Technical Assistance
HYDROLOGY PROJECT

World Bank & Government of The Netherlands funded

Training module # WQ - 34

Absorption Spectroscopy

New Delhi, February 2000

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DHV Consultants BV & DELFT HYDRAULICS

with
HALCROW, TAHAL, CES, ORG & JPS

Table of contents

		Page
1	**Module context**	**2**
2	**Module profile**	**3**
3	**Session plan**	**4**
4	**Overhead/flipchart master**	**5**
5	**Evaluation sheets**	**22**
6	**Handout**	**24**
7	**Additional handout**	**29**
8	**Main text**	**31**

1. Module context

This module introduces the principles of absorption spectroscopy and its applications in chemical analyses. Other related modules are listed below.

While designing a training course, the relationship between this module and the others, would be maintained by keeping them close together in the syllabus and place them in a logical sequence. The actual selection of the topics and the depth of training would, of course, depend on the training needs of the participants, i.e. their knowledge level and skills performance upon the start of the course.

No.	Module title	Code	Objectives
1.	Basic chemistry concepts	**WQ - 02**	• Convert units from one to another • Discuss the basic concepts of quantitative chemistry • Report analytical results with the correct number of significant digits.
2.	Basic aquatic chemistry concepts	**WQ - 24**	• Understand equilibrium chemistry and ionisation constants. • Understand basis of pH and buffers • Calculate different types of alkalinity.
3.	Major ions in water	**WQ - 28**	• Know the major ions in water and their sources • Understand the significance of major ion concentrations
4.	Advanced aquatic chemistry: solubility equilibria	**WQ - 29**	• Explain the principles of chemical equilibrium • Define solubility product and explain how this relates to water quality assessment • Define the octanol-water partition coefficient and explain how this relates to water quality assessment.
5.	Behaviour trace compounds in the aquatic environment	**WQ - 31**	• Give examples of trace contaminants and explain their pollutant properties • Explain behaviour of trace contaminants in aquatic environment

2. Module profile

Title	:	Absorption Spectroscopy
Target group	:	HIS function(s): Q2, Q3, Q5, Q6
Duration	:	One session of 60 min
Objectives	:	After the training the participants will be able to: • understand the principle of absorption spectroscopy • explain the use of absorption spectroscopy for chemical analyses
Key concepts	:	• absorption spectroscopy • spectrophotometers • applications
Training methods	:	Lecture, exercises, discussion, demonstration of instruments
Training tools required	:	Board, flipchart, OHS
Handouts	:	As provided in this module
Further reading and references	:	• Chemistry for environmental engineers - C. N. Sawyer, P. L. McCarty & G. F. Parkin, McGraw - Hill, Inc., 1994 • Standard methods for the examination of water and wastewaters, AWWA, 19th edition, 1995

3. Session plan

No	Activities	Time	Tools
1	***Preparations***		
2	***Introduction***: • Ask participants to state different instruments for water quality analysis • Define colorimetric analysis	10 min	OHS
3	***Absorption Spectroscopy: theoretical concepts and definitions:*** • Relation between color and light absorption • Wavelength of Maximum absorption • Beer-Lambert Law	20 min	OHS
4	***The Spectrophotometer*** • Main components of instrument	10 min	OHS
5	***Measurement*** • General Procedures • Standard curve • Parameters that can be measured	10 min	OHS
6	***Exercise***		
7	***Wrap up and Evaluation***	10 min	

4. Overhead/flipchart master

OHS format guidelines

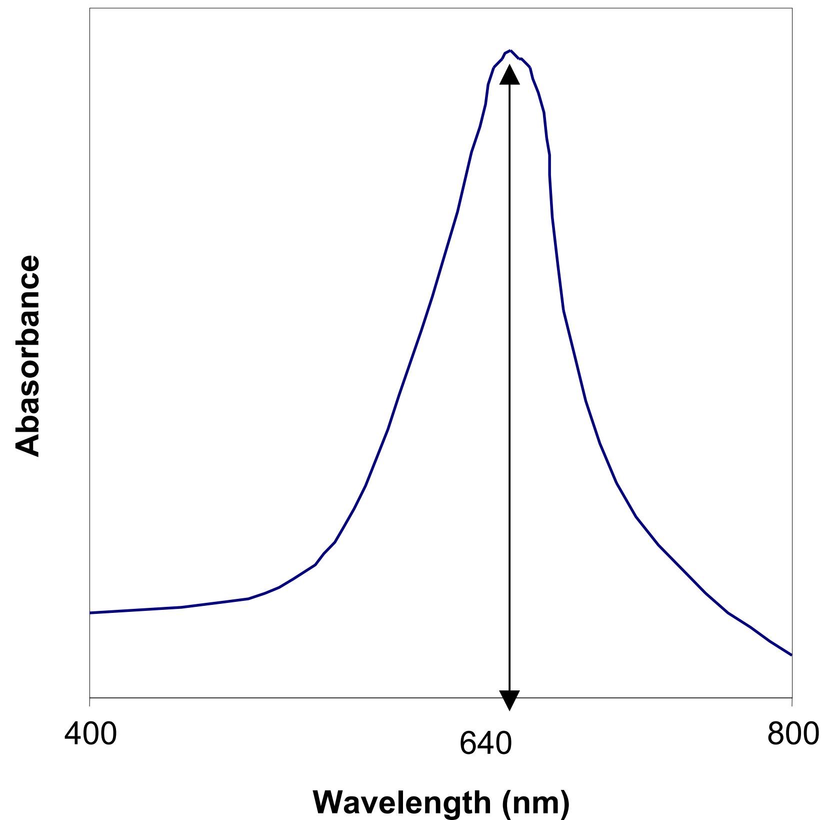
Type of text	Style	Setting
Headings:	OHS-Title	Arial 30-36, with bottom border line (not: underline)
Text:	OHS-lev1 OHS-lev2	Arial 24-26, maximum two levels
Case:		Sentence case. Avoid full text in UPPERCASE.
Italics:		Use occasionally and in a consistent way
Listings:	OHS-lev1 OHS-lev1-Numbered	Big bullets. Numbers for definite series of steps. Avoid roman numbers and letters.
Colours:		None, as these get lost in photocopying and some colours do not reproduce at all.
Formulas/Equations	OHS-Equation	Use of a table will ease horizontal alignment over more lines (columns) Use equation editor for advanced formatting only

