Spectrophotometer

Spectrophotometer is an instrument of absorbance measurement of a solution at one or more wavelength. The spectrophotometer produces light of limited wavelength for interaction with the sample.

❖ Uses of spectrophotometer:

Uses in clinical tests that comprises the determination of the following

a-value of blood sugar

b-creatinine concentration

c- bilirubin concentration

d- uric acid, urea (kidney function)

e- concentration of some enzyme.

f- concentration of proteins in blood.

❖ Principle of Spectrophotometer

The spectrophotometer technique is to measure light intensity as a function of wavelength

❖ The following components are common to all spectrophotometers: -

1. **Light source**: a) A "tungsten filament lamp" is used for the visible.

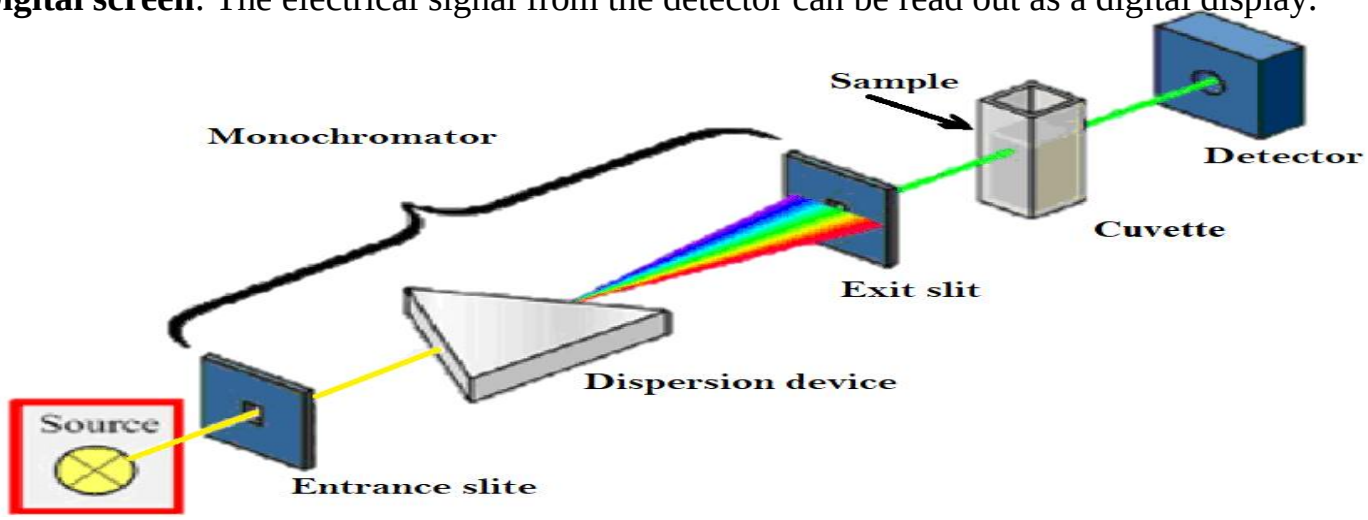
b) a hydrogen or deuterium-discharged lamp is used for the U.V region.

2. **Monochromator**: Allow certain wave length band to penetrate through it (prism, slits).

3.**Cuvette**: it is the transport container for sample which be square and made of glass or plastic for range (400-700 nm) and of quartz for measurement below 400 nm and UV rang.

4. **Detector**: Measures light intensity by converting the light signal into an electric signal.

5. **Digital screen**: The electrical signal from the detector can be read out as a digital display.



❖ Operate of a spectrophotometer

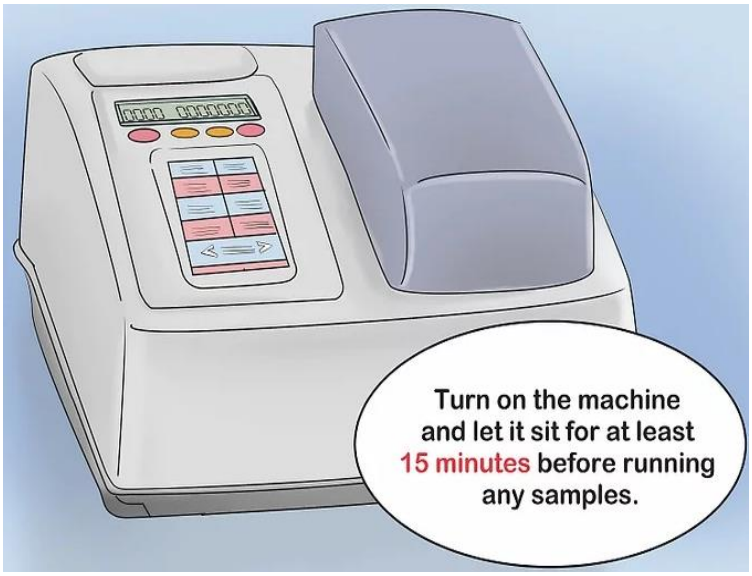
Part 1: Preparing the Samples

1-Turn on the spectrophotometer.

