

Spectrophotometry

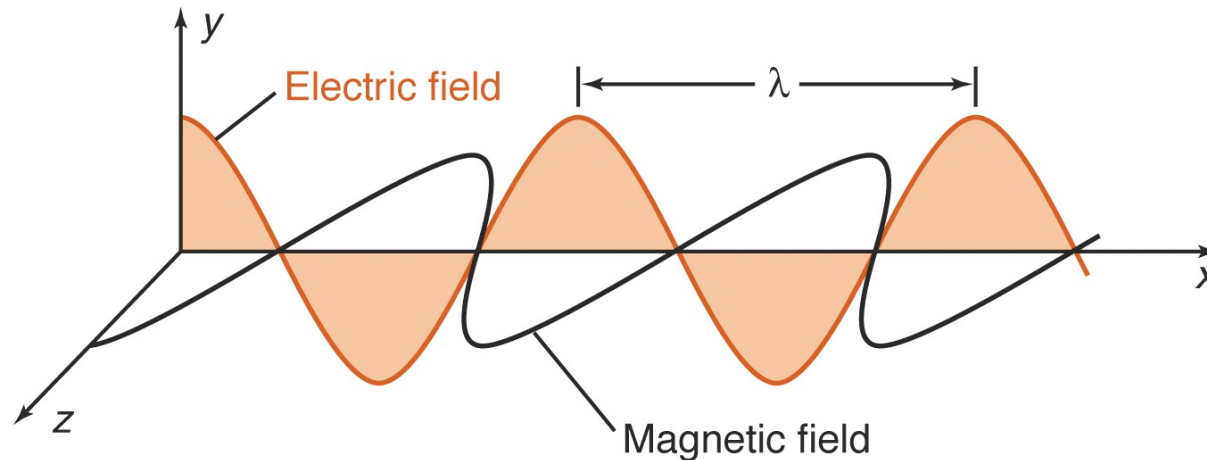


Objectives

- Well understand the properties of light
- Well understand the energy levels of various kinds
- Well understand the types of spectroscopy and their origins
- Understand the application of each types of spectroscopy

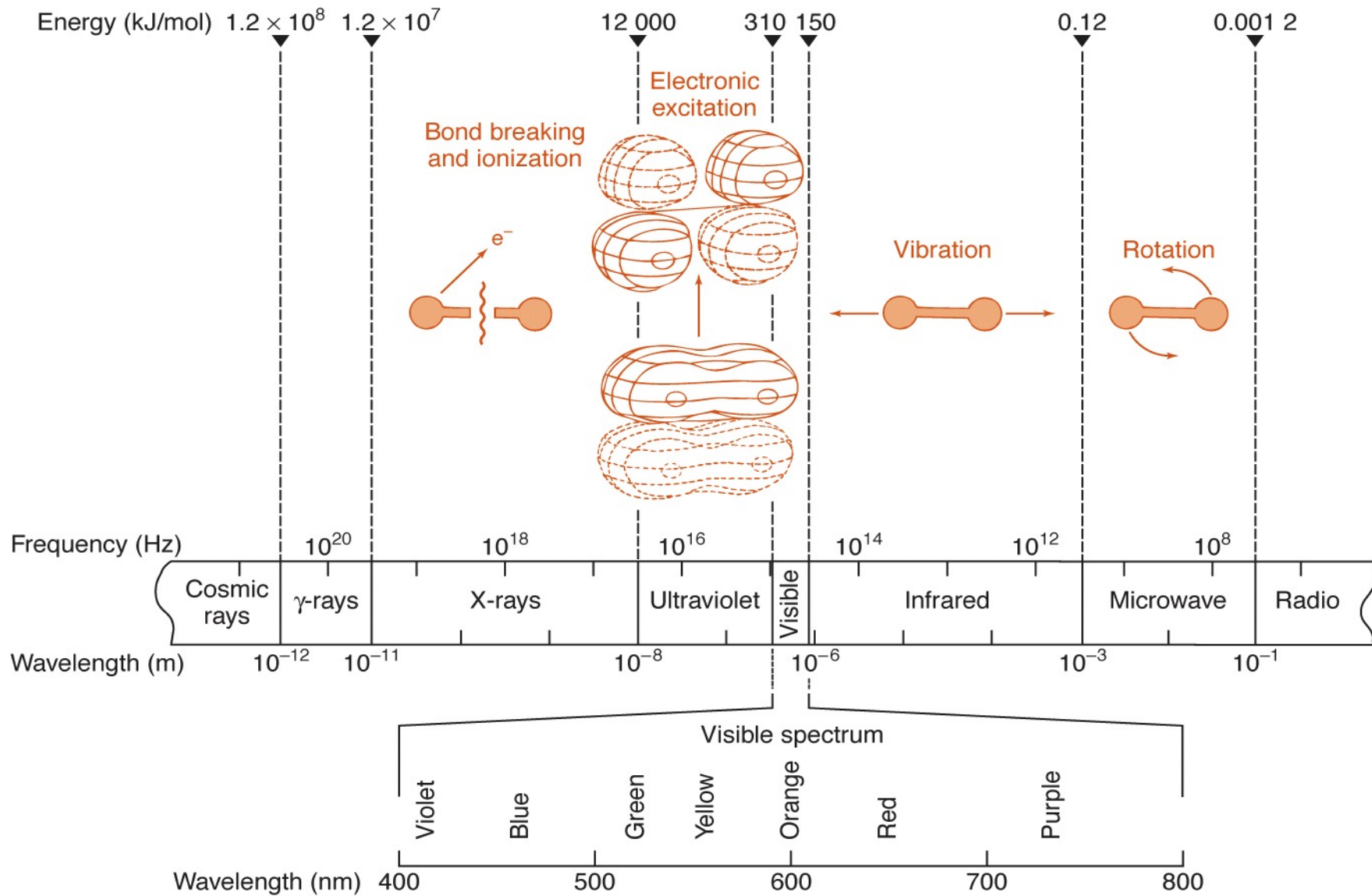
Properties of Light

- Light can be considered as particles and waves
- The light wave is electric and magnetic field



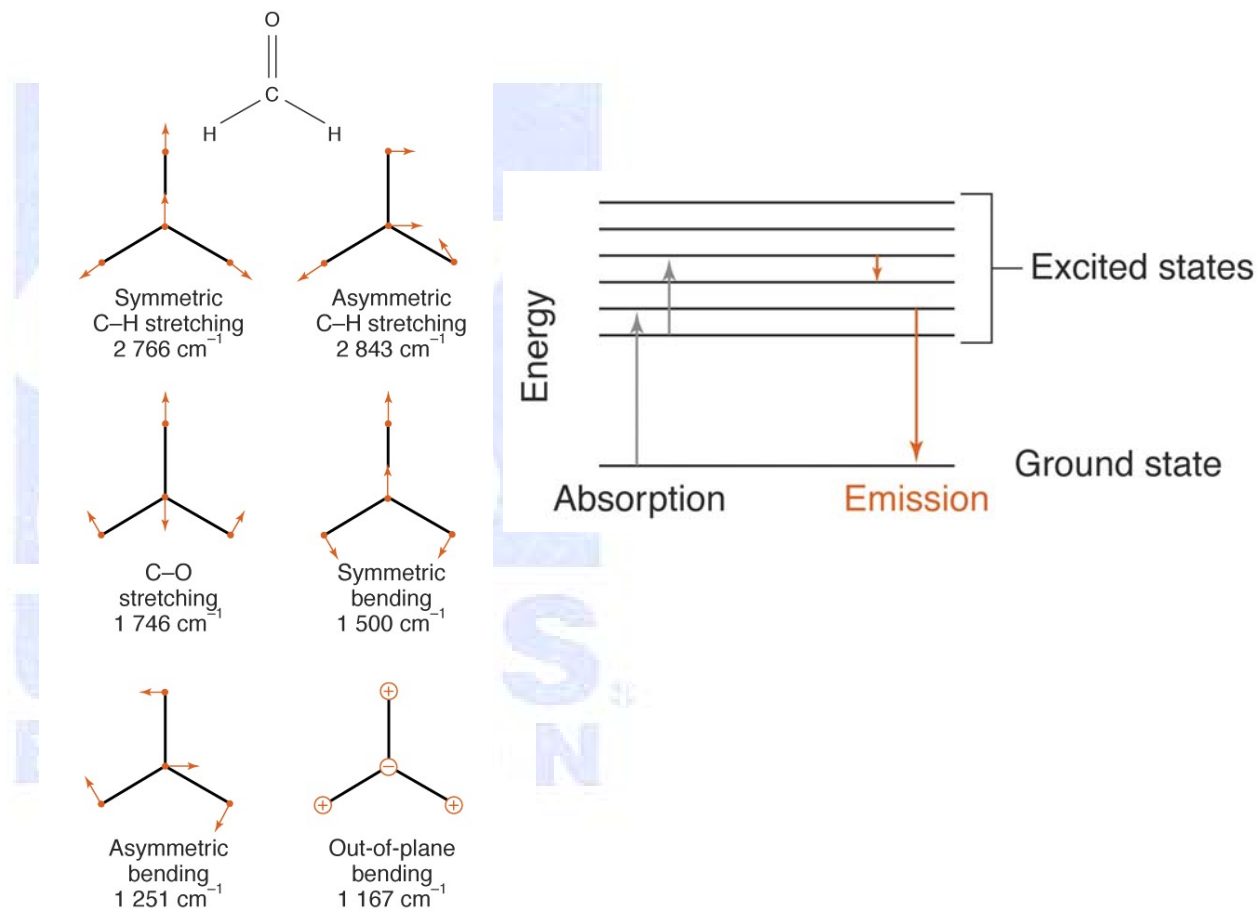
$$\nu \text{ (Frequency)} \lambda \text{ (wavelength)} = c \text{ (light speed)}$$

- Photons: $E = h \text{ (Planck's constant)} \nu \text{ (Frequency)}$
 $h = 6.626 \times 10^{-34} \text{ Js}$

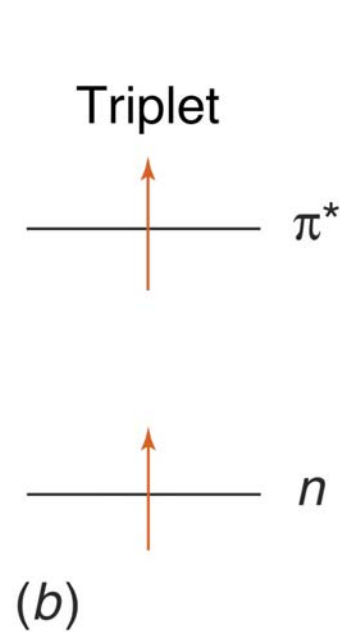
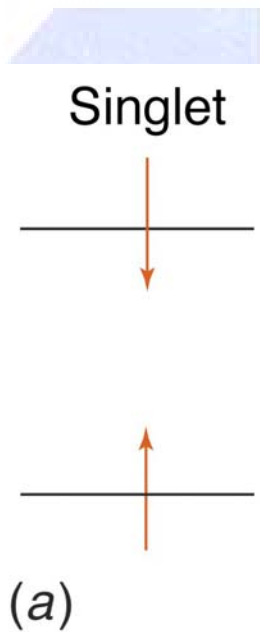
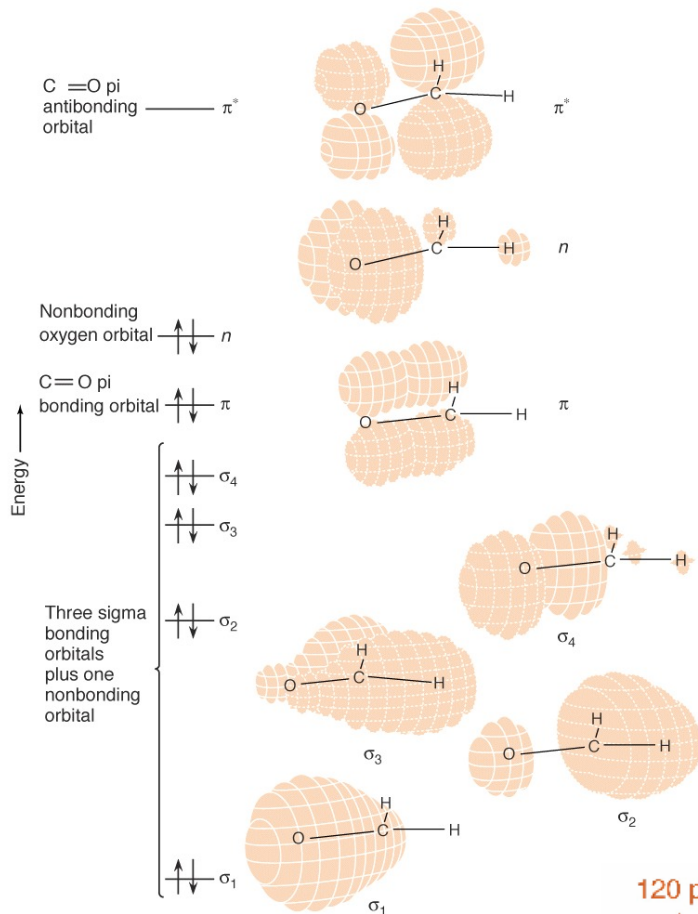