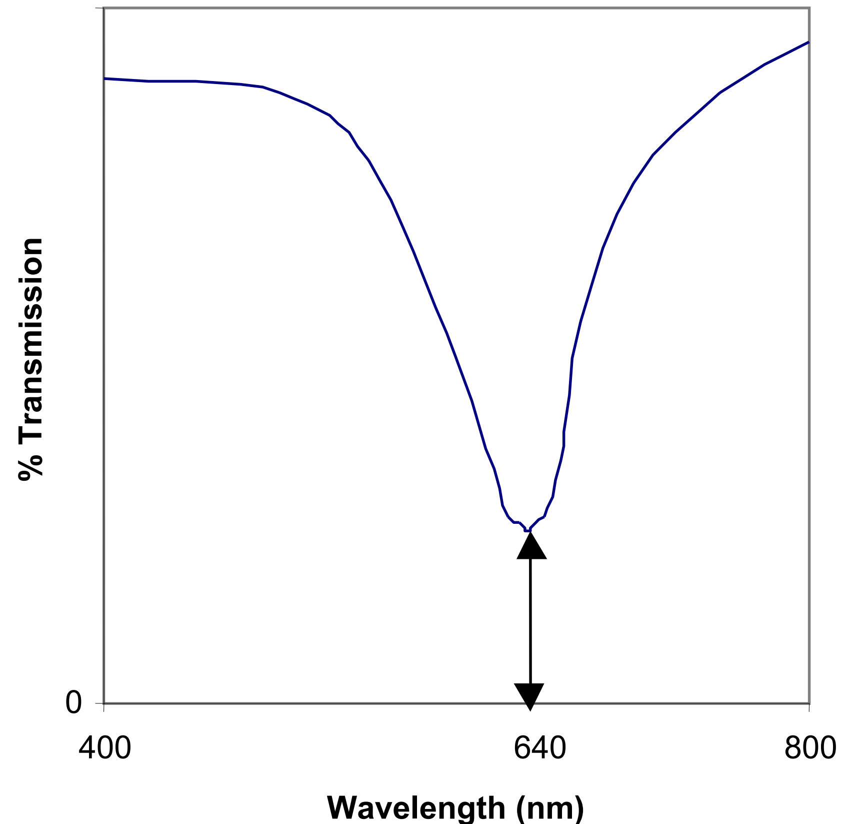
Absorption Spectroscopy: Introduction (1)

- Most widely used method
- Compounds absorb light radiation
  - *directly or after reaching with added chemicals*
  - *visible range: colorimetry*
  - *UV & IR: compound may be colourless*
- PO_4^{3-} forms coloured molybdenum blue, absorb 640 nm
- NO_3^- colourless, absorb 220 nm

Absorption Spectroscopy: Introduction (2)

- Phosphate has maximum light ABSORPTION at λ = 640

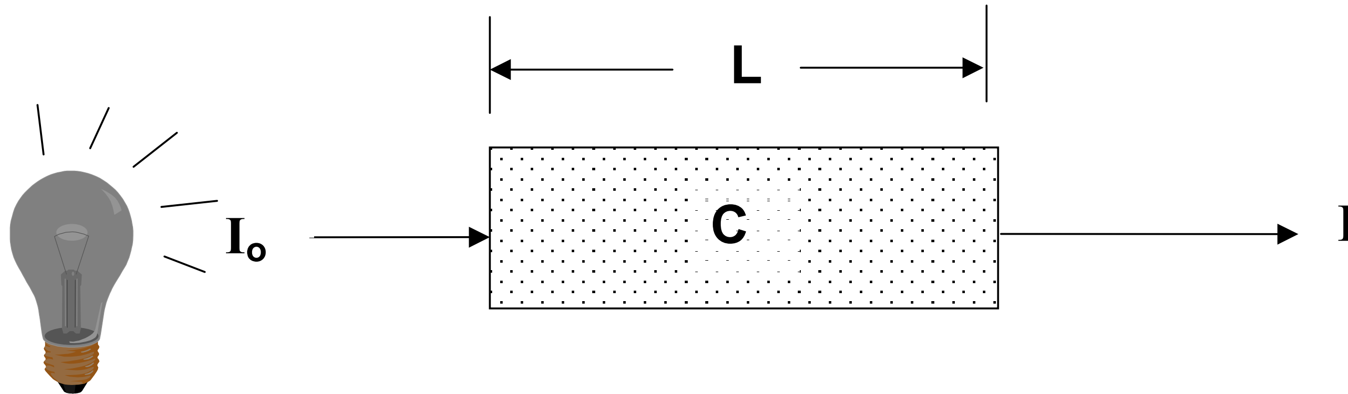




Beer-Lambert Law (1)

