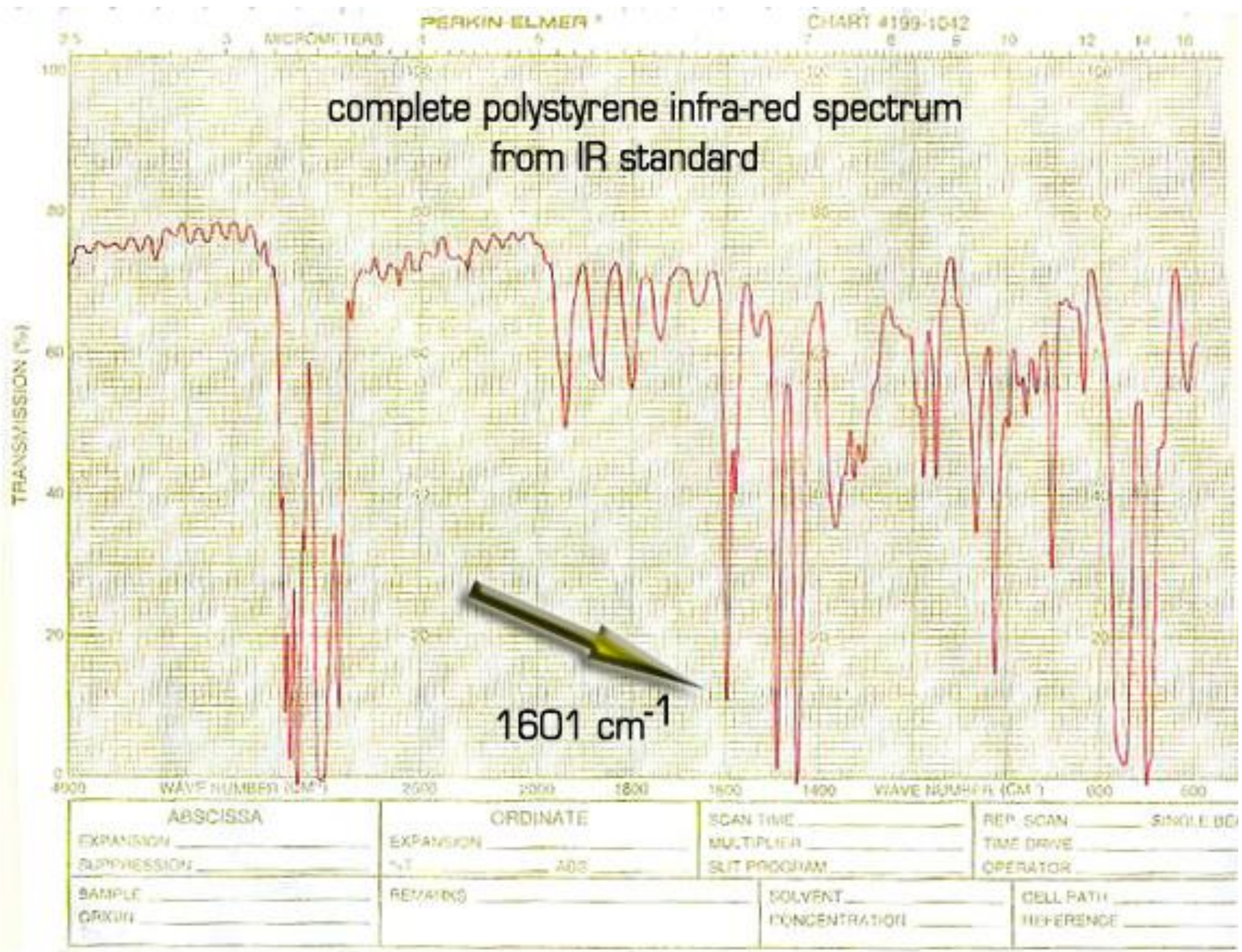
Very similar to UV-VIS, but there are some technical difficulties in working with IR radiation:



- glass+quartz absorb IR
  - useless for constructing sample cells.

- Instead use alkali halide crystals.
  Large NaCl or KBr crystals cut + polished to give disks. Crystal lattices do vibrate, but need much more energy than IR radiation to do so, $\therefore$ disks transmit 100% IR radiation.

