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Print version

CEE 772: Instrumental Methods in Environmental Analysis

Lecture #4

Spectroscopy: Absorbance and Structure

(Skoog, Chapt. 14)

(pp. 329-345)

(Harris, Chapt. 19)
(pp.510-519, 523-530)

David Reckhow

Recap L#3 (Aarthi's addendum)

- Error

	Systematic Errors	Random Errors
What?	Fluctuations around true value	
Nature	Predictable (consistently high of consistently low)	Unpredictable
Causes	Improper calibration of instrument (Instrumental, method, personal errors)	Difficulty taking measurements (hard to pin-point is most cases)
Correction?	Possible with calibrations	Can't be corrected easily. However, statistics on errors may be helpful.

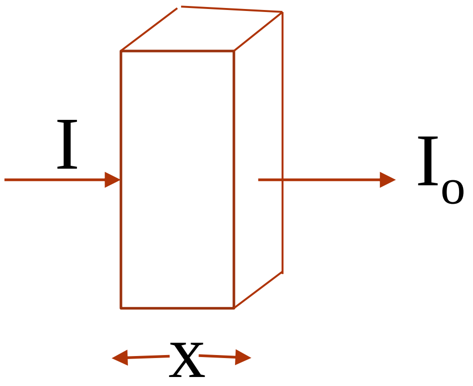
Recap L#3 (Aarthi's addendum)

- Uncertainty & precision
- Detection limits

Sensitivity	Smallest measurement that can be detected on an instrument (related to detection limit)
Selectivity	Ability of an instrument/method to only detect the target analyte in the presence of several other similar analytes.
Resolution	Smallest change in a measurable variable to which the instrument will respond (closeness to true value; better resolution if closer to true value)

Recap L#3 (Aarthi's addendum)

-Beer-Lamberts Law



$A = -\log_{10}(T) = -\log_{10}(I/Io)$

$T = e^{-A}$

$A = acx = €cx$

C= concentration (mg/L or M)

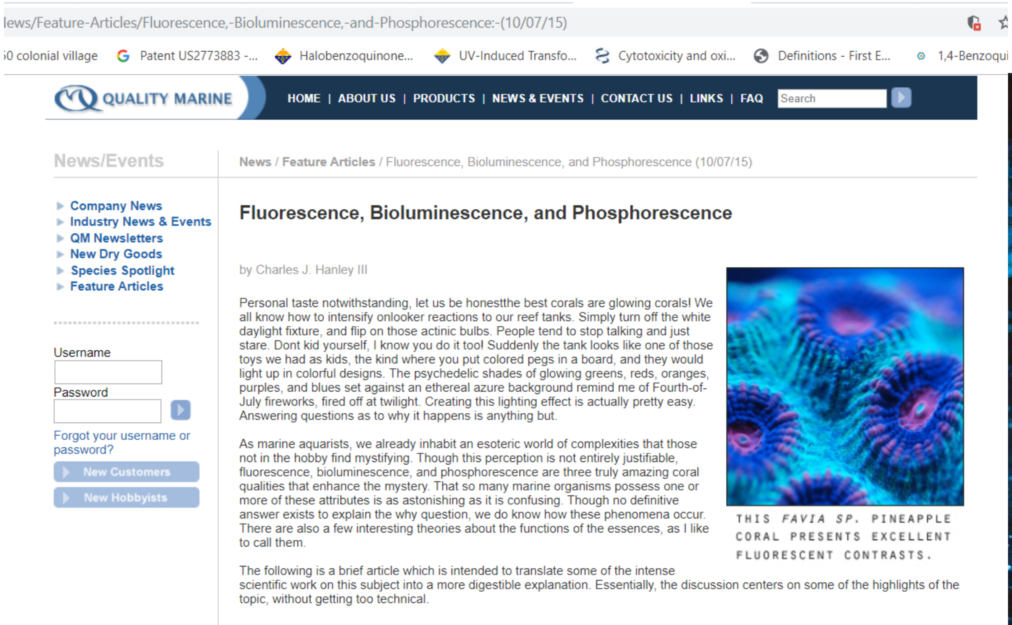
X= path length (cm)

a and € are both absorptivity coefficients when C is expressed as mg/L or M respectively; € most commonly referred to as molar absorptivity coefficient

Recap L#3 (Aarthi's addendum)

	Fluorescence	**Phosphorescence**
What?	Molecular Luminescence methods	
Electron spin	does not change in electron spin, which results in	there is a change in electron spin
Excited state duration	short-live electrons (<10^{-5} s) in the excited state of fluorescence	a longer lifetime of the excited state (second to minutes).
Wavelengths	Both occur at wavelengths longer than excited radiation	
Examples	Fluorescent lights and neon signs, highlighter pens	Glow in the dark stars, paint used to make star murals.

Recap L#3 (Aarthi's addendum)



News / Feature Articles / Fluorescence, Bioluminescence, and Phosphorescence (10/07/15)

Fluorescence, Bioluminescence, and Phosphorescence

by Charles J. Hanley III

Personal taste notwithstanding, let us be honestthe best corals are glowing corals! We all know how to intensify onlooker reactions to our reef tanks. Simply turn off the white daylight fixture, and flip on those actinic bulbs. People tend to stop talking and just stare. Dont kid yourself, I know you do it too! Suddenly the tank looks like one of those toys we had as kids, the kind where you put colored pegs in a board, and they would light up in colorful designs. The psychedelic shades of glowing greens, reds, oranges, purples, and blues set against an ethereal azure background remind me of Fourth-of-July fireworks, fired off at twilight. Creating this lighting effect is actually pretty easy. Answering questions as to why it happens is anything but.

As marine aquarists, we already inhabit an esoteric world of complexities that those not in the hobby find mystifying. Though this perception is not entirely justifiable, fluorescence, bioluminescence, and phosphorescence are three truly amazing coral qualities that enhance the mystery. That so many marine organisms possess one or more of these attributes is as astonishing as it is confusing. Though no definitive answer exists to explain the why question, we do know how these phenomena occur. There are also a few interesting theories about the functions of the essences, as I like to call them.

THIS *FAVIA SP.* PINEAPPLE CORAL PRESENTS EXCELLENT FLUORESCENT CONTRASTS.

The following is a brief article which is intended to translate some of the intense scientific work on this subject into a more digestible explanation. Essentially, the discussion centers on some of the highlights of the topic, without getting too technical.

Vieques, Puerto Rico (Bioluminescence Bay) (Bioluminiscence and phosphorescence are not the same!!!)

Let’s get clear on some interchangeably used terms here (Aarthi’s addendum)

Spectroscopy is the study of radiated energy and matter to determine their interaction, and it does not create results on its own.

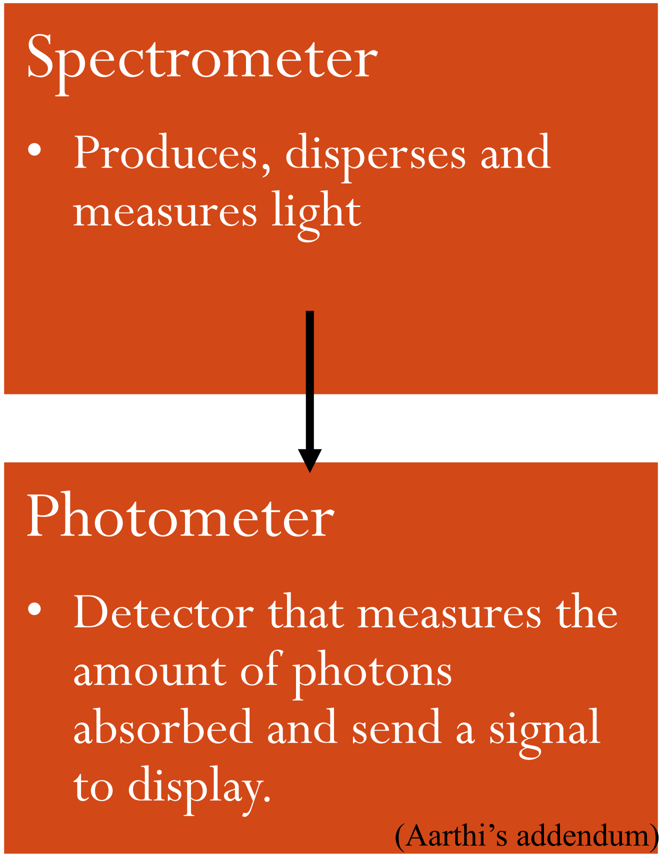
Spectrometry is the application of spectroscopy so that there are quantifiable results that can then be assessed.

NIST definition of **Spectrophotometry**

" *the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. While relatively simple in concept, determining the reflectance or transmittance involves careful consideration of the geometrical and spectral conditions of the measurement*."