$$\text{Light Transmission}\,(T) = \frac{I}{I_0} = 10^{-KCL}$$

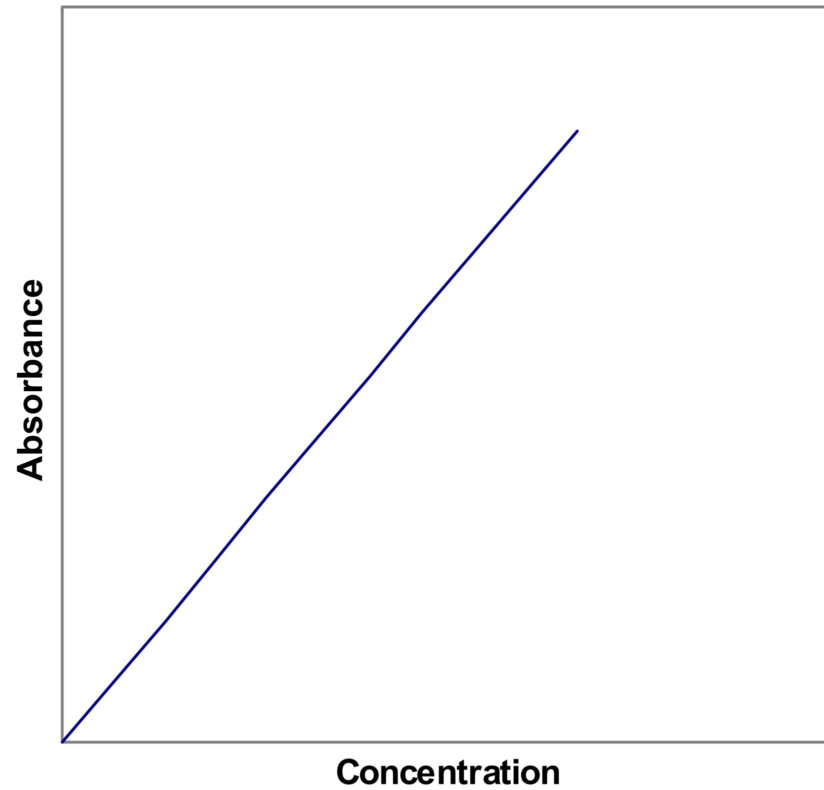
$$\text{Light Absorbance}\,(A) = \log\left(\frac{I_0}{I}\right) = KCL$$