Energy levels of a compound

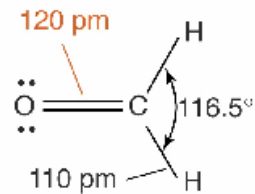
- Types of energy states
 - Electronic
 - Vibrational
 - Rotational
- Energy states



Electrons on the energy state

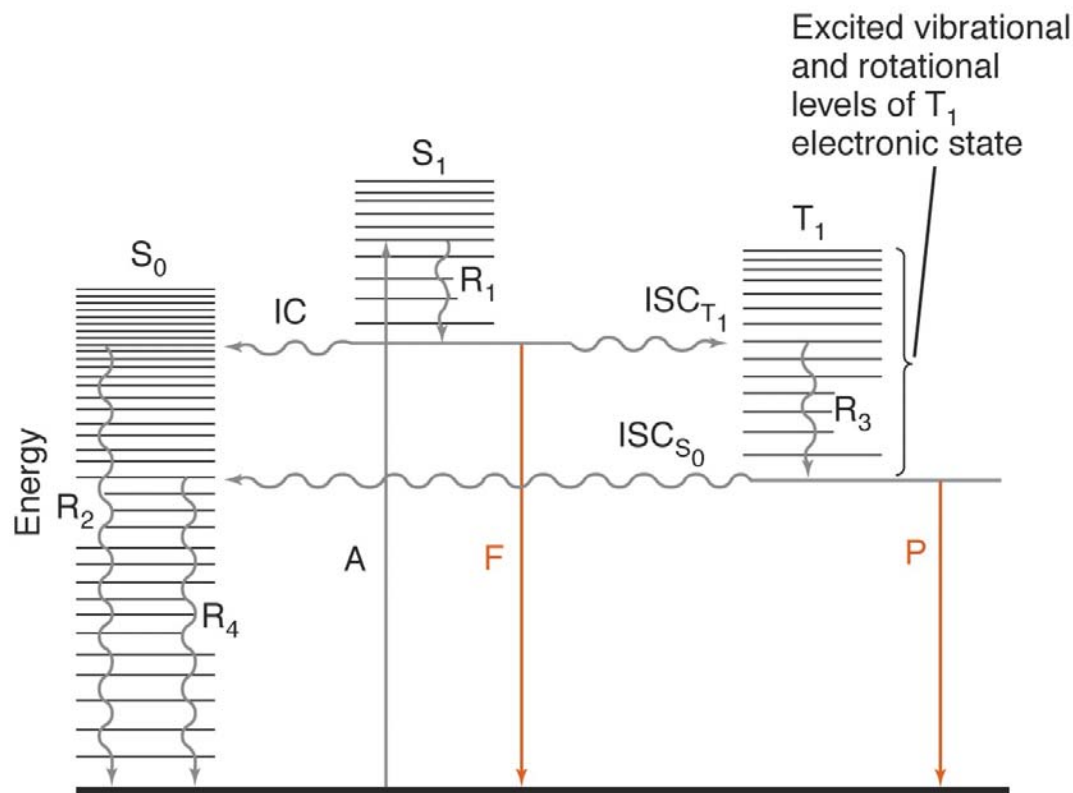


Molecular orbital diagram for formaldehyde



The interaction between light and compounds

- Electrons can be excited by the absorption of light to from the lower energy level to higher energy states.
- The electrons on higher energy states will find their way back to lower energy states and give away energy in the forms of heat (**relaxation**) or light (called **Luminescence: Fluorescence -rapid and Phosphorescence - slow**).

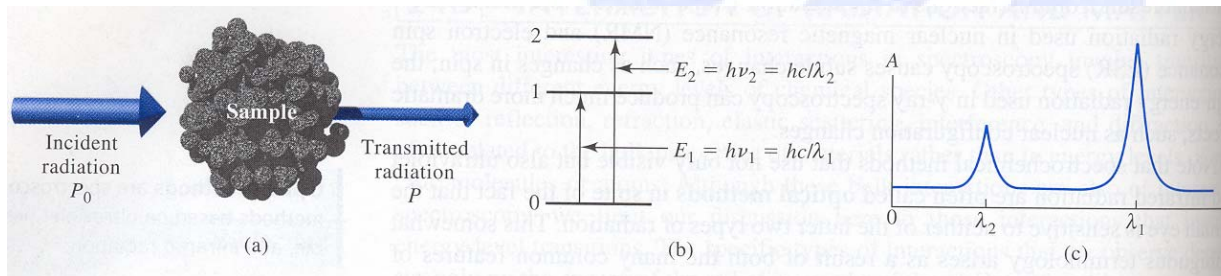


IC: internal conversion

ISC: intersystem crossing

Two kinds of techniques

- To detect the absorption of light:
absorption spectroscopy.
 - Atomic absorption, change electronic state of an atom e.g. from s orbital (ground) to p orbital.



- Molecular absorption, change a energy state of a molecule: electronic (UV-Visible), vibrational and rotational transitions (IR).

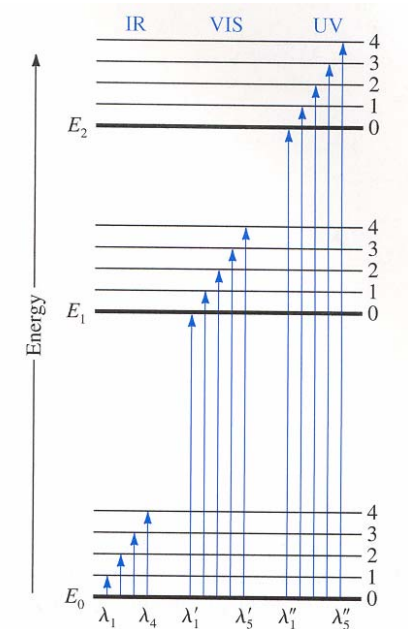
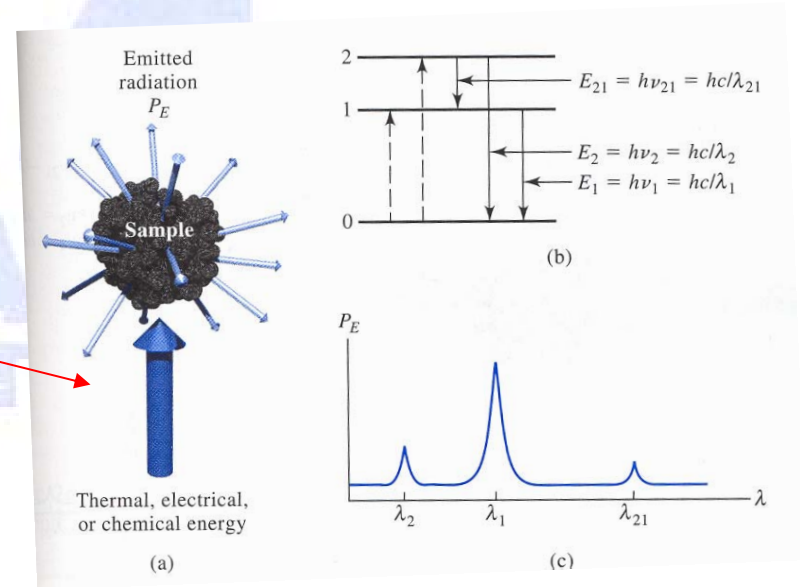
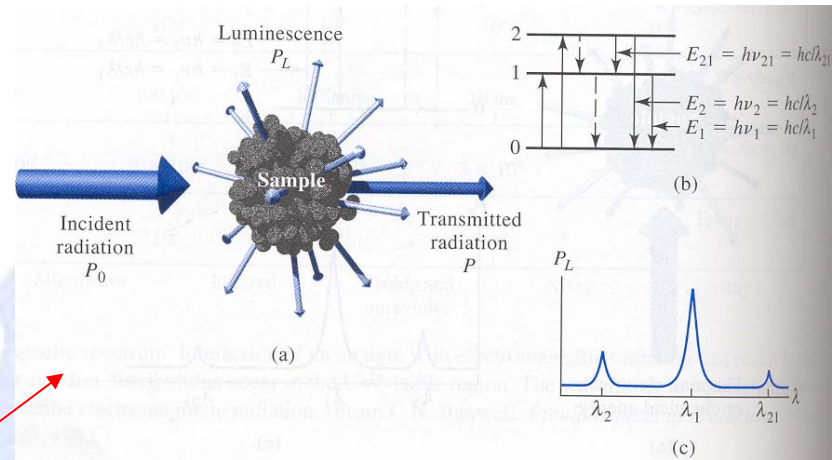


Figure 24-12 Energy-level diagram showing some of the energy changes that occur during absorption of infrared (IR), visible (VIS), and ultraviolet (UV) radiation by a molecular species. Note that with some molecules, a transition from E_0 to E_1 may require UV radiation instead of visible radiation. With other molecules, the transition from E_0 to E_2 may occur with visible radiation instead of UV radiation.

- To detect the luminescence of light (Fluorescence and Phosphorescence):
emission spectroscopy

- By external electromagnetic radiation source or by bombardment with electrons; heating in a plasma, a flame or an electric arc; irradiation with a beam of light.