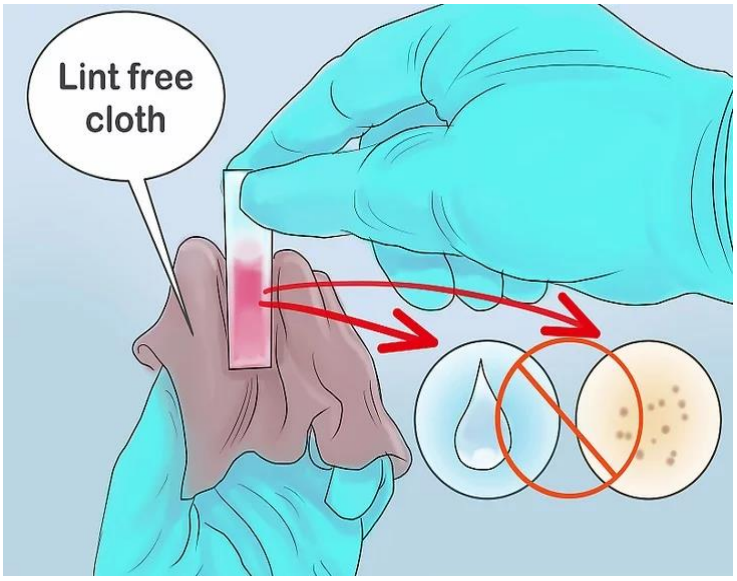
2-Clean the cuvettes from inside and outside.

3-Load the proper volume of the sample into the cuvette.

4-Prepare a control solution (**standard solution**).



step 1



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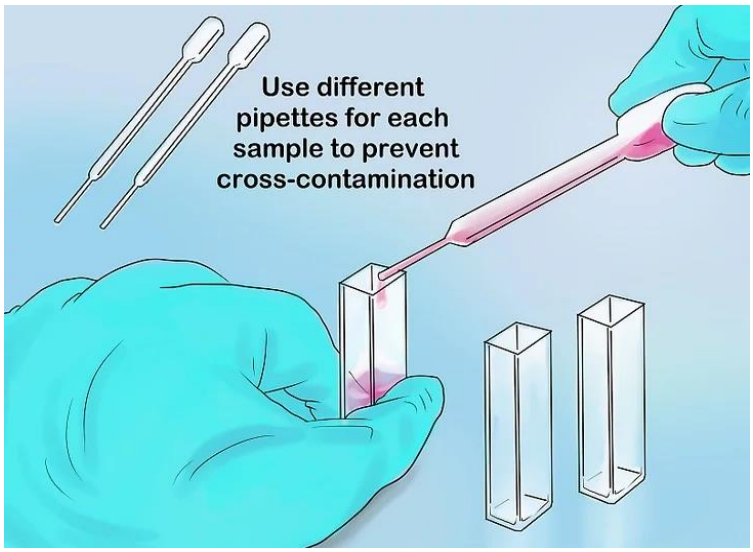


step 3

step 4

Part 2: Running the Experiment

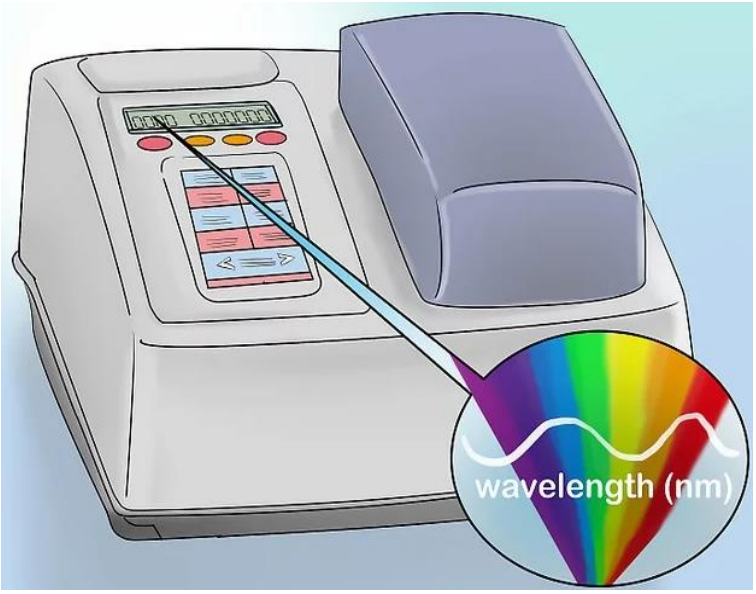
1-Choose and set the wavelength of light to analyze the sample with.