Spectrophotometry

- “Procedure that uses light to measure chemical concentration”- Dr. Dave Reckhow



Spectrometer

- Produces, disperses and measures light

Photometer

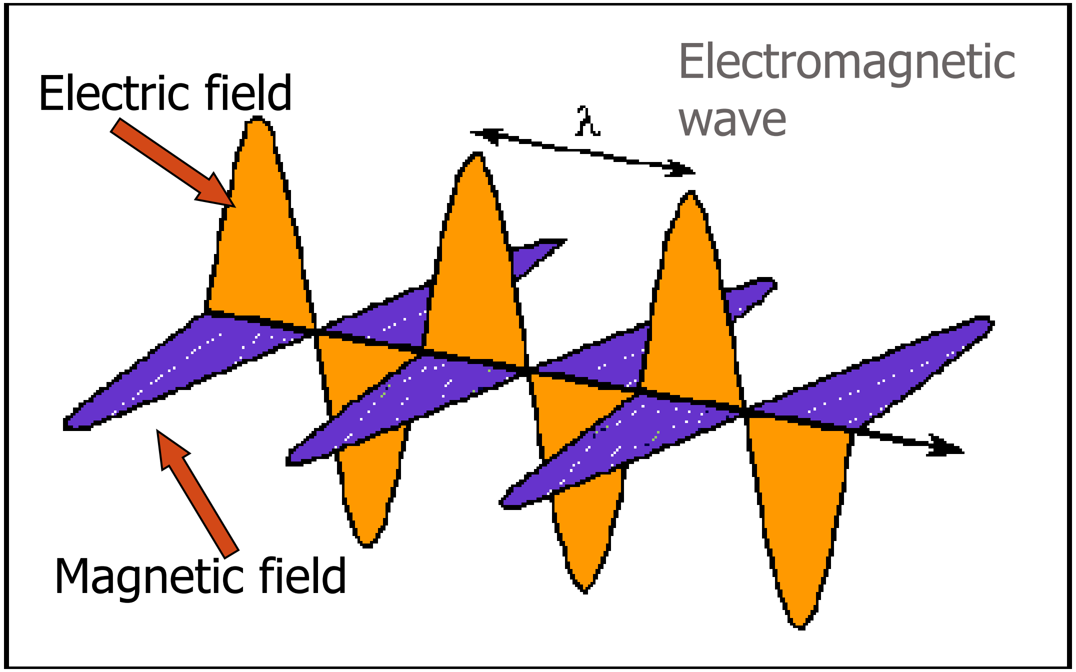
- Detector that measures the amount of photons absorbed and send a signal to display.

(Aarthi’s addendum)

Spectrophotometry

- "Procedure that uses light to measure chemical concentration"- Dr. Dave Reckhow
  - Properties of Light
  - Interaction of light with matter
  - Atom & Light Energy

Properties of Light



λ (wave length): crest to crest distance between waves

ν(frequency, s^{-1}): number of complete oscillations that the wave makes each second

1oscillation/second= Hertz (Hz)

$$\boxed{\nu*\lambda=c}$$

c (speed of light): 2.998 X 10^8 m/s

$$\boxed{E=h*\nu}$$

E=Energy carried by each photon

h=Planck's constant ($6.63*10^{-34}$ J.s)

ν=Frequency (s^{-1})

What happen when light strikes a sample?

- Some light is transmitted through the sample
- Some light is absorbed by the material
- Some light is reflected at each surface
- Some light is scattered to the side

Beer-Lambert's Law

$$\boxed{A=\varepsilon.l.c}$$

A= Absorbance of radiation

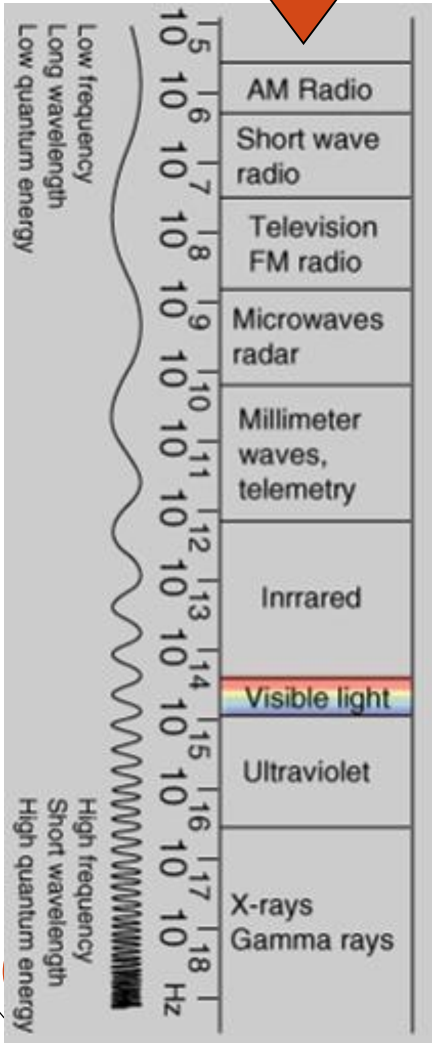
ε= Molar extinction coefficient or molar absorptivity ($M^{-1}.cm^{-1}$)

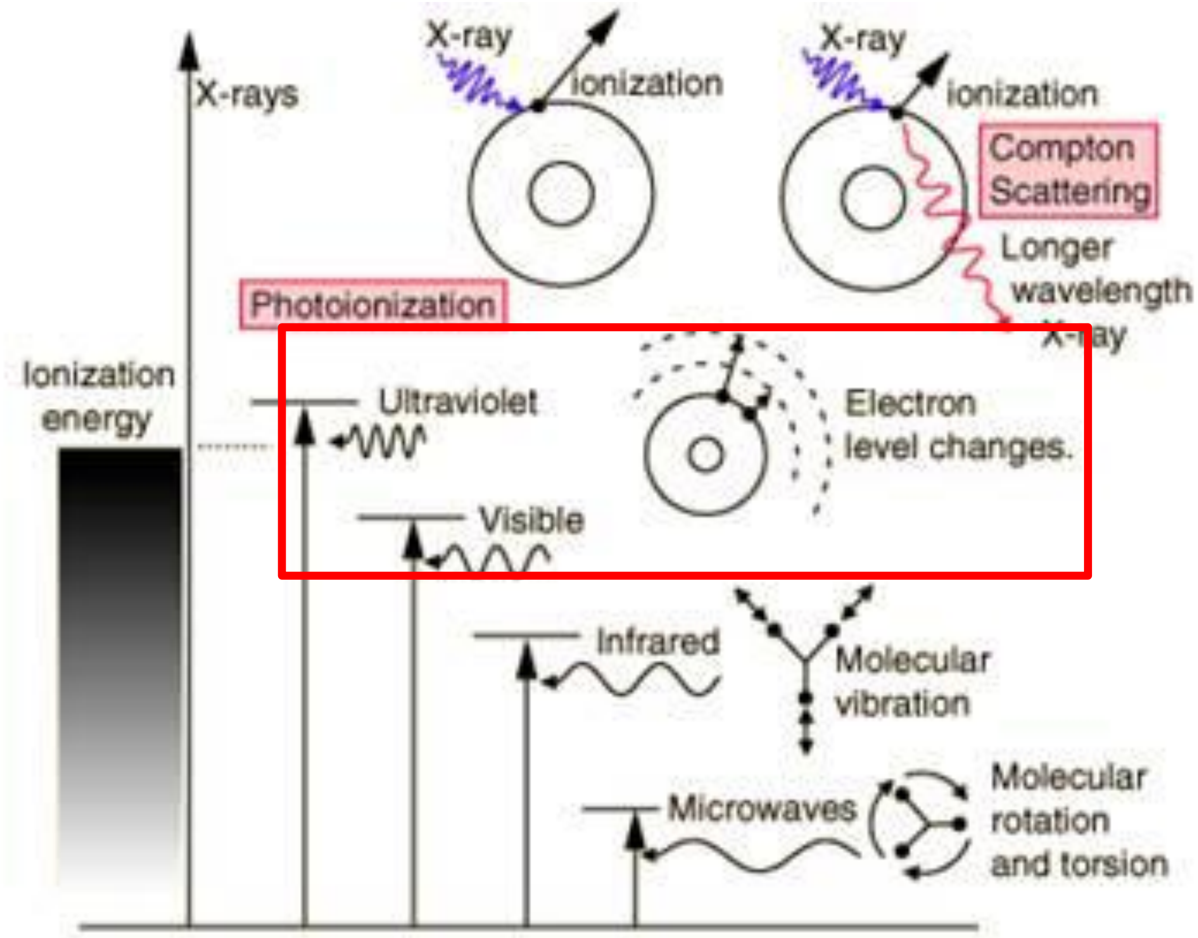
l=path length (cm)

C=concentration (M)

Electromagnetic spectrum

Interaction of radiation with matter





Review-
Atom & Light Energy

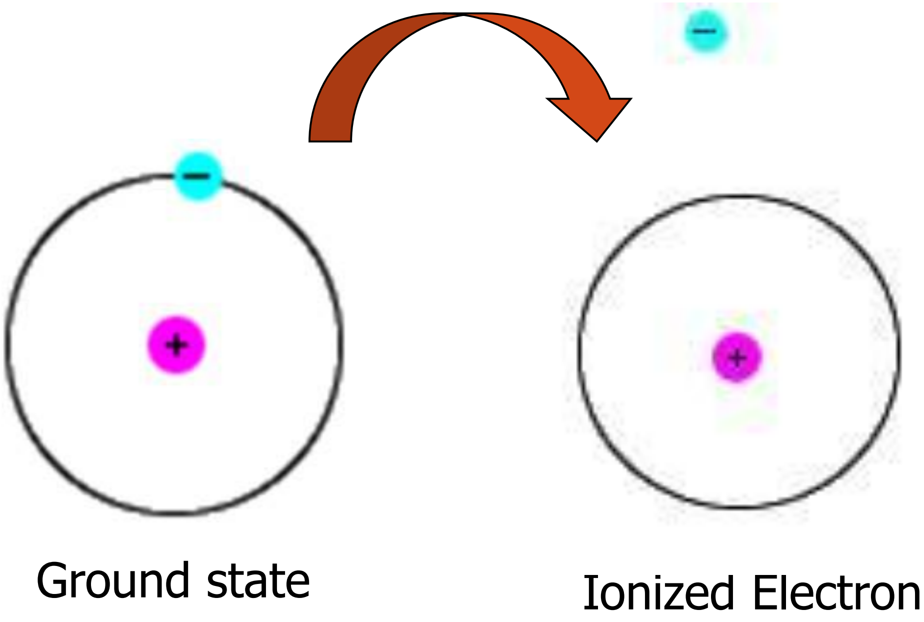
Atom: Building block of a matter (protons (+), neutrons, electrons (-))