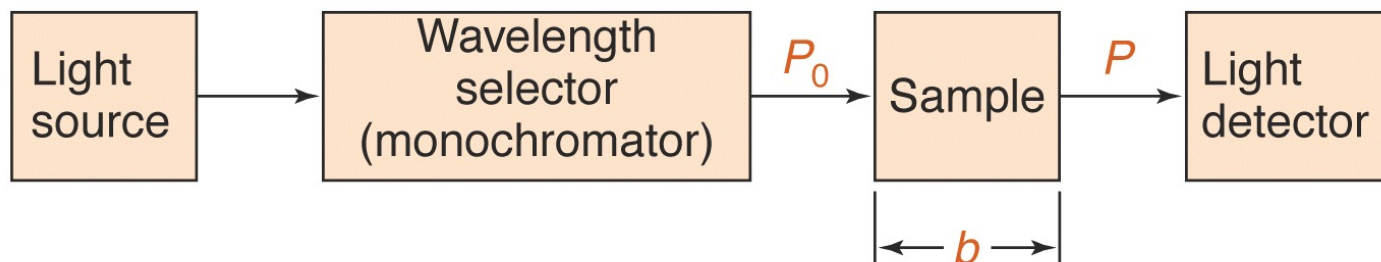


Spectrophotometry



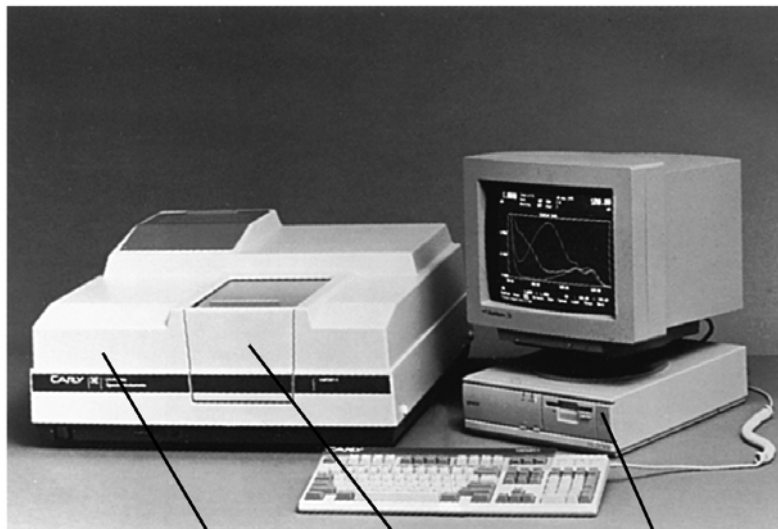
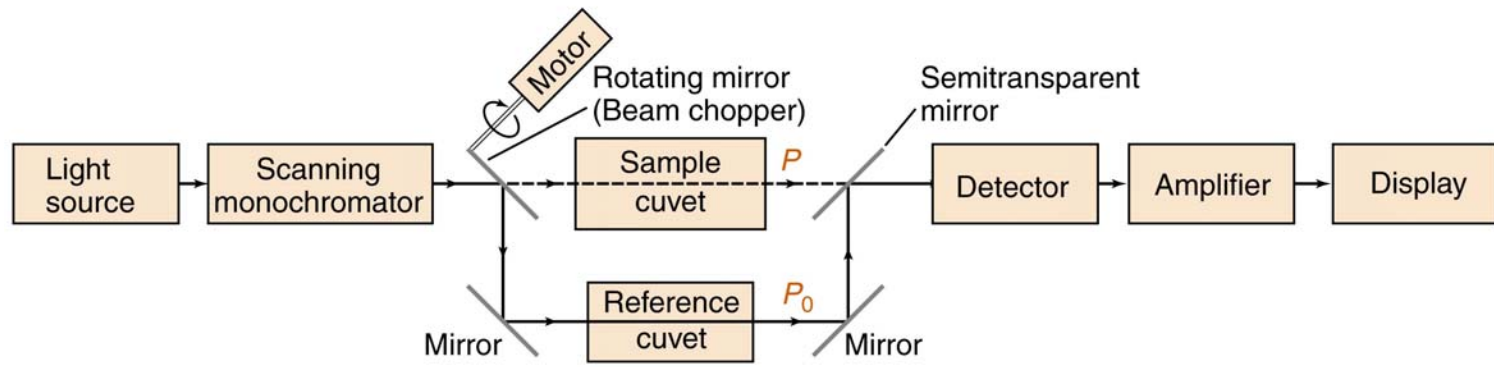
Absorption Spectroscopy

- Single beam absorption spectrophotometry



- Transmittance and absorbance
Transmittance T : the fraction of the irradiance (P) of the original light: $T = P/P_0$
Absorbance A : $A = \log(P_0/P) = -\log T$
- Beer's law: $A = \epsilon bc$

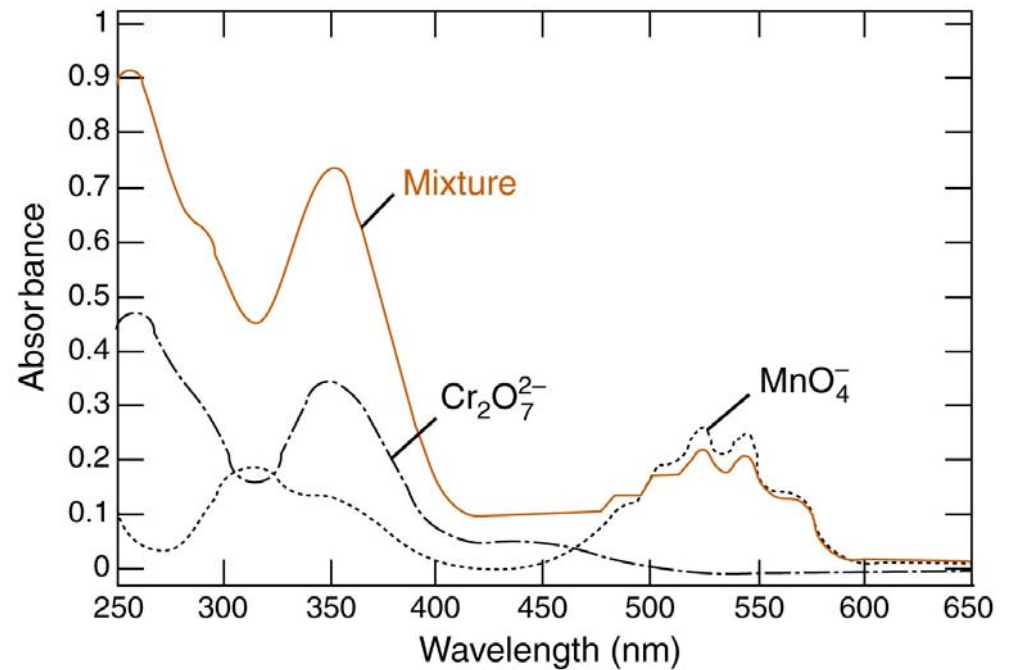
Example: Ultraviolet and Visible spectroscopy (UV-visible)



Source, monochromator, detector

Sample compartment

Display and controls



Example: Ultraviolet and Visible spectroscopy (UV-visible)

Mn(III) SPECIES IN MULTIPLY RECHARGEABLE MnO_2 703

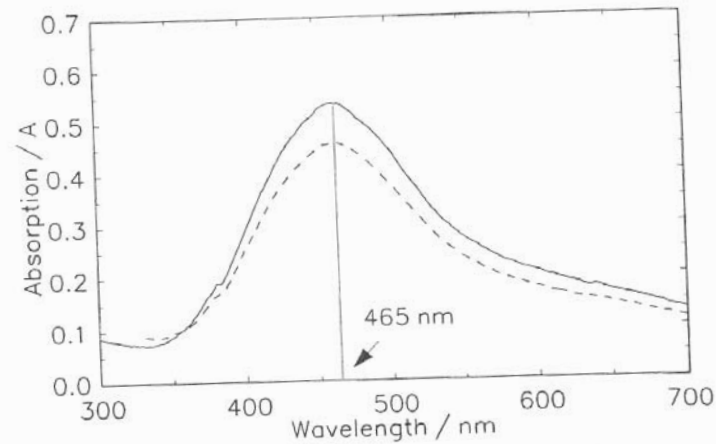


Fig. 10. U.v.-visible spectra of soluble Mn(III) species; solid line for 37% discharge; dashed line for 37% on recharge of 2e capacity of a CM MnO_2 electrode.

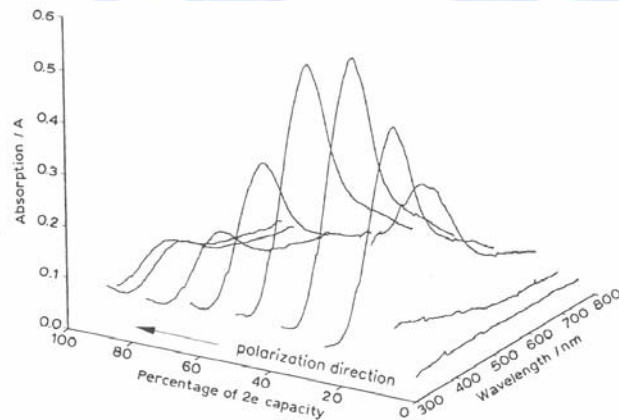
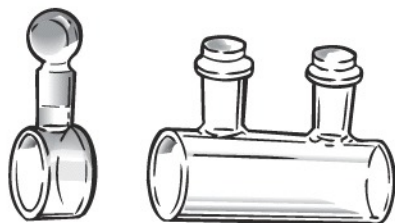


Fig. 11. The change of optical absorption spectrum of Mn(III) species with percentage discharge in the discharge process at a CM MnO_2 /Lonz graphite mixture (1:4).

