



Clinical Chemistry
Spectrophotometric
Lab.5

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Spectrophotometric

The spectrophotometer is a device designed to measure the amount of light absorbed by a solution at various wave lengths and determine the transmitted light.

The Configuration and Parts of the System:

All spectrophotometers have the following fundamental parts:

- A light source.
- The dispersion devices (a prism or grating).
- Focusing devices (lenses, slits, mirrors) .
- A device for holding the sample (cuvettes).
- A photoelectric cell for measuring light intensity (detectors).
- Display devices.



Figure1:Spectrophotometric device

Precautions to be taken into account when using the spectrophotometer:

- 1-The device must not be opened to unstable electricity.
- 2-The wavelength designated for reading must be determined.
- 3-The device must be set to zero on the Blank solution.
- 4- It must be taken into account the cleanliness of the cuvette and place it in the device correctly (so that the transparent side on the side edge of the cuvette is opposite the light source of the device).
- 5-The transparent side on the side edge of the cuvette must not be touched by hand, wipe and print before placing it.
- 6- It must be taken into account that the solution in the cuvette is not less than the minimum reading (1 ml) and that there are no bubbles.
- 7-Close the place to put the cuvette when reading.

Some important terms

Kits: commercially prepared packages containing the reagents needed for a particular test with standard and accompanying leaflet.

Leaflets: It is a very important paper leaflet attached to each awareness kit. It is necessary before starting any calibration to read the attached leaflet carefully because it contains all the interaction instructions such as (temperature, quantity of reagent, amount of serum, duration of incubation, method of work)

Micropipette: they are micro pipettes through which small amounts of serum, reagents or liquids are taken (they have different measurements from 5 microliters up to 5ml).
(1ml=1000 microliter)

Control: It is a special solution that contains all the elements or substances in the serum and has a specific and known value found in a special paper (similar to the serum but manufactured to ensure the quality of work) and the control must be done for all daily analyses before starting the work of clinical chemistry analyses according to the type of analysis where there are two types of control:

*pathogenic

* non pathogenic

Standard: It is a solution of known concentration with extreme accuracy. Therefore, careful work must be done during the preparation of standard solutions, because they will be used later to determine the concentrations of unknown substances.

Factor: It is the result of dividing the standard solution by its absorbance. The factor is made every day and is used to get rid of re-reading the absorbance of the stander every time the sample is measured.

Principle of kits: It is the mechanism of action of the kit and its reading is necessary to know how the reaction takes place and what is the compound or complex that causes the color produced at the end of the reaction.

Reagent composition: It means the components of the working solutions and the concentration of each solution, from which we know the standard concentration that is included in the extract of the factor or in the general equation.

Storage & Stability: It contains notes about the storage of working solutions and their stability after opening the box or after mixing the solutions if they are solutions that are not ready for use directly, and there are notes if they are stored away from light and others.

* They must be read and understood to know how the solutions are stored and stabilized after opening.

Reagent preparation: In it he writes how to prepare working solutions or writes that working solutions are ready for use directly.

Samples: It means what forms can be used, for example, serum, plasma, or Urine.

Materials required: In it he writes, for example, the devices required to complete the analysis, the wavelength used, and the temperature required.

Procedure: And in it you write the method of making the kits through a small table and steps.

Calculation: In it, the equation is used to extract the final product and how to convert units as well.

Reference values : In it, the natural values for women, men, children and newborns are written, and it varies from one kit to another.

Calculate factor and concentration

$$f = \frac{C}{\text{Abs. of standard}}$$

$$x = \frac{\text{Abs. of Test}}{\text{Abs. of standard}} \times C$$

$$x = \frac{\text{Abs. of Test}}{\text{bs. of standard}} \times C$$

$$x = \text{Abs. of Test} \times f$$