Updated: 9 September 2019

[Print version]

CEE 772: Instrumental Methods in Environmental Analysis

Lecture #3

Statistics: Detection Limits

Spectroscopy: Beer's Law & Electronic Transitions

(Skoog, Chapts. 6 & 13)

(pp.116-120, 134-140, 300-312)

(Harris, Chapt. 1)

(pp.1-20)

Errors: Random or Indeterminate

- Causes of "Noise"
- Result of a large number of small errors which cannot be easily isolated from each other
- They occur over short time scales and may be nearly random
- Can use classical statistics with these, because of their nearly-independent and random nature

Errors: Systematic or Determinant

- Instrumental Errors
    - Corrected by calibration
  - Changes in line voltage
  - Increases in resistances due to oxidation of electrical contacts
  - Changes in temperature
  - Vibration of optical elements
  - Induced currents from nearby power lines

Errors: Systematic or Determinant

- Method Errors
    - Non-ideal chemical & physical behavior
    - Some may also be accounted for by calibration, standard addition, etc.
  - Incomplete reactions
  - Unwanted side reactions (interferences)
  - Contamination of reagents
- Personal errors
    - Lack of concentration on the part of the analyst
    - Sometimes creates outliers
  - Mis-reading instrument or apparatus
  - Transposing numbers, error in calculations
  - Addition of incorrect volume

Error vs Uncertainty (Aarthi's addendum)

Error (measure of bias)
- Determinate (systematic)
  - Can be corrected
- Indeterminate (Random)
  - Statistical testing

Uncertainty (range of values)
- Used for both determinate and indeterminate cases
- Different from precision (repeatability)

10±0.02 mL

Detection Limit, Sensitivity & Resolution

- Detection Limit
  - The minimum concentration (or weight) of analyte that can be detected at a known confidence level
  - Minimum distinguishable signal (S_m)

$$S_m = \bar{S}_{b1} + k\left(SD_{b1}\right)$$

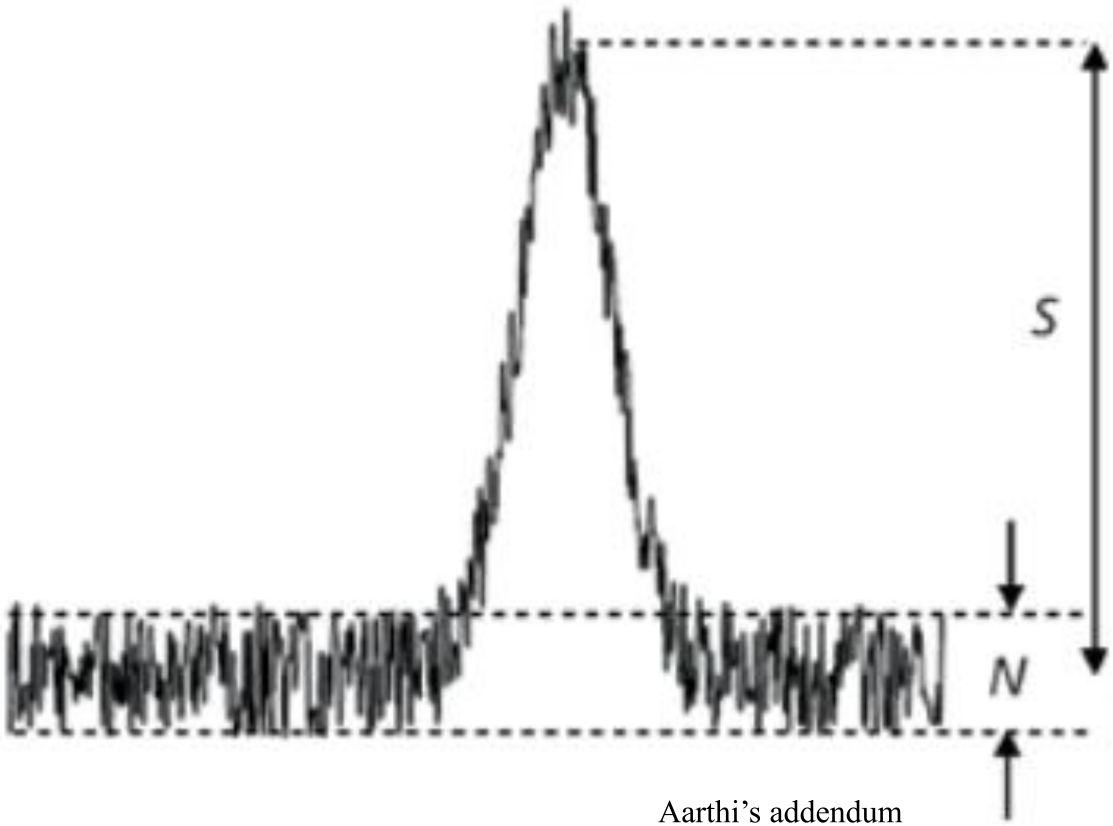
Mean blank signal; s.d. of blank signal

    - Often, k=3 for 95% confidence interval (non-gaussian)
  - Detection limit (C_m)

$$C_m = \frac{S_m - \bar{S}_{b1}}{m}$$

Slope of standard curve

Detection Limit, Sensitivity & Resolution

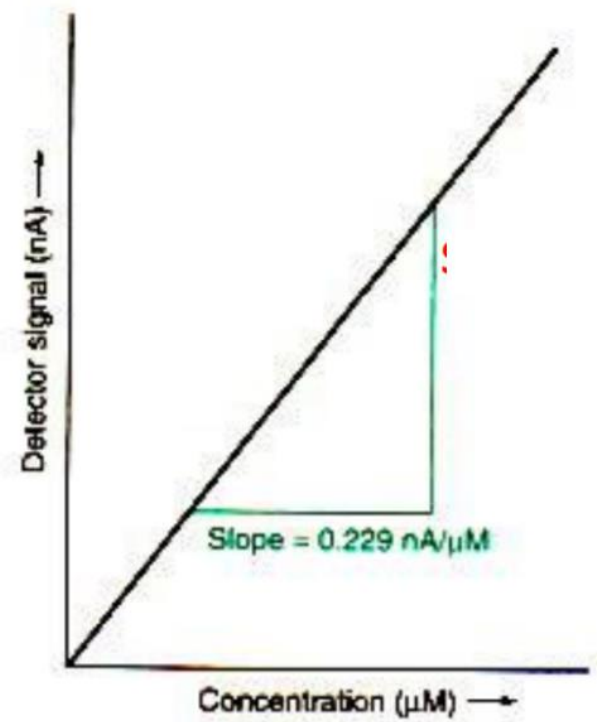


Acceptable S/N > 3 in chromatography

Aarthi's addendum

Detection Limit, Sensitivity & Resolution

- Sensitivity



Ability to distinguish small differences in concentration

- Calibration Sensitivity: slope of a calibration curve at the concentration of interest
- Analytical Sensitivity: response to noise ratio (change in detector signal)

slope

$$\gamma = m / s_s$$

s.d. of signal

https://www2.chemistry.msu.edu/courses/cem434/Lecture%202.pdf

Detection Limit, Sensitivity & Resolution

- Resolution (Aarthi's addendum)
  - Closeness to true value; closer the value better the resolution
  - smallest unit of measurement that can be indicated by an instrument.
  - Different for different instruments

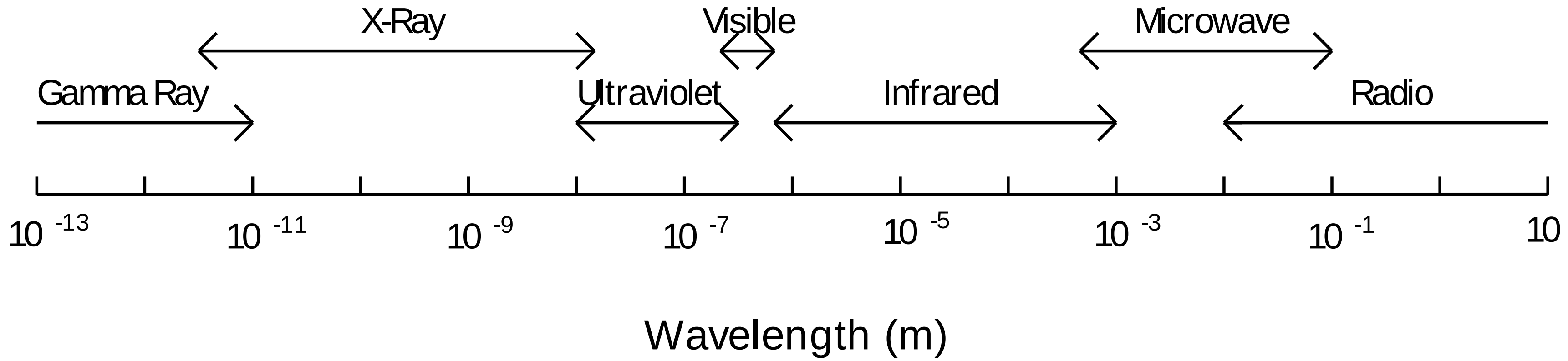
Sensitivity is the smallest amount of **difference** in quantity that will change an instrument's reading.

