Chapter 11

Spectrophotometer

GMDN Code	36411	36411	36411
ECRI Code	15-082	15-083	15-084
Denomination	Spectrophotometer, ultraviolet	Spectrophotometer, ultraviolet, visible	Spectrophotometer, visible

The word *spectrophotometer* is derived from the Latin word *spectrum*, which means *image*, and the Greek word *phos* or *photos*, which means light. The spectrophotometer is one of the main diagnostic and research instruments developed. It uses the properties of light and its interaction with other substances. Generally, light from a lamp with special characteristics is guided through a device, which selects and separates a determined wave length and makes it pass through a sample. The light intensity leaving the sample is captured and compared with that which passed through the sample. Transmittance, which depends on factors such as the substance concentration is calculated from this intensity ratio.

PHOTOGRAPH OF SPECTROPHOTOMETER

Conventional spectrophotometer

Photo courtesy of Beckman Coulter

PURPOSE OF THE EQUIPMENT

The spectrophotometer is used in the laboratory for determining the presence or concentration of a substance in a solution, thus allowing a qualitative or quantitative analysis of the sample.

OPERATION PRINCIPLES

As a basic principle, light is considered to be a form of electromagnetic energy. In space, it has a constant and universal velocity [C] of approximately 3 x 10^8 m/s. In any other medium (transparent) through which light passes, its velocity will be slightly lower and can be calculated by the following equation:

$$v_0 = \frac{C}{n}$$

Where:

v_0= Velocity at which light passes through the medium

n = Medium refraction index: whose value oscillates, in general, between 1.0 and 2.5.

The electromagnetic energy has a very wide range of wavelengths. Some examples are shown in the following table:

Type of electromagnetic energy	Range of wavelength
Radio waves	From a few meters to a few kilometres
Radar waves	From 1 to 10 cm
Infrared waves	From 1 to 10 microns (10^{-6} m)
Visible light	From 300 to 700 nm (nanometres)
X rays	From 0.1 to 0.5 Å (Angstrom)
Gamma rays	Approximately 0.0012 Å (Angstrom)

Upon passing or interacting with diverse mediums, light undergoes a series of phenomena. Among these are featured reflection, refraction, diffraction, absorption, diffusion, polarization and other phenomena measured by various instruments and devices. The table below shows the wavelength ranges used for carrying out spectrophotometry tests.

Section of the lighting spectrum	Range of wavelength
Ultraviolet	10–200 nm (nanometres)
Near ultraviolet	200–280 nm
Visible light	380–780 nm
Near infrared	780–3 000 nm
Mid infrared	3 000–20 000 nm
Far infrared	30 000–300 000 nm

With regard to the interaction of light with matter, Figure 27 assists in clarifying the complexity of phenomena that occur.

The diagram in Figure 27 shows that the incidental radiation [Io] can undergo a series of transformations. It can be reflected [Ir], transmitted [It], diffused [Id], absorbed and directly emitted as fluorescence [If]. The phenomena on which spectrophotometry is based are mainly absorption and transmission. In order to understand how, it is necessary to take Beer Lambert's law into account.

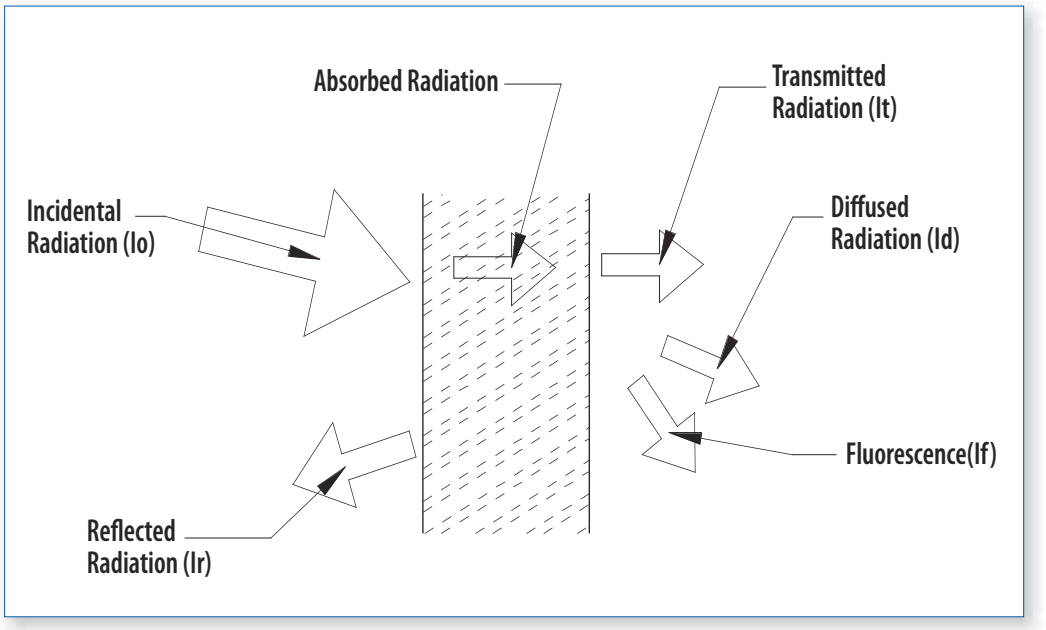
Beer Lambert's Law. Also known as Beer's law or Beer Lambert Bouguer's law, it identifies the relationship between the concentration of the sample and the intensity of light transmitted through it. With regard to the law mentioned, there are two implicit concepts: transmittance [T] and absorbance [A].

