NEPHAR 201 Analytical Chemistry II

Chapter 5 Ultraviolet/Visible molecular absorption spectrometry

Assist. Prof. Dr. Usama ALSHANA

	1 [14/09]	Introduction
	2 [21/09]	An introduction to spectromet methods
	3 [28/09]	Components of optical instruments
July 1	4 [05/10]	Atomic absorption and emissi spectrometry
	5 [12/10]	Ultraviolet/Visible molecular absorption spectrometry
ablication of the second	6 [19/10]	Infrared spectrometry
	7	Quiz 1 (12.5 %)
	[26/10]	Chromatographic separations
San Alar	8 [02- 07/11]	MIDTERM EXAM (25 %)
	9 [09/11]	High-performance liquid
HPLC	10 [16/11]	High-performance liquid chromatography (2)
	11 [23/11]	Gas, supercritical fluid and th layer chromatography
	12 [30/11]	Capillary electrophoresis
	13	Quiz 2 (12.5 %)
	[07/12] 14 [14/12]	Revision
	15 [21-	FINAL EXAM (50%)

Week	Торіс	Reference Material	Instructor
1 [14/09]	Introduction	Instructor's lecture notes	Alshana
2 [21/09]	An introduction to spectrometric methods	 Principles of Instrumental Analysis, Chapter 6, pages 116-142 Enstrümantal Analiz- Bölüm 6, sayfa 132-163 	Alshana
3 [28/09]	Components of optical instruments	 Principles of Instrumental Analysis, Chapter 7, pages 143-191 Enstrümantal Analiz- Bölüm 7, sayfa 164-214 	Alshana
4 [05/10]	Atomic absorption and emission spectrometry	 Principles of Instrumental Analysis, Chapter 9, pages 206-229, Chapter 10, pages 230-252 Enstrümantal Analiz- Bölüm 9, sayfa 230-253, Bölüm 10 sayfa 254-280 	Alshana
5 [12/10]	Ultraviolet/Visible molecular absorption spectrometry	 Principles of Instrumental Analysis, Chapter 13, pages 300-328 Enstrümantal Analiz- Bölüm 13, sayfa 336-366 	Alshana
6 [19/10]	Infrared spectrometry	 Principles of Instrumental Analysis, Chapter 16, pages 380-403 Enstrümantal Analiz- Bölüm 16, sayfa 430-454 	Alshana
_	Quiz 1 (12.5 %)		
7 [26/10]	7 [26/10] Chromatographic separations	 Principles of Instrumental Analysis, Chapter 26, pages 674-700 Enstrümantal Analiz- Bölüm 26, sayfa 762-787 	Alshana
8 [02- 07/11]	MIDTERM EXAM (25 %)		
9 [09/11]	High-performance liquid chromatography (1)	• Principles of Instrumental Analysis, Chapter 28,	Alshana
10	High-performance liquid chromatography (2)	 Pages 725-767 Enstrümantal Analiz- Bölüm 28, sayfa 816-855 	Alshana
11 [23/11]	Gas, supercritical fluid and thin- layer chromatography	 Principles of Instrumental Analysis, Chapter 27, pages 701-724, Chapter 29 pages 768-777 Enstrümantal Analiz- Bölüm 27, sayfa 788-815, Bölüm 29 sayfa 856-866, Bölüm 28 sayfa 848-851 	Alshana
12 [30/11]	Capillary electrophoresis	 Principles of Instrumental Analysis, Chapter 30, pages 778-795 Enstrümantal Analiz- Bölüm 30, sayfa 867-889 	Alshana
13	Quiz 2 (12.5 %)		Alshana
[07/12] 14	Extraction techniques	Instructor's lecture notes Instructor's lecture notes and from the above given	Alshens
[14/12]	Kevision	materials	Aisnana
15 [21- 31/12]	FINAL EXAM (50%)		

UV/VIS molecular absorption spectrometry

UV/VIS absorption spectrometry:

- is based on the absorption in the region of 160-780 nm of the radiation spectrum,
- > is used for quantitative determination of **inorganic** and **organic** analytes,
- is based on the measurement of absorbance (A) or transmittance (T) of solutions contained in transparent cells having a path length of b cm.
- Concentration (c) of an absorbing analyte is linearly related to absorbance (A) as represented by Beer's Law:

$$A = -logT = log\frac{P_0}{P} = \varepsilon bc$$

Symbol	Meaning	Unit
A	Absorbance	-
Т	Transmittance	_
P ₀	Intensity of incident light	_
Р	Intensity of emergent light	_
8	Molar absorptivity	$L mol^{-1} cm^{-1}$
b	Path length	ст
С	Analyte concentration	$mol \ L^{-1}$

Measurement of Transmittance and Absorbance

Absorbance or transmittance cannot be measured directly due to:



To solve this problem, the power of the beam transmitted by the analyte solution is compared with the power of the beam transmitted by an identical cell containing only solvent (blank). An experimental transmittance and absorbance are then obtained

with the following equations:

$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0} \qquad A = \log \frac{P_{solvent}}{P_{solution}} \approx \log \frac{P_0}{P}$$



To compensate for radiation loss, a reference cell is used.



Proportionality example

A solution with a concentration of 0.14 M was found to have an absorbance of 0.43. If another solution of the same chemical is measured under the same conditions and has an absorbance of 0.37, what is its concentration?

Solution

For solution 1: $A_1 = \varepsilon_1 b c_1$

For solution 2:

$$A_2 = \varepsilon_2 b c_2$$

$$\Rightarrow \qquad \frac{A_1}{A_2} = \frac{\varepsilon_1 b c_1}{\varepsilon_2 b c_2} \qquad \Longrightarrow \qquad \frac{A_1}{A_2} = \frac{c_1}{c_2}$$

$$\implies \frac{0.43}{0.37} = \frac{0.14 \ M}{c_2} \implies c_2 = 0.12 \ M$$

The Graphical Method

- The graphing method is called for when several sets of data involving STANDARD SOLUTIONS are available for concentration and absorbance. This is probably the most common way of Beer's law analysis based on experimental data collected in the laboratory.
- Graphing the data allows you to check the assumption that Beer's Law is valid by looking for a straight-line relationship for the data.



If the following absorbance values were obtained for standard solutions using a UV/VIS spectrophotometer, what is the concentration of a 1.00 cm (path length) sample that has an absorbance of 0.60?

Concentration (M)	Absorbance
0.10	0.12
0.20	0.27
0.30	0.41
0.40	0.55
0.50	0.69

Solution

- 1. Plot a calibration graph of Absorbance (y-axis) versus Concentration (x-axis).
- 2. Obtain a linear equation for the best-fit line.
- 3. Find the unknown concentration using that equation which gives an absorbance of 0.60.



The **coefficient of determination**, (R^2) is a number that indicates how well data fit a statistical model – sometimes simply a line or a curve. An R^2 of 1.00 indicates that the regression line perfectly fits the data, while an R^2 of 0 indicates that the line does not fit the data at all. The closer to 1.00, the better.

Application of Beer's Law to Mixtures

- Beer's law applies to a medium containing more than one kind of absorbing substance.
- Provided that there is no interaction among the various species, the total absorbance for a multicomponent system is given by:

$$A_{total} = A_1 + A_2 + \dots + An$$
$$= \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \dots + \varepsilon_n b c_n$$

where, the subscripts refer to absorbing components 1, 2, ... and n.

Assumptions in derivation of Beer's Law

- 1. Monochromatic incident radiation (all **molecules absorb** light of one λ),
- 2. Absorbing **molecules act independently** of one another i.e, at low concentration,
- 3. Path length is uniform (all rays travel the same distance in sample),
- 4. No scattering,
- 5. Absorbing medium is optically homogeneous,
- 6. Incident **beam is not** large enough to cause **saturation of the detector**,
- 7. All **rays** are **parallel** to each other and perpendicular to surface of medium.