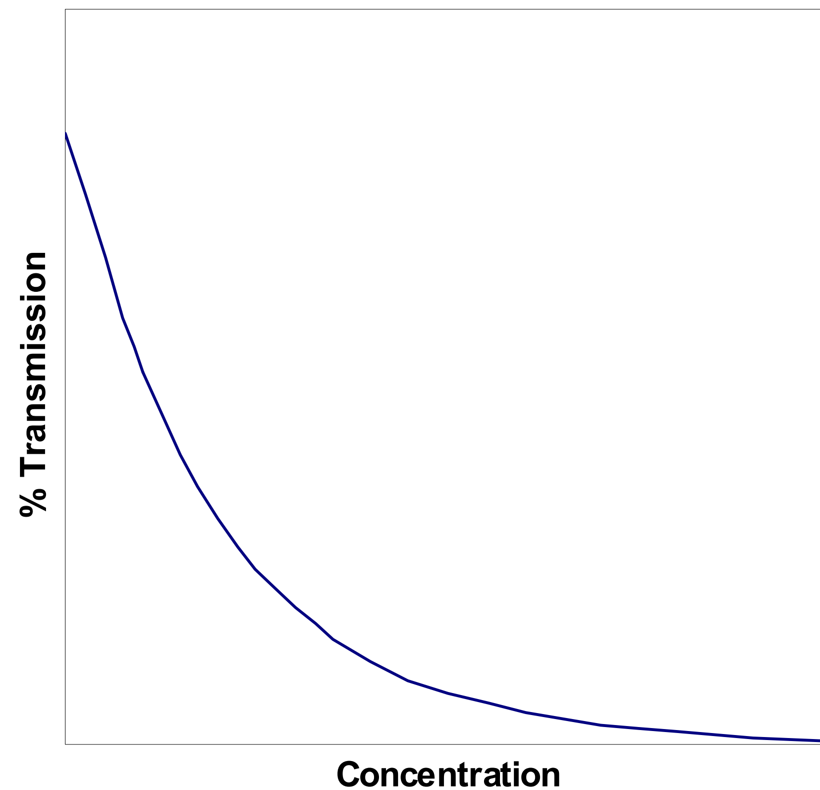


Beer-Lambert Law

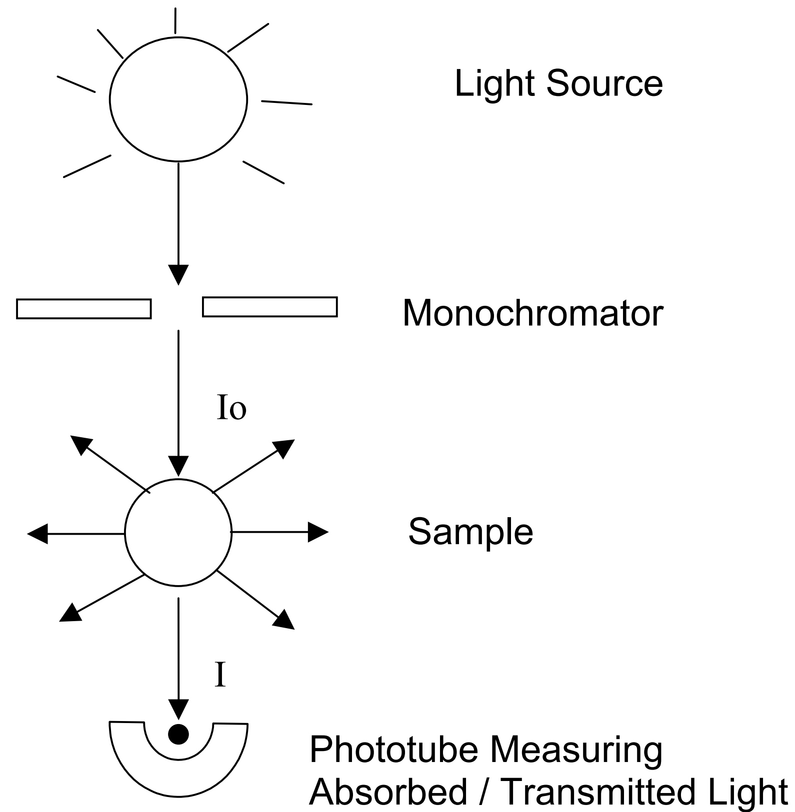
- Relates light Absorbance (A) to Concentration (C) and the length (L) of light path
- K is a constant,depends on the compound and wavelength.
- As Concentration (C) increases, light Absorbance (A) increases, *linearly*
- As Concentration (C) increases, light Transmission (T) decreases, *exponentially*

Beer-Lambert Law (3)





Spectrophotometer (1)



Spectrophotometer

Spectrophotometer (2)

- Light source
  - *UV:* λ *= 200-400 nm*
  - *Visible:* λ *= 400-800 nm*
- Detector and readout
  - *Displays absorbance (optical density) and percent transmission (transmittance)*
- One light source and detector can not be used for all wavelengths

Spectrophotometer (3)

- Wavelength selection
  - *filters (photometers)*
  - *monochromator (spectrophotometers)*
- Monochromatic light
  - *validity of Beer-Lambert Law*
  - *less interference from substance absorbing at other wavelengths*
  - *increases sensitivity*

Spectrophotometer (4)

- Sample containers, cells, cuvettes
  - *transparent to wavelengths of interest*
  - *quartz or fused silica : UV to 2 μm in IR*
  - *Silicate glass: > 350 nm to 2 μm in IR*
  - *Plastic: visible region*
  - *Polished NaCl or AgCl: >2μm*
- Matched cuvettes

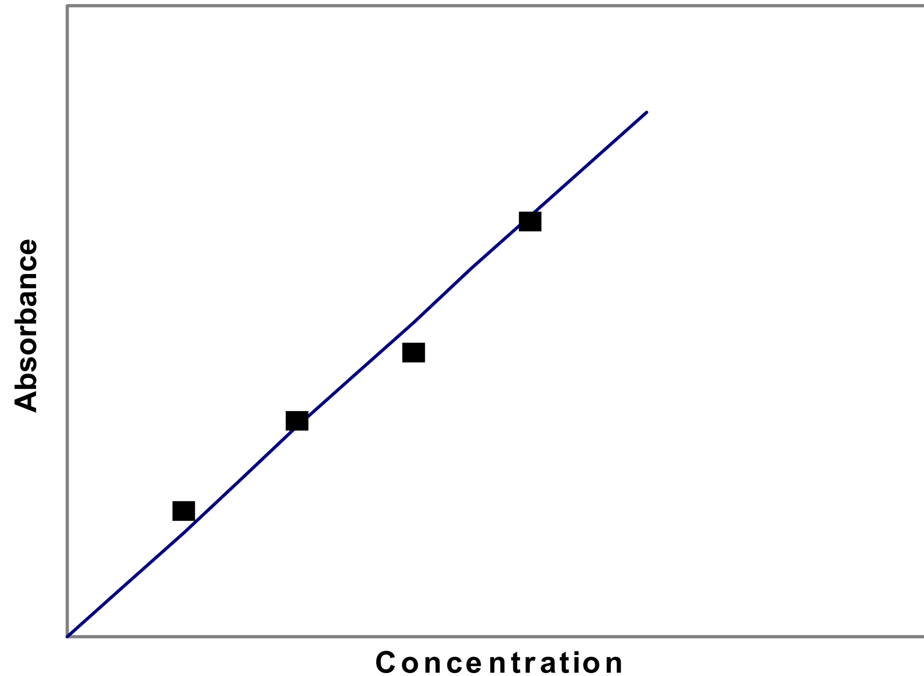
Measurement (1)