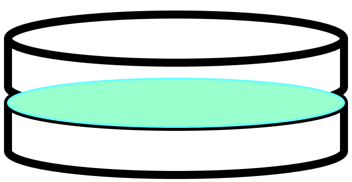
- But! NaCl dissolves in water! Cannot record IR spectra for aqueous solutions! Normally use organic solvents - $CHCl_3$ or CCl_4, or pure liquid film etc.

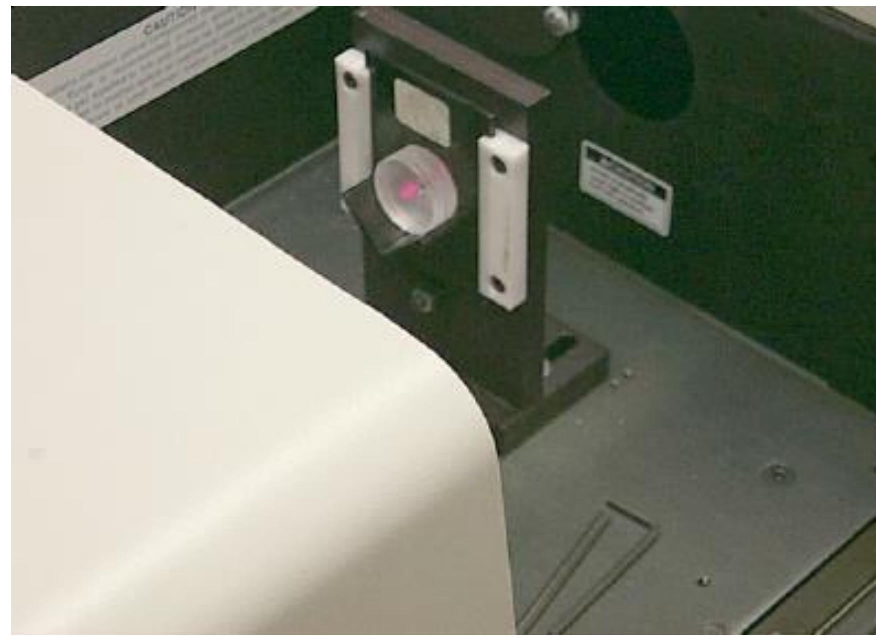

complete polystyrene infra-red spectrum
from IR standard
1601 cm-1
PERKIN-ELMER
CHART #199-1042
MICROMETERS
TRANSMISSION (%)
WAVE NUMBER (CM-1)
ABSCISSA
EXPANSION
SUPPRESSION
ORDINATE
SCAN TIME
MULTIPLIER
SLIT PROGRAM
REP. SCAN
SINGLE BEAM
TIME DRIVE
OPERATOR
SAMPLE
ORIGIN
REMARKS
SOLVENT
CONCENTRATION
CELL PATH
REFERENCE