2-Calibrate the machine with the **blank solution** (To zero the device).

3-Remove the blank and test the calibration.

4- Measure the absorbance of the **standard.**

5- Measure the absorbance of your experimental **sample**.



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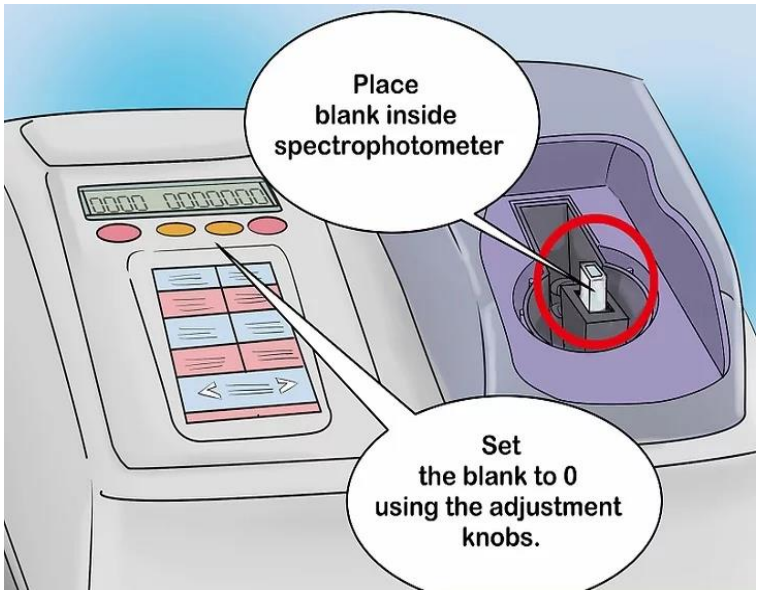

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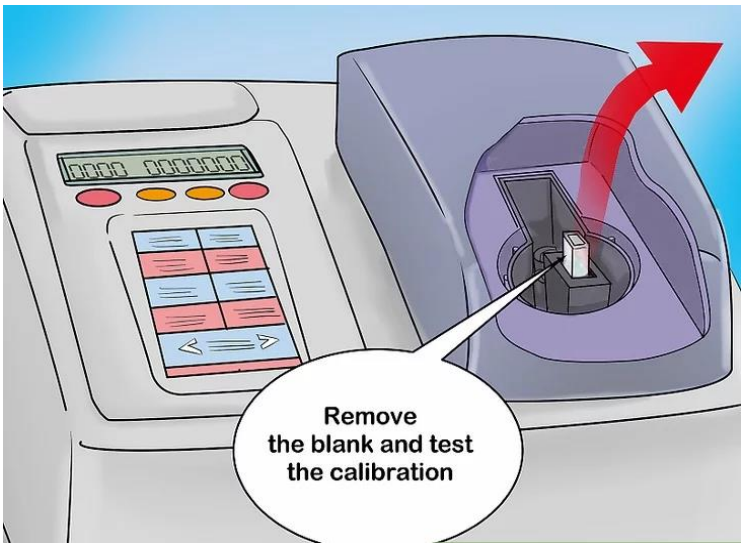

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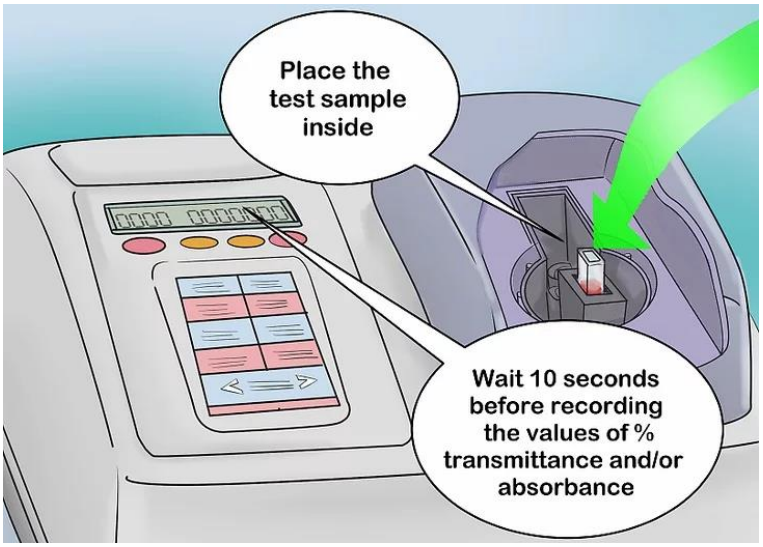

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