Limitations (deviations) of Beer's Law



1. Real Limitations

- a) Beer's law is good for dilute analyte solutions only.
- High concentrations (> 0.01 M) will cause a negative error since as the distance between molecules becomes smaller, the charge distribution will be affected which alters the molecules ability to absorb a specific wavelength.
- The same phenomenon is also observed for solutions with high electrolyte concentration, even at low analyte concentration. The molar absorptivity is altered due to electrostatic interactions.

b) In Beer's law we assume that molar absorptivity (ϵ) is a constant.

However, ε is dependent on the refractive index and the refractive index is a function of concentration. Therefore, ε will be concentration dependent. However, the refractive index changes very slightly for dilute solutions and thus we can practically assume that ε is constant.

c) In some cases, the molar absorptivity (ϵ) changes widely with concentration, even at dilute solutions.

Therefore, Beer's law is never a linear relation for such compounds, examples include methylene blue.

2. Chemical Deviations

This factor is an important one which largely affects linearity in Beer's law. It originates when an analyte dissociates, associates, or reacts with the solvent. For example, an acid base indicator (HIn) when dissolved in water will partially dissociate according to its acid dissociation constant (K_a).

$$HIn_{(aq)} \implies H^+ + In_{(aq)}^-$$

Calculated absorbance data for various indicator concentrations

c _{HIn} , M	[HIn], M	[In ⁻], M	A_{430}	A_{570}	
2.00×10^{-5}	0.88×10^{-5}	1.12×10^{-5}	0.236	0.073	
4.00×10^{-5}	2.22×10^{-5}	1.78×10^{-5}	0.381	0.175	
8.00×10^{-5}	5.27×10^{-5}	2.73×10^{-5}	0.596	0.401	
12.0×10^{-5}	8.52×10^{-5}	3.48×10^{-5}	0.771	0.640	
$16.0 imes 10^{-5}$	11.9×10^{-5}	$4.11 imes 10^{-5}$	0.922	0.887	



Chemical deviations from Beer's Law for unbuffered solutions of the indicator HIn

3. Instrumental Deviations

a) Beer's law is good for monochromatic light only since ϵ is wavelength dependent.

It is enough to assume a dichromatic beam passing through a sample to appreciate the need for a monochromatic light.

Deviation from Beer's Law with polychromatic light. The absorber (analyte) has the indicated molar absorptivities at the two wavelengths λ' and λ'' .



Why is it critical to use λ_{max} for measuring absorbance?

Because at λ_{max} (Band A in the figure below) measurements show little deviation since ϵ does not change much throughout the band. On the other hand, using other regions of the spectrum (e.g., band B) gives large deviations due to strong dependence of ϵ on wavelength.



The effect of polychromatic radiation upon Beer's Law.

b) Stray (lost) Radiation

- Stray radiation resulting from
 scattering or various
 reflections in the instrument
 will reach the detector without
 passing through the sample.
- High concentrations of the analyte would scatter more radiation resulting in higher deviations and lead to negative absorbance errors.



Deviation from Beer's Law brought about by various amounts of stray radiation.

INSTRUMENTATION



UV/VIS Spectrophotometer

① Sources of radiation in UV/VIS

Continuum light sources are used in commercial UV/VIS spectrophotometers which include:

- Deuterium (D_2) or hydrogen (H_2) lamps (UV: 160 375 nm),
- Tungsten (W) filament lamps (UV-VIS, NIR: 300 2500 nm),
- Xenon (Xe) arc lamps (UV-VIS: 150 800 nm).



W lamps

D₂ Lamp



(a) A deuterium lamp of the type used in spectrophotometers and (b) its spectrum. The plot is of irradiance E_{λ} (proportional to radiant power) versus wavelength. Note that the **maximum intensity occurs at about 225 nm**. Typically, **instruments switch from deuterium to tungsten at about 350 nm**.