- Beer-Lambert Law forms basis of measurment:

1. Prepare samples to make coloured compound
2. Make series of standard solutions, known concentrations
3. Set Spectrophotometer to λ of maximum light absorption
4. Measure light absorption of standards
5. Plot standard curve: Absorption vs. Concentration

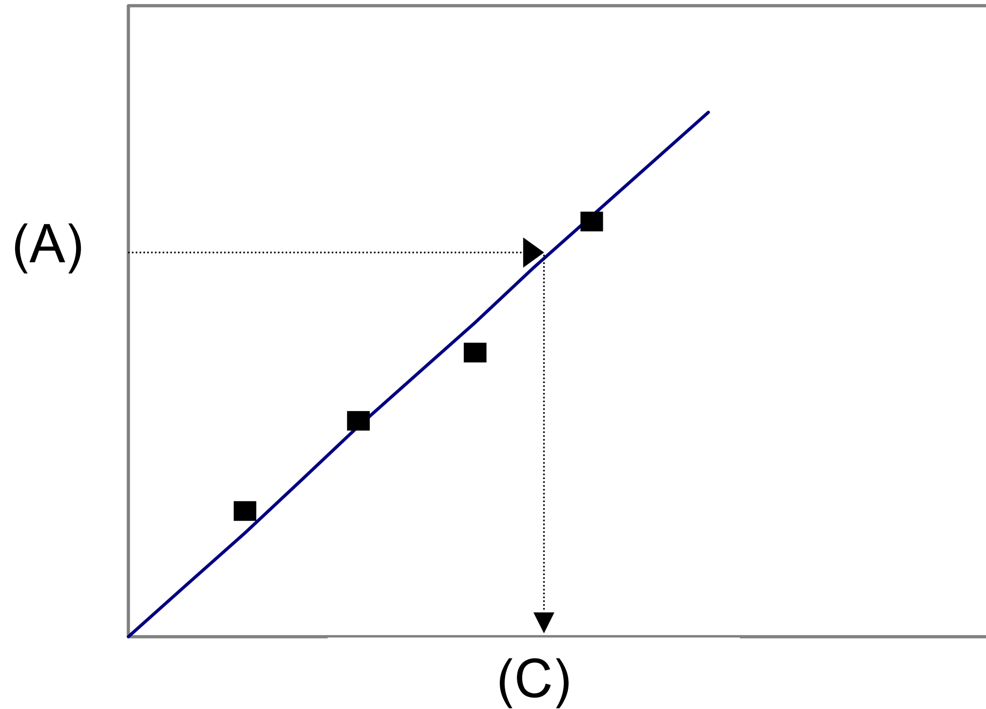
Measurement (2)

- Standard plot: Linear relation between Absorbance & Conc.



Measurement (3)

- Measure (A) of unknown, read (C) from Standard plot



Calibration by standard addition

- When sample matrix influences absorption
- Read absorbance of unknown sample
- Add a known standard to another aliquot and read absorbance
- From change in absorbance due to known standard, calculate the sample conc.
- Assumes Beer-Lambert Law holds

Standard Addition: Example

- 25mL sample aliquot: absorbance = 0.428
- 25mL sample aliquot + 1mL std containing 5.0μg: absorbance = 0.517
- Correction for dilution: (26/25) x 0.517 = 0.538
- Absorbance due to 5.0μg: 0.538-0.428 = 0.110
- Therefore sample contains: (5.0/0.11) x 0.428 = 19.5μg

Absorption Spectroscopic: Measurements (1)

- Many parameters measured with spectrophotometer

Parameter	λ of Maximum absorption
Boron	540
Chlorophyll a	750, 664, 665
Flouride	570
Iron	510
Manganese	525
NH_3-N	640
NO_3-N	220
o-PO_4	880
Silica	815
Sulphate (Note: Nephelometry is preferred method)	420

Absorption Spectroscopic Measurements (2)

- Wide applicability
- High sensitivity: detection limit 10^{-5}M to 10^{-4}M
- Moderate to high selectivity
- Good accuracy: relative error 1 to 3%
- Ease and convenience, lend to automation

5. Evaluation sheets

6. Handout

Absorption Spectroscopy: Introduction (1)

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- Ease and convenience, lend to automation

Add copy of Main text in chapter 8, for all participants.

7. Additional handout

These handouts are distributed during delivery and contain test questions, answers to questions, special worksheets, optional information, and other matters you would not like to be seen in the regular handouts.

It is a good practice to pre-punch these additional handouts, so the participants can easily insert them in the main handout folder.