IR Sample Preparation

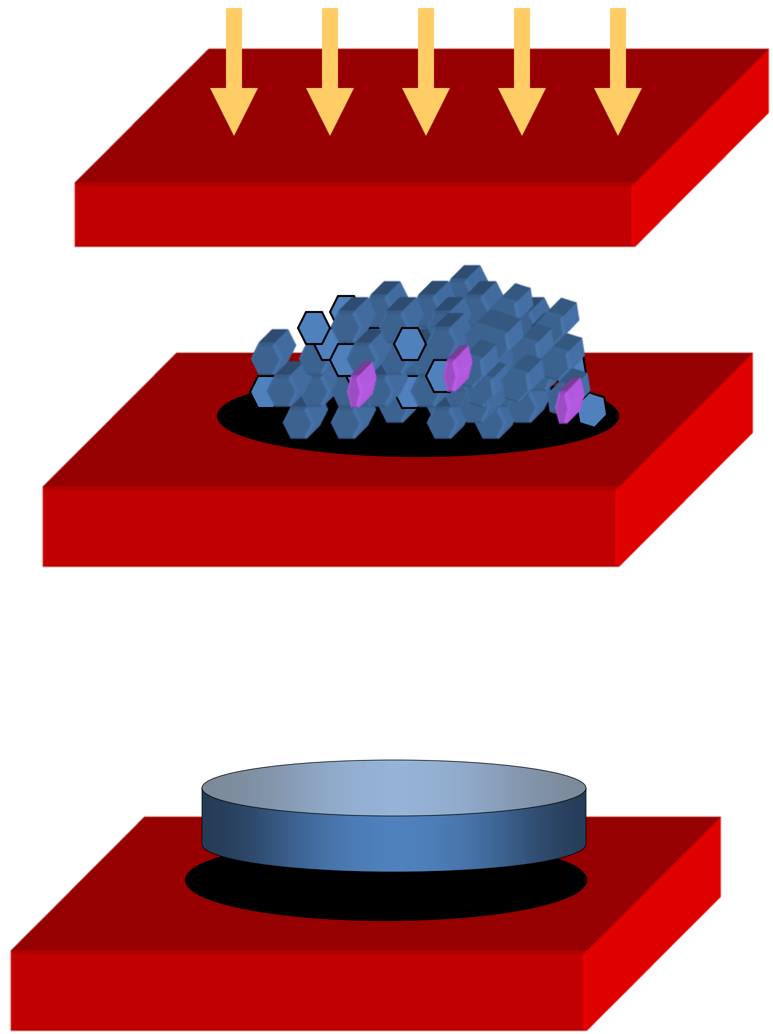
- Most flexible system for analysing all 3 states of matter (solid, liquid, gas)
- “Neat” (analysis of liquids/oils)
- Nujol mull or Pellet (analysis of solids)
- Thin Cell (analysis of dissolved solid samples - solutions)
- Long Cell (analysis of gases)

Preparing a “Neat” IR Sample

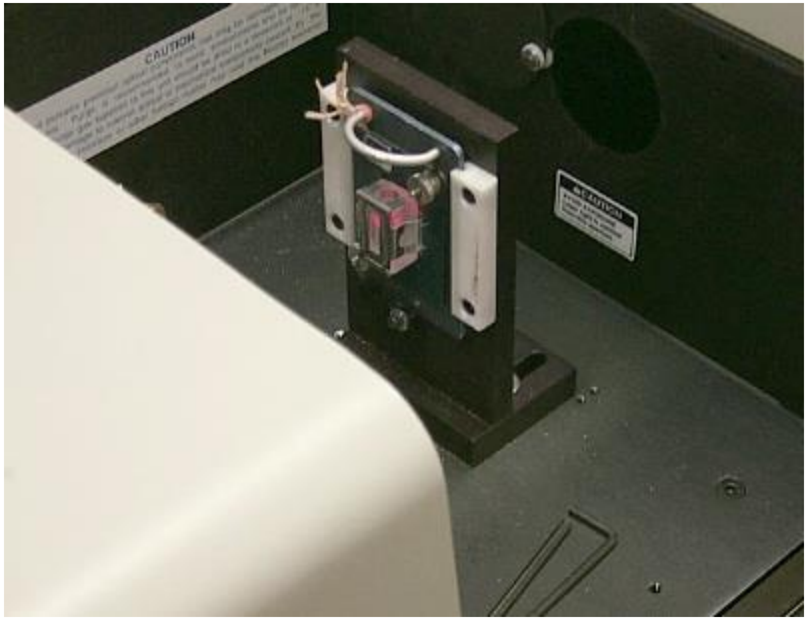




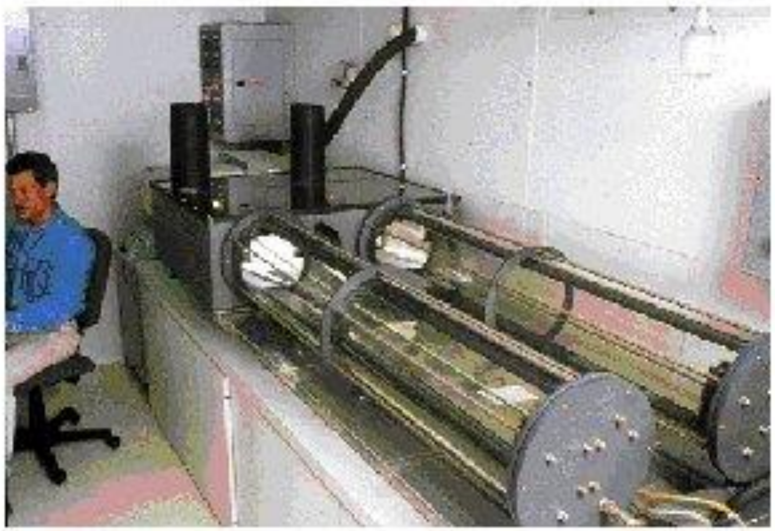
Preparing a KBr Disk



IR Liquid Sample Cell



IR Gas Sample “Cell”



Recall: Fingerprint of Molecule

- No two molecules will give exactly the same IR spectrum (except enantiomers).