The transmittance [T] is the fraction of the incidental light of determined wavelength passing through the sample.

$$T = \frac{I_t}{I_o}$$

Where:
I_t = intensity of the transmitted radiation
I_o = intensity of the incidental radiation

Figure 27. Interaction of light with matter



The percentage of transmittance [%T] can be expressed by the following equation:

$$\%T = \frac{I_t}{I_o} \times 100$$

The concentration of light absorbing molecules in a sample is proportional to the absorbance [A] of that sample. It is expressed mathematically as:

$$A = \varepsilon \times l \times c$$

Where:

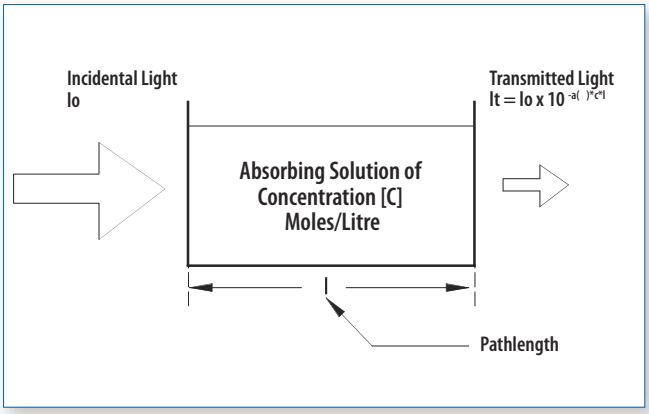
A = Absorbance measured
ε = Molecule absorbance coefficient [litres/moles/cm]
l = Distance of the trajectory traversed (path length) by the light in the sample
c = Sample concentration [moles/litres]

Absorbance [A] is related to transmittance [T] through the following equation:

$$A = \log_{10}\frac{1}{T} = \log_{10}\frac{I_o}{I_t} = \log_{10} 10^{\varepsilon \times c \times l} = \varepsilon \times c \times l$$

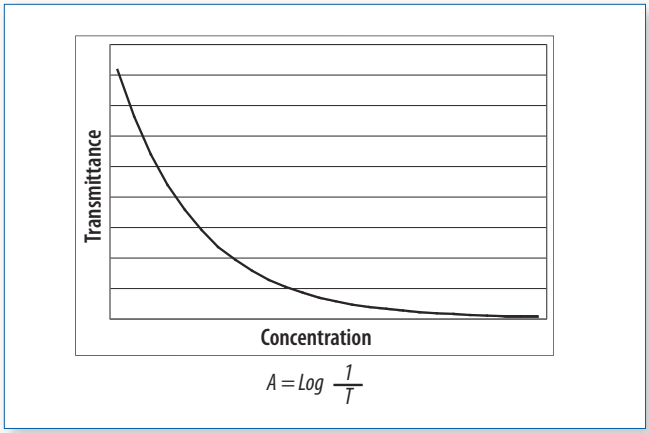
The following diagram explains the phenomenon of absorbance:

Figure 28. Absorbance phenomenon

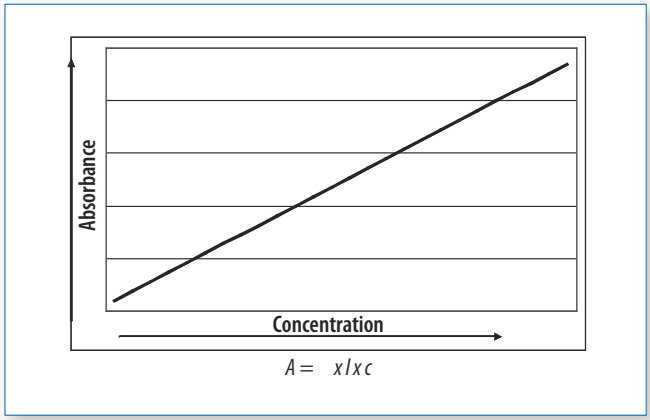


The graphs presented next demonstrate how absorbance [A] and transmittance [T] vary as a function of the concentration [C] according to Beer Lambert's law.

Transmittance graph



Absorbance graph



In conclusion it can be inferred that by increasing the concentration of a substance, the transmittance is decreased and, upon increasing the concentration of the substance, absorbance is increased.

The linearity of Beer Lambert's law is affected if the following conditions occur.

1. Displacement of the sample's chemical balance as a function of the concentration.
2. Deviations in the absorbance coefficients, greater concentrations than 0.01 M due to electrostatic interaction between nearby molecules.
3. Changes in the refraction index at high concentrations of the analyte.
4. Diffusion of light due to particles in the sample.
5. Fluorescence or phosphorescence of the sample.
6. Non-monochromatic radiation.

SPECTROPHOTOMETER COMPONENTS

The diagram shown in Figure 29 describes the relationship between the different components of a spectrophotometer. The most important are the following.

1. The light source
2. The monochromator
3. The sample carrier
4. The detector system
5. The reading system

These are the basic spectrophotometer components, not covering novel technology incorporated by manufacturers in advanced models. A brief explanation of these basic parts is shown in Figure 29.

Light source

Depending on the type of spectrophotometer, the light source can be a tungsten lamp for visible light or a deuterium arc lamp for ultraviolet light. Some manufacturers have designed spectrophotometers with long lasting xenon intermittent lamps emitting light in the visible and ultraviolet ranges. The lamp(s) come factory-assembled on a base that ensures a fixed position, to maintain optical adjustment and focus when operating or when replacing the bulb. The typical radiating energy emitted from a tungsten lamp is between 2600 and 3000°K (Kelvin degrees).

Monochromator

The monochomator is a set of elements used to disperse white light into waves of different wavelengths, one of which is used in the sample reading. In general, it has an entry crevice or groove which limits the light radiation produced by the source and confines it to a determined area; a set of mirrors for transmitting light through the optic system; an element for separating the light radiation wavelengths (which may be a prism or a diffraction (or transmission) grating); and an exit opening for selecting the wavelength required to illuminate the sample. Diffraction gratings have the advantage of eliminating the non-linear dispersion and being insensitive to changes in temperature.

Sample holder

This device holds the sample(s) to be analysed. There are various sample holder types to accommodate different spectrophotometer models and sample volumes: these come as cuvettes, microcells, microplates, test tubes and continuous flow cells, etc. In conventional spectrophotometers, the holder is a cell or cuvette of rectangular shape. Cuvettes are made of glass to read in the range of 340 to 1000 nm and others of silica to read in the visible range of 220 to 340 nm. There are also cuvettes and other sample holder types (e.g. microplates) in plastic such as styrene or polystyrene which are disposable.