Topics Covered

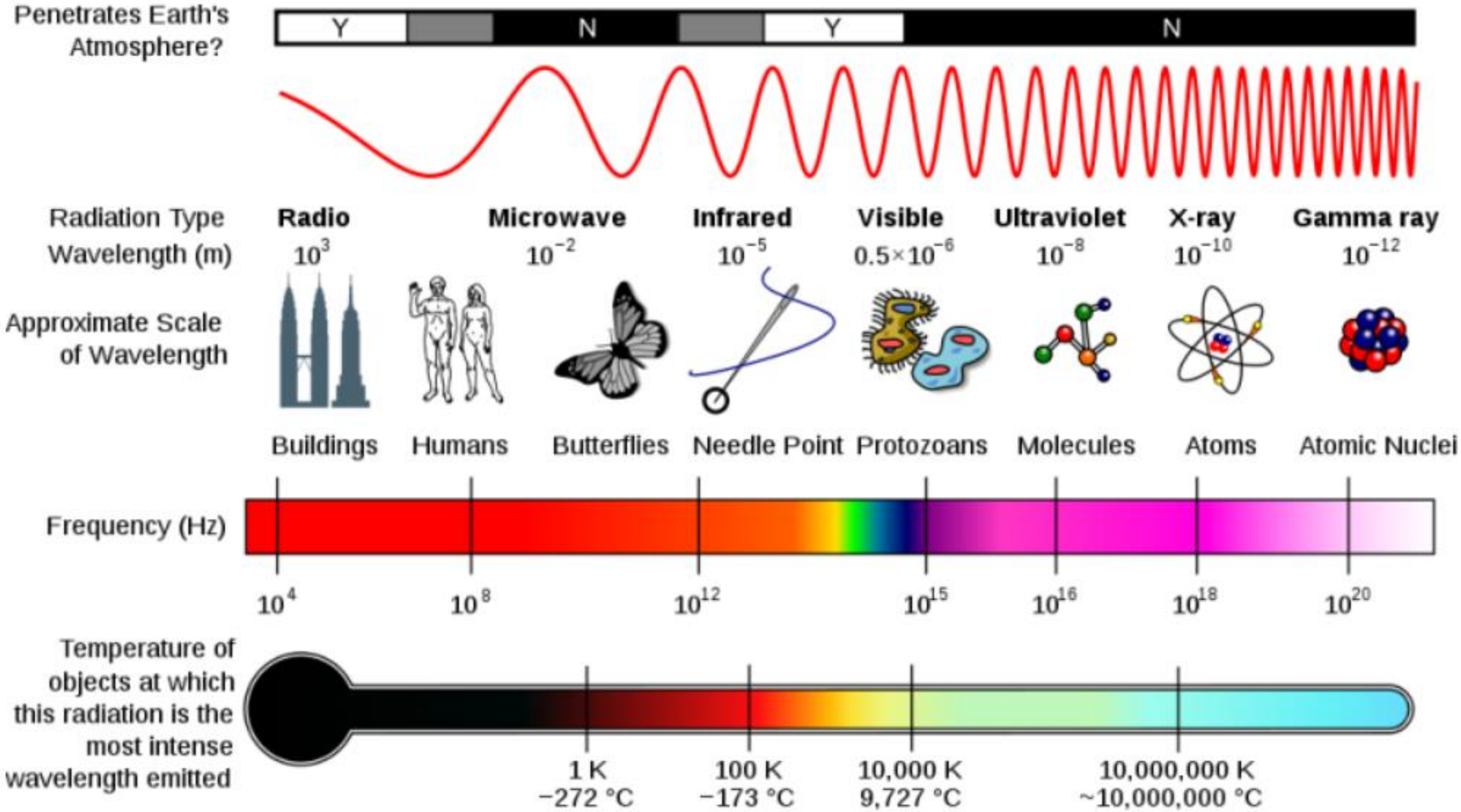
- Beer's Law
- Spectra
- Structure and Absorbance
- Standard Curves

Light

- The electromagnetic spectrum

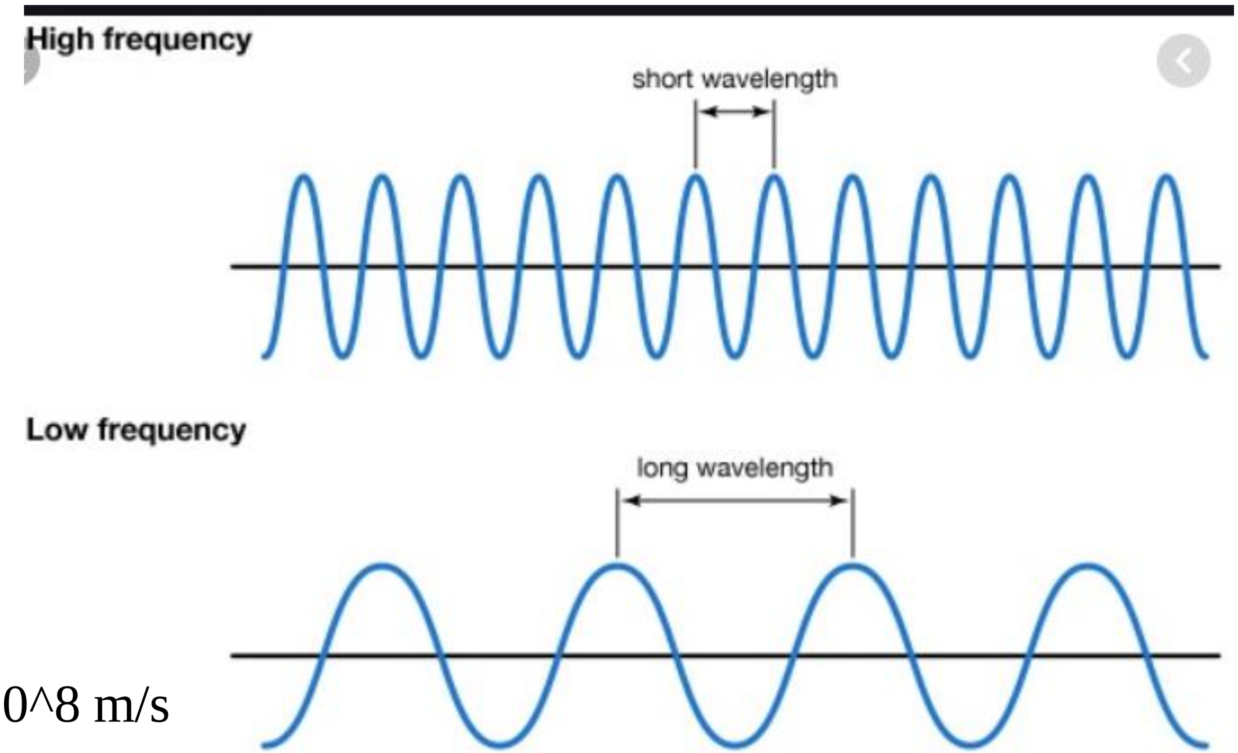


Light



https://earthsky.org/space/what-is-the-electromagnetic-spectrum

Light



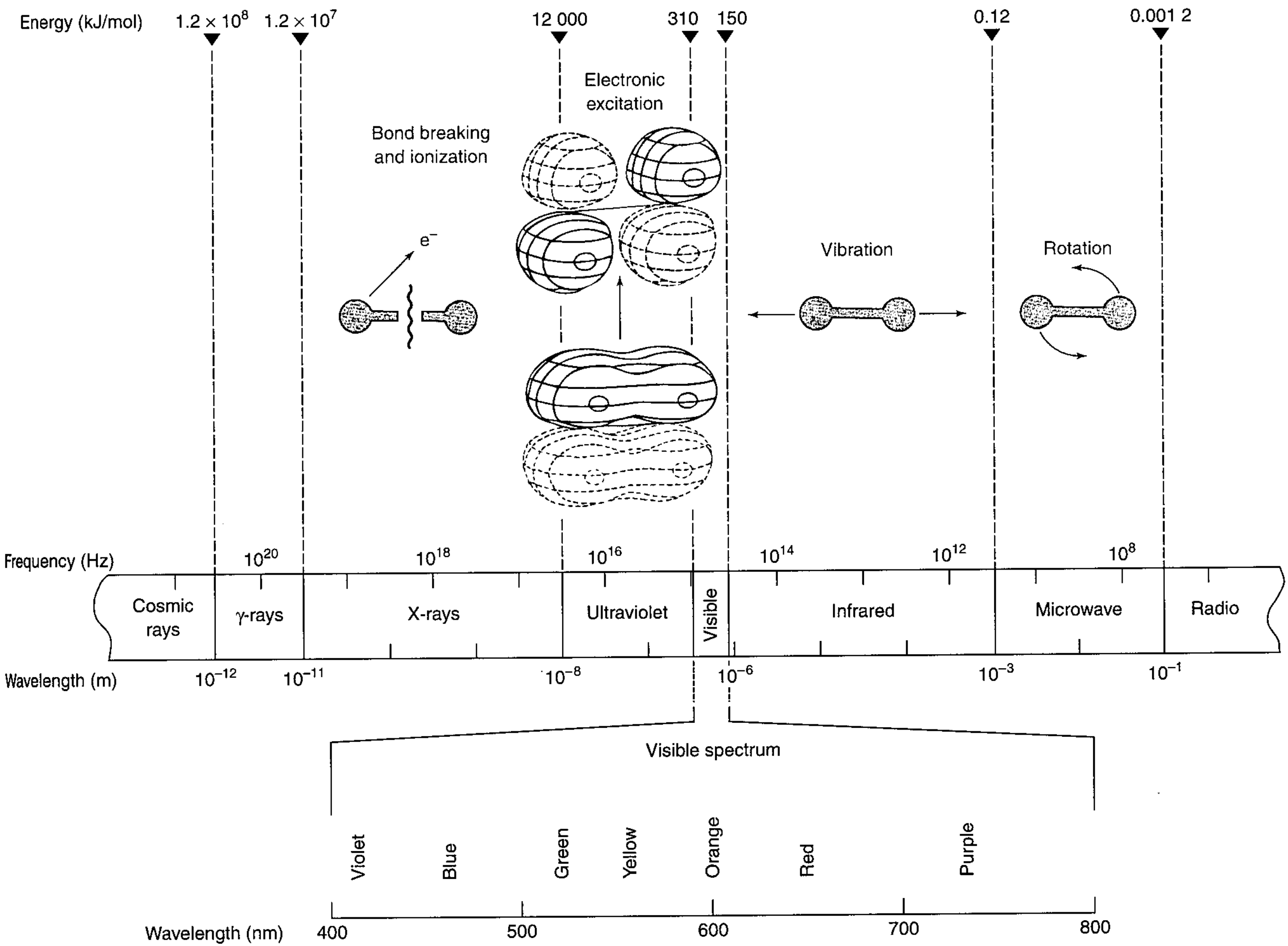
$C = \lambda \upsilon$

C= speed of light = 3X 10^8 m/s

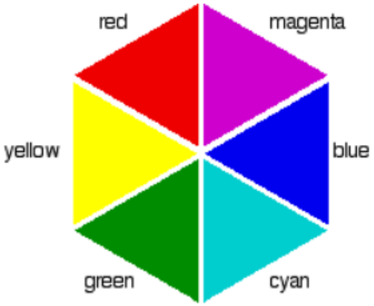
$E = h \lambda$

h= Planck's constant=

$6.62607004 \times 10^{-34}\ m^2\ kg / s$


Energy (kJ/mol)
1.2 × 10^8
1.2 × 10^7
12 000
310
150
0.12
0.001 2
Electronic excitation
Bond breaking and ionization
e⁻
Vibration
Rotation
Frequency (Hz)
10^20
10^18
10^16
10^14
10^12
10^8
Cosmic rays
γ-rays
X-rays
Ultraviolet
Visible
Infrared
Microwave
Radio
Wavelength (m)
10^−12
10^−11
10^−8
10^−6
10^−3
10^−1
Visible spectrum
Violet
Blue
Green
Yellow
Orange
Red
Purple
Wavelength (nm)
400
500
600
700
800