W lamp

(a) A tungsten lamp of the type UV/VIS used in spectrophotometers and its spectrum (b) Intensity of the tungsten source is usually quite low at wavelengths shorter than about 350 nm. Note that the intensity reaches a maximum in the near-IR region of the spectrum (~1200 nm in this case).



2 Sample holders

- Sample holders must be made of a material that is transparent to radiation in the spectral region of interest.
- UV-VIS cuvettes must not absorb in the UV-VIS region.
- The most **common cell length is 1.0 cm**, though 0.1-10 cm are present.







Wavelength, nm 10	00 20	00 4	00 70	0 1000	200	0 40	00 70	00 10,00	0 20,0	00 40,00
Spectral region	VAC	UV	Visible ,	NEA	R IR	4	IR			FAR IR
(a) Materials for			L.F.							
lenses, and prisms	F		Fused silica or quartz							
1		L	Corex	glass						
			Silicate glass			-				
	-				NaCl					
				k	Br					
	-				 TlBr (or TlI				
					6		ZnSe			

Materials for spectroscopic instruments

Location of Sample Cell

- In all photometers and scanning spectrophotpmeters described above, the cell has been positioned after the monochromators. This is important to decrease the possibility of sample photodecomposition due to prolonged exposure to all frequencies coming from the continuum source.
- However, the sample is positioned before the monochromator in multichannel instruments like a photodiode array spectrophotometers. This can be done without fear of photodecomposition since the sample exposure time is usually less than 1 s. Therefore, it is now clear that in UV-VIS where photodecomposition of samples can take place, the sample is placed after the monochromators in scanning instruments while positioning of the sample before the monochromators is advised in multichannel instruments.



A photodiode is a **semiconductor** device that converts light into current. The current is generated when photons are absorbed in the photodiode. Photodiodes may contain optical filters, built-in lenses, and may have large or small surface areas. Photodiodes usually have a slow response time as their surface area increases. The common, traditional **solar cell** used to generate electric solar power is a large area photodiode. Photomultiplier tubes, members of the class of vacuum tubes, are **extremely sensitive detectors** of light in the ultraviolet, visible, and near-infrared ranges of the electromagnetic spectrum. These detectors multiply the current produced by incident light by as much as **100 million times** in multiple dynode stages, enabling (for example) individual photons to be detected when the incident flux of light is very low.





Three Si and one Ge (top) photodiodes.

Photomultiplier tube (PMT)

Types of UV/VIS Instruments



1 Single-Beam Instruments



- The cuvette with a **blank** (i.e., the **solvent**) is placed in the instrument and a reading is taken and is **considered as 100% T**.
- The cuvette is replaced with the sample containing the analyte and a reading is taken. The **observed % T is for the analyte** (from which absorbance can be calculated).

2 Double-Beam Instruments

(a) Double-beam-in-space



- Light is split by a V-shaped mirror and directed towards both reference cell (blank) and sample cell at the same time.
- Two detectors are used that subtract the blank from sample.
- Advantages over single-beam:
 - 1. Compensates for fluctuations in source intensity and drift in detector,
 - 2. Better design for continuous recording of spectra.

2 Double-Beam Instruments



- Light is split in-time by a sector mirror and directed towards both reference cell (blank) and sample cell one after the other but very rapidly.
- **One detector** is used that subtracts the blank from sample.
- Offers the same advantages as for the double-beam-in-space.

3 Multichannel Instruments

- Photodiode array detectors (DAD) are used (multichannel detectors can measure all wavelengths dispersed by grating simultaneously).
- Advantage: scan spectrum very quickly "snapshot" < 1 s.
- Powerful tool for studies of transient intermediates in moderately fast reactions.
- Useful for kinetic studies.
- Useful for qualitative and quantitative determination of the components exiting from a

liquid chromatographic column.



DAD



Diagram of a multichannel spectrophotometer based on a grating monochromator and a photodiode array detector (DAD).