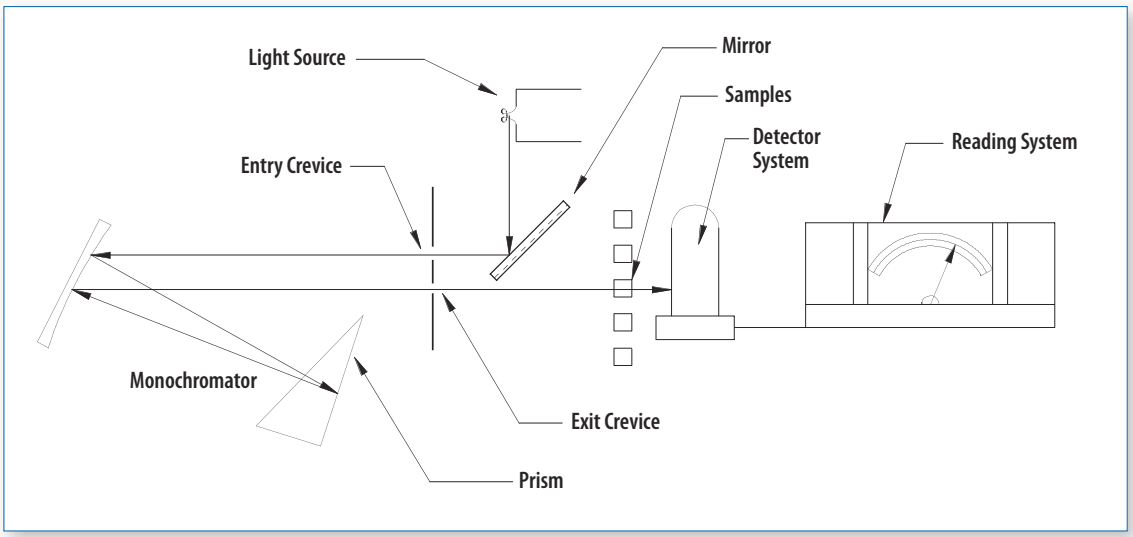
Detector system

The detection system can be designed with photocells, phototubes, photodiodes or photomultipliers. This depends on the ranges of wavelength, the sensitivity and the required speed of response. The detection system receives light from the sample and converts it into an electrical signal proportional to the energy received. This electrical signal can be processed and amplified to be interpreted by the reading system. A summary of advantages and disadvantages of devices normally used in detection systems is included in the following table (see opposite).

Reading system

The signal which leaves the detector goes through various transformations. It is amplified and transformed until its intensity becomes a proportional transmittance/absorbance percentage. There are analogous reading systems (displaying results on a reading scale) or digital ones (showing results on a screen).

Figure 29. Spectrophotometer components



Advantages and disadvantages of common detection devices

Device	Advantages	Disadvantages
Photocells	Economic.	Limited wave lengths between 400 and 750 nm.
	Small.	Low sensitivity.
	Robust.	Respond slowly to change in light intensity.
	Do not need energy sources nor signal amplifiers.	Wear out.
		Signal is dependent on the temperature.
Phototubes	Function between 190 and 650 nm. Also between 600 and 1000 nm.	Require calibrations depending on the temperature of the environment where the equipment is installed.
		Wear out with high levels of illumination.
Photodiodes	No movable mechanical parts.	
	Acquire spectral data simultaneously.	
	Wide dynamic range.	
	Excellent reproducibility of wavelengths.	
Photomultipliers	More sensitive than phototubes and photocells.	Can burn if day light penetrates them while in operation.
	Work on wider ranges of wavelengths.	Very expensive.
	Rapid responses to changes in light intensity.	Need a high voltage source.
	Do not become worn out like photocells.	Used only in specialized spectrophotometers.
	Can be made with sensitivity in the whole range of ultraviolet and visible light. (From 190 to 900 nm).	

Analogous indicators traditionally bear the name *meters*. Their exactitude depends among other factors, on the length and the number of divisions of the scale (the more divisions, the more exact it is). Their main disadvantage is that they can be incorrectly read, due to the operators' fatigue or errors identifying scales when there are several.

Digital indicators usually show results on a screen as illuminated alpha numerals. This makes reading errors less likely.

INSTALLATION REQUIREMENTS

For the correct functioning of a spectrophotometer, the following is required:

1. An electric supply source that complies with the norms and standards used in the country. In American countries, voltages of 110 V and frequencies of 60 Hz are generally used. Other parts of the World require 220-230V/50-60 Hz.
2. A clean, dust free, environment.
3. A stable work table away from equipment that generate vibrations (centrifuges, agitators).

SPECTROPHOTOMETER MAINTENANCE

Spectrophotometers are very specialized and costly equipment. Their integrity depends to a great extent on the way they are installed and used. Their direct environment and the quality of the electricity services constitute factors of prime importance for the equipment to function according to specifications. Routine maintenance required vary in complexity, ranging from careful cleaning of components to specialized procedures carried out by a trained specialized technician or engineer with the technical information for different manufacturers' models and designs. Following manufacturer's instructions and careful use will guarantee a prolonged operational life. In recent models, manufacturers have incorporated automatic routines of calibration and verification.