8. Main text

Contents

1.	**Introduction**	**1**
2.	**Theory**	**1**
3.	**The Spectrophotometer Instrument**	**3**
4.	**General Measurement Procedures**	**4**
5.	**Overview of individual methods**	**5**

Absorption Spectroscopy

1. Introduction

Absorption Spectroscopic methods of analysis rank among the most widespread and powerful tools for quantitative analysis. The use of a spectrophotometer to determine the extent of absorption of various wavelengths of *visible* light by a given solution is commonly known as *colorimetry.* This method is used to determine concentrations of various chemicals which can give colours either directly or after addition of some other chemicals.

As an example, in the analysis of phosphate, a reaction with orthophosphate is made, to form the highly coloured molybdenum blue compound. The light absorption of this compound can then be measured in a spectrophotometer.

Some compounds absorb light in other than the visible range of the spectrum. For example, nitrates absorb radiation of 220 nm wave length in the UV region.

2. Theory

Absorption Spectroscopic methods of analysis are based upon the fact that compounds ABSORB light radiation of a specific wavelength. In the analysis, the amount of light radiation absorbed by a sample is measured. The light absorption is directly related to the concentration of the coloured compound in the sample.

The wavelength (λ) of Maximum Absorption is known for different compounds. For example, the coloured compound formed for analysis of Phosphate (molybdenum blue) has maximum light absorption at λ= 640 nm. Conversely, a minimum amount of light is *transmitted* through the compound at λ= 640 nm. This is shown schematically in Figure 1.

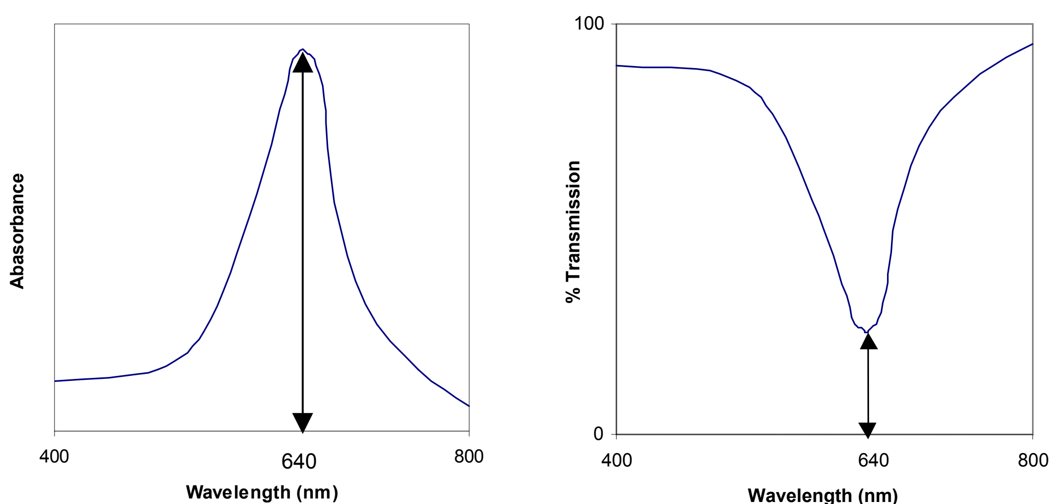


Figure 1: Light Absorption and Transmission by Phosphate-molybdenum blue compound. Schematic diagram showing maximum light absorption (and minimum light transmission) at λ= 640 nm.

The Beer-Lambert Law

The Beer-Lambert Law is illustrated in Figure 2. The Absorbance (or optical density) and Transmission (or Transmittance) of light through a sample can be calculated by measuring light intensity entering and exiting the sample.

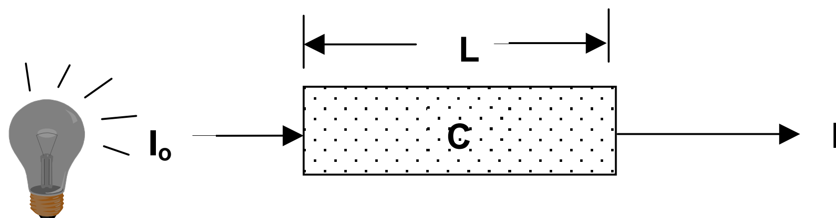


Figure 2: Light energy of Intensity 'I_o' passes through a sample with concentration 'C'. Some light energy is absorbed by the sample. The amount of light energy exiting the sample has Intensity 'I'

The following terms are defined:

- Light Intensity entering a sample is "I_o"
- Light Intensity exiting a sample is "I"
- The Concentration of analyte in sample is "C"
- The length of the light path in glass sample cuvette is "L"
- "K" is a constant for a particular solution and wave length

The Beer-Lambert Law is given by the following equations:

$$\text{Light Absorbance (A)} = \log\left(\frac{Io}{I}\right) = KCL$$

$$\text{Light Transmission (T)} = \frac{I}{I_o} = 10^{-KCL}$$

Plots of absorbance and transmission versus concentration of the analyte in sample according to the above equations is shown in Figure 3.