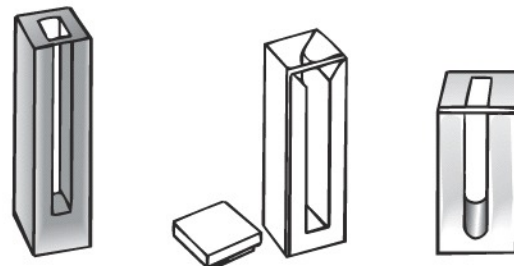
Standard
1-cm path



Cylindrical



Micro cells



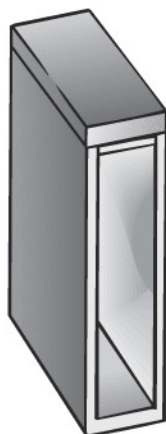
5-mm
path



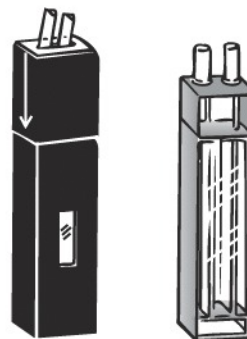
1-mm
path



20-mm path



Flow

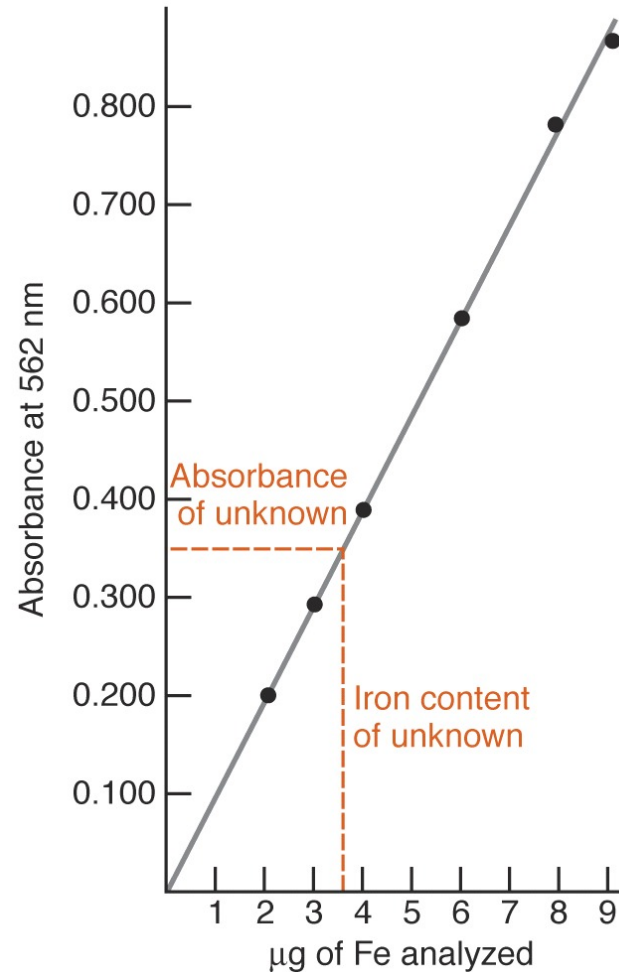


Thermal

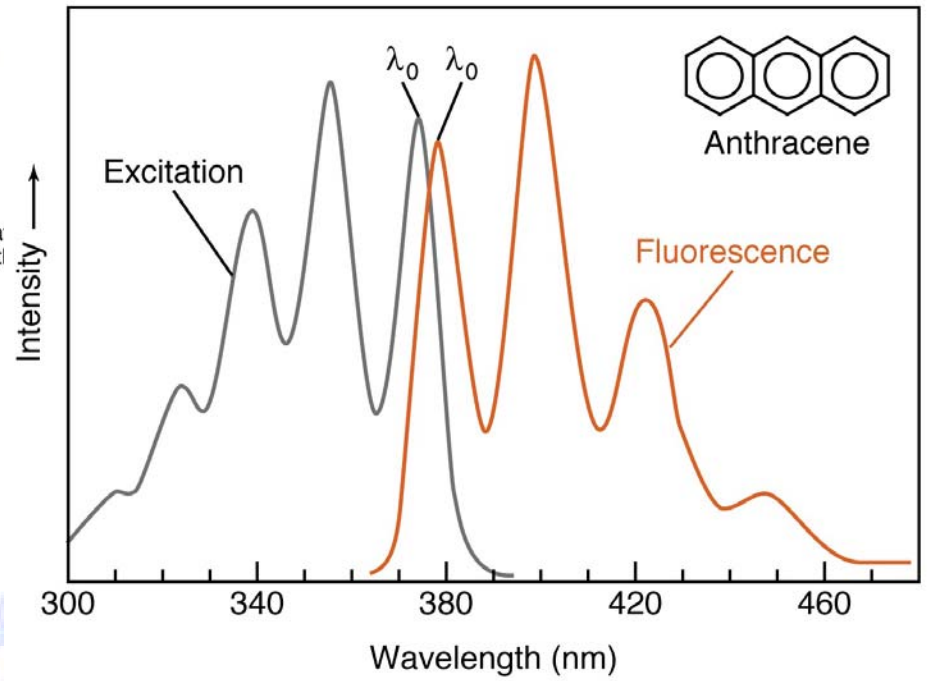
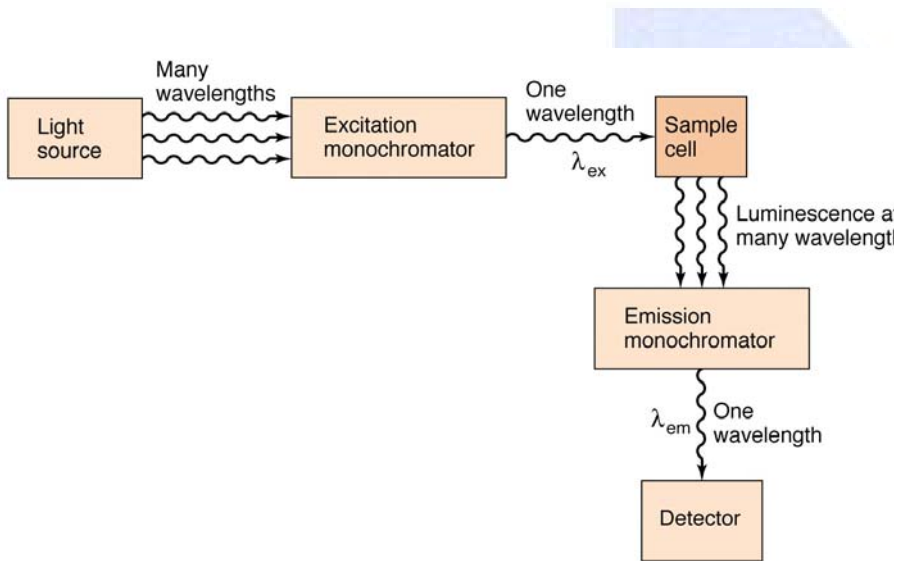


Analysis using UV-Visible

- Beer's law: $A = \sum \epsilon_i b c_i$
- Find the wavelength for unique and maximum absorption
- Generating calibration curve
- Measure the unknowns
- Calculate the concentration out of calibration curve



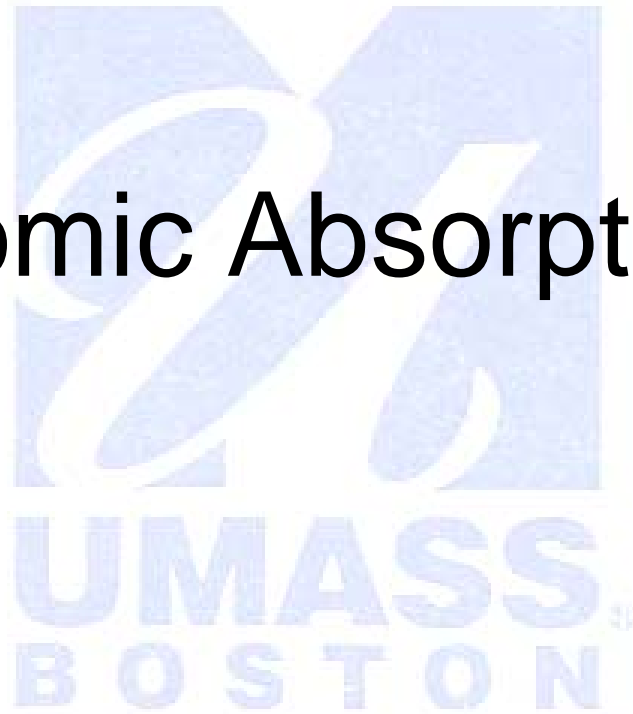
Luminescence



Luminescence

- Excitation spectrum: variable excitation wavelength and detect the strength of fix wavelength of emission light
- Emission spectrum: fix wavelength for excitation wavelength and detect the full spectrum of emission light

Atomic Absorption



Atomic Spectroscopy

- Sample is vaporized and the substance is decomposed into **atoms** in a flame, furnace, or plasma (when a gas is hot enough e.g. 6000K, it will contain ions and free electrons). The concentration and types of atoms in the vapor are measured by emission or absorption of the unique
 - Atomic absorption
 - Atomic emission
 - Atomic fluorescence

Origin of the atomic spectroscopy

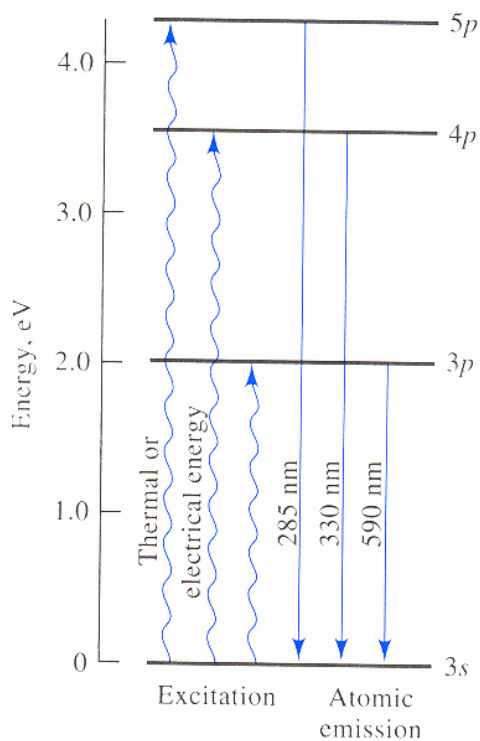
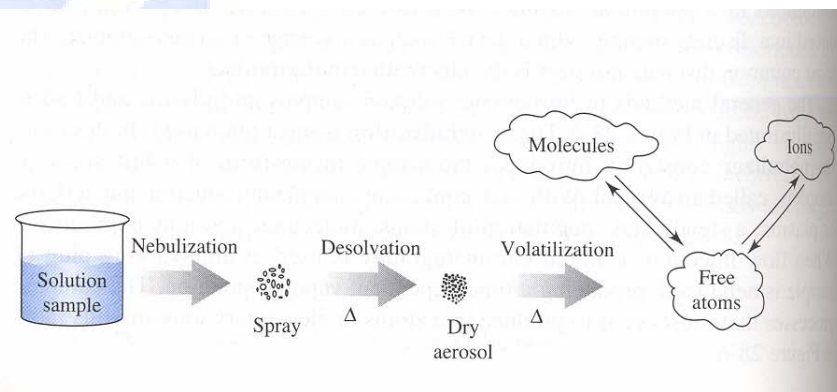


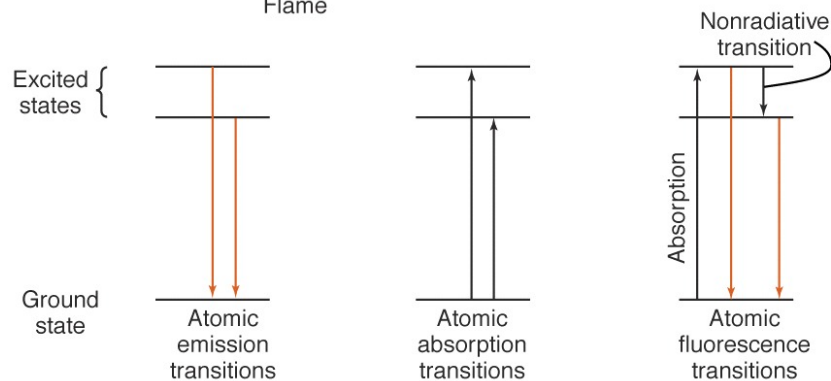
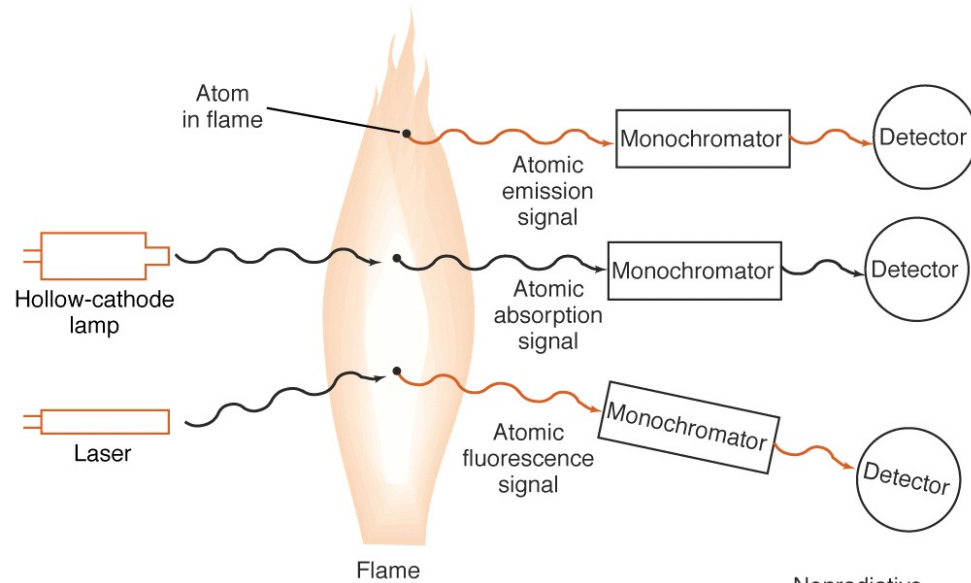
Figure 28-1 Origin of three sodium emission lines.



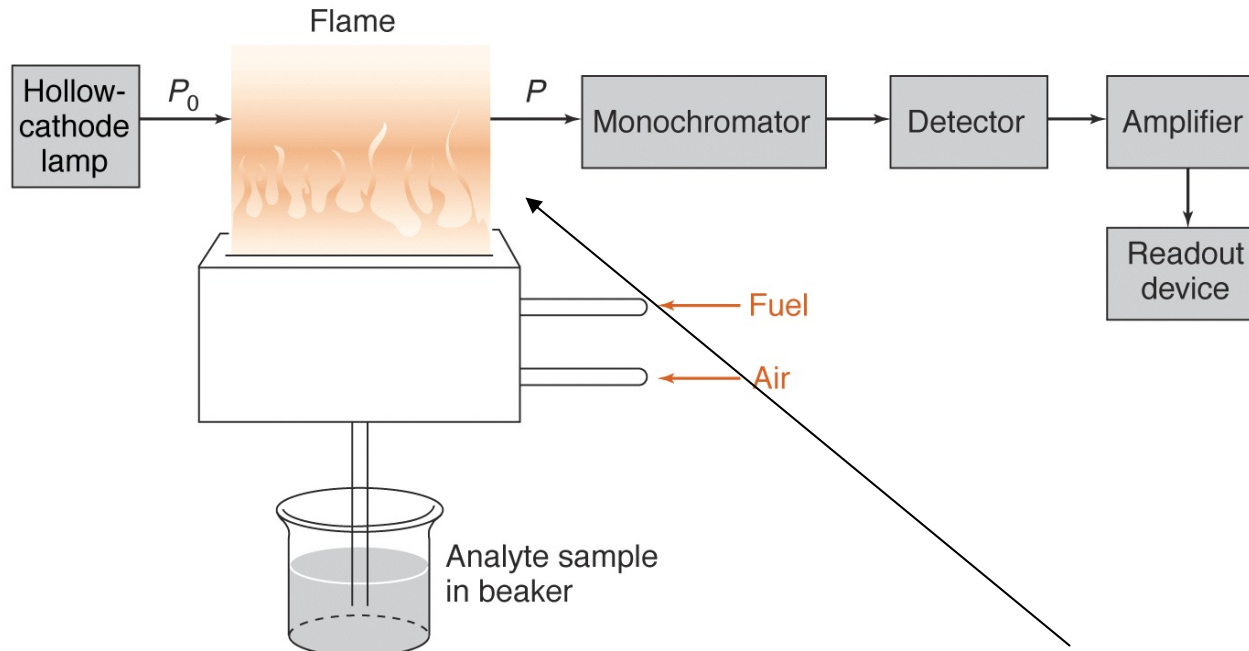
Figure 28-6 Processes leading to atoms, molecules, and ions with continuous sample introduction into a plasma or flame. The solution sample is converted into a spray by the nebulizer. The high temperature of the flame or plasma causes the solvent to evaporate, leaving dry aerosol particles. Further heating volatilizes the particles, producing atomic, molecular, and ionic species. These species are often in equilibrium, at least in localized regions.