In this document general maintenance recommendations applicable to a wide range of spectrophotometers are presented. It is emphasized that specialized routines can only be performed according to the specific manufacturer's recommendations for each particular model. General routine maintenance for a spectrophotometer in good condition and the frequency of estimated checks are as follows:

Inspection of the instrument's surroundings

Frequency: Annually

The area in which the spectrophotometer is installed must be inspected visually and tested electrically in order to guarantee the safety of the operator. The inspection covers the electrical installation and the installation area (physical infrastructure related to the spectrophotometer).

Electrical installation

It must be verified and tested for ensuring the following:

1. There is an electrical outlet or receptacle with a ground pole.
2. The receptacle is in good condition and is no further than 1.5 m from the spectrophotometer.
3. The voltage is of an appropriate level and must not vary by more than 5% of the voltage specified on the equipment's plate.
4. The receptacle's polarity is correct.

These tests must be done by an electrical technician or an engineer and results must be recorded to allow follow-up over time.

Installation area

1. Check that there is free space around the spectrophotometer for two purposes. First, for the connecting cables to pass without hindrances and for other components or support equipment (e.g. the voltage stabilizer). Second, to allow adequate ventilation of the equipment when it is in operation.
2. Test the integrity of the counter, its state and cleanliness.
3. Verify that there is no equipment installed that can transmit vibrations in proximity. (E.g. centrifuges).
4. Verify that it is not affected by excessively humid conditions, dust or high temperatures. The appropriate room temperature for the operation of the spectrophotometer generally ranges between 10 and 40 °C.
5. Avoid installing the equipment where it receives direct solar radiation.
6. Do not install the equipment where there are magnetic fields or intense electromagnetic radiation.
7. Ensure installation area is free from the influence of gases and corrosive substances.

Visual inspection of the equipment

Frequency: Every six months

The spectrophotometer must be inspected visually to verify that the state and integrity of its components are maintained in accordance to the manufacturer's specifications. The most important aspects are cited next:

1. Check that the structure of the work table supporting the spectrophotometer is in good condition.
2. Test the general structure of the spectrophotometer. Verify that buttons or control switches and mechanical closures are mounted firmly and that their identification labels are clear.
3. Ensure that accessories are clean, not showing cracks and that their functional state is optimal.
4. Confirm that mechanical adjustment parts (nuts, screws, bolts, etc.) are adjusted and are in good condition.
5. Check that electrical connectors do not have cracks or ruptures, that they are joined correctly to the line.
6. Verify that cables are not showing signs of splicing, that they are not frayed and that they do not have worn-out insulation.
7. Check that cables securing devices and terminals are free of dust, filth or corrosion. These same cables must not be worn out or show signs of deterioration.
8. Check that the grounding system (internal and external) is standardized, of approved type, functional and correctly installed.
9. Ensure that circuit switches or interrupters, the fuse box and indicators are free from dust, filth and corrosion.
10. Check the external electrical components for signs of overheating.

General maintenance

Cleaning of spills

In case of a leak in the sample holder or carrier, the spill must be cleaned according to the following procedure:

1. Turn off the spectrophotometer and disconnect the cable from the electrical feed.
2. Use a syringe for cleaning the sample holder. Absorb as much liquid that can possibly be extracted.
3. Dry the sample holder with a medicinal cotton bud.
4. Use lens paper or a clean piece of soft textured cloth for cleaning the window of the photocell.
5. Clean the exterior of the instrument with a piece of cloth moistened with distilled water. Include the screen, control and keyboard in the cleaning.

Cleaning of quartz cuvettes

It is recommended to carry out the following procedure to maintain quartz cuvettes in good condition:

1. Wash the cuvettes using a diluted alkaline solution such as NaOH 0.1 M and a diluted acid such as HCl, 0.1 M.
2. Rinse cuvettes several times with distilled water. Always use clean cuvettes to take absorbance measurements.
3. Conduct rigorous and careful cleaning procedures on cuvettes if samples used can deposit films. Some manufacturers recommend using special detergents for cleaning cuvettes.

Battery changes

Various models of spectrophotometers use batteries to memorize data associated with the analysis, such as date and time. The procedure to change the battery is similar

in the various equipment. Following this procedure is recommended:

1. Verify that the low battery indication appears on the instrument's screen.
2. Turn off the spectrophotometer.
3. Disconnect the electrical feed cable.
4. Open the battery compartment and remove the worn-out batteries.
5. Clean the electrical contact points.
6. Install new batteries with the same specifications as the originals.
7. Close the compartment.
8. Reconnect the equipment.
9. Adjust the date and time information.