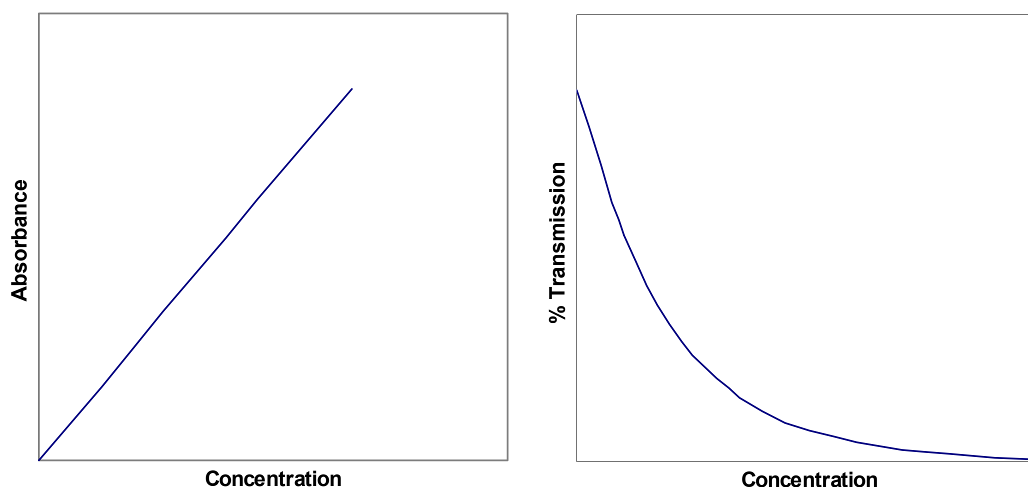


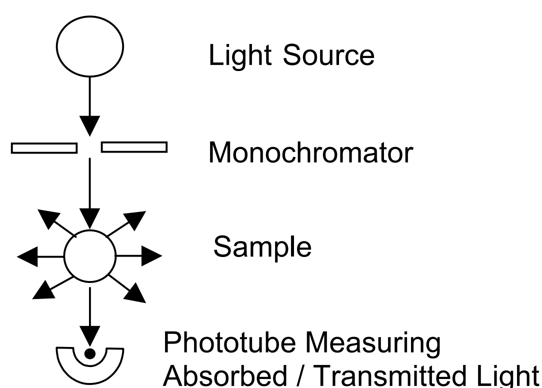
Figure 3: Beer-Lambert Law relates the amount of light Absorbance (A) by a solution to the Concentration (C) of a compound in solution and the length of light path:

- **As Concentration (C) increases, light Absorption (A) increases, linearly**
- **As Concentration (C) increases, light Transmission (T) decreases, exponentially**

3. The Spectrophotometer Instrument

All spectrophotometer instruments designed to measure the absorption of radiant energy have the basic components as follows (Figure 4):

(1) a stable source of radiant energy (Light);
(2) a wavelength selector to isolate a desired wavelength from the source (filter or monochromator);
(3) transparent container (cuvette) for the sample and the blank;
(4) a radiation detector (phototube) to convert the radiant energy received to a measurable signal; and a readout device that displays the signal from the detector.



Spectrophotometer

Figure 4: Components of a spectrophotometer

The energy source is to provide a stable source of light radiation, whereas the wavelength selector permits separation of radiation of the desired wavelength from other radiation. Light radiation passes through a glass container with sample. The detector measures the energy after it has passed through the sample. The readout device calculates the amount of light absorbed by the sample displays the signal from the detector as absorbance or transmission.

The spectrophotometers, which are used for such measurements may vary from simple and relatively inexpensive colorimeters to highly sophisticated and expensive instruments that automatically scan the ability of a solution to absorb radiation over a wide range of wavelengths and record the results of these measurements.

One instrument cannot be used to measure absorbance at all wavelengths because a given energy source and energy detector is suitable for use over only a limited range of wavelengths.

True linearity between absorbance and concentration according to Beer-Lambert Law requires the use of monochromatic light. In addition, a narrow band of light ensures a greater selectivity since substance with absorption peaks in other close by wavelengths are less likely to interfere. Further, it increases sensitivity as there is a greatest change in absorbance per increment of change in concentration.

Both filters and monochromators are used to restrict the radiation wavelength. Photometers make use of filters, which function by absorbing large protions of the spectrum while

transmitting relatively limited wavelength regions. Spectrophotometers are instruments equipped with monochromators that permit the continuous variation and selection of wavelength. The effective bandwidth of a monochromator that is satisfactory for most applications is about from 1 to 5 nm.

The sample containers, cells or cuvettes, must be fabricated from material that is transparent to radiation in the spectral region of interest. The commonly used materials for different wave length regions are:

Quartz or fused silica :	UV to 2 μm in 1R
Silicate glass:	Above 350 nm to 2 μm in 1R
Plastic:	visible region
Polished NaCl or AgCl:	Wave lengths longer than 2μm

Cuvettes or cells are provided in pairs that have been carefully matched to make possible the transmission through the solvent and the sample. Accurate spectrophotometric analysis requires the use of good quality, matched cells. These should be regularly checked against one another to detect differences that can arise from scratches, etching and wear. The most common cell path for UV-visible region is 1cm. For reasons of economy, cylindrical cells are frequently used. Care must be taken to duplicate the position of such cells with respect to the light path; otherwise, variations in path length and in reflection losses will introduce errors.

4. General Measurement Procedures

As explained above, the Beer-Lambert Law forms the basis of the measurement procedure. The amount of light radiation absorbed by a compound is directly related to the concentration of the compound.