Atomic spectroscopy



Atomic Absorption (AA)



BOSTON

Flame AA or graphite furnace

Inductively Coupled Plasma (ICP)- Atomic Emission Spectroscopy

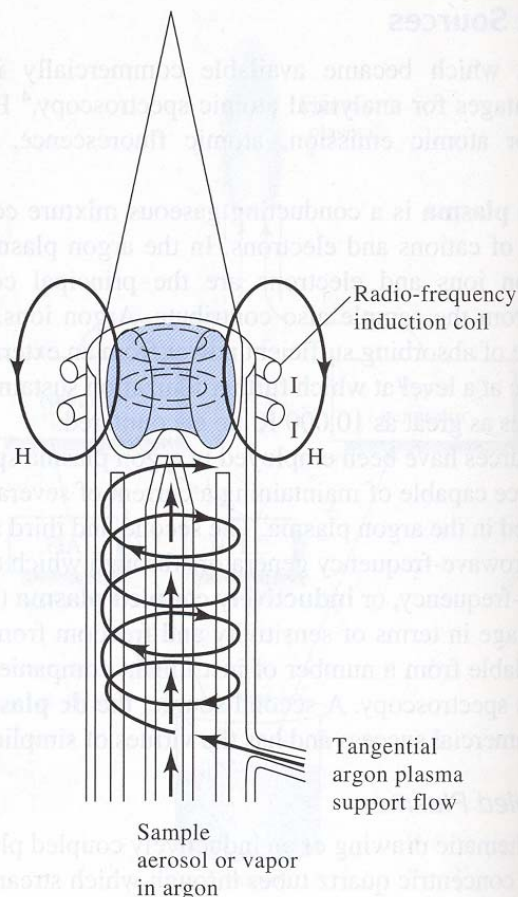
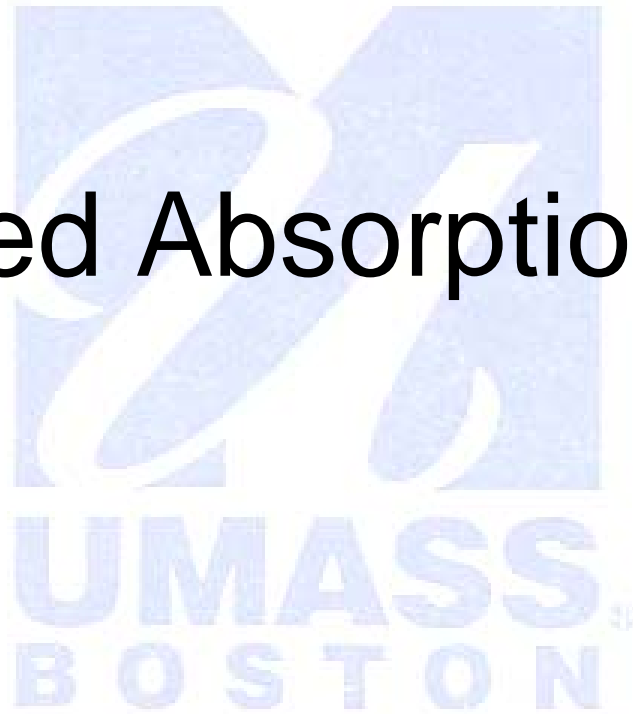
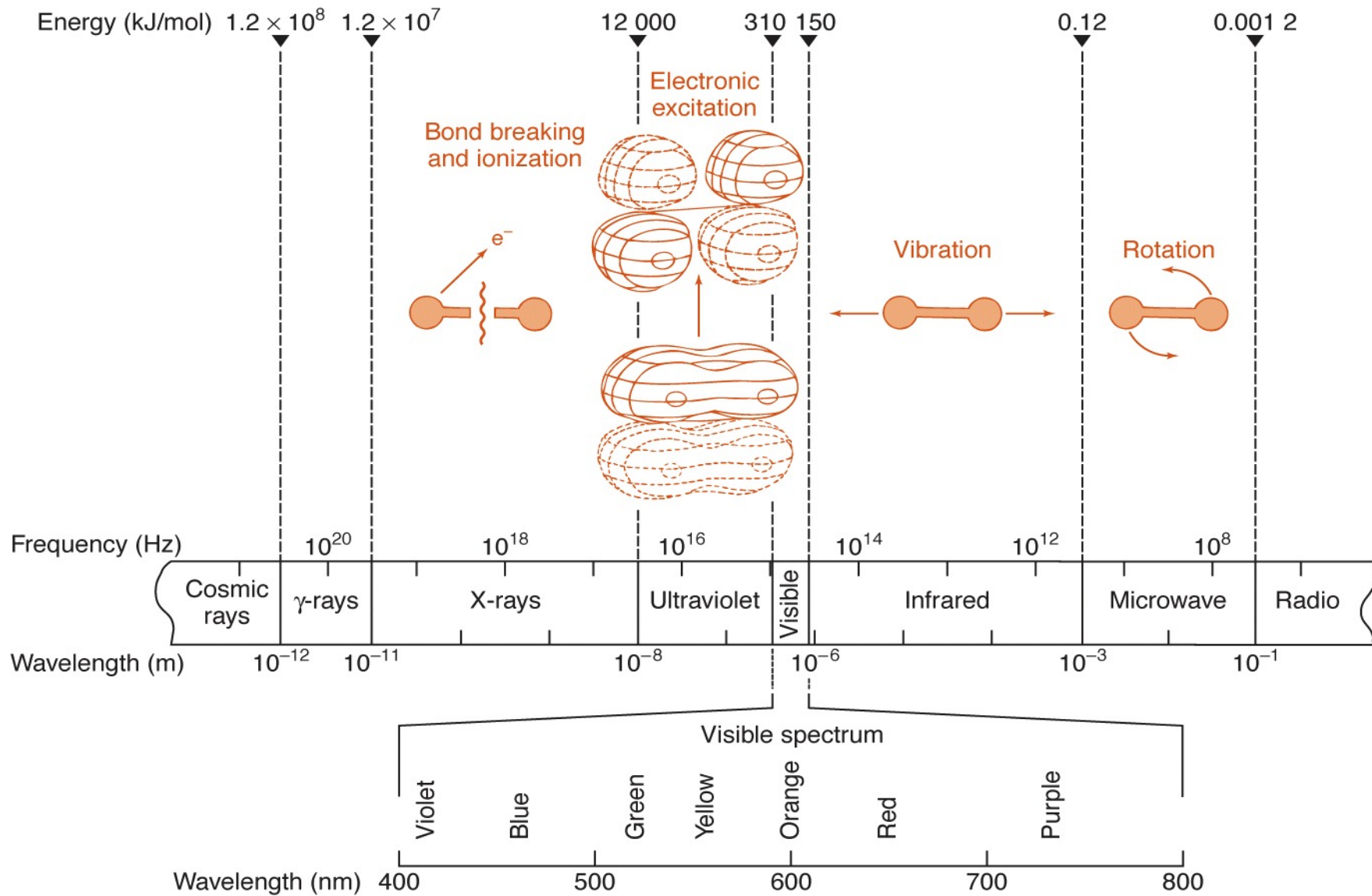


Figure 28-7 Inductively coupled plasma source.
(From V. A. Fassel, *Science*, **1978**, 202, 185.
Reprinted with permission. Copyright 1978 by the
American Association for the Advancement of
Science.)

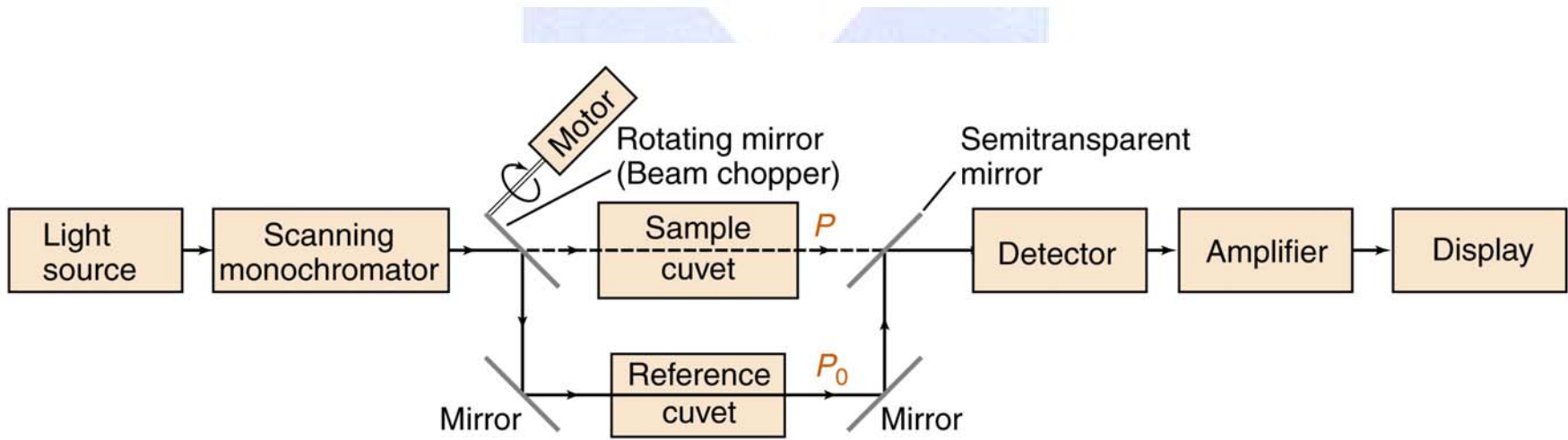
Infrared Absorption (IR)





Types of IR spectrometers

- Dispersive Infrared Instrument: double-beam layout – wavelength by wavelength with monochromator.
- Fourier Transform Instruments
 - Detect all the wavelengths all the time with interferometer which produce interference patterns that contain the infrared spectral information.



UMASS
BOSTON

Interferometer

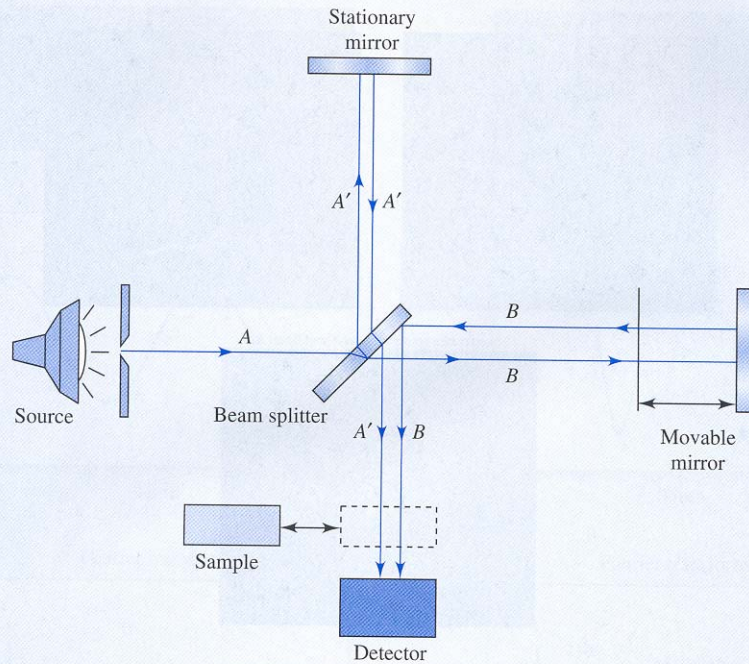


Figure 25F-6 Diagram of a Michelson interferometer. A beam from the light source at left is split into two beams by the beam splitter. The two beams travel two separate paths and converge on the detector. The two beams A' and B converge in the same region of space and form an interference pattern. As the movable mirror on the right is moved, the interference pattern shifts across the detector and modulates the optical signal. The resulting reference interferogram is recorded and used as a measure of the power of the incident beam at all wavelengths. An absorbing sample is then inserted into the beam, and a sample interferogram is recorded. The two interferograms are used to compute the absorption spectrum of the sample.