Wavelength and Color



Also called "complementary color"

Wavelength of absorbance maximum (nm)	**Color Absorbed**	**Color Remaining**
380-420	Violet	Green-yellow
420-440	Violet-blue	Yellow
440-470	Blue	Orange
470-500	Blue-green	Red
500-520	Green	Purple
520-550	Yellow-green	Violet
550-580	Yellow	Violet-blue
580-620	Orange	Blue
620-680	Red	Blue-green
680-780	Purple	Green

Transmittance

- Beer/Lambert's Law

$$I = I_o e^{-\gamma x}$$

  – Sum of scattering cross section and absorption coefficient

$$\gamma = \tau + \kappa$$

  – Absorption coefficient

$$\kappa = {}^{ac}\!/_{2.303}$$

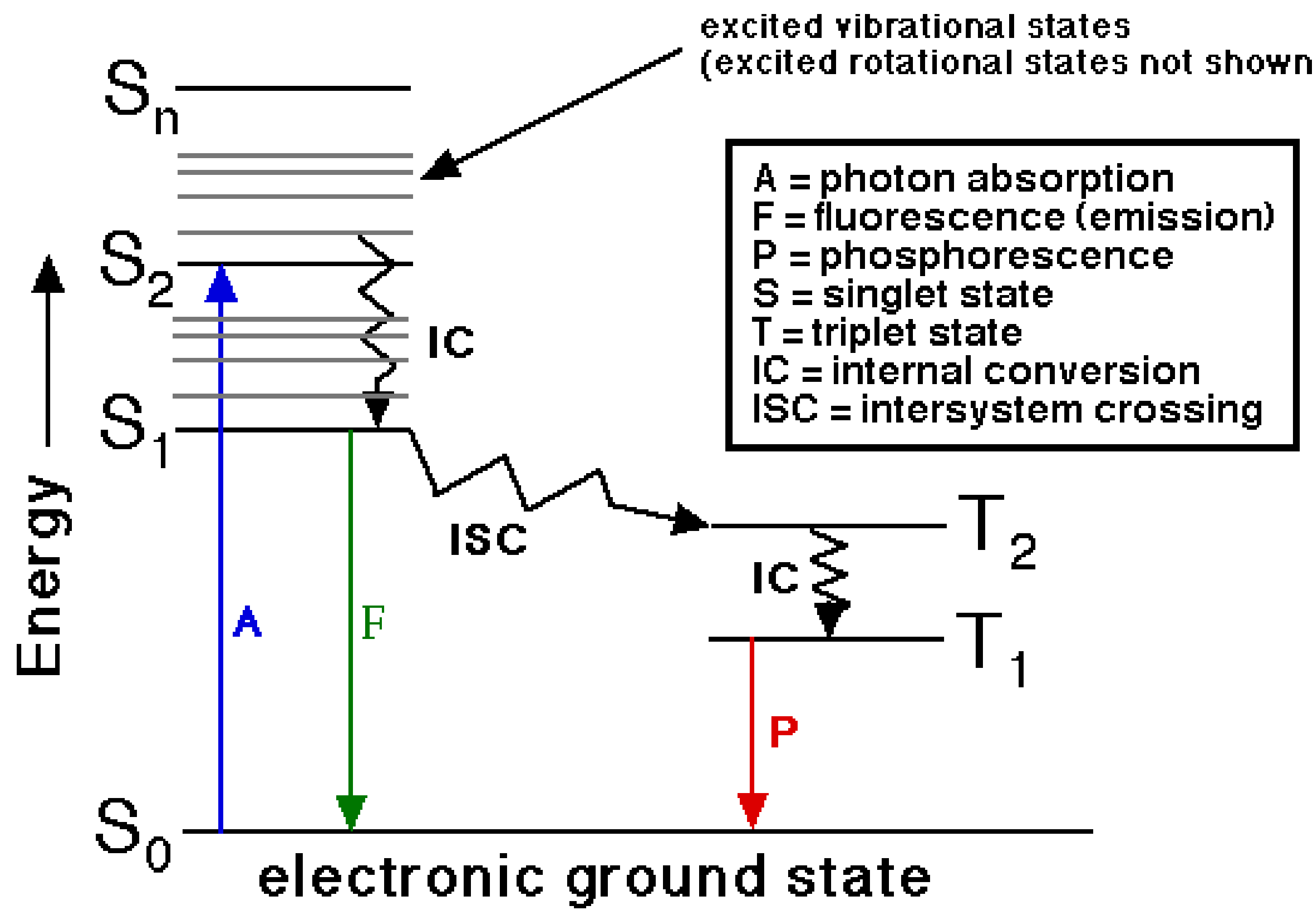
Absorbance

- Transmittance
- Absorbance
  - A = -log(T)
  - A = acx

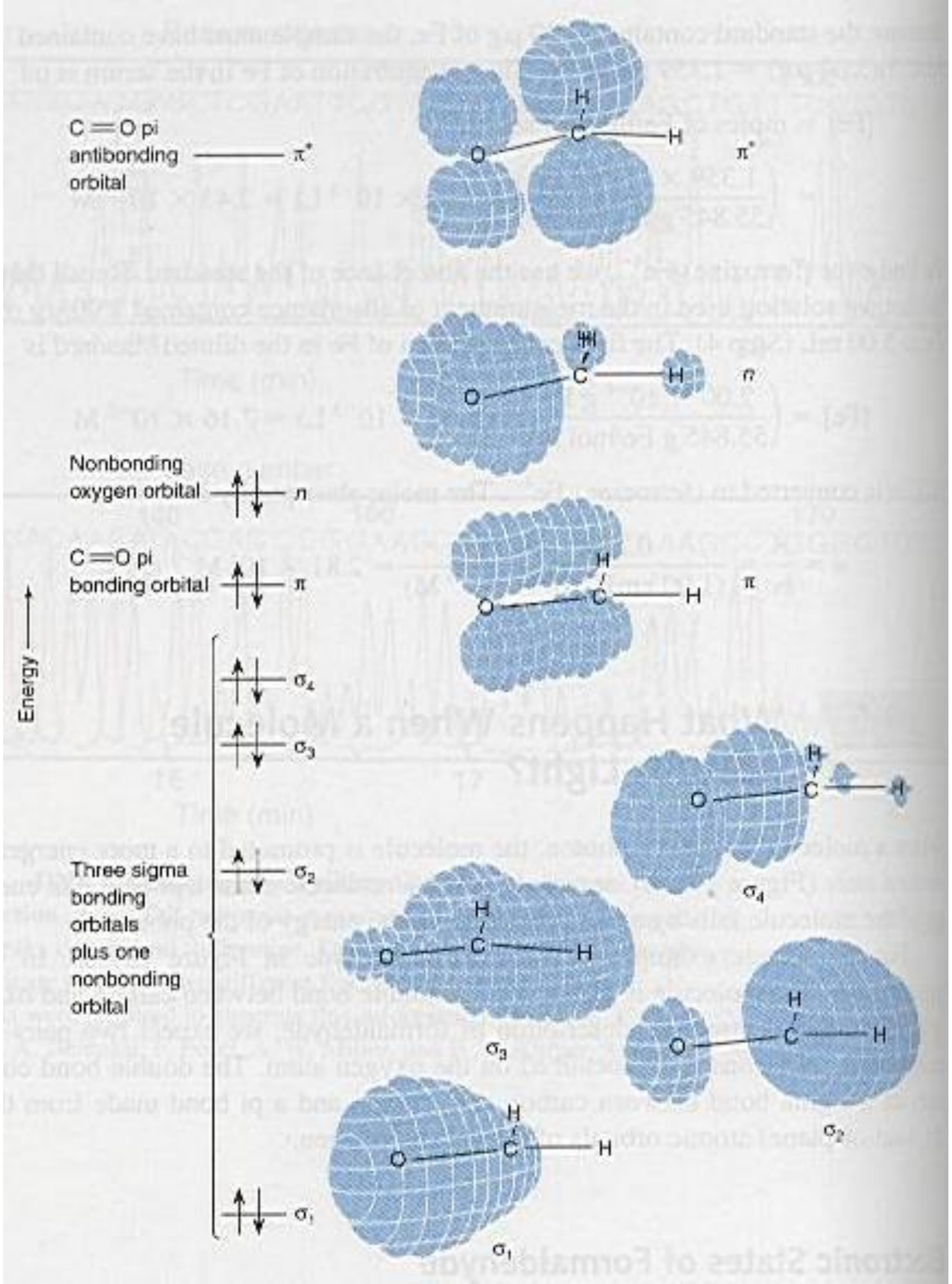
$$T \equiv I / I_o = e^{-acx/2.303}$$

- Absorptivity
  - a: absorbance per mg/L concentration
  - ε: absorbance for 1 mole/L concentration
    - Molar absorptivity ($L\ cm^{-1}\ mol^{-1}$)