The general measurement procedure consists of 5 steps:

1. Prepare samples to make coloured compound
2. Make series of standard solutions of known concentrations and treat them in the same manner as the sample for making coloured compounds
3. Set spectrophotometer to λ of maximum light absorption
4. Measure light absorbance of standards
5. Plot standard curve: Absorbance vs. Concentration, as shown in Figure 5

Once the standard plot is made, it is simple to find the concentration of an unknown sample: Measure the absorption of the unknown, and from the standard plot, read the related concentration (Figure 6).

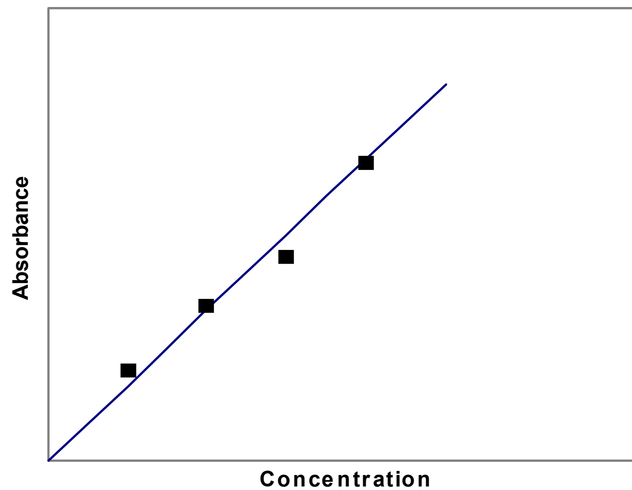


Figure 5: Plot of the standard curve: showing the linear relation between light absorption and concentration of the standards

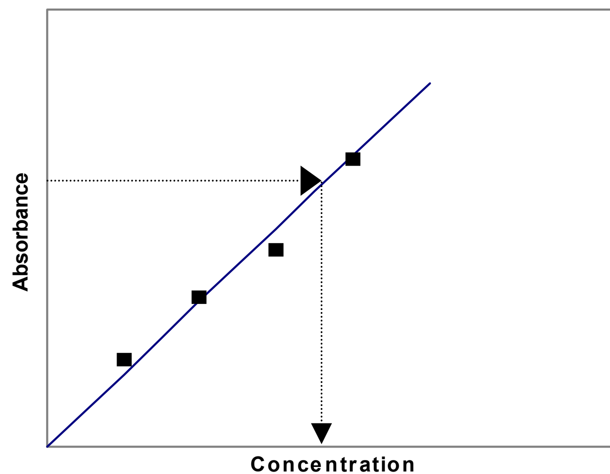


Figure 6: Finding the concentration of an unknown sample from the standard curve.

Due to the fact that the overall composition of the sample is seldom the same as that of the calibration standard, in some cases, the absorption characteristics of the two may differ. Where such discrepancy is suspected, the standard addition approach may be used. Here, a known amount of analyte is added to a second aliquot of the sample. The difference in absorbance is used to calculate the analyte concentration of the sample as illustrated in Example 1.

Example 1

A 25 mL sample after treatment with reagents to generate colour for measurement of phosphate yielded absorbance of 0.428. Addition of 1.00 mL of a solution containing 5.0μg phosphorus to a second 25 mL aliquot and development of colour resulted in an absorbance of 0.517. Calculate μg phosphorus in each mL of sample.

Solution:

Correct absorbance for dilution:

Corrected absorbance = 0.517 (26.0/25.0) = 0.538

Absorbance caused by 5μg phosphorus = 0.538 - 0.428 = 0.110

Therefore, phosphorus in the sample = (5.0/0.11) 0.428
= 19.5μg, or 19.5/25 = 0.7μg/mL

5. Overview of individual methods

The general procedure can be followed for all spectrophotometer analyses. For analysis of specific compounds, the method of preparation of the colored compound, and the wavelength of maximum light absorption will vary. An overview is given in Table 1.

Table 1 Overview of specific methods used for analysis of water quality parameters, and the wavelength of maximum light absorption

Parameter	λ	Method Name	SAP
Aluminum	535	Eriochrome Cyanine R Spectrophotometric	1.30
Boron	540	Curcumin Spectrophotometric	1.3
Chlorophyll a	750, 664, 65	Acetone Extraction Spectrophotometric	1.5
Flouride	570	SPADNS Spectrophotometric	1.11
Iron	510	Phenanthroline Spectrophotometric	1.13
Manganese	525	Persulphate Spectrophotometric	1.34
NH_3-N	640	Phenate Spectrophotometric	1.15
NO_3-N	220, 275	UV Spectrophotometric	1.16
NO_2-N	543	Sulphanilamide Spectrophotometric	1.17
o-PO_4	880	Ascorbic Acid Spectrophotometric	1.20
Total P	880	Digestion + Ascorbic Acid Spectrophotometric	1.39
Silica	815	Ammonium Molybdate Spectrophotometric	1.38
Sulphate	420	NOTE: preferred method for sulphate analysis is with nephelometer	1.26

Spectrophotometric analysis has
- wide applicability
- high sensitivity: detection limit 10^{-5}M to 10^{-4}M range
- moderate to high selectivity
- good accuracy: relative error 1 to 3%
- ease and convenience, lends to automation