Protons+neutrons= nuclei

Neutral atom: protons=electrons

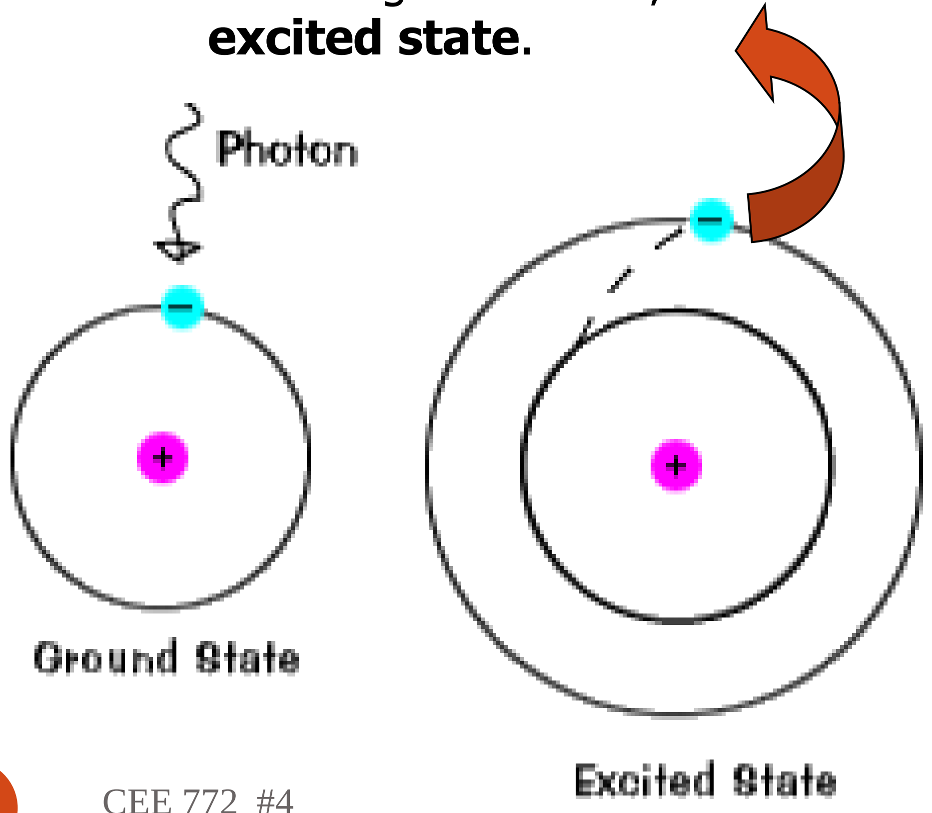
Ground State of an electron is the state of lowest energy for that electron.

Ionized electron formed as a result of loss or gain of electron

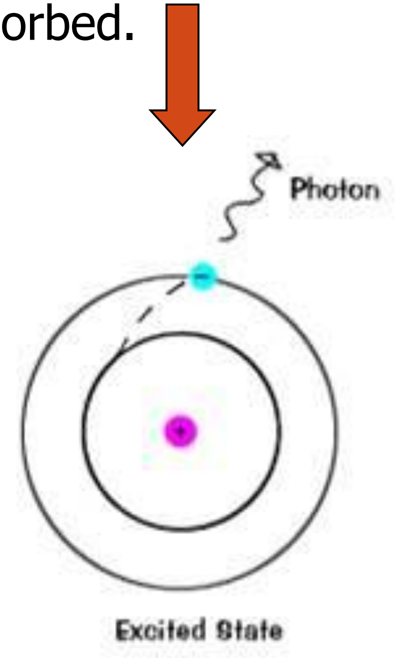


Review-
Atom & Light Energy

When an electron temporarily occupies an energy state greater than its ground state, it is in an **excited state**.



Electrons do not stay in excited states for very long - they soon return to their ground states, emitting a photon with the same energy as the one that was absorbed.



Electronic transitions

- Types of photon-absorbing electrons in organic molecule
  - Electrons that participate directly in bond formation between atoms
  - Non-bonding or unshared electrons that are localized about such atoms as oxygen, the halogens, sulfur, and nitrogen

- Types of transitions
  - $\sigma \rightarrow \sigma^*$
  - $\pi \rightarrow \pi^*$
  - $n \rightarrow \sigma^*$
  - $n \rightarrow \pi^*$

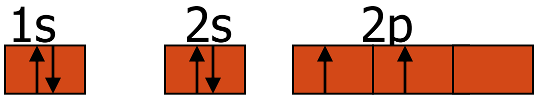
Bonding (π , σ)(stabilize, low energy) & anti bonding orbitals (π* , σ*)((higher energy)

Review- Quantum numbers

- Principal quantum number (n)
  - Defines the size and the energy of an orbital (n=1, 2, 3, etc)
    - n=1 (ground state)
    - n>1 (excited state)
- Angular quantum number (l)
  - Defines the shape of the orbital (l=0 to n-1)
    - l=0 (s), l=1 (p), l=2 (d), l=3 (f), l=4 (g)
- Magnetic quantum number (m)
  - Defines the orientation of the orbital (m=-1 to +1)
- Spin magnetic quantum number (m_s)
  - Defines the direction of an electron (m_s=-1/2 or +1/2)
    - +1/2 for spin up
    - -1/2 for spin down

Review-Electron configuration

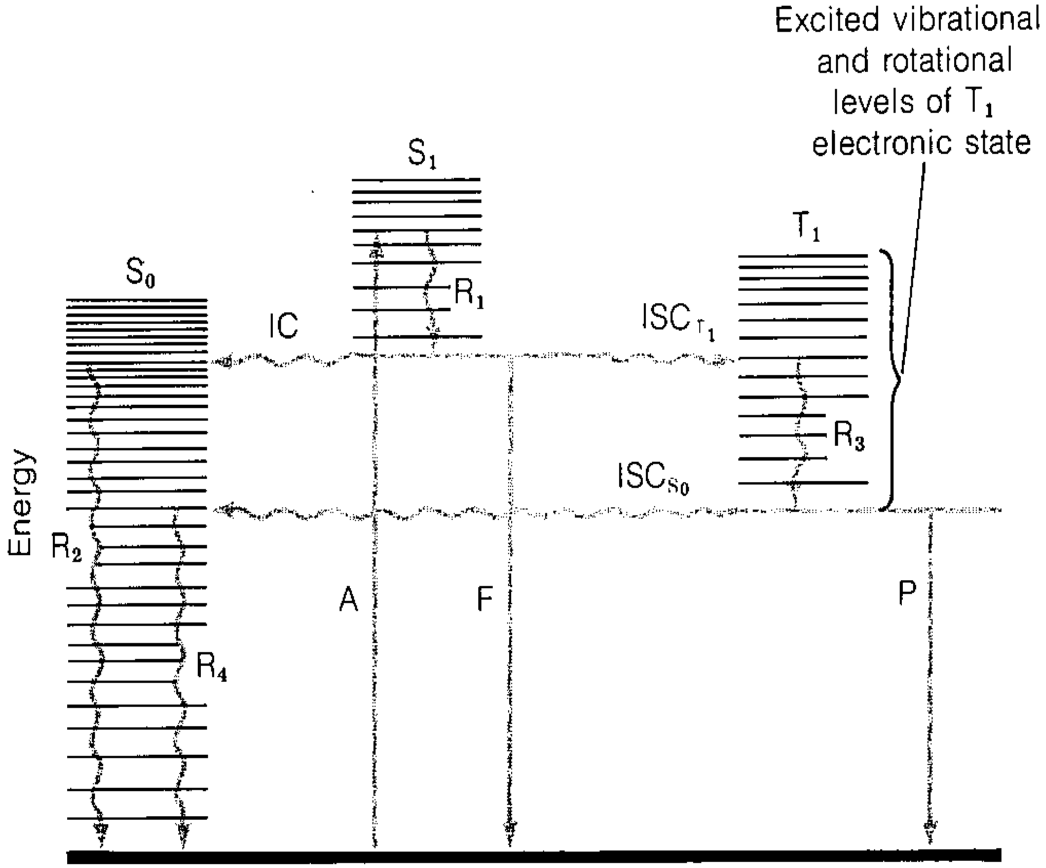
- Orbital with the lowest energy is filled first (1s orbital), orbital in the second shell (n=2) is filled next and so on…
  - $^{6}C\ 1s^2\ 2s^2\ 2p^2$
    - 1st shell has 1 orbital (1s)
    - 2nd shell has 4 orbital (1s and 3p)



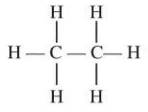
- Molecular Orbital (interaction between atomic orbitals creates a bonding and antibonding molecular orbitals)
  - H_2
  - O_2