Energy Absorption & Bonding



Partial energy diagram in a photoluminescence system


C═O pi antibonding orbital
π*
Nonbonding oxygen orbital
n
C═O pi bonding orbital
π
Energy
σ4
σ3
σ2
σ1
Three sigma bonding orbitals plus one nonbonding orbital
O
C
H
σ4
σ3
σ2
σ1

EVE Spectrophotometers

	Bausch & Lomb to Milton Roy to:			ThermoSpectronic; ThermoElectron		Perkin-Elmer			Hewlett-Packard	Hach
Characteristic	**Spec 20**	**Spec 21D**	**Spec 70**	**Genesys 20**	**Genesys 10UV**	**Model 111**	**Lambda 3A**	**Lambda 3B**	**Diode Array**	**DR/4000U**
Location & condition[1]		**Marst 24**	**3rd fl storage**	**Marston 24**	**304 Elab II 213 Elab II**	**3rd fl storage**	**308 Elab II 24 Marston**	**24 Marston**	**308 Elab II**	**304 Elab II**
Optical system	Single beam	Single beam	Single beam	Single beam	Split beam	Single beam	DoubleBeam	Double Beam	Diode Array	Single Beam
Monochromator	Grating			Grating	Grating		Holographic Grating	Holographic Grating	Holographic Concave Grtn.	Seya-Namioka split-beam
Groove Density	600/mm			1200/mm	1200/mm		1440/mm	1440/mm		1200/mm
Detector	Phototube			Solid state			Photo-multiplier	Photo-multiplier	328 Photo-diodes	
Lamp(s)	Tungsten			Tungsten	Xenon		Tungsten-Br Deuterium	Tungsten-Br Deuterium	Deuterium[b]	Tungsten, Deuterium
Readout	Analog	Ditigal	Analog	Digital	Digital	Analog	Digital	Digital	Digital	Digital
Cell Holder	0.5-1" tube			1 cm & tubes		multi-position	0.1-10cm	0.1-10cm	0.1-10cm	1-10 cm
Wavelength Range	340-625 nm[*]	Vis	Vis	325-1100nm	190-1100nm	UV/Vis	190-900 nm	190-900nm	190-820nm	190-1100nm
Wavelength Accuracy	2.5 nm			2.0 nm	1 nm		0.5 nm	0.3 nm	2 nm	1 nm
Wavelength Precision	1.0 nm			0.5 nm	0.5 nm		0.2 nm	0.1 nm	0.05 nm	0.1 nm
Effective Bandwidth	20 nm			8 nm	5 nm		<2 nm	1 nm	2 nm	4 nm
Photometric Accuracy	2.5 %T			0.003 A; 1% (0.3A up)	0.5% T		0.3 %T[d], 0.005 A,	0.3 %T[d], 0.005 A	0.3 %T[d], 0.005 A	
Photometric Precision	1 %T						0.15 %T[d], 0.002 A	0.15 %T[d], 0.002 A		0.001 A
Stray Light	< 0.5%[+]			<0.1%T	<0.1%T		<0.05%	0.02%	<0.05%	<0.05%T
Baseline Flatness							0.005 A	0.002 A	0.001 A	
Noise				<0.002 A	<0.002 A		<0.0005 A	<0.0003 A	<0.0002 A	
Zero Abs Stability				<0.003A/hr	<0.001A/hr		<0.0005A/hr	<0.0005A/hr	<0.001A/hr	

