FAQs

1. What is Colorimetry?

The Colorimetry has proven to be the most versatile, reliable and widely used of all laboratory instruments in clinical chemistry.

Colorimetry is the quantitative measurement of how much a chemical substance absorbs light by passing a beam of light through the sample using a spectrophotometer. The spectrophotometer has proven to be the most versatile, reliable and widely used of all laboratory instruments in clinical chemistry. The majority of clinical chemistry procedures have been developed to produce a colored end-product which can be detected and measured by some sort of photometer.

2. Explain the principle of Colorimetry.

The concept of Colorimetry is the beam of light that is radiated toward the sample is made up of a stream of photons. When photons encounter molecules in the sample, the molecules may absorb some of them, reducing the number of photons in the beam of light and decreasing the intensity of the detected signal. From the absorbance, the concentration of the sample solution can be determined from the Beer-Lambert Law, which states that there is a linear relationship between the absorbance and concentration of a sample. According to the Beer-Lambert Law, absorbance is the product of the extinction coefficient, the length that light passes through the sample and the concentration of solute.

3. Explain the different types of Colorimetry.

There are two kinds of Colorimetry: single beam and double beam. A double beam Colorimetry compares the light intensity between two light paths, one path containing a reference sample and the other the test sample. A single beam Colorimetry measures the relative light intensity of the beam before and after a test sample is inserted.

4. Explain the theory of Colorimetry.

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 colored 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 640 nm.

The Beer-Lambert Law The Absorbance and Transmission of light through a sample can be calculated by measuring light intensity.

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

Light Absorbance (A) = $\log (I_0/ I) = €bc$

Light Transmission (T) = $I/I_0 = 10^{-€bc}$

The following terms are defined:

- Light Intensity entering through the sample is"I_0"
- Light Intensity retained in sample is "I"
- The concentration of analyte in sample is "C"
- The length of the light path in glass sample cuvette is "b"
- "€" is a constant called molar extinction coefficient.
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5. What are the conditions for true linearity in Colorimetry?

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. And very dilute solutions lead to linearity.

6. Explain the Instrumentation of Colorimetry.

All spectrophotometer instruments are designed to measure the absorption of radiant energy have the basic components as follows

- a stable source of radiant energy or Light source

- a wavelength selector to isolate a desired wavelength from the source also called filter or monochromator.
- transparent container called cuvette for the sample and the blank
- Radiation detector ie phototube to convert the received radiant energy to a measurable signal; and
- A readout device that displays the signal from the detector.
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7. Write a note on Cuvettes.

Cuvettes also called as cells are provided in pairs that have been carefully matched to make possible the transmission through the solvent and the sample. The 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 IR
Silicate glass: Above 350 nm to IR
Plastic: visible region
Polished NaCI or AgCI: Wave lengths longer than 2 μm

8. What is General Measurement Procedures for Colorimetry.

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:
- Prepare samples to make colored compound
- Make series of standard solutions of known concentrations and treat them in the same manner as the sample for making colored compounds
- Set spectrophotometer to λ of maximum light absorption
- Measure light absorbance of standards
- Plot standard curve: Absorbance vs. Concentration

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.

9. What are the applications of Colorimetry in food dyes ?
Identifying Food Dyes with Colorimetry:

The term Colorimetry analyzer of food is used for various Colorimetry sensors, devices, instruments, probes and testers dedicated to measuring physical and chemical parameters characterizing ingredients in food products and beverages.

10. How Colorimetry analyzers of food in food manufacturing industries are useful?

1. checking the quality of food;
2. monitoring of the food production process;
3. providing data necessary for production control;
4. specification of food products necessary for their labelling;
5. precise classification of food products enabling their better pricing

11. How analysis of wine done through Colorimetry?

A laboratory for analysis is a basic essential in most wineries today to allow wine to be tested and analyzed for taste and quality. this can be used throughout this process to perform a more specific enzymatic analysis. UV Colorimetry is favored because it is a non-destructive way to test liquids, such as wine, without disturbing it in anyway. Although the Colorimetry analysis is optional, it is important for many wineries to record the properties of their wines for their own purposes

However, wine analysis does not have to wait for the lab. It can actually begin on the vine. NIR spectroscopy can be used in this instance to determine color quality and phenols just through the skin of grapes in the vineyard. NIR spectrophotometer is able to determine: ethanol, glucose and fructose, maleic acid, volatile acid, total acid and pH in finished wine or under fermentation.

12. Why wine is quality is good as it ages?

Phenols are chemical compounds found in alcohol that contribute to the body and structure of the wine. As the wine ages, these compounds will oxidate. The physical and chemical changes, such as this oxidation, that wine goes through over time can affect its quality and therefore need to be evaluated. In regard to color, a white wine may begin to look more gold than green as it ages and a red wine appears more brown.

13. How estimation of protein is done by Colorimetry analysis

Since proteins absorb light at a specific wavelength, a Colorimetry can be used to directly measure the concentration of a purified protein in solution. It is important to note that direct UV measurement at 280 nm yields highly reproducible measurements since no reagents are added to the protein solution and the protein of interest was not modified or inactivated during the process. It also produces quick results since the sample does not need to be incubated in order to complete the process.

14. What are the chemical compositions of protein?

The chemical composition of the protein i.e. the number and type of amino acids present, will affect its absorption. Since a sample protein's absorption at 280 nm will depend on the amount of the amino acids tyrosine and tryptophan, it is very much possible that proteins of similar molecular weight will have different absorbance values due to their different tyrosine and tryptophan content.

15. What are the applications of Colorimetry in food content?

Vis/NIR spectroscopy has been successfully used in the production of cheese, eggs, milk, butter, beer, wine, fruit juices, baked goods, cereal, jams and jellies, nutritional supplements, fish, and more. Some of the more common applications include measuring:

- Fat levels
- Protein content
- Moisture content
- Particle size
- Sugar content
- Brix and acidity levels
- Blend analysis

16. what are Steps for operating the photoelectric colorimeter:

1. Choose the glass filter recommended (see table below) in the procedure and insert in the filter.
2. Fill two of the cuvette with blank solution to about three-fourth and place it in the cuvette slot.
3. Switch on the instrument and allow it to warm up for 4 – 5 minutes.
4. Adjust to zero optical density.
5. Take the test solution in another cuvette and read the optical density.
6. Take the standard solution in varying concentration and note down the optical density as S1, S2, S3, S4, S5 and so on.

7. A graph is plotted taking concentration of standard solution versus the optical density.
8. From the graph the concentration of the test solution or the unknown solution can be calculated.