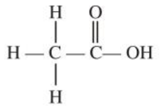
Energy Absorption & Bonding

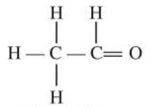
- A=absorbance
- F=fluorescence
- P=phosphorescence
- IC=internal conversion
- ISC=intersystem crossing
- R=vibrational relaxation

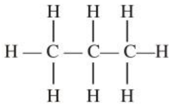


Electronic Molecular Energy Levels


ethane

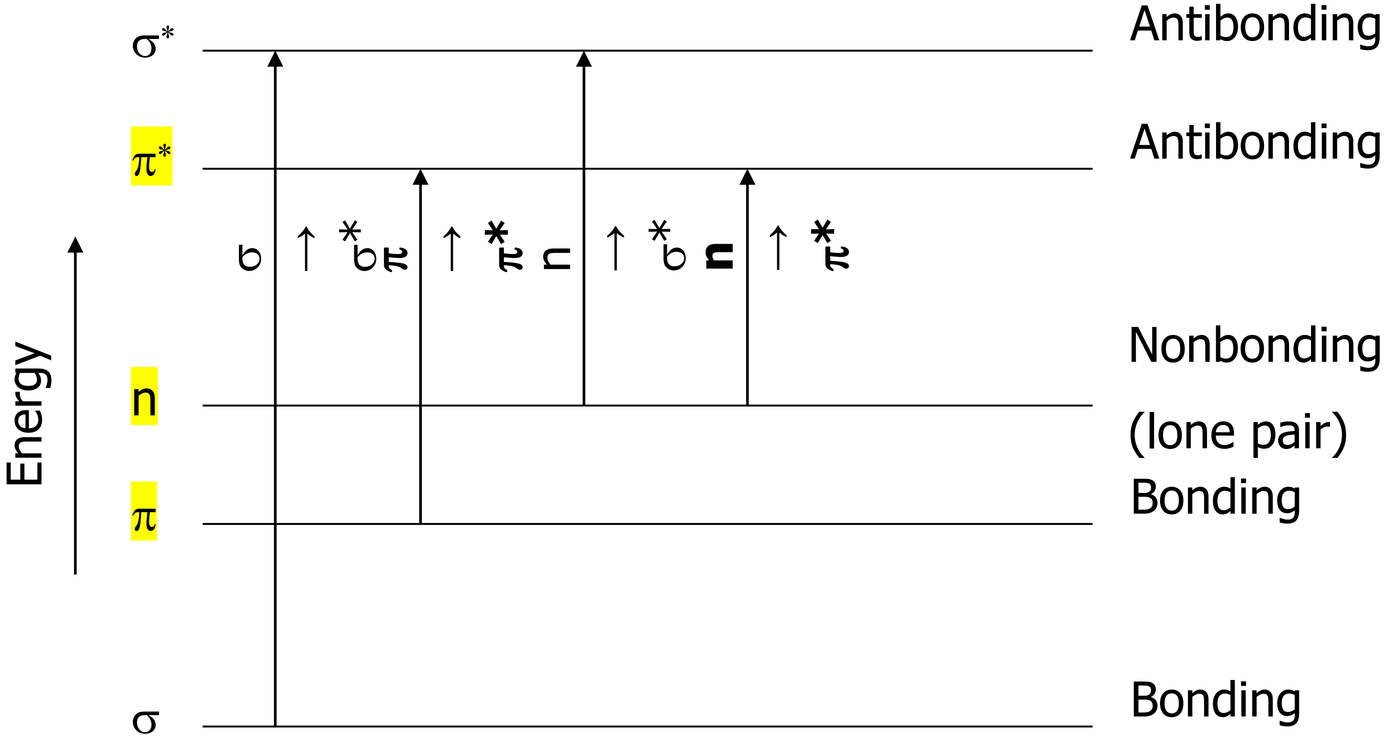

CH_3COOH
Acetic Acid


CH_3CHO
Acetaldehyde


$CH_3—CH_2—CH_3$
Propane


Ethylene


acetylene



The most applications of absorption spectroscopy to organic compounds are based upon transitions for n or π electrons to the π* excited state. Both transitions requires the presence of unsaturated functional group to provide the π orbitals.

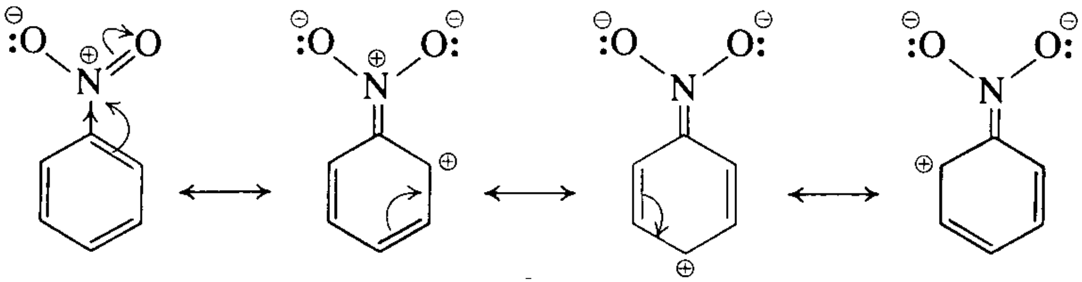
Difference between two types of transitions: $\pi \longrightarrow \pi^*$ & n $\longrightarrow \pi^*$

- Molar absorptivity for peak associated with n $\rightarrow \pi^*$ transition are low (10 to 100 $M^{-1}.cm^{-1}$). For $\pi \rightarrow \pi^*$ transition, ε range from 1000 to 10000 $M^{-1}.cm^{-1}$

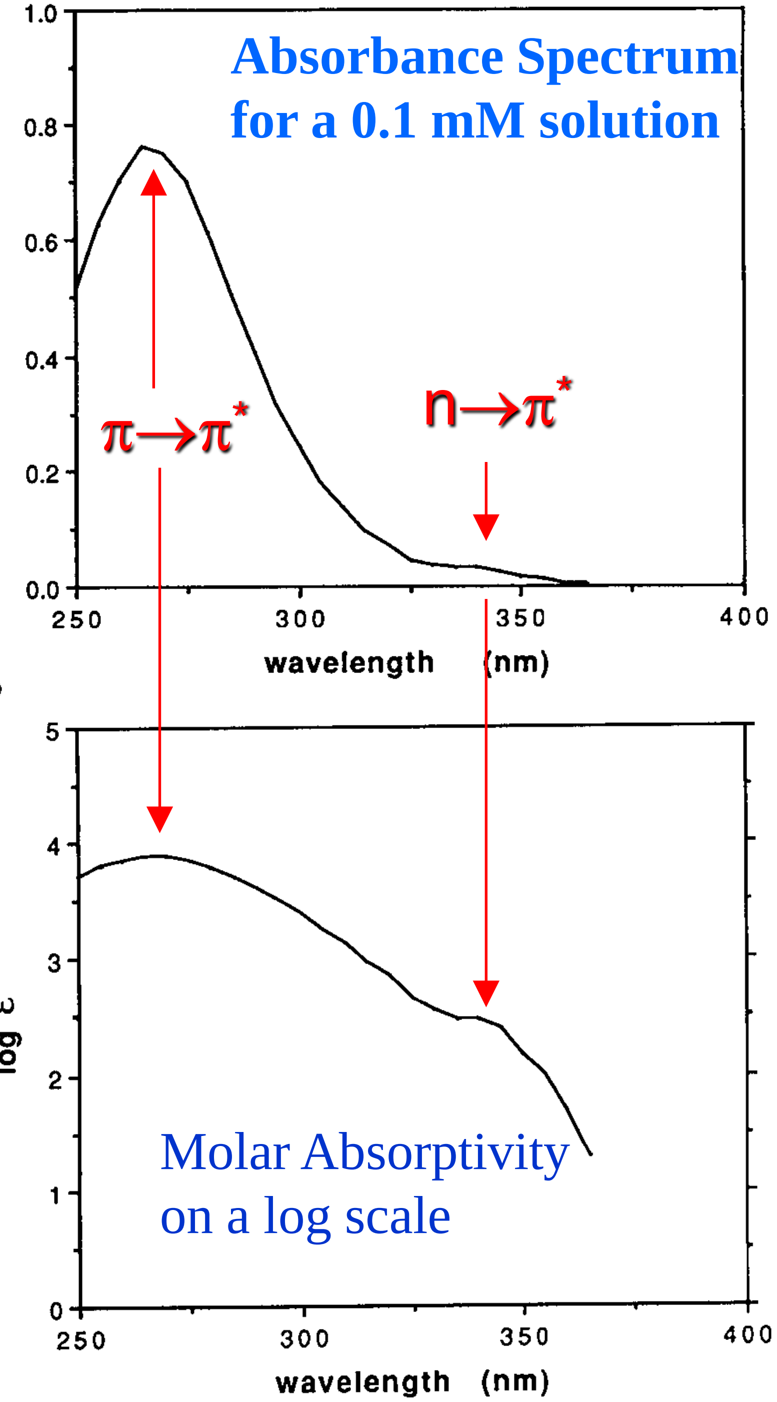
- Effect of solvent
  - Peaks associated with n $\rightarrow \pi^*$ transition are shifted to shorter wavelength (hypsochromic shift) with increasing polarity of solvent
  - Peaks associated with $\pi \rightarrow \pi^*$ transition are shifted to longer wavelength (bathochromic shift) with increasing polarity of solvent