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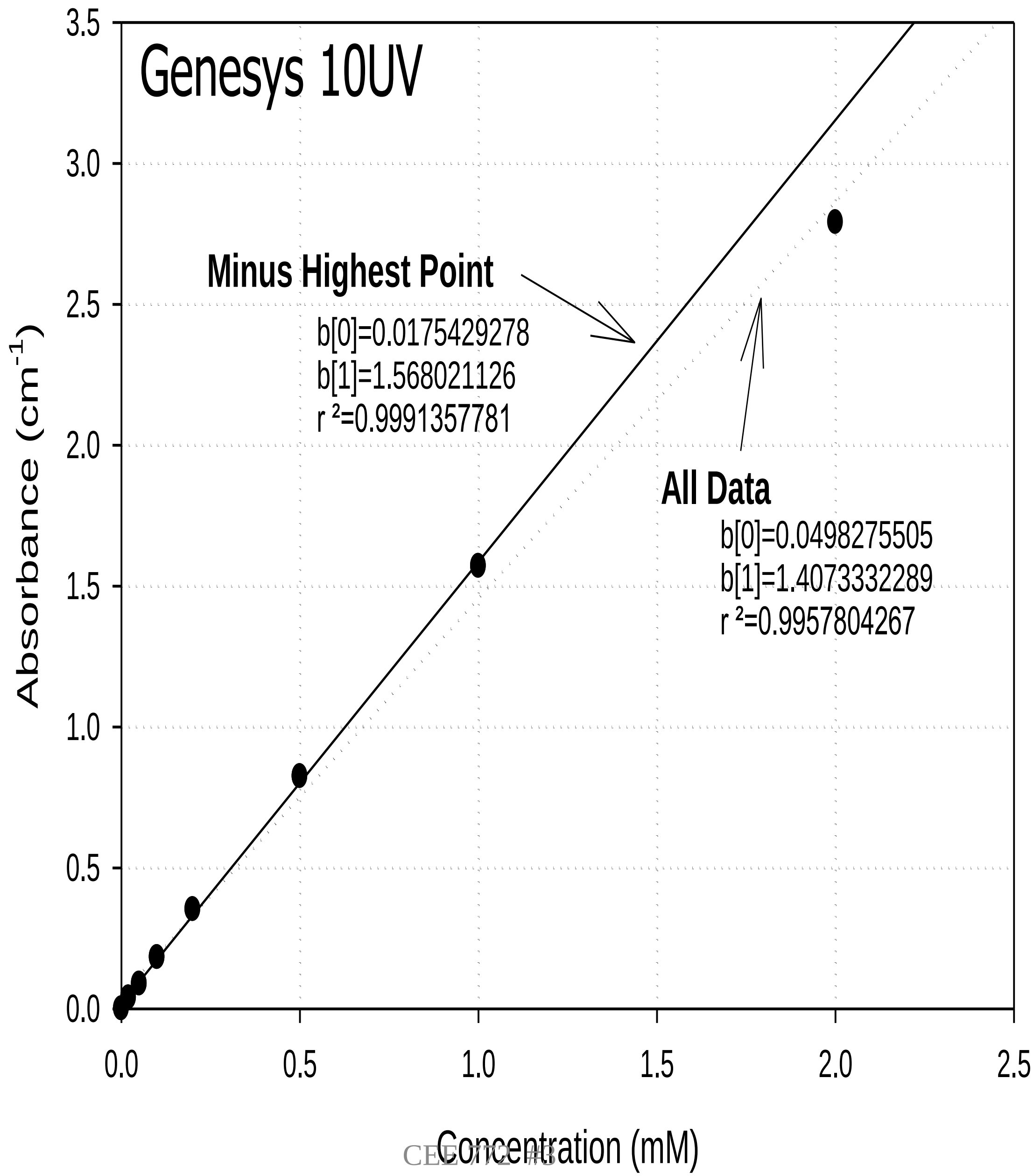
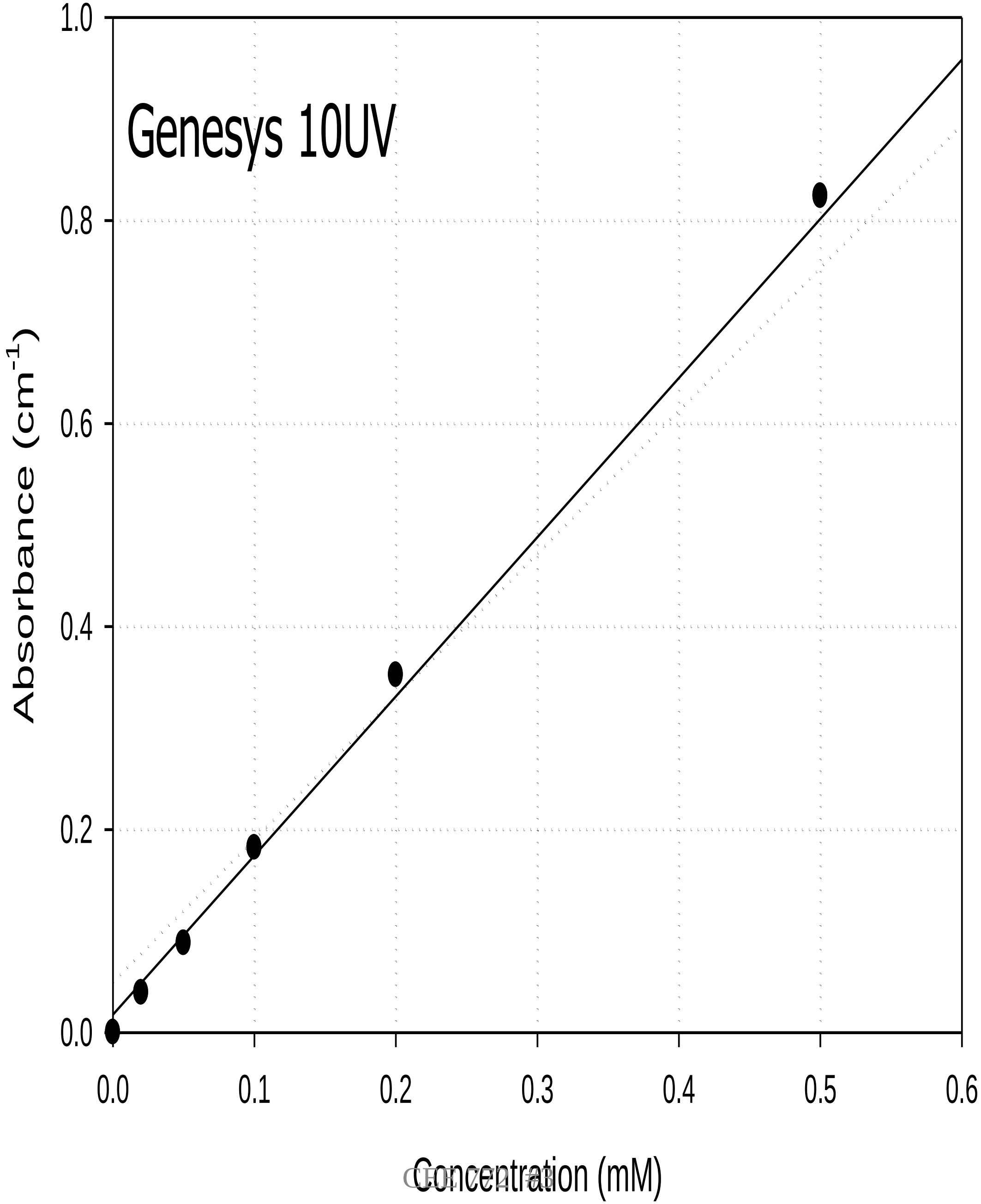
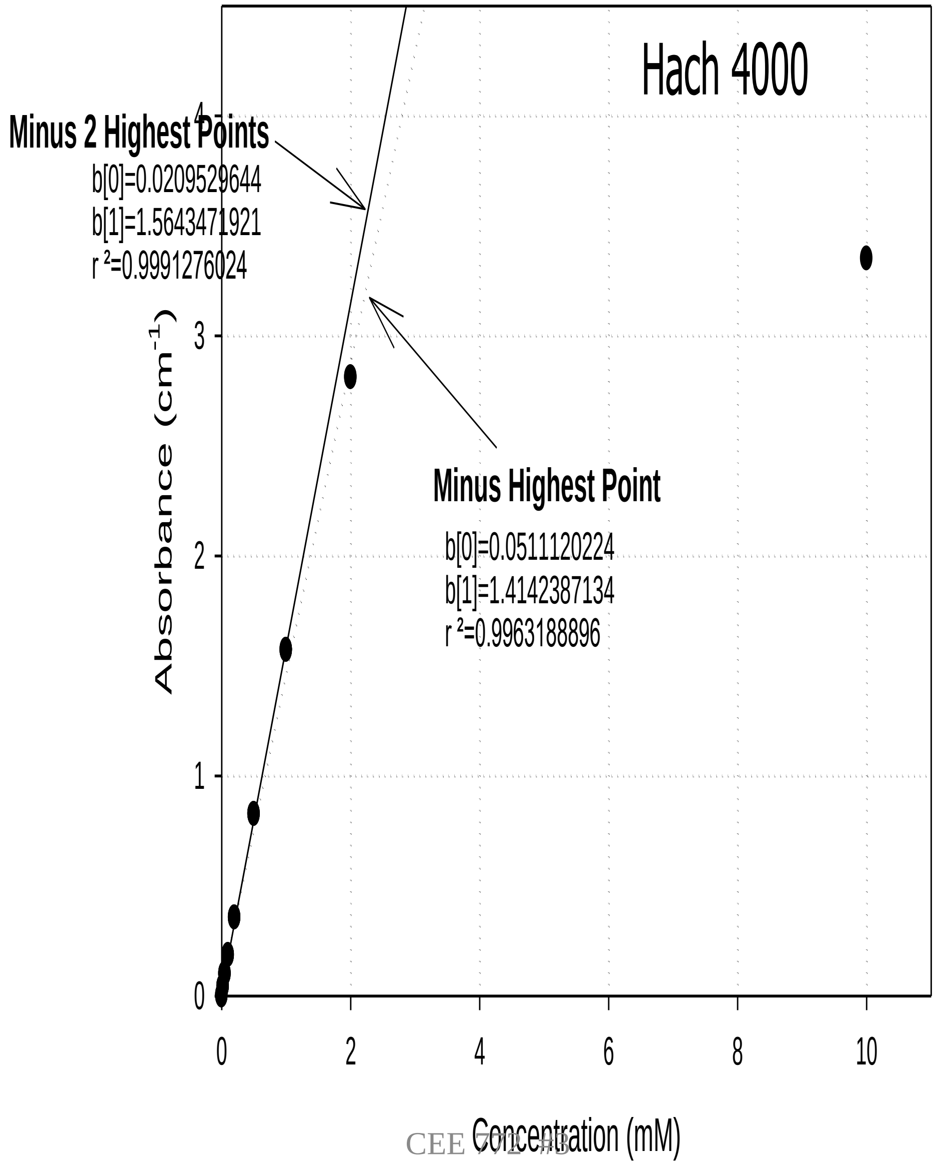
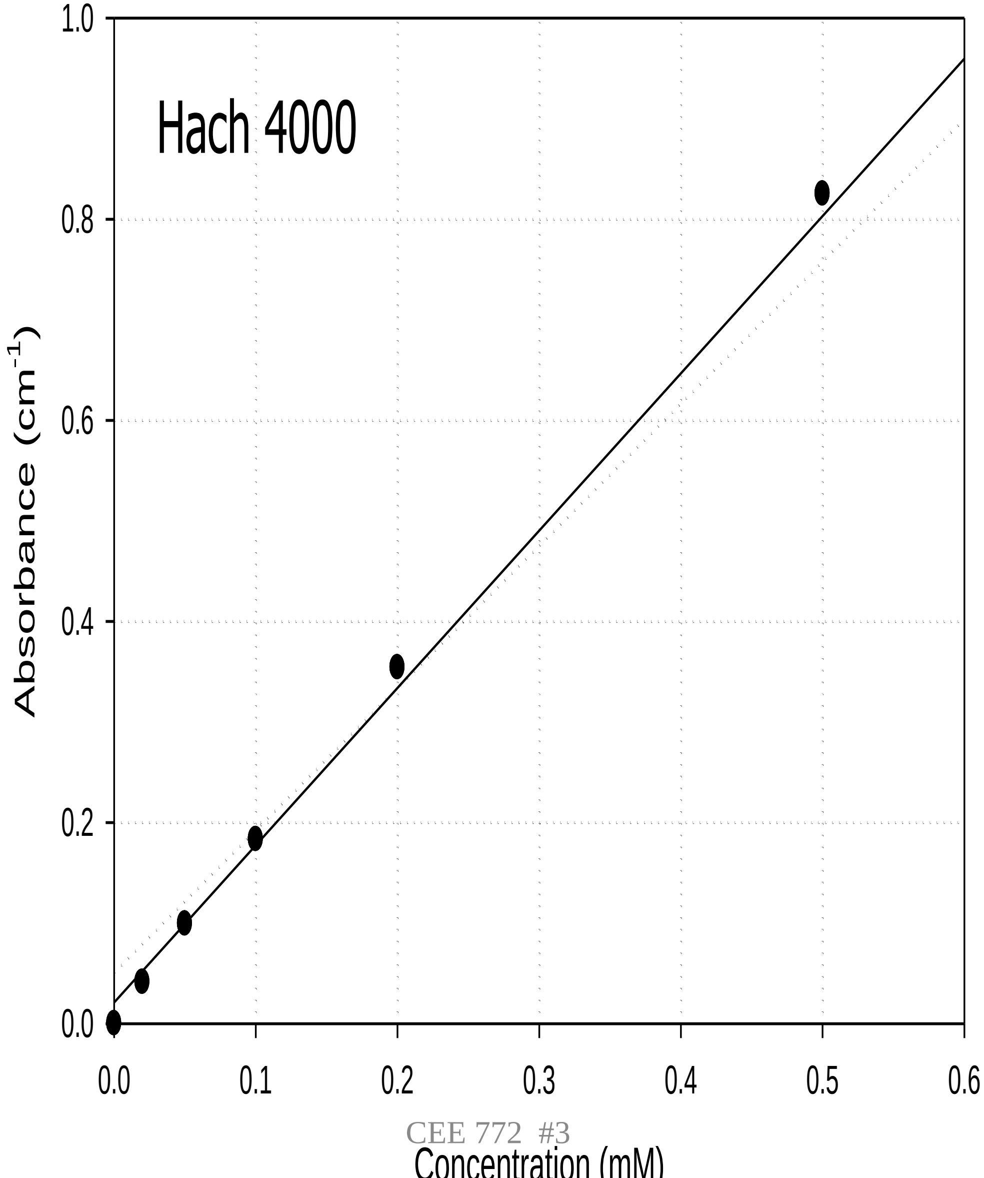
1 Green=good operating condition; blue=some operational problems; red=currently not operating


Genesys 10UV
Minus Highest Point
b[0]=0.0175429278
b[1]=1.568021126
r ²=0.9991357781
All Data
b[0]=0.0498275505
b[1]=1.4073332289
r ²=0.9957804267
Absorbance (cm-1)
Concentration (mM)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5


Genesys 10UV
Absorbance (cm-1)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mM)


Hach 4000
Minus 2 Highest Points
b[0]=0.0209529644
b[1]=1.5643471921
r ²=0.9991276024
Minus Highest Point
b[0]=0.0511120224
b[1]=1.4142387134
r ²=0.9963188896
Absorbance (cm-1)
0
1
2
3
4
0
2
4
6
8
10
Concentration (mM)

