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Colorimeter

Hello,,,, Welcome to the lecture series of Food Science and Technology. The present lecture on the topic entitled colorimeter. This topic comprises with the following five sections,

1. Introduction
2. Basic Principle
3. Instrumentation and Methodology
4. Applications
5. Conclusion

Introduction

In physical and analytical chemistry, **colorimetry** is a technique "used to determine the concentration of colored compounds in solution. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance in a specific wavelength of light. To use the colorimeter, different solutions must be made, including a control or reference of known concentration. The color or wavelength of the filter chosen for the colorimeter is extremely important, as the wavelength of light that is transmitted by the colorimeter has to be the same as that absorbed by the substance being measured.

A colorimeter is a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops upon introducing a specific reagent into a solution. There are two types of colorimeters — color densitometers, which measure the density of primary colors, and color photometers, which measure the color reflection and transmission.

Color is the attribute of visual perception consisting of any combination of chromatic and achromatic content. This attribute can be described by chromatic color names such as yellow, orange, brown, red, pink, green, blue, purple, etc., or by achromatic color names such as white, grey, black, etc., and qualified by bright, dim, light, dark or by combinations of such names.

Perceived color depends on the spectral distribution of the color stimulus, on the size, shape, structure and surroundings of the stimulus area, on the state of adaptation of the observer's visual system, and on the person's experience of prevailing and similar situations of observation.

2. Basic Principle

The colorimeter is based on Beer-Lambert's law, according to which the absorption of light transmitted through the medium is directly proportional to the medium concentration.

In a colorimeter, a beam of light with a specific wavelength is passed through a solution via a series of lenses, which navigate the colored light to the measuring device. This, analyses the color compared to an existing standard. A microprocessor then calculates the absorbance or % of transmittance. If the concentration of the solution is greater, more light will be absorbed, which can be identified by measuring the difference between the amount of light at its origin and that after passing through the solution.

In order to determine the concentration of an unknown sample, several sample solutions of a known concentration are first prepared and tested. The concentrations are then plotted on a graph against absorbance, thereby generating a calibration curve. The results of the unknown sample are compared to that of the known sample on the curve to measure the concentration.

Beer-Lambert's Laws: According to Beer's law when monochromatic light passes through the colored solution, the amount of light transmitted decreases exponentially with increase in concentration of the colored substance. $I_t = I_o e^{-kC}$

- Lambert's Law According to Lambert's law the amount of light transmitted decreases exponentially with increase in thickness of the colored solution.

$I_t = I_o e^{-kt}$

Therefore, together Beer-Lambert's law is: $I_E/I_o = e^{-KCT}$

where,

I_E = intensity of emerging light

I_o = intensity of incident light

e = base of neutral logarithmic exponential function

K = a constant

C = concentration

T = thickness of the solution

3. Instrument and Methodology

Apparatus:

The instrument use to measure color is colorimeter. This appartus will comprise of the following parts:

1. light source
2. filter (the device that selects the desired wave length)
3. cuvette-chamber (the transmitted light passes through compartment wherein the solution containing the colored solution are kept in cuvette, made of glass or disposable plastic)
4. detector (this is a photosensitive element that converts light into electrical signals)
5. Galvanometer (measures electrical signal quantitatively)

Steps for operating the photoelectric colorimeter:

1. Choose the glass filter recommended 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 stabilize 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. Plot a graph by 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.

Color and Illuminance Measurement

In many applications involving the measurement of color or of the color temperature of self-emitting light sources, the same measurement geometry is used as for illuminance. Appropriate absolute calibration of the measuring system allows the illuminance of a

reference plane in Lux to be determined in addition to the colorimetric parameters. If the incident light is falling diffusely, this measurement requires the measuring system to have a field of view adapted to the cosine function. Only in this way the laws for the incidence of diffuse radiation from one or more sources of radiation can be satisfied. Detectors used to determine absolute illuminance must therefore have a cosine spatial function as their measurement geometry. If the incident radiation is not parallel, the accuracy of the cosine function is critically important to the result of the measurement. Classifies the quality of devices for measuring illuminance also called as luxmeters or photometers according to the accuracy of their measurement into:

- Devices of class A, with a total uncertainty of measurement of 7.5 % for precise measurements.
- Devices of class B, with a total uncertainty of measurement of 10 % for operating measurements.

Design of Colorimeter

The three main components of a colorimeter are a light source, a cuvette containing the sample solution, and a photocell for detecting the light passed through the solution. The instrument is also equipped with either colored filters or specific LEDs to generate color. The output from a colorimeter may be displayed by an analog or digital meter in terms of transmittance or absorbance.

In addition, a colorimeter may contain a voltage regulator for protecting the instrument from fluctuations in mains voltage. Some colorimeters are portable and useful for on-site tests, while others are larger, bench-top instruments useful for laboratory testing.

4. Applications of colorimeter

Colorimeters are widely used to monitor the growth of a bacterial or yeast culture. They provide reliable and highly accurate results when used for the assessment of color in bird plumage. They are used to measure and monitor the color in various foods and beverages, including vegetable products and sugar. Certain colorimeters can measure the colors that are used in photo copy machines, fax machines and printers.