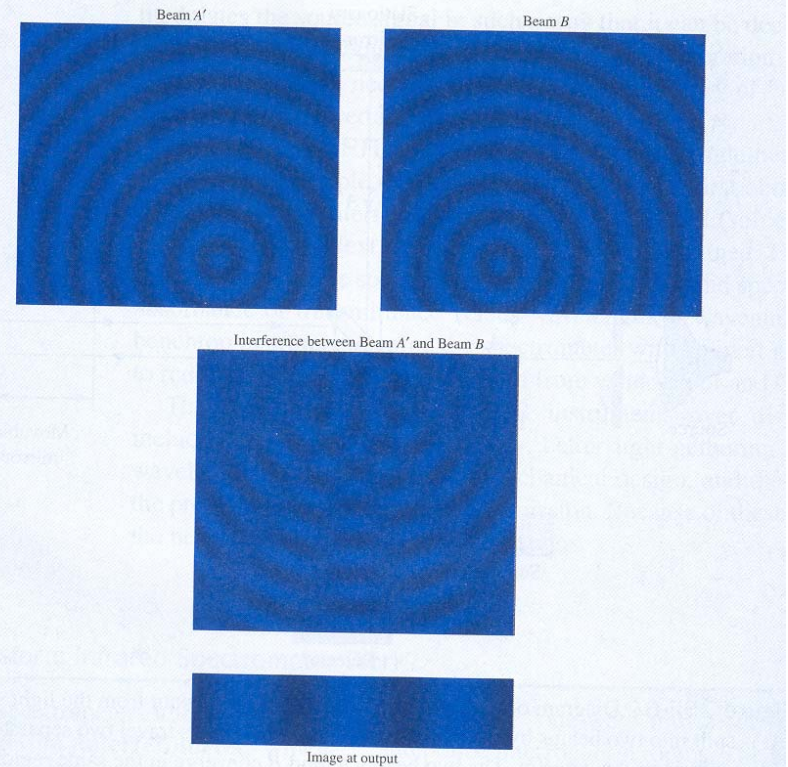


Figure 25F-7 A two-dimensional representation of the interference of two monochromatic wavefronts of the same frequency. Beam A' and beam B at the top form the interference pattern in the middle, and the two wavefronts constructively and destructively interfere. The image shown at the bottom would appear at the output of the Michelson interferometer perpendicular to the plane of the two-dimensional interference pattern.

Advantages of FTIR

- Better speed
- Sensitivity
- Light-gathering power
- Accurate wavelength calibration
- Simpler mechanical design

Infrared Absorption Spectroscopy

- Powerful tool for identifying pure organic and inorganic compounds – qualitative
 - IR can excite vibrational and rotational transitions.
- Less satisfactory for quantitative purpose

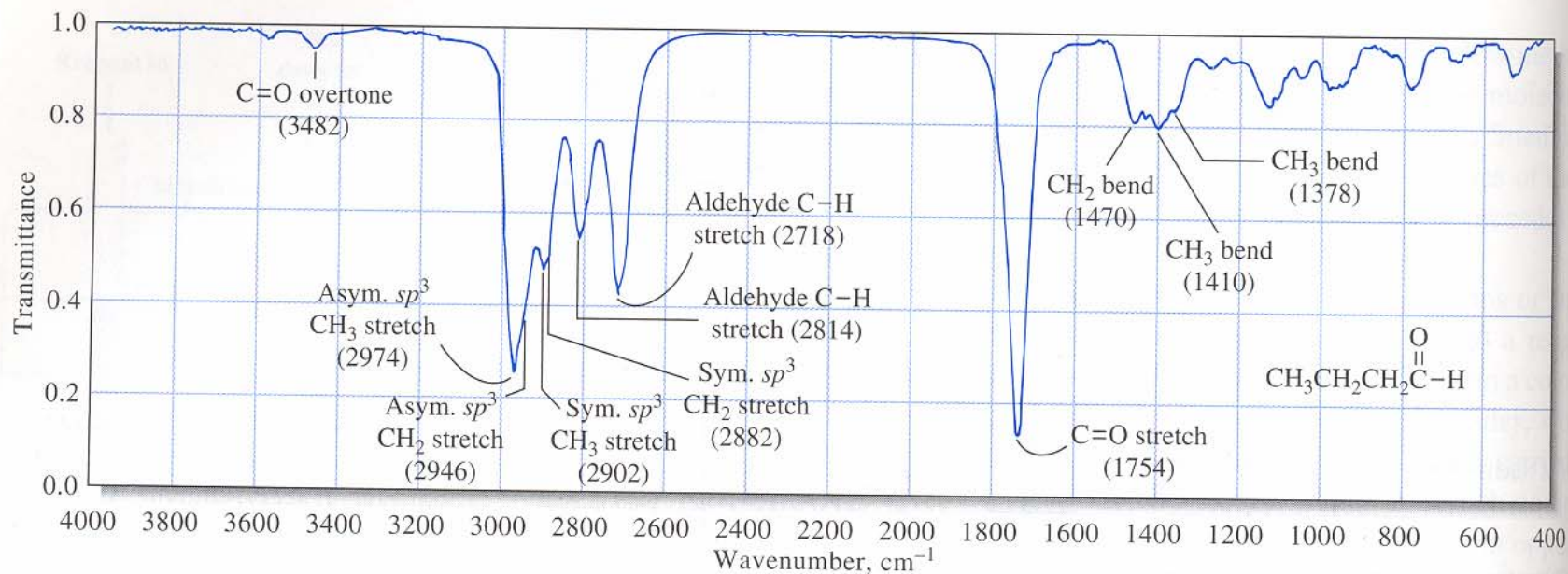


Figure 26-20 Infrared spectrum for *n*-butanal (*n*-butyraldehyde). The vertical scale is plotted as transmittance, as has been common practice in the past. The horizontal scale is linear in wavenumbers, which is proportional to frequency and thus energy. Most modern IR spectrometers are capable of providing data plotted as either transmittance or absorbance on the vertical axis and wavenumber or wavelength on the horizontal axis. IR spectra are usually plotted with frequency increasing from right to left, which is a historical artifact. Early IR spectrometers produced spectra with wavelength increasing from left to right, which led to an auxiliary frequency scale from right to left. Note that several of the bands have been labeled with assignments of the vibrations that produce the bands. (Data from NIST Mass Spec Data Center, S.E. Stein, director, "Infrared Spectra" in NIST Chemistry WebBook, NIST Standard Reference Database Number 69, P. J. Linstrom and W. G. Mallard, Eds. March 2003, National Institute of Standards and Technology, Gaithersburg, MD 20899 [<http://webbook.nist.gov>].)