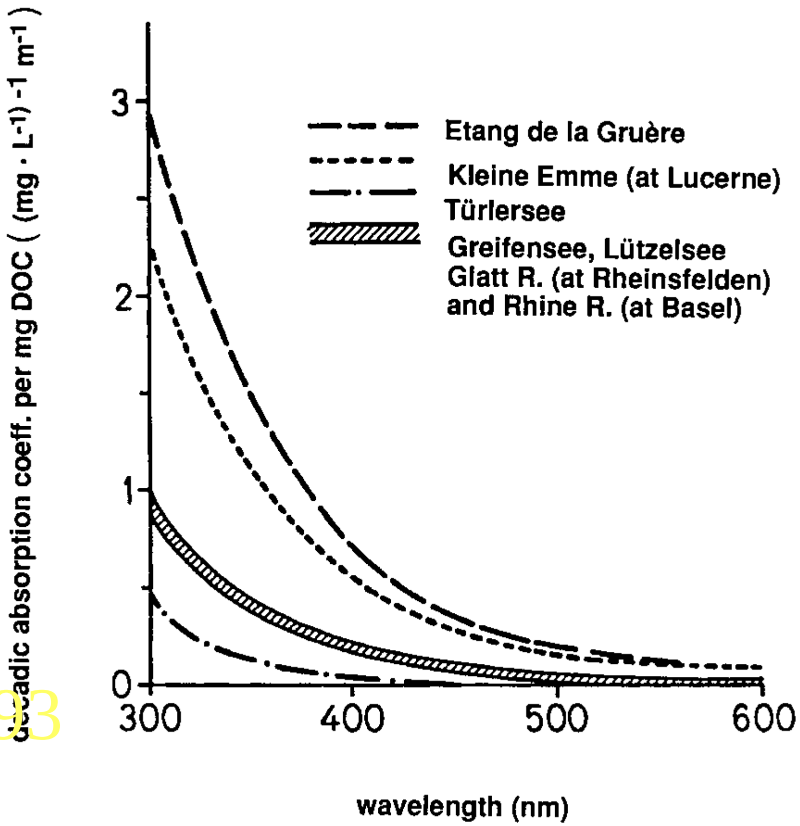

Hach 4000
Absorbance (cm-1)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (mM)

Functional Groups

Compound Type	Formula	Example	λ_{max} (nm)
Alkene	RCH=CHR'	Ethylene	193
Alkyne	RC≡CR'	Acetylene	173
Ketone	RR'C=O	Acetone	271
Aldehyde	RHC=O	Acetaldehyde	293
Carboxyl	RCOOH	Acetic Acid	208
Nitrile	RC≡N	Acetonitrile	<160
Nitro	RNO_2	Nitromethane	271
Amide	$RCONH_2$	Acetamide	208

Background NOM

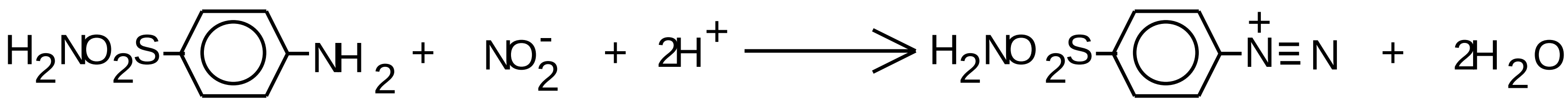
- Specific Absorbance of water samples from several Swiss lakes and rivers



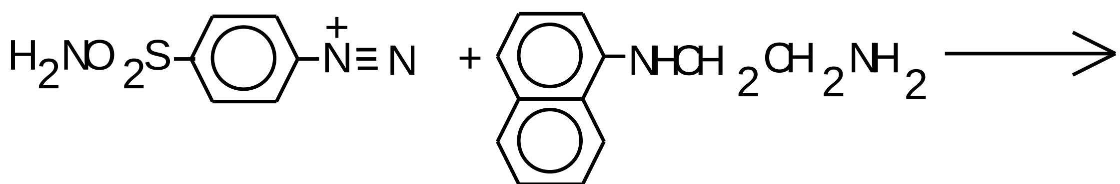
From: Schwarzenbach et al., 1993

Nitrite method

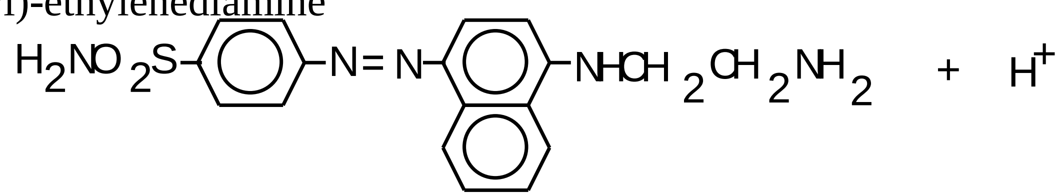
- diazotization



sulfanilamide



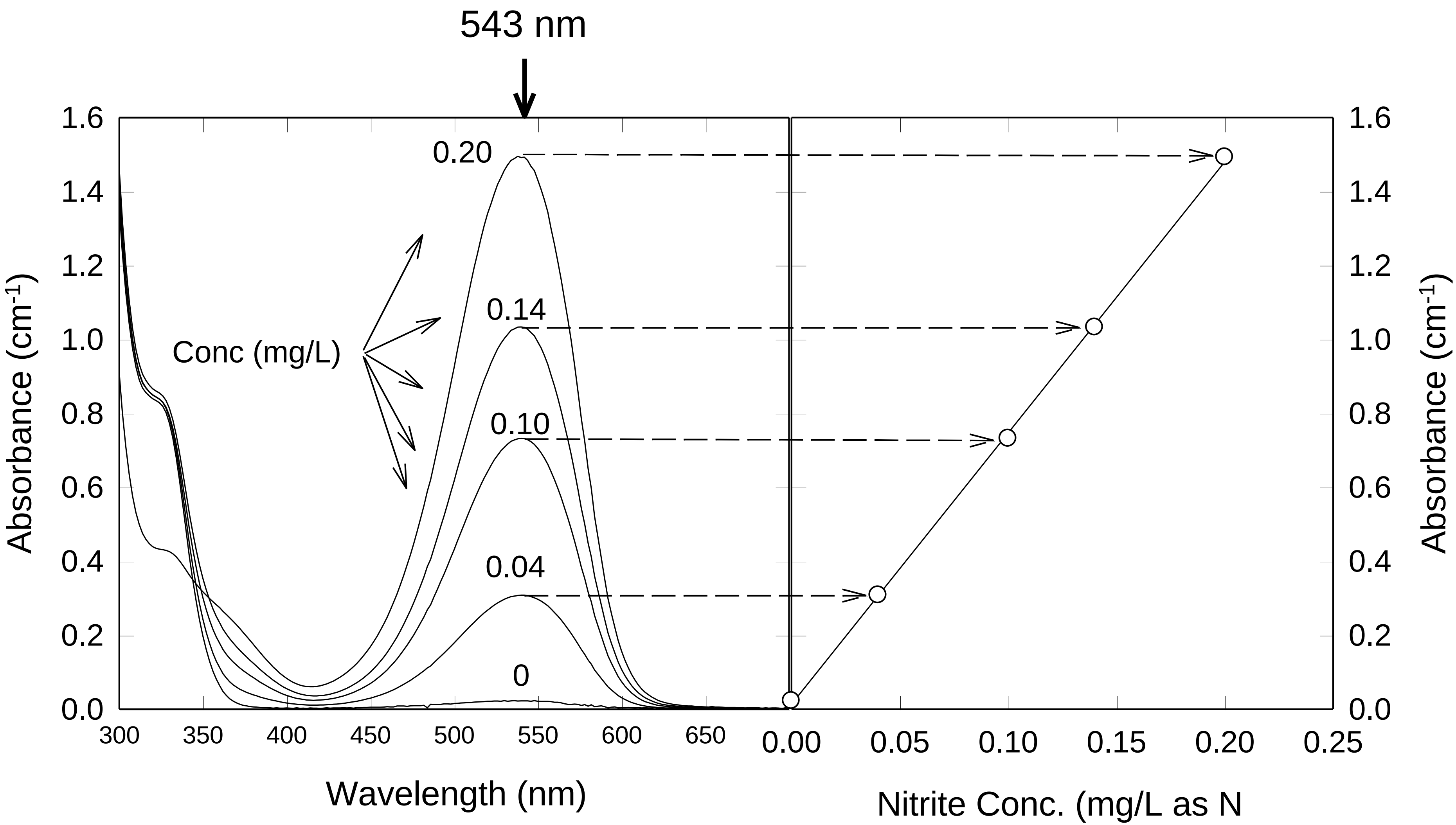
coupling reagent [N-(1-naphthyl)-ethylenediamine dihydrochloride].