Absorbance Spectra

- Nitrobenzene in aqueous solution



  - Heavily conjugated with 4 resonance forms



Absorbance Spectrum for a 0.1 mM solution

π→π*

n→π*

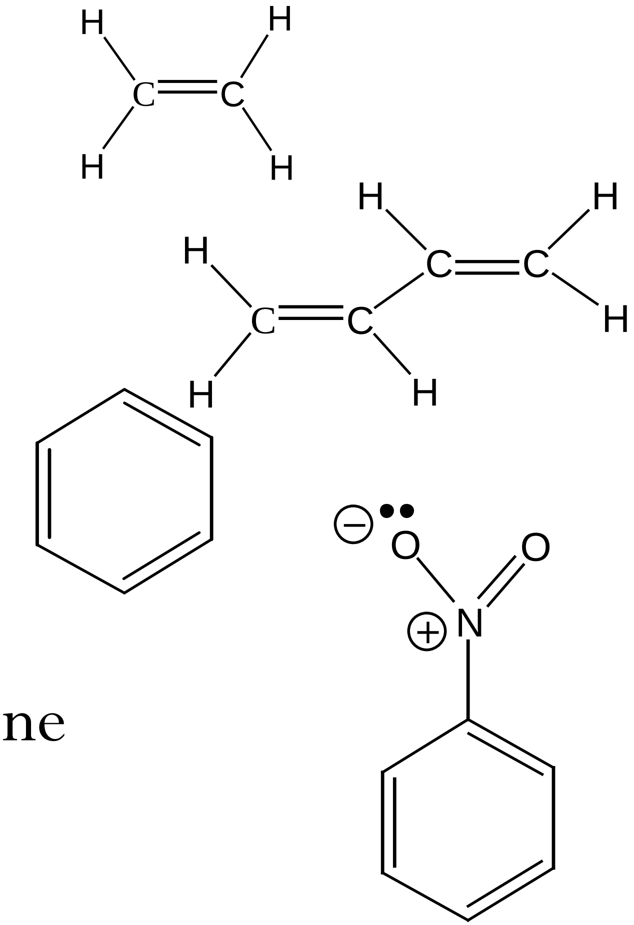
Molar Absorptivity on a log scale

Graph from: Schwarzenbach et al., 1993

Terminology

- **Absorbance (A)** a measure of the amount of radiation that is absorbed
- **Band** Term to describe a uv-vis absorption which are typically broad.
- **Chromophore** Structural unit responsible for the absorption
- **Molar absorptivity (ε)**, absorbance of a sample of molar concentration in 1 cm cell.
- **Extinction coefficient** An alternative term for the molar absorptivity
- **Path length (l)** the length of the sample cell in cm
- **Beer-Lambert Law** $A = \varepsilon . l . c$ (c = concentration in moles / litre)
- $\boldsymbol{\lambda}_{\mathbf{max}}$ The wavelength at maximum absorbance
- $\boldsymbol{\varepsilon}_{\mathbf{max}}$ The molar absorbance at λ_{max}
- **HOMO** **H**ighest **O**ccupied **M**olecular **O**rbital
- **LUMO** **L**owest **U**noccupied **M**olecular **O**rbital

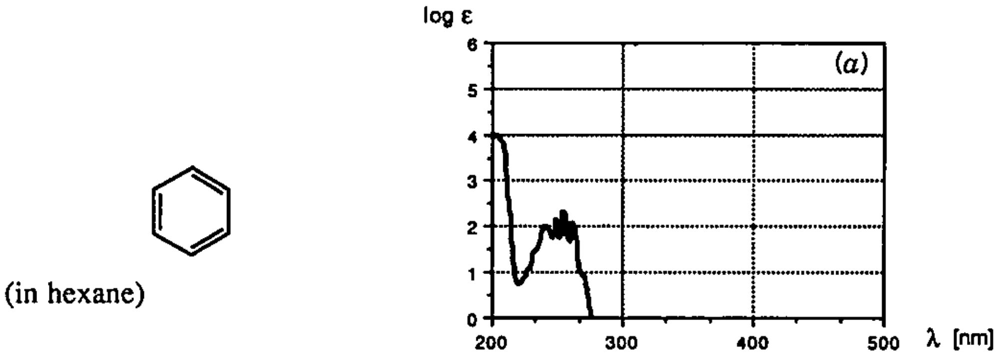
Bathochromic Shift

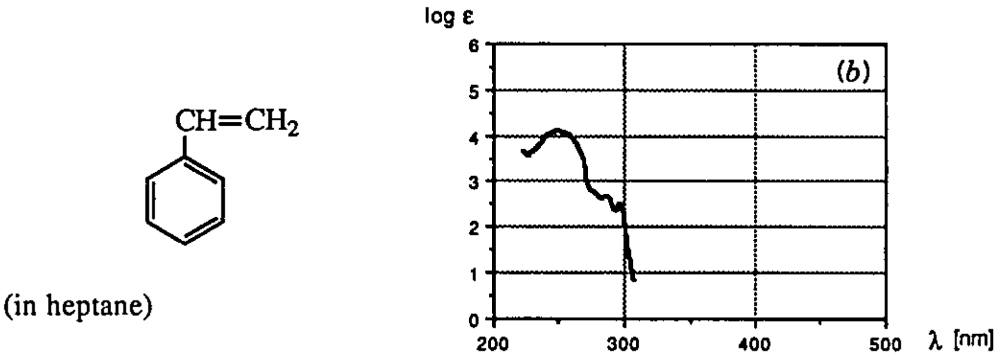


	λ_{max}
Ethylene	190 nm
Butadiene	220 nm
Benzene	255 nm
Nitrobenzene	270 nm

Conjugation

- Impact of double bonds in conjugation with aromatic ring
  - More $\pi \rightarrow \pi^*$ transitions
  - Example
    - Benzene
    - Styrene

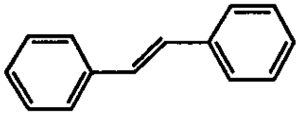




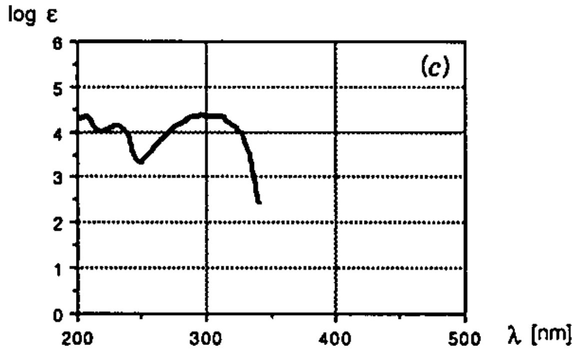
From: Schwarzenbach et al., 1993

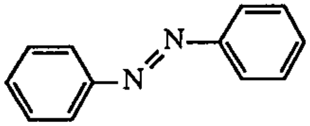
Heteroatoms

- Impact of heteroatoms
  - $n \rightarrow \pi^*$ transitions
    - Longer λ_{max}
  - Example
    - Trans-stilbene
    - Azobenzene

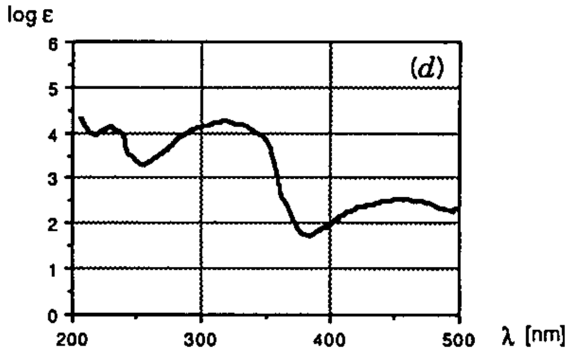


(in ethanol)





(in hexane)

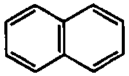


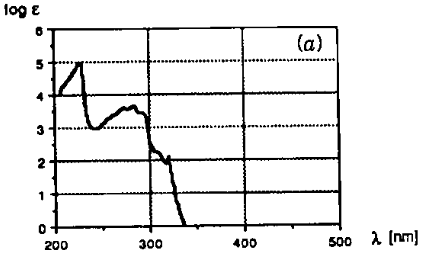
From: Schwarzenbach et al., 1993

Conjugation revisited

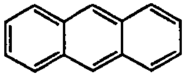
- Impact of increasing conjug
  - $\pi \rightarrow \pi^*$ transitions
    - λ_{max} increases ~30 nm per conjugated bond
      - Bathochromic shift
  - Examples
    - Naphthalene
    - Anthracene
    - Phenanthrene

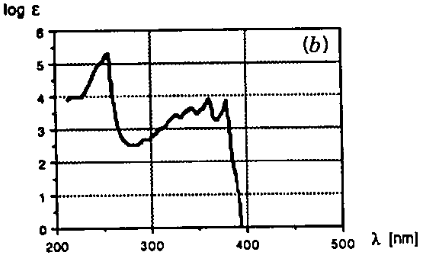
(in hexane)



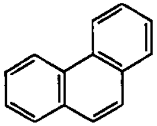


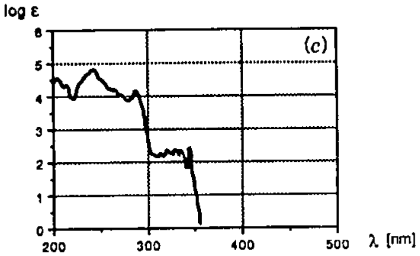
(in hexane)





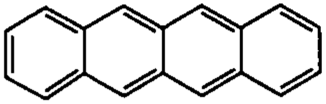
(in hexane)