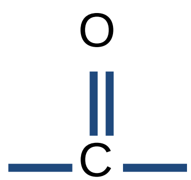
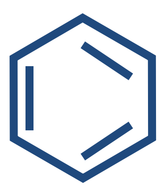
- Simple stretching: 4000-1300 cm^{-1}.
  In this region the bands are characteristic of specific functional groups in a molecule.

- Complex vibrations: 1300 - 600 cm^{-1},
  called the 'fingerprint region'.
  In this portion of the spectrum the energy of the absorption bands varies depending on the structure of a molecule.

Due to the complexity of an IR spectrum, it is unusual to assign every single peak on the spectra.

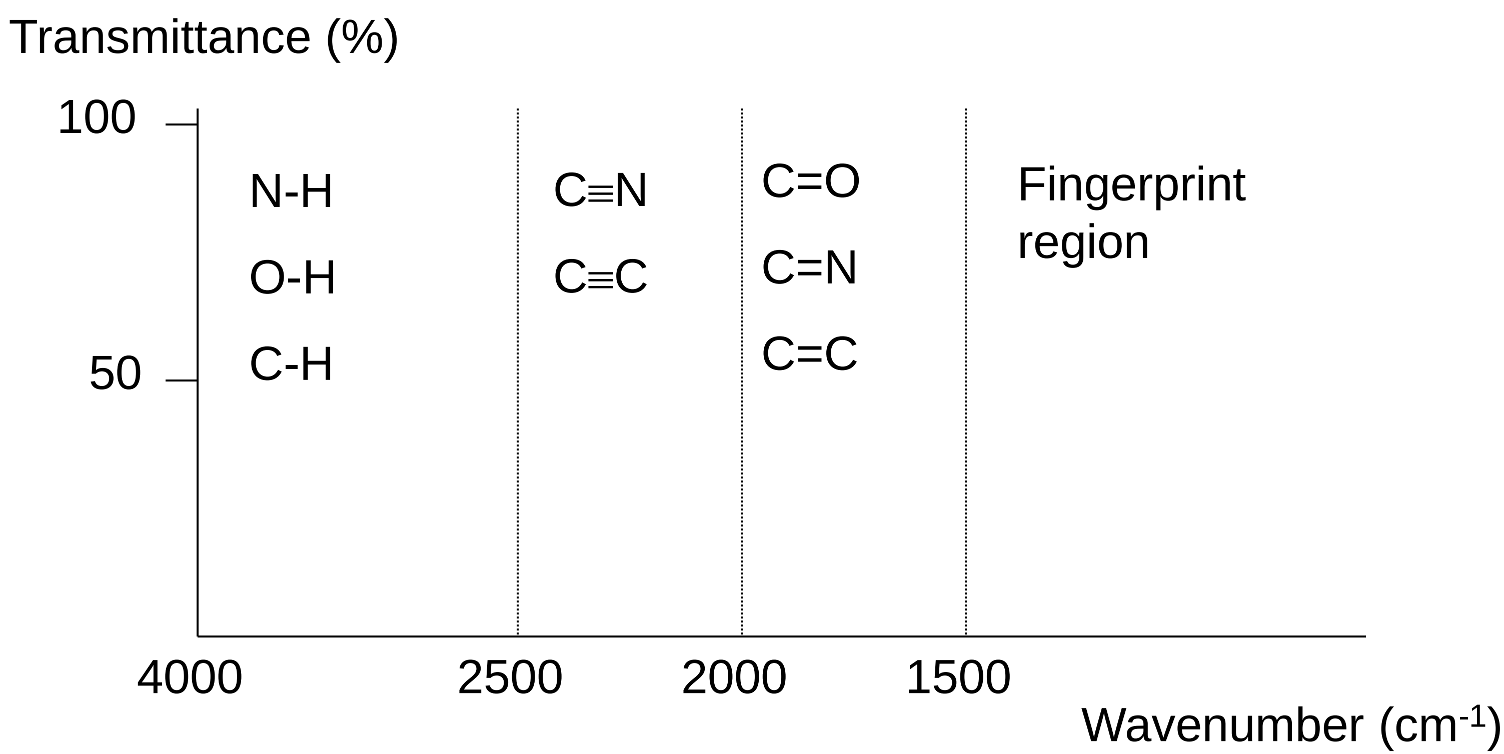
Fortunately we don't need to!