Change of bulb/lamp

The bulb is a consumable with a limited operational life. It must be foreseen that at some point in time, it will be necessary to replace it. Most likely it will burn out, or suffer from internal metallization and evaporation and the light emitted will no longer meet the spectrophotometric processes specifications. Lamp change steps differ for each model and one must always follow the manufacturer's instructions. Common steps are as follows:

1. Verify that the bulb is not functioning or that there is some indication of flaw. In modern equipment, a sign will appear on the screen or an error code. In old equipment, the light will simply no longer work.
2. Turn off the spectrophotometer.
3. Disconnect the feed cable.
4. Undo the screws securing the top of the lamp's compartment.
5. Undo the screws keeping the lamp's mechanism fixed.
6. Undo the screws fastening the electrical connection cable to the lamp (in some equipment, this might not be necessary, as the assembly base has direct contact mechanism to the lamp's contact terminals).
7. Install a new lamp with the same characteristics as the original. Use gloves to avoid getting fingerprints on the surface of the lamp.
8. Reconnect the electrical feed cables to the lamp.
9. Reinstall the screws keeping the lamp in place.
10. Replace the screws securing the lamp's compartment's cover.
11. Reconnect the spectrophotometer.
12. Turn the equipment ON and carry out the equipment's recalibration procedure stipulated by the manufacturer.

Preventive Maintenance

Preventive maintenance of the spectrophotometer must correspond with routines and frequencies recommended by the manufacturer. A series of basic routines which can be performed in the laboratory is presented next:

1. Clean the spectrophotometer externally, including the controls, screens or measurement meters. This can be done using a piece of fine cloth (similar to the texture used in handkerchiefs) dampened with distilled water.
2. Inspect and clean the electrical feed cable.
3. Verify that the lamp is clean and in good state. If it is not functioning, install a new one with the same specifications as the original. In modern spectrophotometers, the lamp's state is detected automatically by software which controls the state and functioning of the equipment making it easy to determine when it is necessary to change the lamp. Change the lamp and carry out the subsequent adjustments following the manufacturer's recommendations.
4. Check the protection fuse. Before opening the compartment where the fuse is housed, check that the spectrophotometer is turned off and check that its contacts are clean and in good condition. If it is necessary, replace by a new one with the same characteristics as recommended by the manufacturer.
5. Put the instrument in the operational configuration.
6. Activate the "on" switch and allow it to warm up for five (5) minutes. Verify that:
   a) The lights or pilot indicators work.
   b) The reading indicators stay on zero (0).
   c) The light source works.
7. Carry out an escaping current test in the "on" and "off" position.
   a) Verify the ground pole and the correct polarity.
   b) Verify the correct polarity without a ground pole.
   c) Verify the inverse polarity without a ground pole.
8. Calibrate the front panel of the spectrophotometer according to the manufacturer's instructions.
9. Measure the equipment's sensitivity.
10. Conduct a test according to Beer's law.
11. Return the spectrophotometer to the initial configuration if the calibration has been successfully completed.

GOOD PRACTICES WHEN USING THE SPECTROPHOTOMETER

1. Calibrate the spectrophotometer every time a set of samples is to be analysed.
2. Keep the cover of the sample holder and compartment closed during the measurement process to ensure adequate reading.
3. Avoid reusing disposable cuvettes.
4. Only use quartz cuvettes for carrying out analysis under 310 nm.
5. Avoid the use of plastic cuvettes if using organic solvents.
6. Use high quality boron silicate glassware for preparing standards. Avoid the use of sodium glass (sodium oxide) whenever possible, as prolonged contact with standards can permeate it and produce erroneous results.

7. Carefully clean the glass cuvettes after use. Discard those that show lines on the clear surface.
8. Use high quality reagents. Those of low quality can cause contamination even in very low concentrations. The diluents used (water or solvents) must be free of impurities.
9. Verify that samples or standards did not degas inside the cuvettes. This phenomenon produces bubbles on the inner surface of the cuvettes and causes errors in the readings.
10. Take into account that not all substances comply with Beer's law. Carry out linearity tests on the range of concentrations to be used. It is recommended to prepare a group of known high standard solutions and verify the results. The phenomena that affects Beer's law are the following:
    a) High concentration by molecular association of ionic species.
    b) Variation in hydration at low concentrations changing the nature of complex ions.
    c) Absorptions that do not comply with the Beer law require graphing results of known standards. This will indicate reading versus the concentration such that the reading of the unknown concentrations can be related to concentrations from the graph.