Mass Spectroscopy and Separation

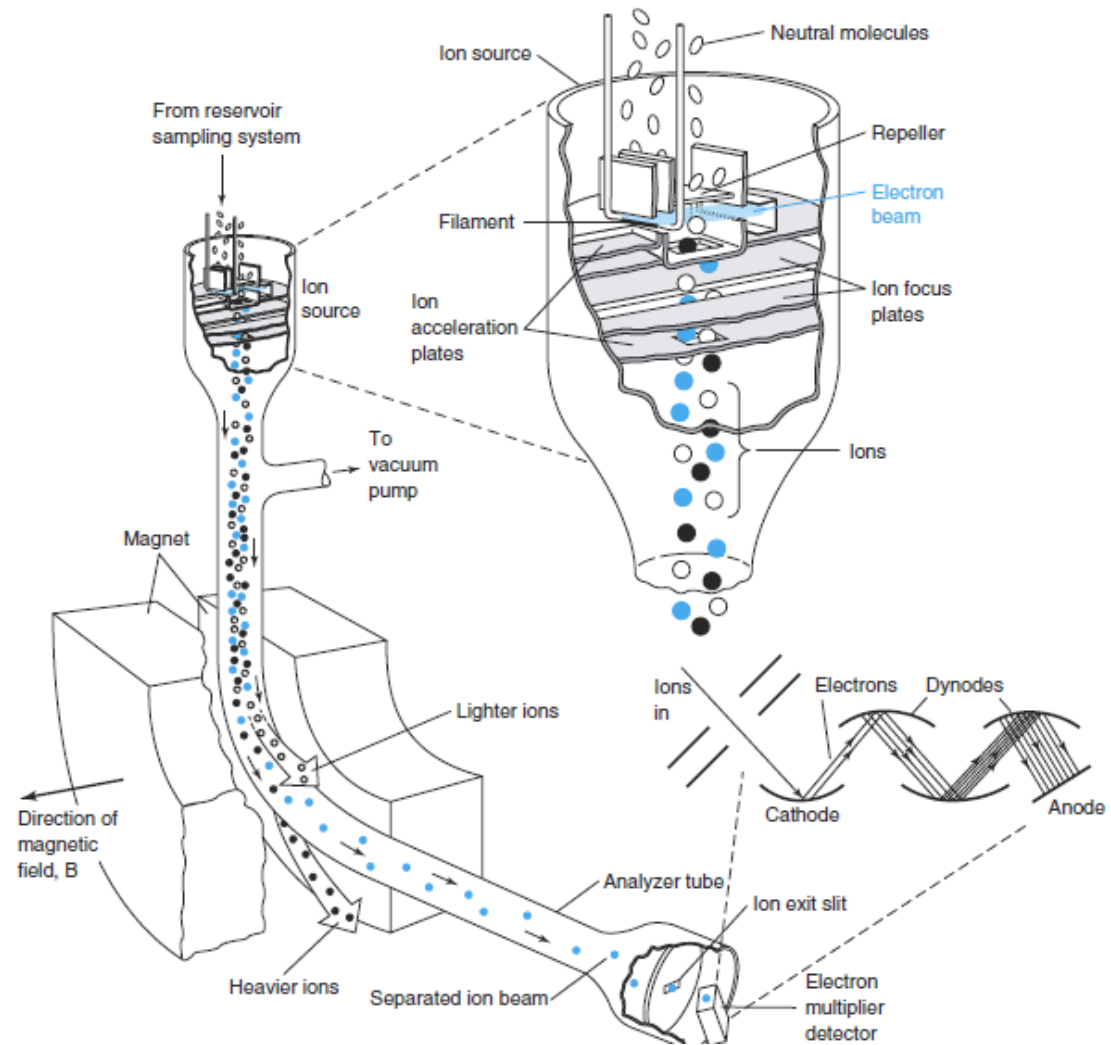


Separation of charged species

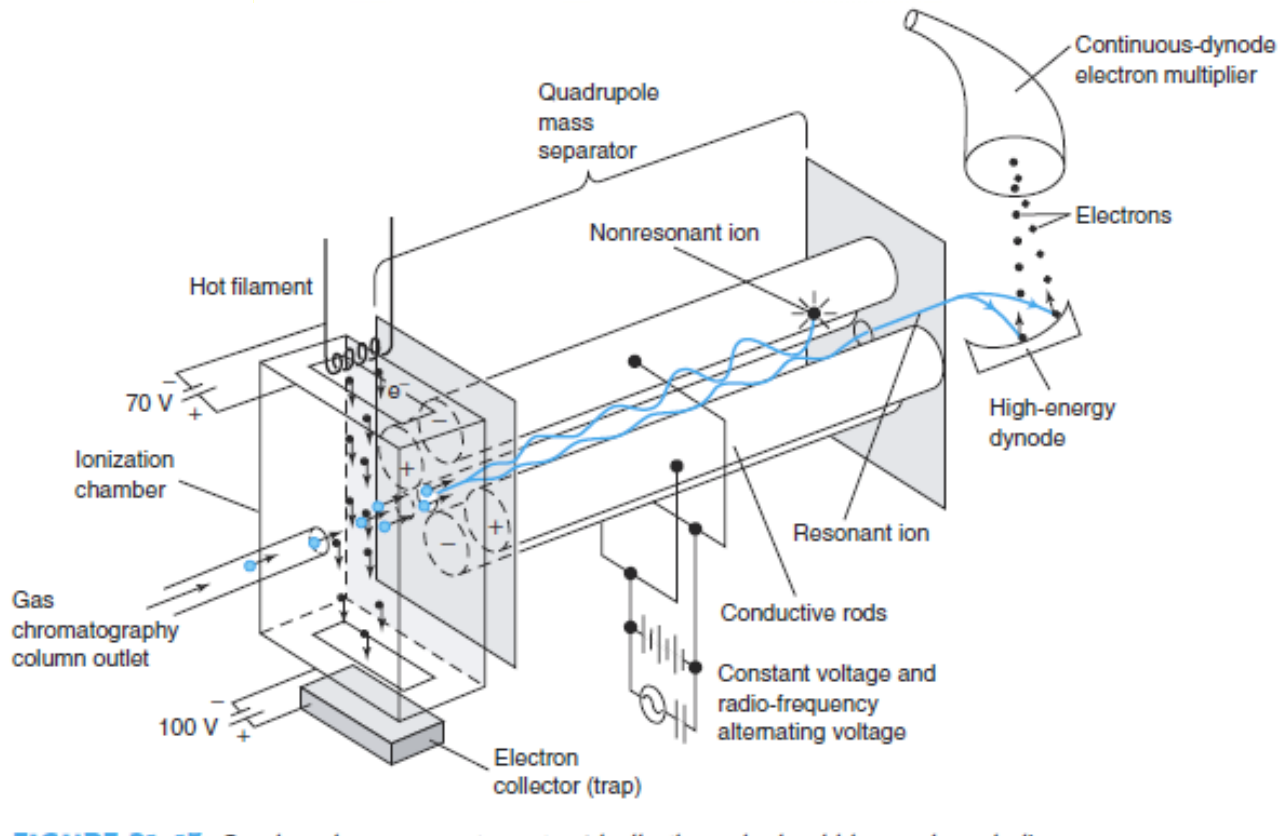
$$\frac{1}{2}mv^2 = zeE$$

$$\frac{mv^2}{r} = zevB$$

$$\frac{m}{z} = \frac{eB^2r^2}{2V}$$



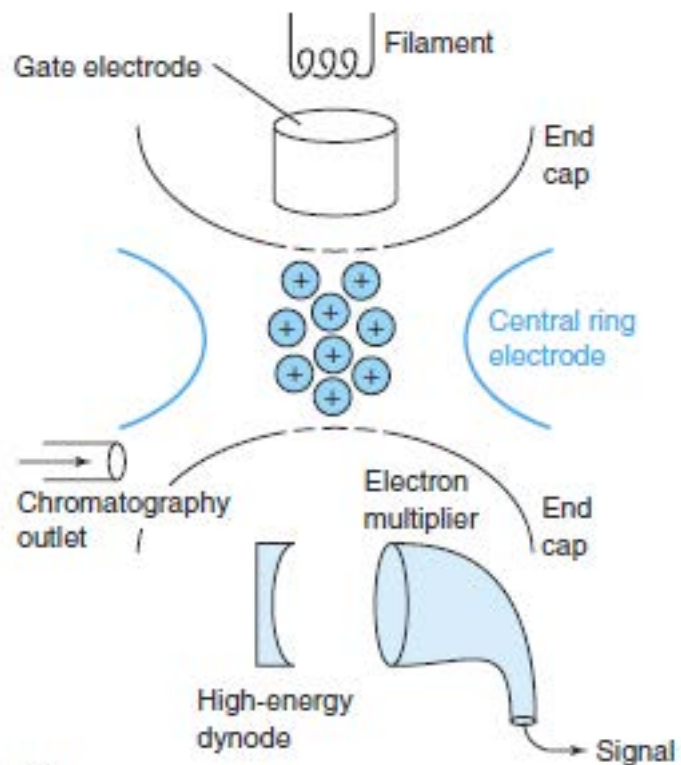
Quadrupole MS



Ion Trap MS

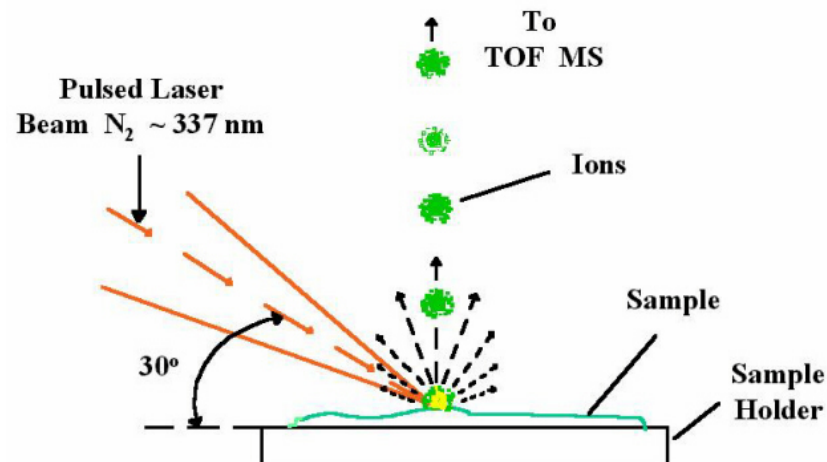
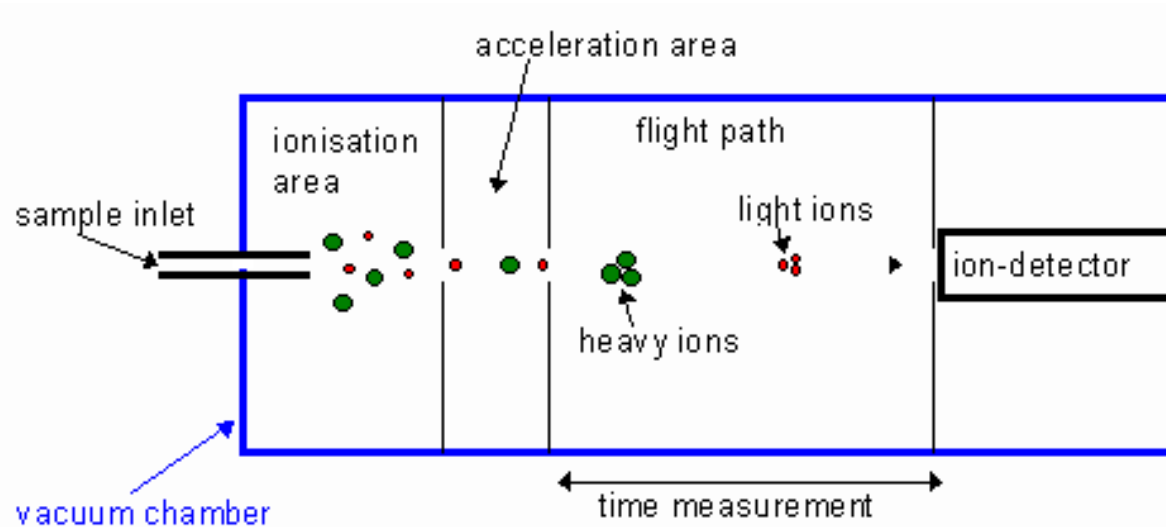


(a)

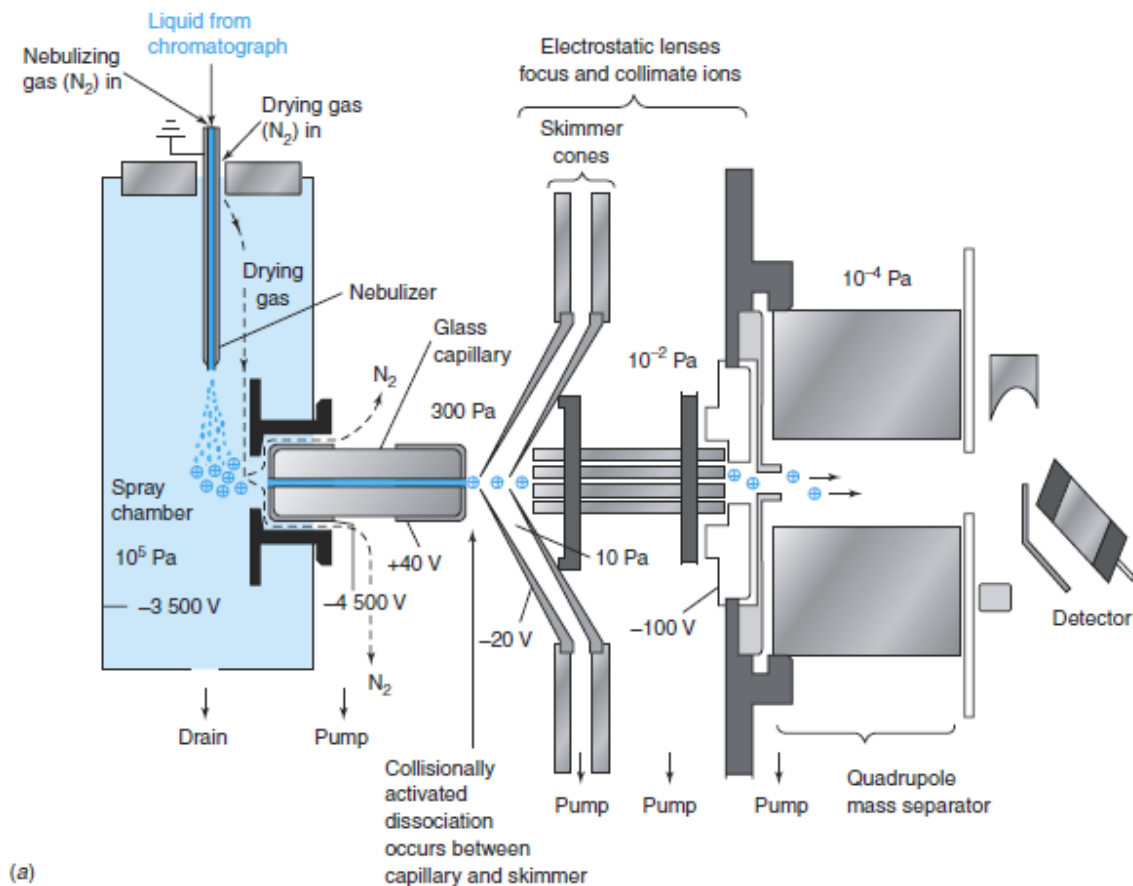


(b)