Most functional groups give rise to characteristic IR absorptions that change little in going from one compound to another

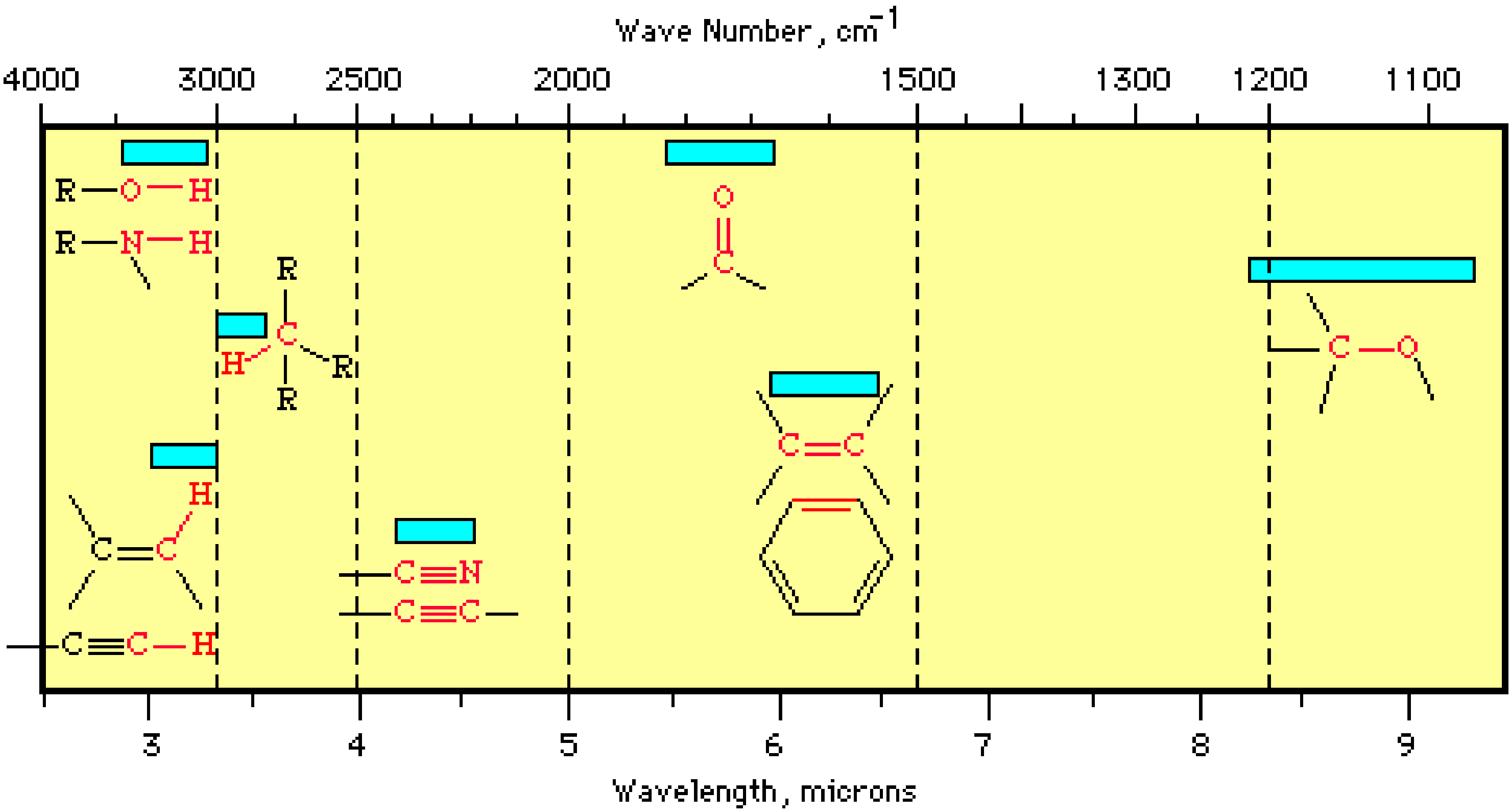
Functional group	absorption frequency
	~ 1690 - 1750 cm^{-1}
	~ 3200 - 3600 cm^{-1}
	~ 1650 - 1670 cm^{-1}
	~ 1600 cm^{-1}