TROUBLESHOOTING TABLE

Automated spectrophotometer[1]

PROBLEM	PROBABLE CAUSE	SOLUTION
The spectrophotometer is without power.	The on and off switch is in the off position.	Move the switch to the on position.
	There is no electric energy in the feed outlet.	Verify the general electric feed. Test that some safety mechanism has not misfired.
	The electric feed cable is not connected well.	Connect the feed cable firmly.
The keyboard's buttons do not respond.	The initialization of the equipment during start-up is incomplete.	Turn off the equipment and switch on again.
	An incorrect command was activated during start-up.	
The serial port RS 232 does not respond.	There was incomplete initialization of the equipment during start-up.	Turn off the equipment and switch on again.
	The interconnection cable is badly connected.	Verify the connection.
The LCD screen is difficult to read.	The contrast control is maladjusted.	Adjust the contrast.
	The base lighting system burnt out.	Call the representative.
The printer is blocked.	There is a paper jam in the printer.	Remove the excess paper with finely pointed tweezers.
		Turn off the equipment, remove the paper and reinstall again.
The printer's paper does not auto feed or advance.	The printer paper is installed erroneously.	Turn off the equipment, reinsert the roll of paper.
	The front edge of the paper is not aligned or folded.	Turn off the equipment. Reinsert the roll of paper. Cut the front edge and realign in the feed system.
	The paper feed control does not respond.	Call the representative.
The cuvette does not enter the sample holder compartment.	The cuvette is of the wrong size.	Use the size of cuvettes specified by the manufacturer.
	The cuvette's adjustment mechanism is incorrectly placed.	Correct the position of the adjustment mechanism.
The reading shows fluctuations.	There are interferences in the light path.	Verify that the cuvette is not scratched.
		Verify that there are no particles floating in the cuvette.
		Rub the optic walls of the cuvette with a piece of clean cloth.
		Verify that the working range selected is appropriate for the sample under analysis.
The reading shows negative values. There is no absorbance reading.	There is no sample.	Add a sample to the solution.
	The cuvette is incorrectly positioned.	Verify the orientation of the cuvette's window.
	The wavelength is erroneously selected.	Adjust the wavelength to the range compatible with the analysis.
	The equipment was erroneously calibrated with a sample instead of a blank solution.	Calibrate with a blank solution or with distilled water.

[1] Instruction Manual, Spectrophotometer, SmartSpecTM 3000, BIO-RAD Laboratories.

Non-automated spectrophotometer[1]		
PROBLEM	**PROBABLE CAUSE**	**SOLUTION**
The source lamp does not light-up.	The filament is broken.	Replace the lamp.
	The safety fuse is burnt out.	Replace the lamp.
	There is resistance in the lamp's filament.	Replace the lamp.
	The voltage is erroneous.	Review the voltage. Check the feed source.
Low readings in the meter or in the galvanometer.	The source lamp is defective.	Replace the lamp.
	The photocell is dirty or defective.	Clean or replace the photocell.
	The amplifying circuit is defective.	Change or repair the amplifying circuit.
	The source lamp's voltage is low.	Adjust the voltage.
Unstable indication of the measurer.	The Zener diode stabilizer is defective.	Replace the Zener diode.

[1] Operation seminar workshop and Maintenance of Spectrophotometers, Maintenance Subregional Project, RE-HS-02, OPS/OMS Agreement.

BASIC DEFINITIONS

Absorption. A physical phenomenon occurring when atoms or molecules of a substance absorb light (photons). The energy of a photon is taken up by another entity, e.g. by an atom whose valence electrons change between two electronic energy levels destroying the photon in the process. The energy absorbed is lost through heat or radiation. **Absorbance** is a mathematical measure of absorption, expressed in **optical density units (OD)**.

Angstrom. A unit of length equal to 10^{-10}m. Its symbol is [Å]. It is used for carrying out measurements of X- or Gamma-rays.

Band width. A wavelength range that a monochromator can transmit.

Diffraction. Phenomenon caused by a change in the directions and intensities of a group of waves after reaching an obstacle, or through a narrow aperture whose size is approximately the same as the wavelength of the waves.

Diffraction grating. A component of the monochromator, also called "transmission grating". It diffracts light and is shaped as a series of parallel fissures carved onto a reflecting surface. It is made by tracer machines protected against vibrations and temperature variations. Gratings used in spectrophotometers are copies of one master grating that usually has more than 1200 fissures per millimetre. Figure 31 demonstrates the phenomenon of diffraction.