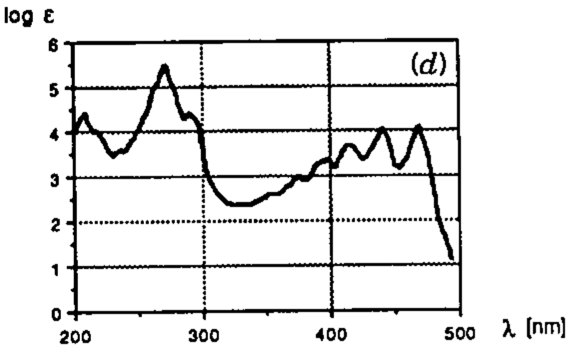


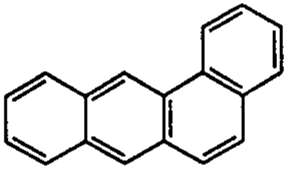
From: Schwarzenbach et al., 1993

- More Examples
  - Naphthacene
  - Benz(a)anthracene

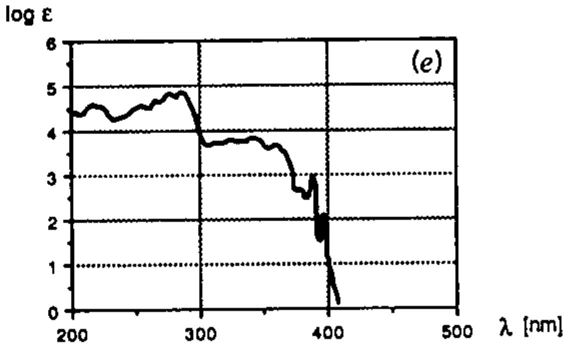


(in heptane)

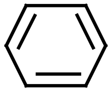




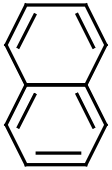
(in heptane)



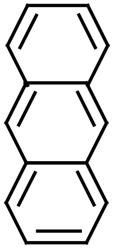
From: Schwarzenbach et al., 1993



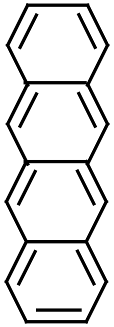
Benzene



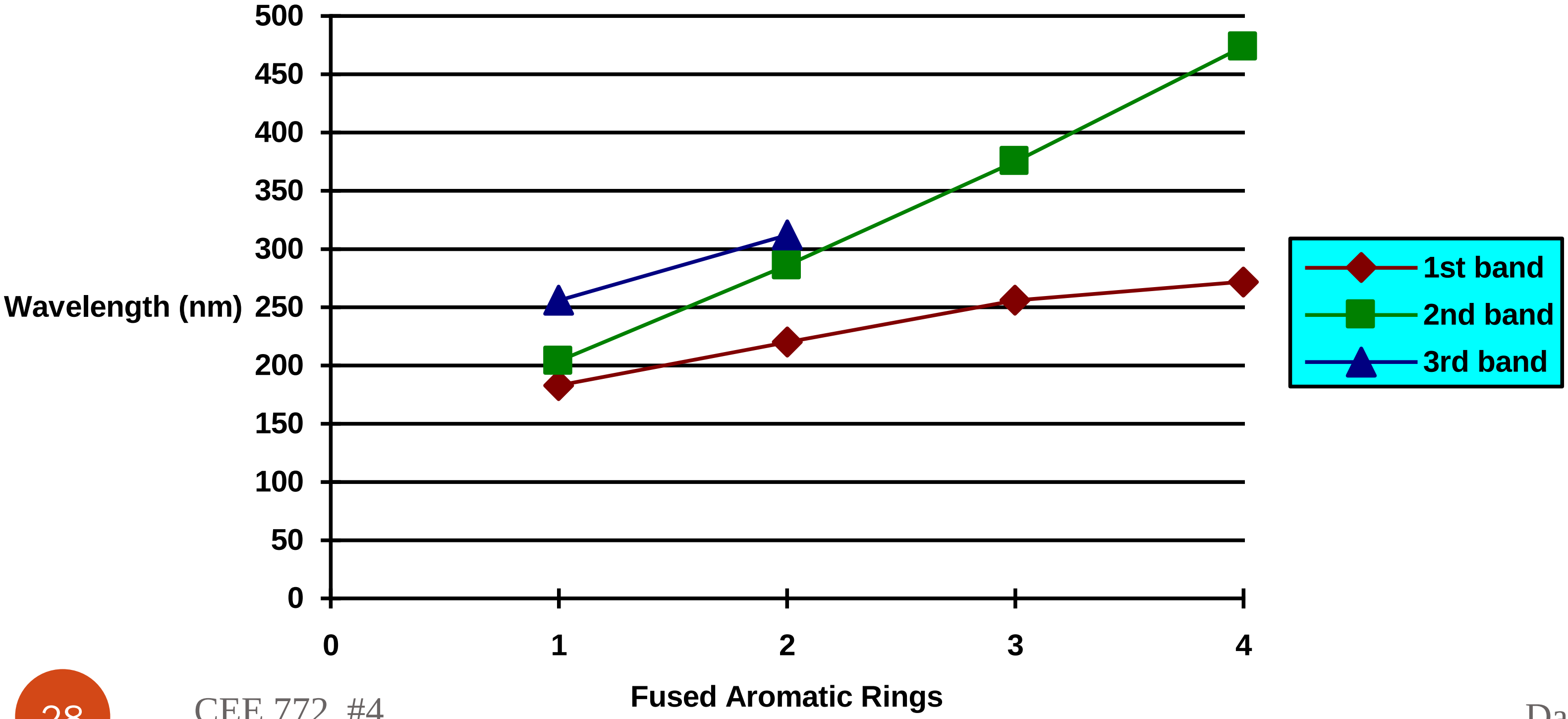
Naphthalene



Anthracene



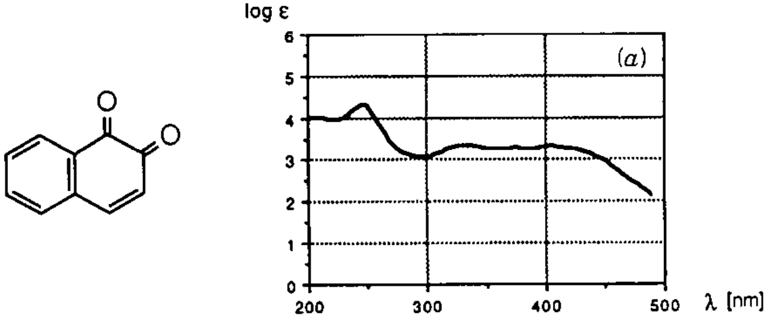
Naphthacene



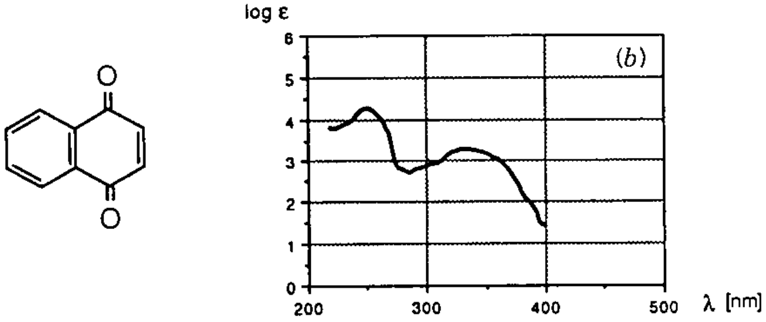
Geometry

- 1,2-Naphthoquinone
- 1,4-Naphthoquinone

(in methanol)



(in methanol)

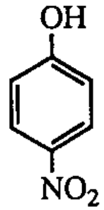


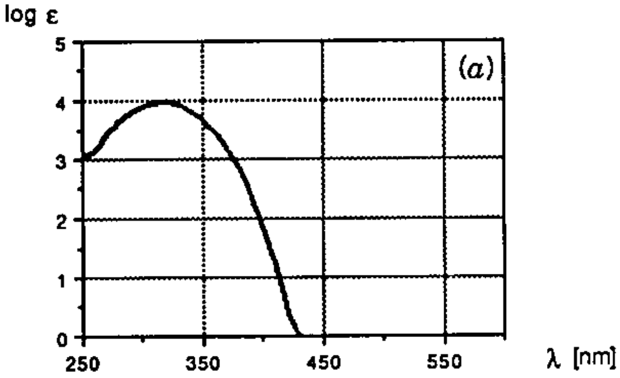
From: Schwarzenbach et al., 1993

pH speciation: Acids

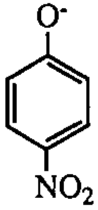
- Deprotonation leads to delocalization of negative charge
  - Bathochromic shift
  - Examples
    - 4-Nitrophenol
    - 4-Nitrophenolate

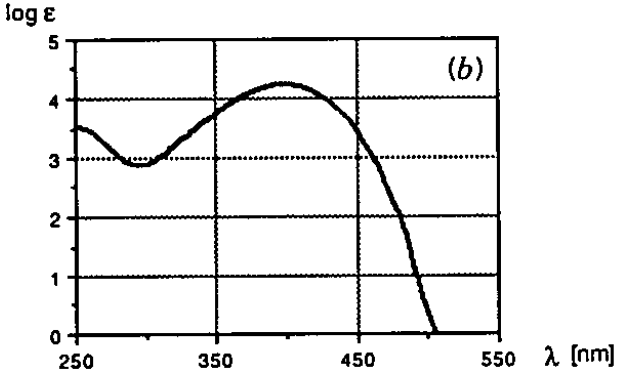
(in water)