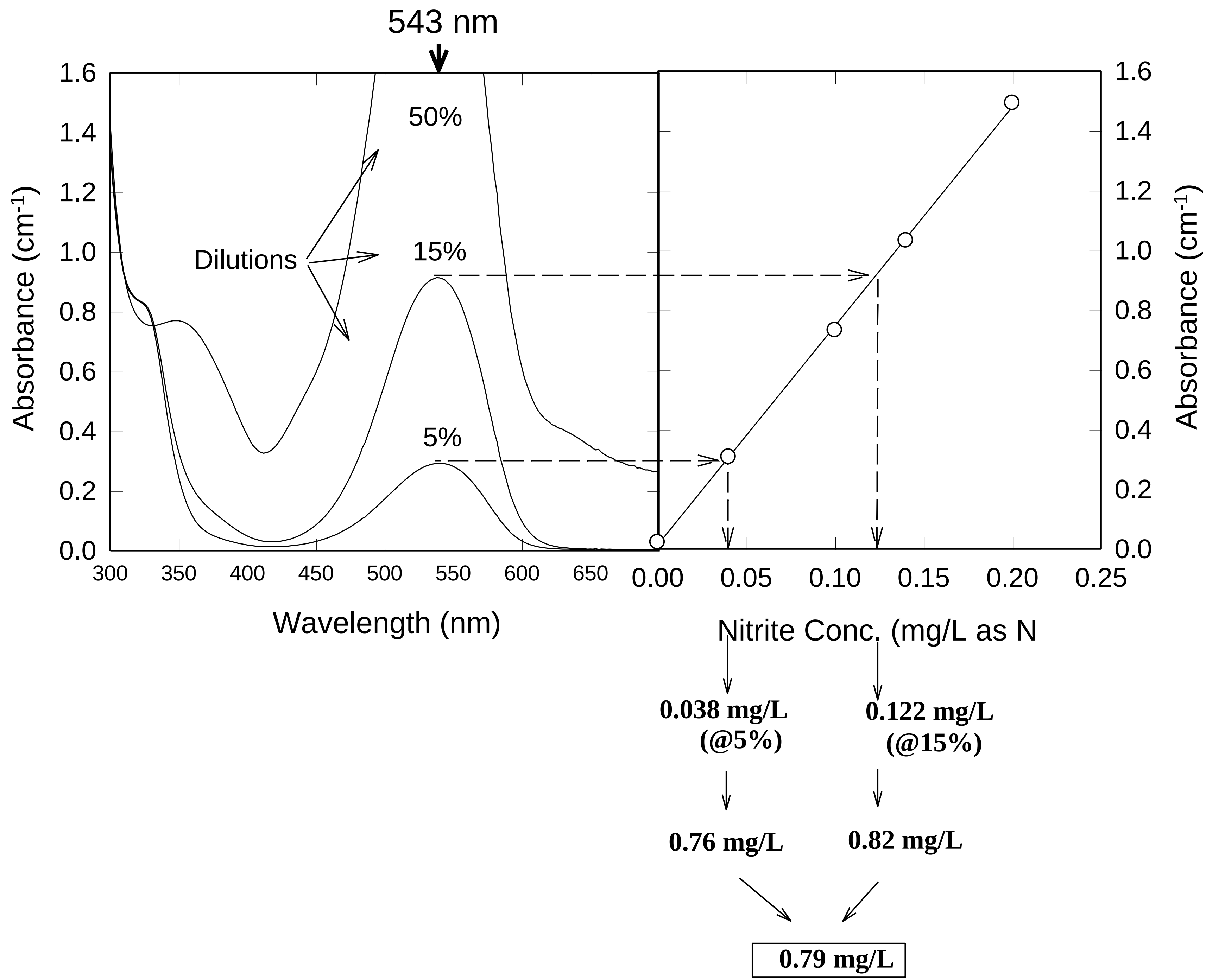


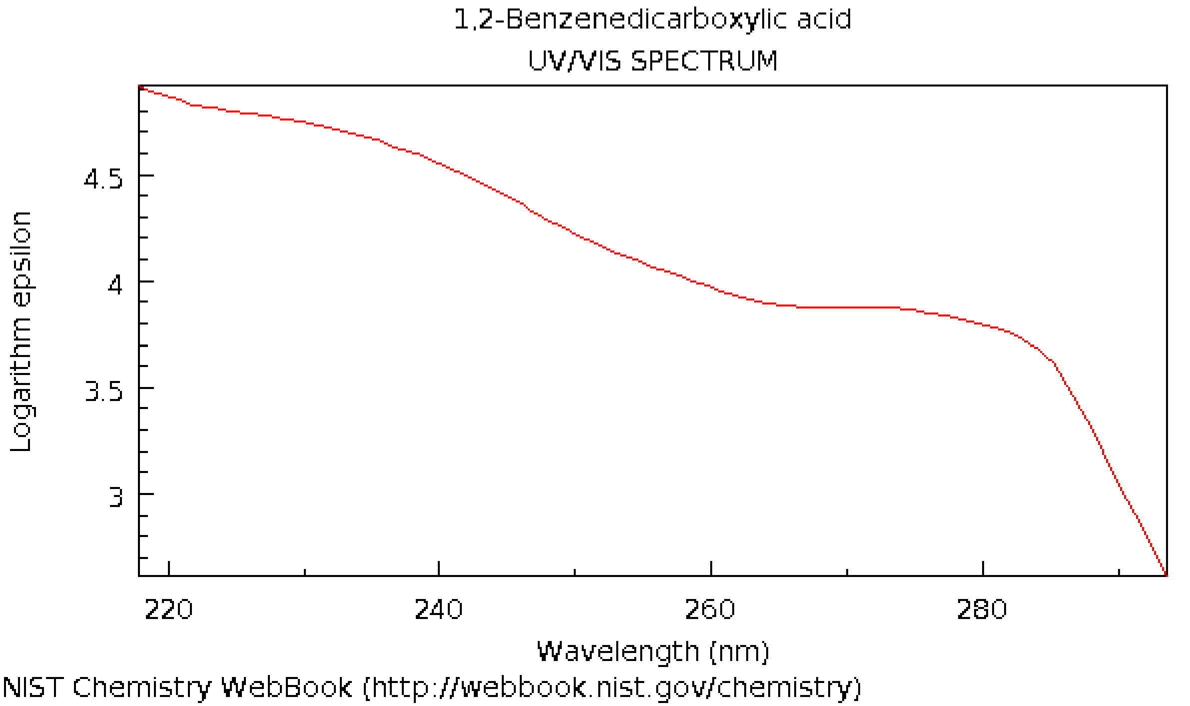
Aromatic amine to diazonium salt.

Nitrite method

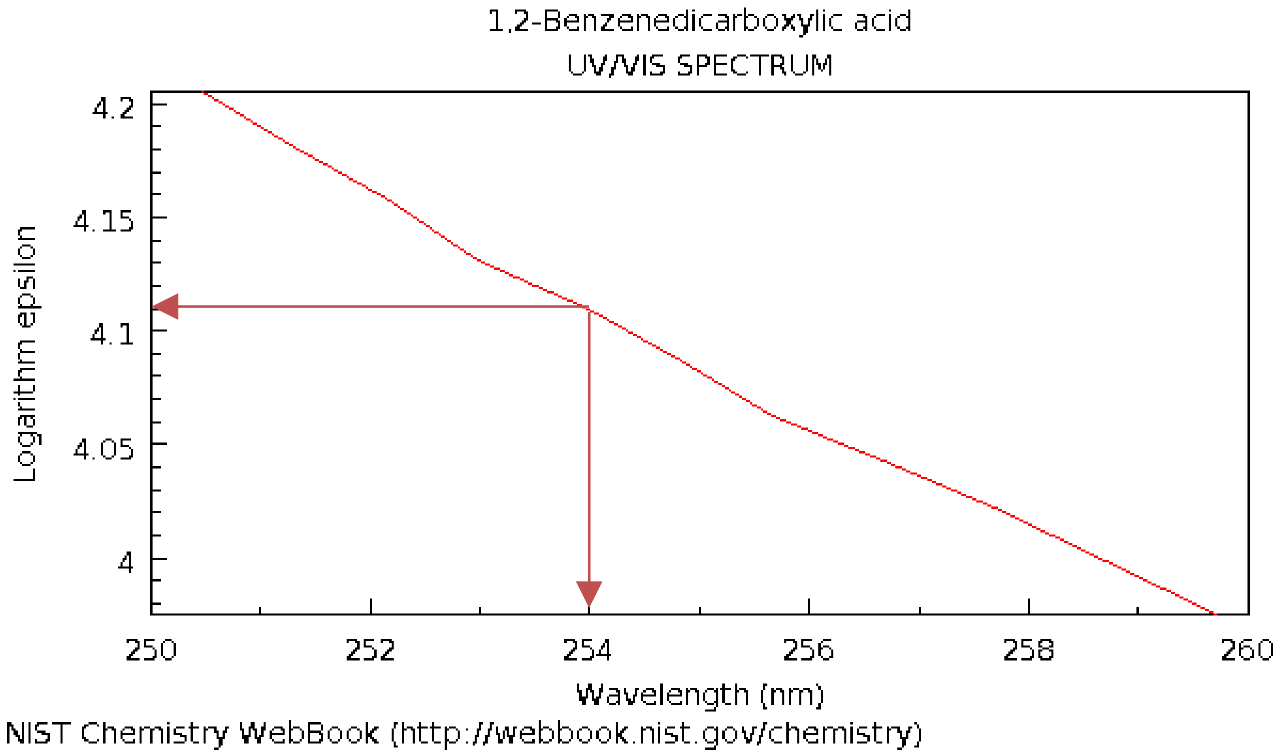
- The NO_2-N is measured colorimetrically following reaction with a diazotizing reagent (sulfanilamide) and a coupling reagent [N-(1-naphthyl)-ethylenediamine dihydrochloride]. A pinkish-purple color develops that is then measured between the wavelengths of 510 and 550 nm; maximum sensitivity is approximately 540 nm (Keeney and Nelson, 1982).

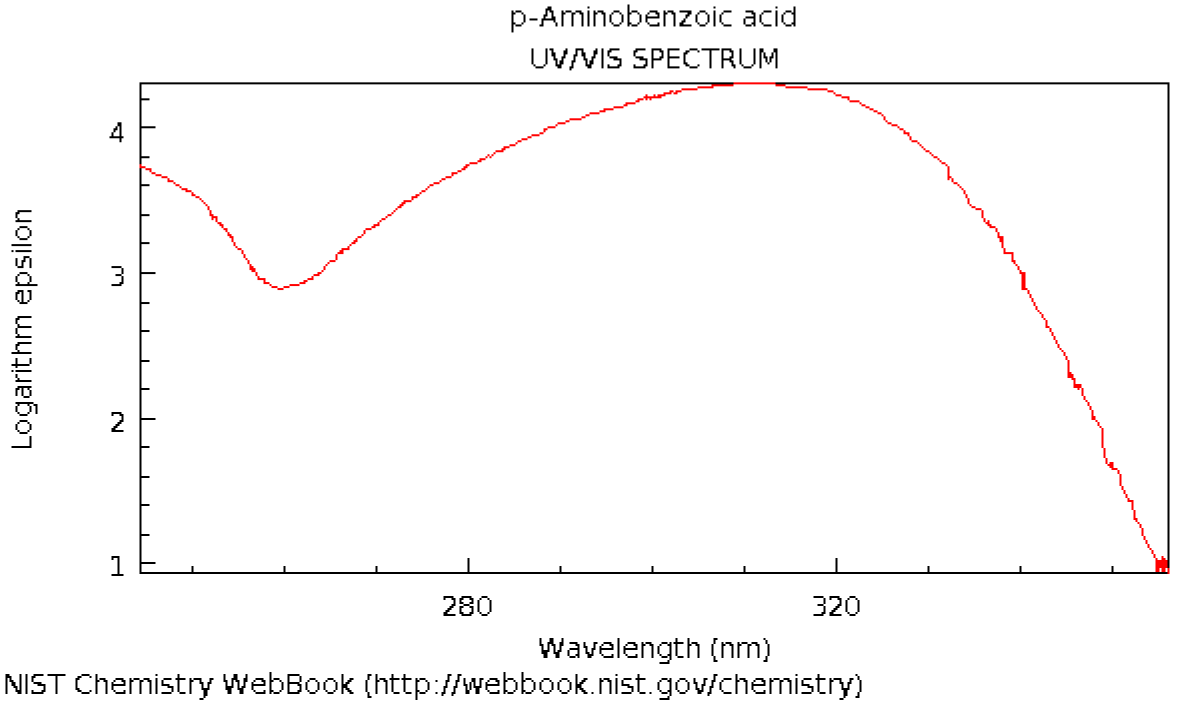

543 nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance (cm-1)
0.20
0.14
0.10
0.04
0
Conc (mg/L)
300
350
400
450
500
550
600
650
Wavelength (nm)
0.00
0.05
0.10
0.15
0.20
0.25
Nitrite Conc. (mg/L as N
Absorbance (cm-1)