As a rough guide, the spectrum can be split into four sections

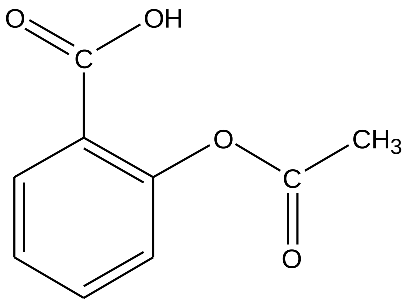
$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$



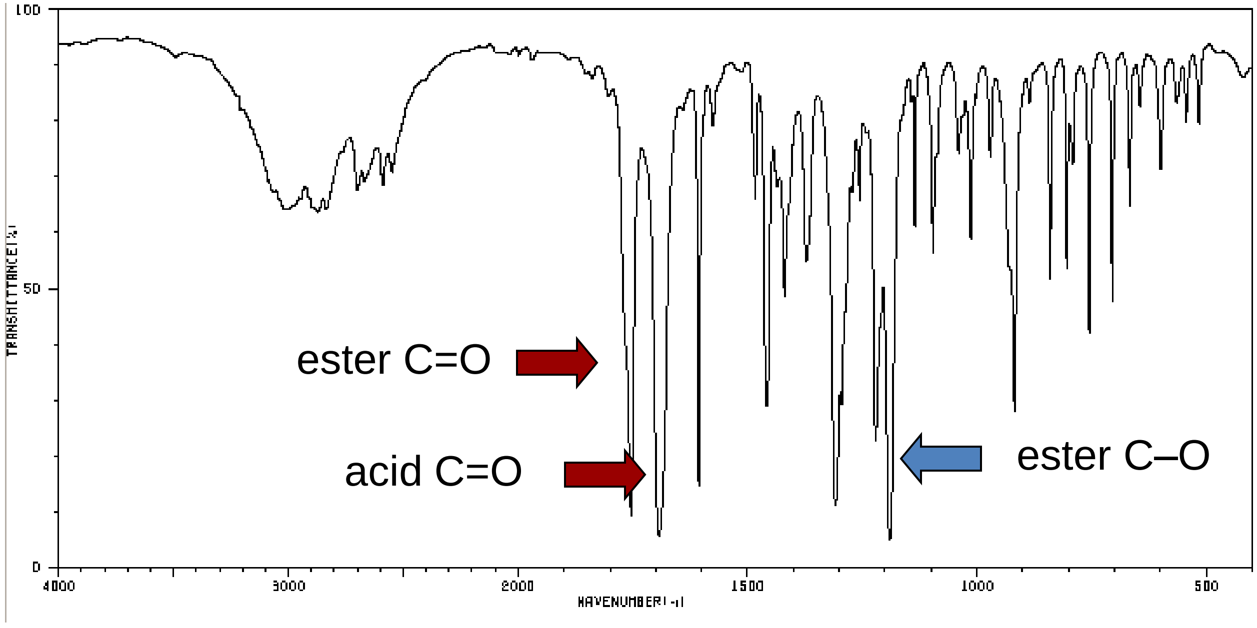
IR Characteristic Vibrations



IR Spectroscopy: Where do you begin?!



Aspirin



<u>Characteristic group vibrations</u>

4000-1300 cm^{-1}

<u>Fingerprint region</u>

1300 - 600 cm^{-1}

So remember to look for peaks to positively identify functional groups, but also remember to look for evidence to discount others.