(in water)

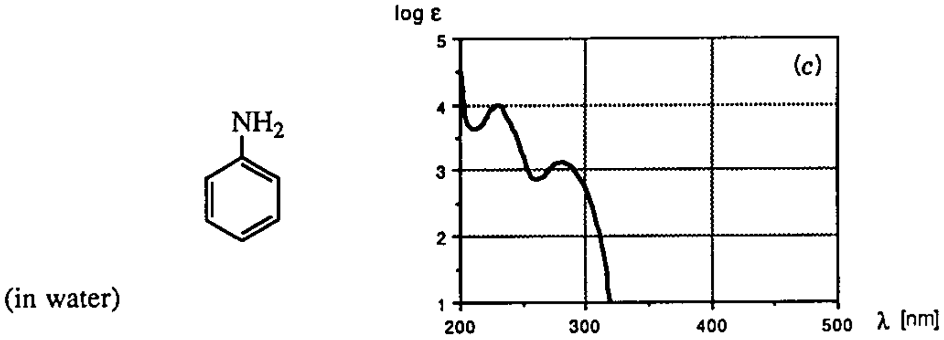


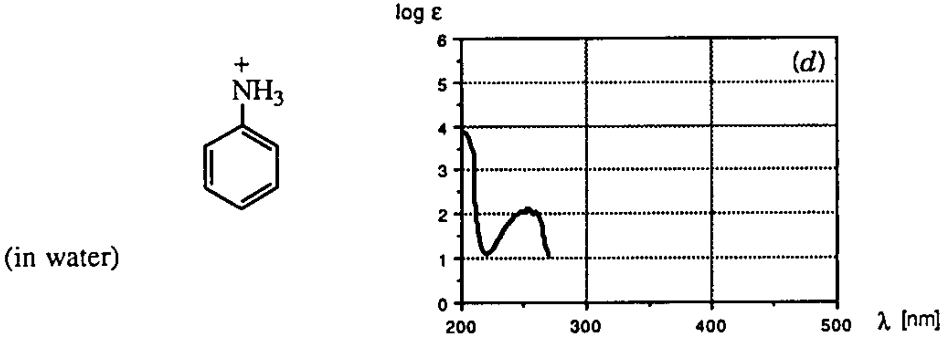


From: Schwarzenbach et al., 1993

pH Speciation: Bases

- Protonation causes loss of available “n” electrons
  - Hypsochromic shift
  - Examples
    - Aniline
    - Anilinium ion

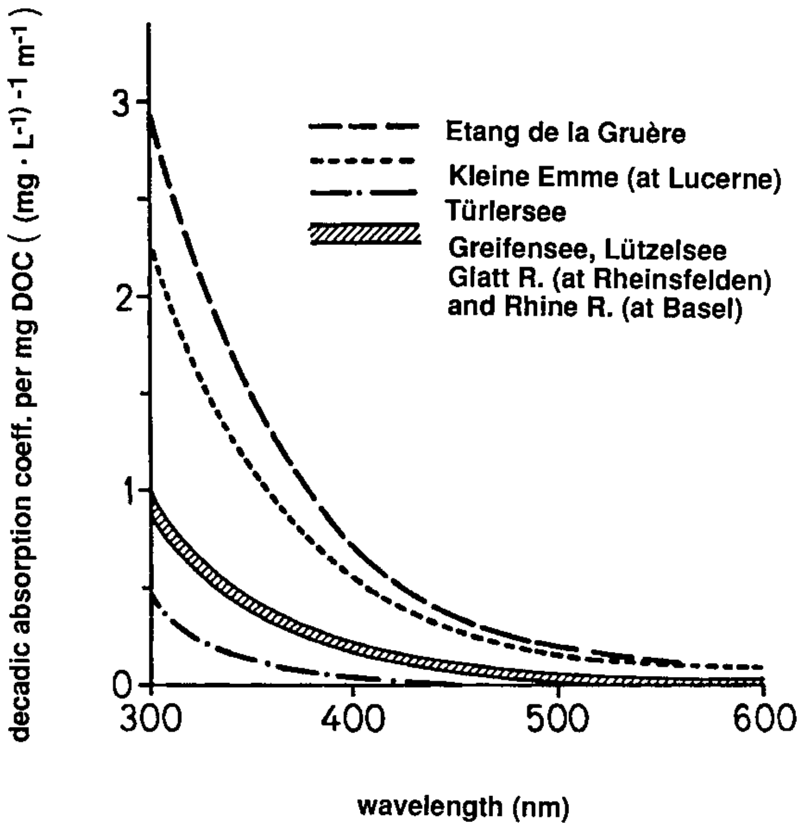




From: Schwarzenbach et al., 1993

Background NOM

- Specific Absorbance of water samples from several Swiss lakes and rivers



From: Schwarzenbach et al., 1993

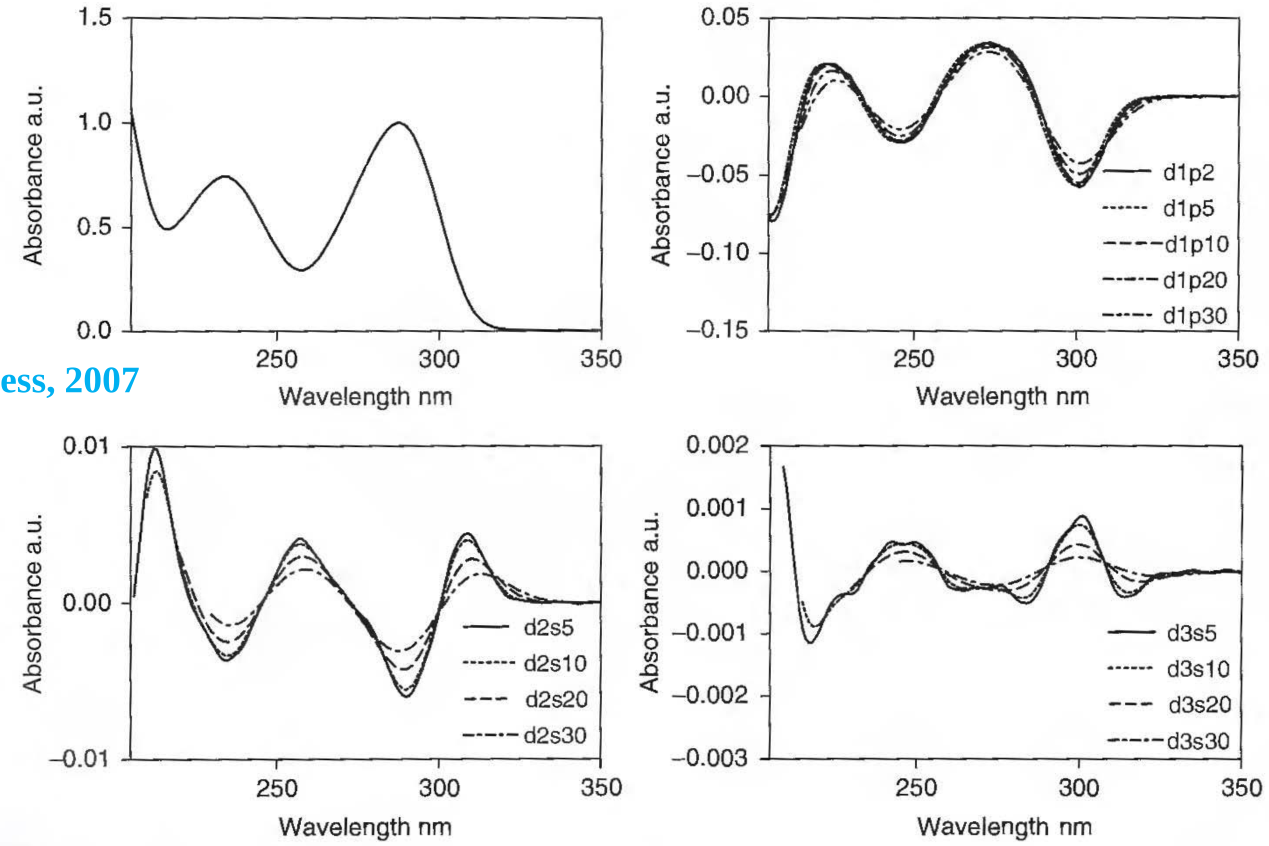
S::CAN

- Field deployable diode array spectrophotometer in a probe-like configuration
  - Can be submersed in flowing water or fitted with a flow-through cell
- Produces a full UV-Vis spectrum
- Algorithms tailored to estimate other paramaters
- Good surrogate for DOC
  - especially when the character of the DOC is reasonably constant
- A very good surrogate for THMFP, HAAFP
  - takes into account reactivity of DOC as well as amount of DOC
- Oxidation processes (ozonation) disrupt relationships between UV and DOC or THMFP