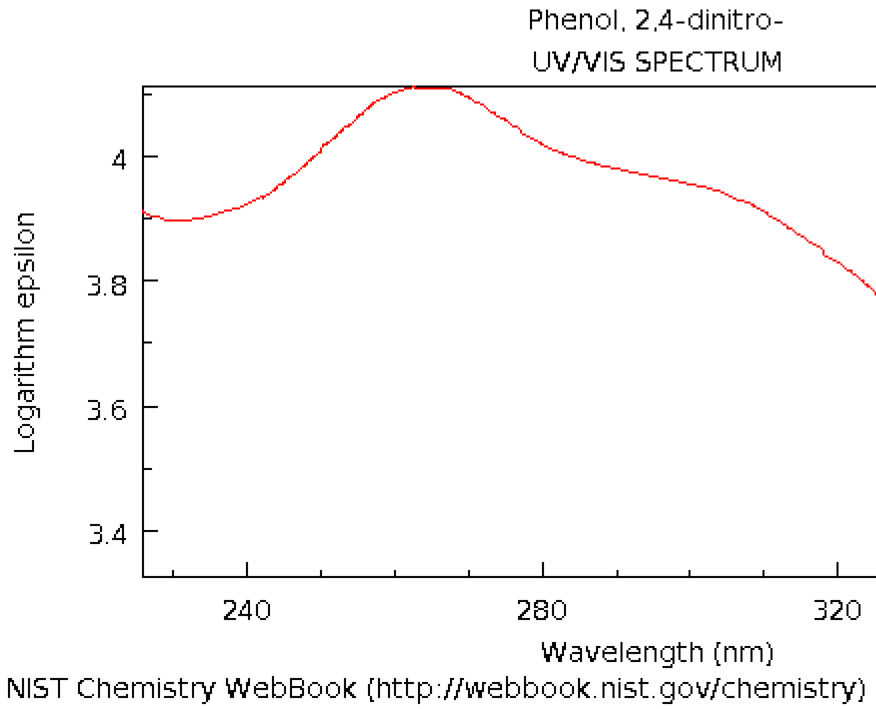

543 nm
50%
15%
5%
Dilutions
Absorbance (cm-1)
Wavelength (nm)
Nitrite Conc. (mg/L as N
0.038 mg/L
(@5%)
0.122 mg/L
(@15%)
0.76 mg/L
0.82 mg/L
0.79 mg/L

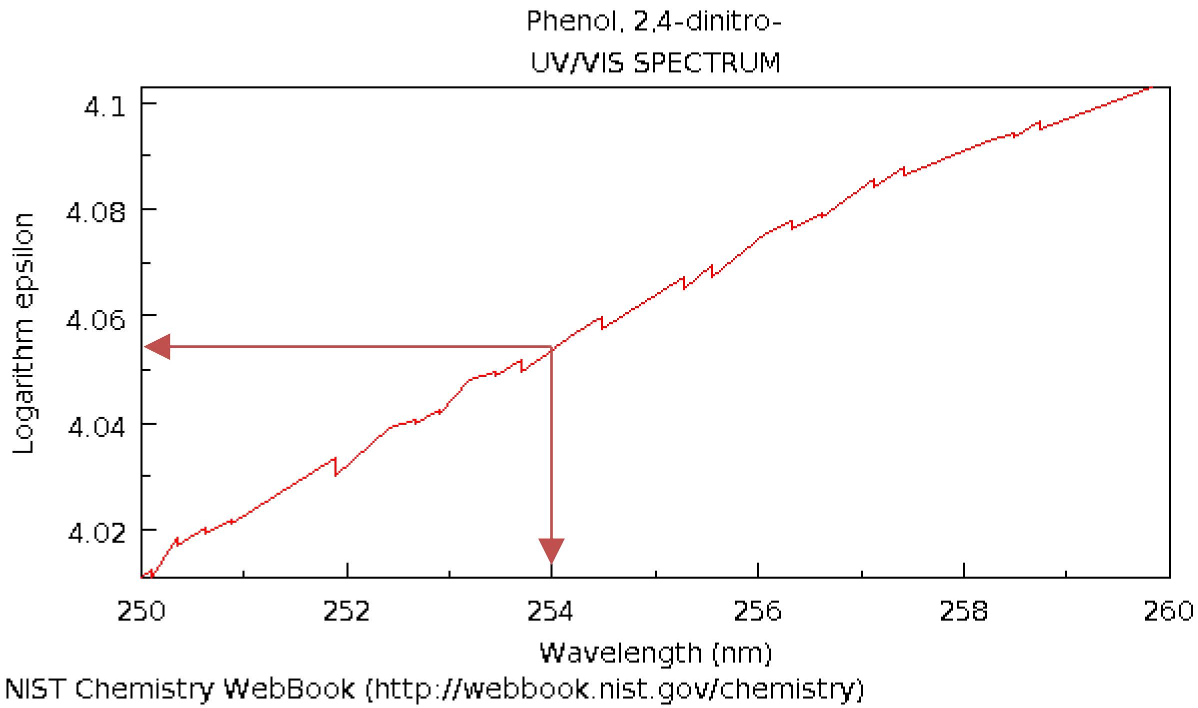


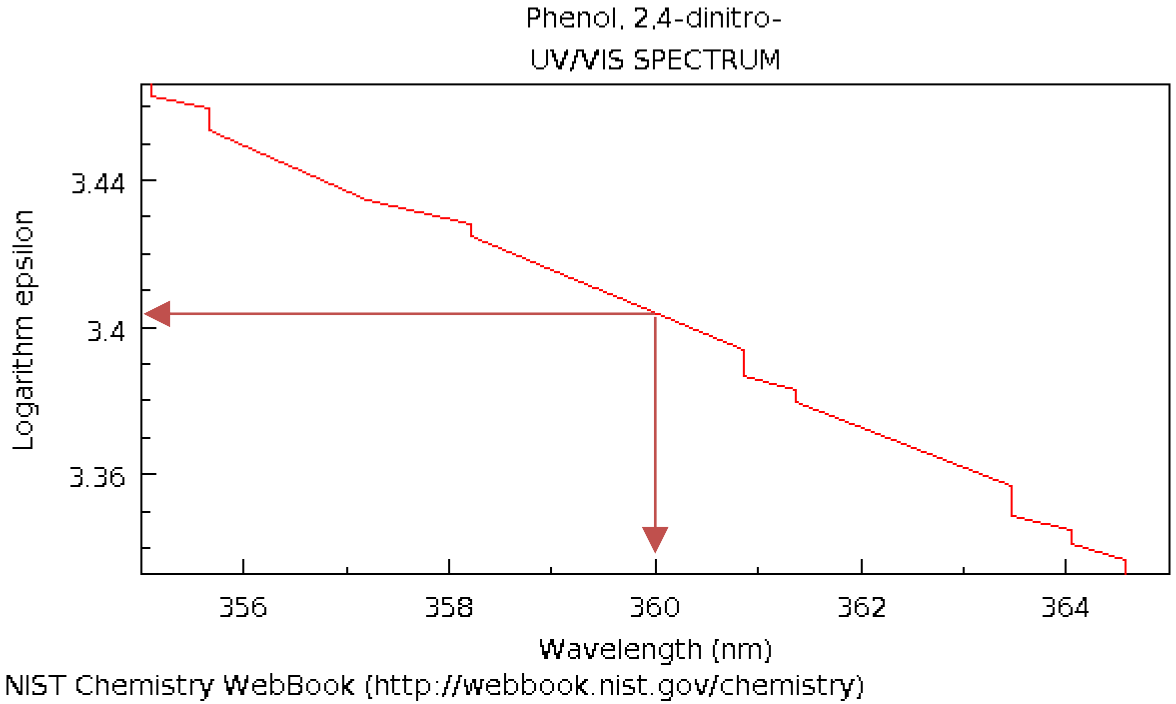
1,2-Benzenedicarboxylic acid
UV/VIS SPECTRUM
Logarithm epsilon
4.5
4
3.5
3
220
240
260
280
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


1,2-Benzenedicarboxylic acid
UV/VIS SPECTRUM
Logarithm epsilon
4.2
4.15
4.1
4.05
4
250
252
254
256
258
260
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


p-Aminobenzoic acid
UV/VIS SPECTRUM
Logarithm epsilon
4
3
2
1
280
320
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


Phenol, 2,4-dinitro-
UV/VIS SPECTRUM
Logarithm epsilon
4
3.8
3.6
3.4
240
280
320
360
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


Phenol, 2,4-dinitro-
UV/VIS SPECTRUM
4.1
4.08
4.06
4.04
4.02
Logarithm epsilon
250
252
254
256
258
260
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)


Phenol, 2,4-dinitro-
UV/VIS SPECTRUM
3.44
3.4
3.36
Logarithm epsilon
356
358
360
362
364
Wavelength (nm)
NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

- To next lecture