If the reflection angle [δ] is known as well as the width [d] of the fissures, the wavelength [λ] can be determined according to the following equation:

$$\sin\delta = \frac{n\lambda}{d}$$

Intensity [I_v]. The amount of light emitted by a source in a particular direction per unit of time. More generally, a measurement of the average energy flow per unit of time. To get the intensity, the energy per unit of volume is multiplied by the speed at which the energy moves. The resulting vector is the energy by square surface per unit of time.

Molar extinction or absorptivity coefficient [ε]. Measures how strongly a chemical species absorbs light at a determined wavelength. It is an intrinsic property of the chemical species. When there is more than one absorbing species in a solution, the absorbance is the sum of the absorbance values for each individual species. The absorbance at a given wavelength of a mixture of species X, Y ... is given by

$$A = \int \left[C_x \times \varepsilon_x + C_y \times \varepsilon_y + \ldots\right]$$

Where A is the absorbance of the mixture.

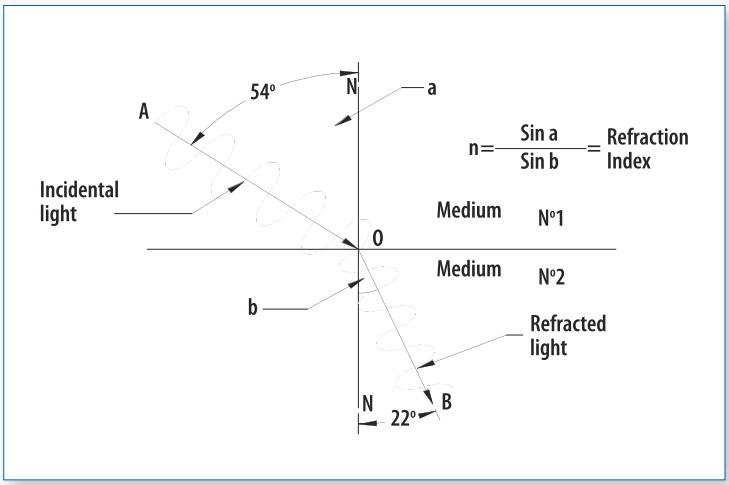
Nanometre. A unit of length corresponding to 10^{-9} m (a thousand millionth of a metre). It is identified by the symbol [nm]. It is used for measuring visible or ultraviolet light wavelengths.

Path length. The distance covered by visible or ultraviolet light through a sample in an analytical cell (cuvette or well).

Refraction. A change of direction that occurs when a ray of light reaches the interface between two media.

The light cuts at an angle [a] and refracts at an angle [b] upon changing propagation medium.

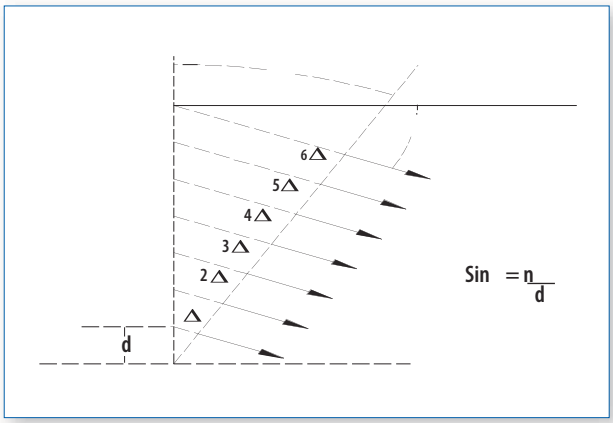
Figure 30. Refraction of light



Spectrophotometry. A method of chemical analysis based on the absorption or attenuation of light of a specified wavelength or frequency by matter. The light interacts with specific features of the molecular species being analyzed: the light absorbed depends on the wavelength, the concentration of the species and the trajectory. This allows determining properties such as the concentration of substances, which in the field of basic health, serves to perform a multitude of analysis for determining the health status of a patient.

Wavelength. The distance between crests of a wave. It determines the nature of the different forms of radiant energy in the electromagnetic spectrum. For electromagnetic waves, the wavelength in meters is calculated by the speed of light divided by frequency (number of peaks passing through a certain point in a determined time).

Figure 31. Diffraction grid



Δ = difference in wavelength between two adjacent slots (fissures).