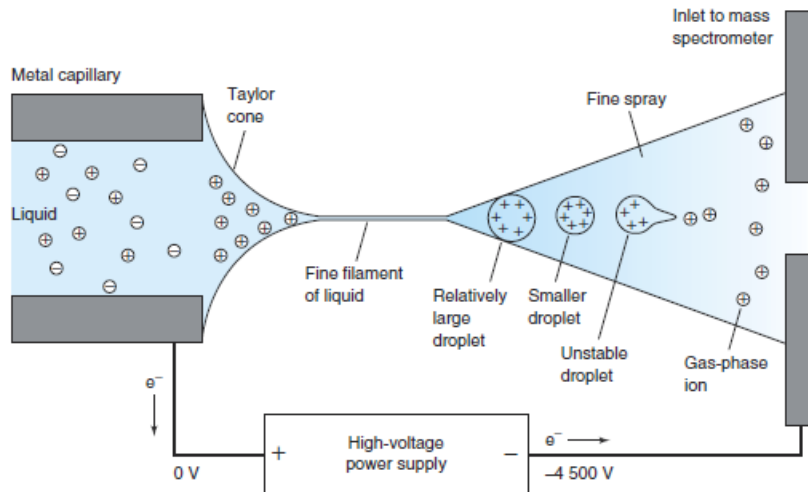
Time of Fly



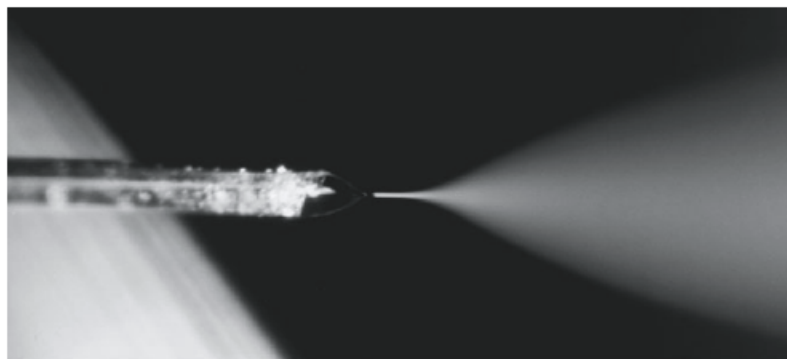
Electrospray



Electrospray

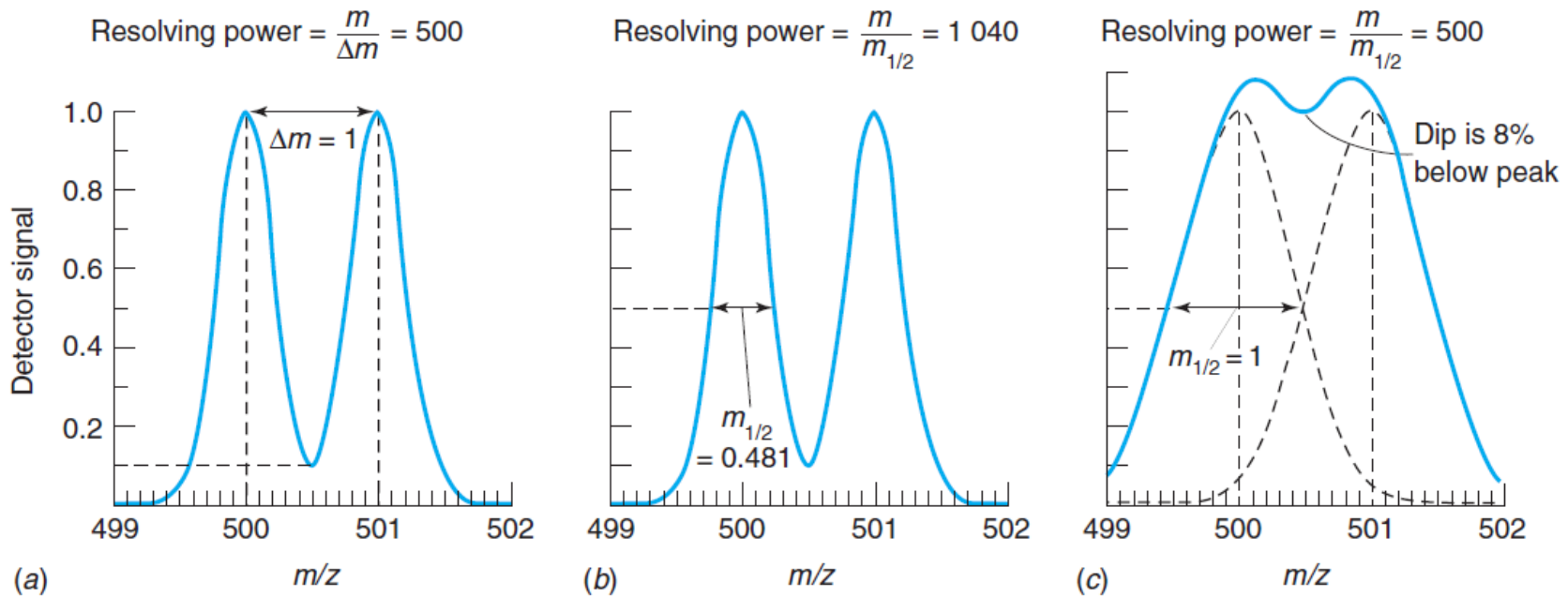


(b)



(c)

Resolving power



$$\text{Resolving power} = \frac{m}{\Delta m} \quad \text{or} \quad \frac{m}{m_{1/2}}$$

Figure 21-5a

Figure 21-5b

Chromatography

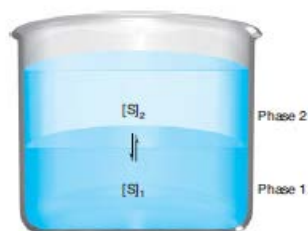


FIGURE 22-1 Partitioning of a solute between two liquid phases.

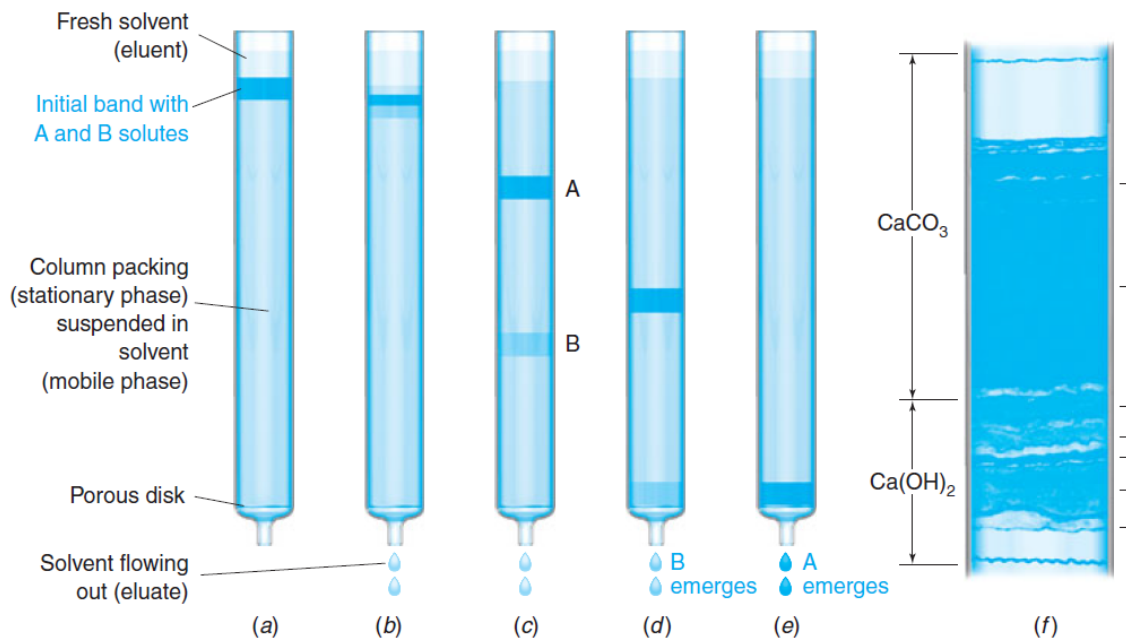
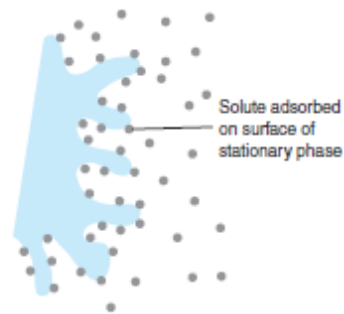
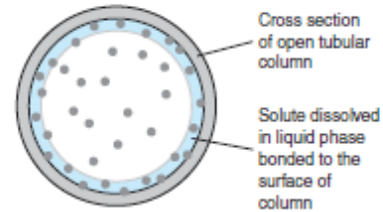


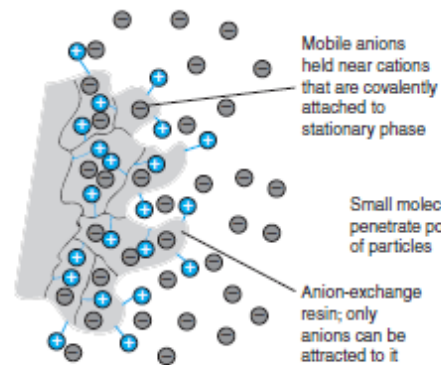
FIGURE 22-5 The idea behind chromatography: solute A, with a greater affinity than solute B for the stationary phase, remains on the column longer. Panel *f* is a reconstruction of the separation of pigments from red paprika skin from the work of L. Zechmeister in the 1930s. Bands marked by horizontal lines are different pigments. The lower stationary phase is Ca(OH)_2 and the upper stationary phase is CaCO_3 . [Panel *f* from L. S. Ettre, "The Rebirth of Chromatography 75 Years Ago," *LCGC* 2007, 25, 640.]



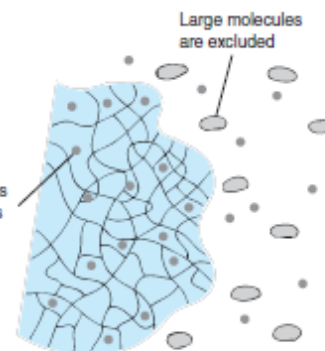
Adsorption chromatography



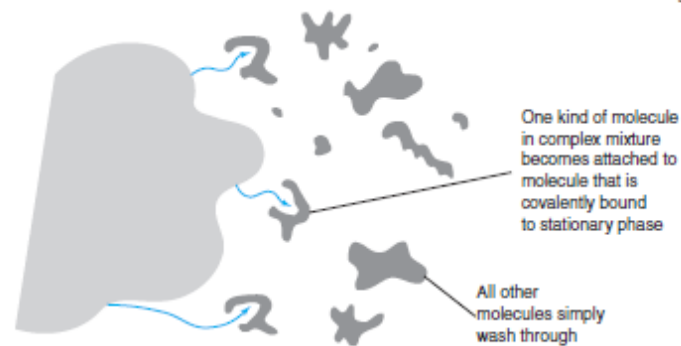
Partition chromatography



Ion-exchange chromatography

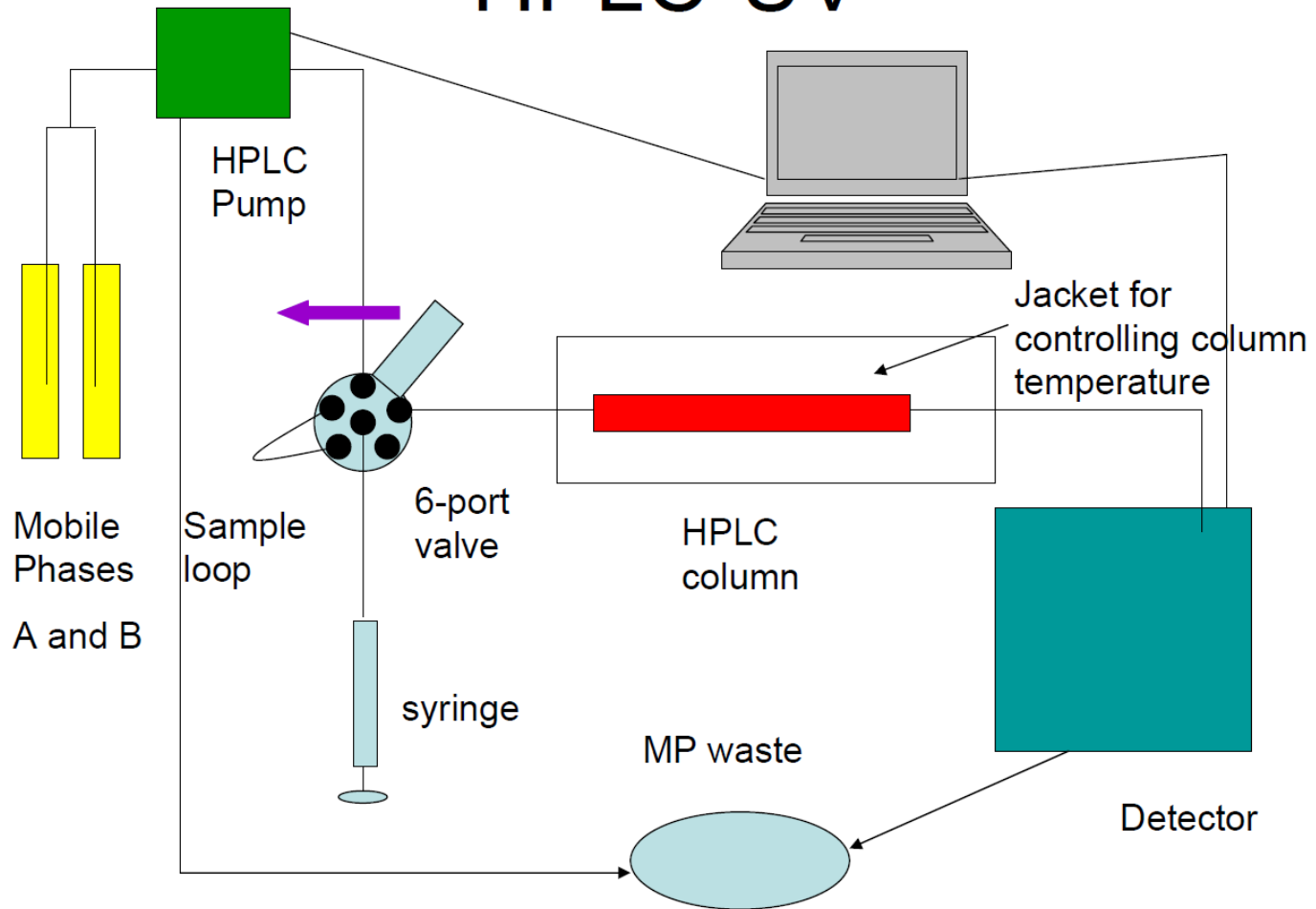


Molecular exclusion chromatography



Affinity chromatography

HPLC-UV



RP-HPLC Separation of a Tryptic Digest of BSA