Commercial field probe

Derivative Spectroscopy

- Derivatives can be used in various algorithms

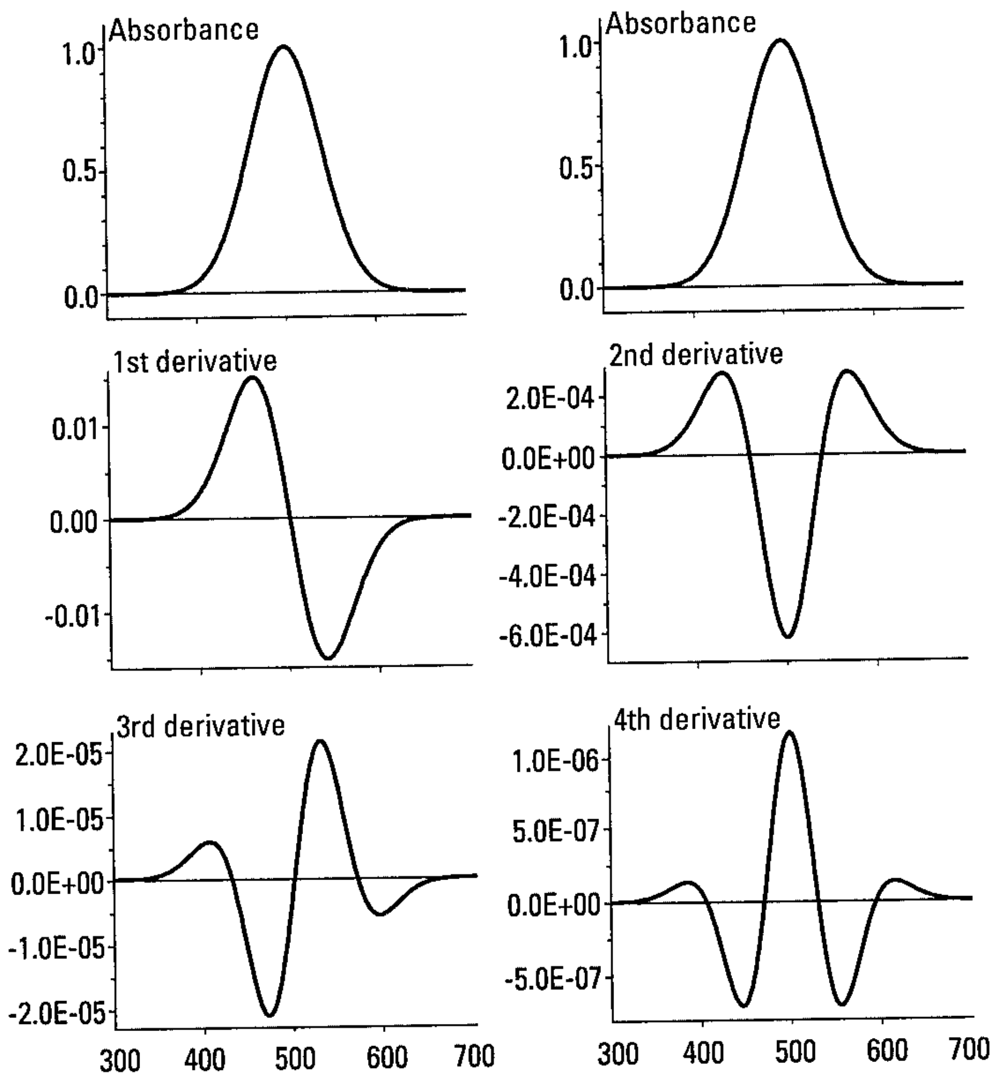


Thomas & Burgess, 2007

FIGURE 2. *Spectrum of uric acid, and derivative spectra (first, second and third) calculated with several differentiation steps (2, 5, 10, 20 and 30 nm). For example: d2s10 is the second derivative spectrum for a diffferentiation step of 10 nm.*

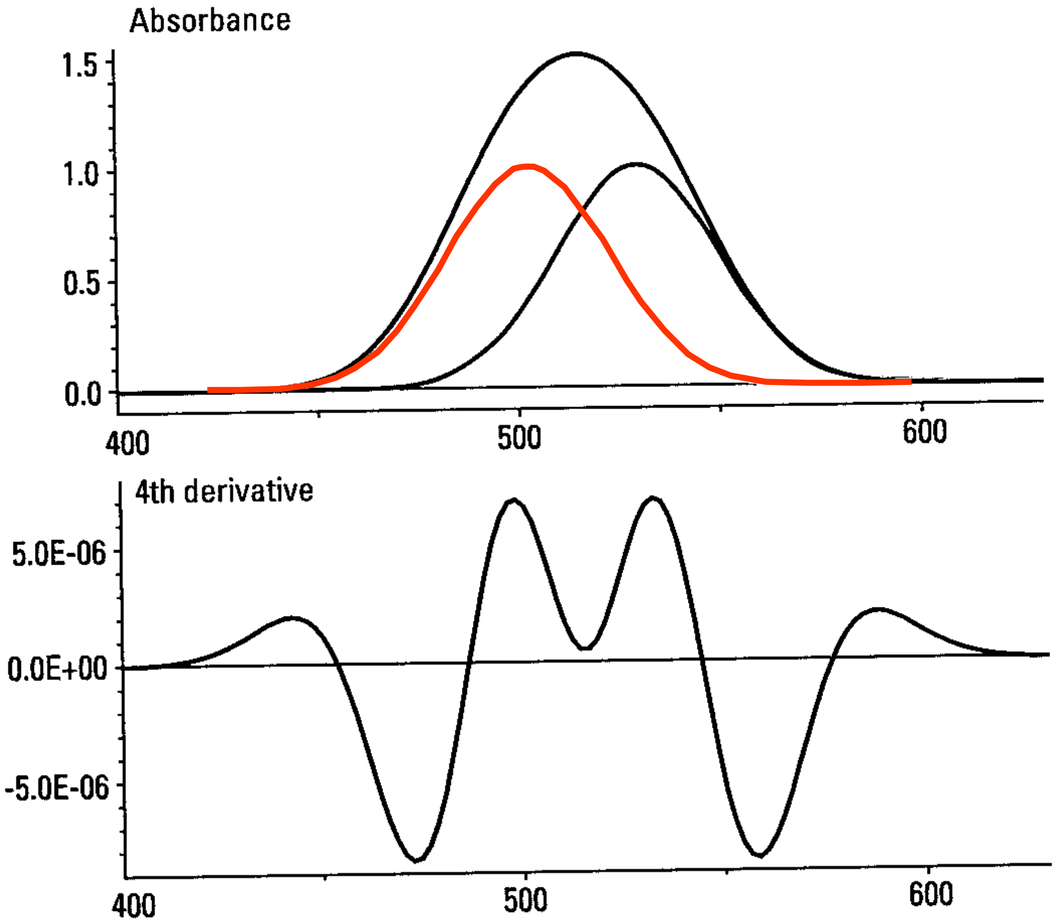
Derivative Spectra

- Derivative of absorbance with respect to wavelength
- Some features
  - the 2nd derivative shows a negative peak at the λ_{max}
  - the 4th derivative shows a positive peak at the λ_{max}



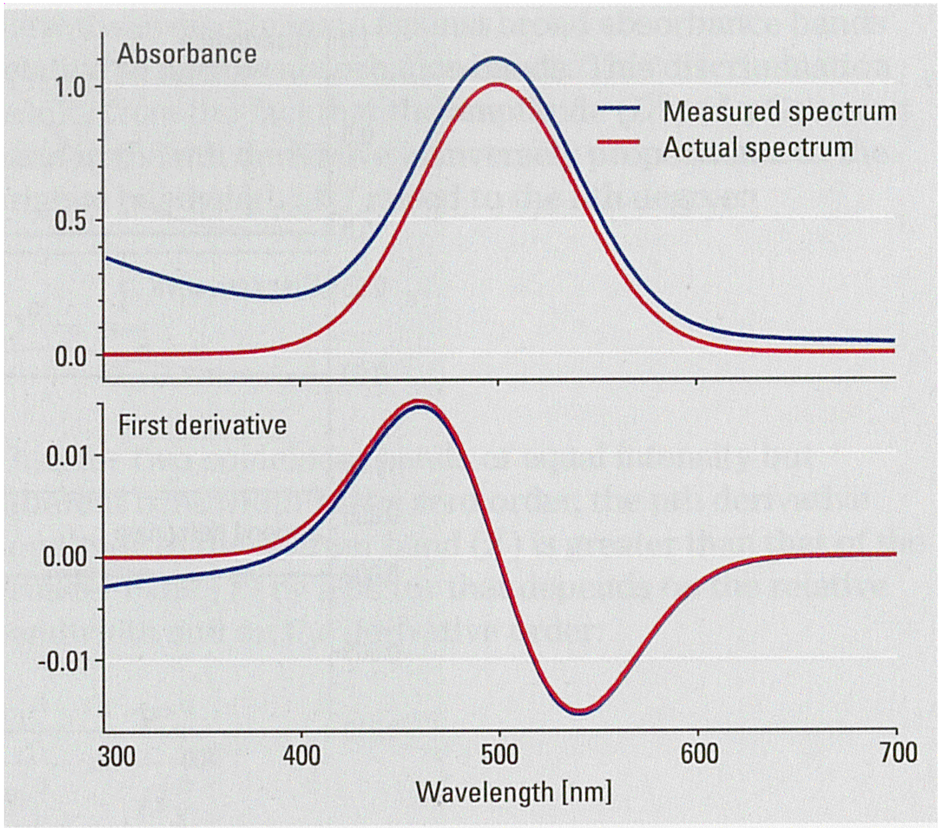
Applications of Derivative Spectroscopy 1

- Resolution of overlapping spectral bands
  - Spectra must be relatively free of noise



Applications of Derivative Spectroscopy 2

- Removal of background interference, e.g., scattering
  - +9.2% error in peak height
  - -1.1% error in max to min of 1^{st} derivative



- To next lecture