A Colorimetric Method for the Estimation of Glucose in Solution

One useful and often used way of obtaining concentration of chemicals in a solution, if it has color is by colorimetric method. If the analyte is not colored an appropriate reagent must be added that reacts with the analyte to produce a colored compound.

In this experiment, the method for determining concentration of sugar is based upon the color that forms when sugar reduce 3,5-dinitrosalicyclic acid to 3-amino-5-nitrosalicyclic acid.

This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions: Oxidation of aldehyde group in to carboxylic group and Reduction of 3,5-dinitrosalicylic acid to 3-amino,5-nitrosalicylic acid.

In this experiment sucrose is provided which is the non-reducing sugar, does not undergo reaction with 3,5-dinitrosalicyclic acid. Therefore the sucrose and complex carbohydrate must be broken down into simple sugars like glucose first. The hydrolysis can be done by boiling the sample with hydrochloric acid, and then the pH is adjusted to give a basic solution, under which conditions are good for reducing sugar.

This method is a straightforward modification of the original DNS method for glucose analysis.

The sugars in the soft drink are at too high concentration for this method. So dilutions must be carried out before carrying out analysis.

The measurement of transmittance is made by determining the ratio of the intensity of incident and transmitted light passing through pure solvent and sample solutions as a function of wavelength. The logarithm of the reciprocal of the transmittance is called the absorbance

$A = \log (1 / T)$

According to Beer's law, the absorbance of a solution should be zero if there is none of the absorbing species present in sloution. A blank solution that does not contain analyte being analyzed but have the same composition as the solution can be used to calibrate the machine into zero reading absorbance. Then the machine can be used to find concentration of other solution.

The Beer-Lambert Law is only obeyed when the standard curve is linear for reasonably dilute solutions.

PROCEDURE:

Preparation of sucrose standard solutions

· Prepare 1000 mg/L of standard solution by suitable dilution of the stock solution.

· 2:10 dilutions of the stock solution as follows, 2.0 ml of the stock solution pipette into a clean 10 ml test tube and add distilled water to calibrate mark of 10 ml. Cover the tube and shake well to mix. In a similar fashion, prepare 4, 6, and 8 ml dilutions in 10 ml standard flask.

· Prepare five standards from the original stock solution and the four dilutions as follows:

- Pipette out 2.0 ml of each sucrose standard solution into test tubes.
- Add 2.0 ml of 6 M HCl into each test tube and placed in a boiling water bath for 10 minutes.
- The test tubes are removed carefully, pipette 0.8 ml of 2.5 M NaOH, 2.0 ml of 0.0050 M 3,5-dinitrosalicyclic acid into each test tube.
- Then placed the tubes in a boiling water bath for 5 minutes.
- Set the timer when the tubes placed in the hot water so that each tube to stay in the water for the same amount of time.
- Remove the test tubes from the boiling water bath at the proper time and quickly placed in ice-water bath for 10 minutes.
- Wash the couvette with some of the blank or distilled water solution and dry couvette and then placed 80% solution filled couvette into the spectrophotometer.
- The measurement of the standard solution was taken and the abundance was recorded.
- Preparation of unknown sample solution like Beverages
  Dilutions of beverages were prepared as follows:

- 0.1, 0.2, 0.4, 0.6, and 0.7 in series of 100 ml standard flask
- Treated 2.0 ml aliquots of the diluted samples in the same manner that you did for the standards.
- Record the absorbance for the diluted samples.

Determination of Phosphatase Enzymes in Plants

Phosphatase enzymes are widespread in nature and may be easily extracted from germinating seeds. They serve to remove phosphate groups from a wide range of organic phosphates and thus make available a metabolic pool of phosphate ions.

The accurate estimation of phosphate is a complex process so this protocol relies on a substrate which yields a product which is easily quantified by colorimetric methods.

Phenolphthalein phosphate has the property of being colourless in alkaline solution, whereas free phenolphthalein produces a pink colour. The amount of enzymatic activity can thus be estimated by the intensity of colour produced from a standard reaction mixture, when sodium carbonate solution is added.

The phosphatase enzyme has an optimum pH of around 5 so the pH of the reactions should be kept below 9.2. Any buffer system can be used, including the buffer tablets. The practical makes use of basic laboratory apparatus, although you may need to borrow a colorimeter from the chemists.

Since the reaction is stopped by the sodium carbonate the tubes can be stored in the refrigerator for several days before the colour intensity is assessed. This can also be used for reassessment of the accuracy of the technique. If duplicates are done for each variable under investigation the colorimeter readings for each pair should be identical.

Determination of iron in food products

Some metal ions in very low concentration show the strong colors by the formation of complex with ligand. These complexes are the result of the interaction of a Lewis acid and a Lewis base. The ideal color-forming reagent should be stable, selective and react rapidly to form soluble, highly colored complexes. The colored complex should have a high absorptivity and be free from variations in color due to minor changes in pH or temperature.

In general organic colorimetric reagents are considerably more sensitive than inorganic ones. They give more intense colors and are therefore frequently used for trace analysis. With many organic reagents, it is possible to determine concentrations at the ppm level.

2,2'-Bipyridyl forms an intensely red complex with ferrus ion that means iron(II) which may be exploited to determine iron concentrations in the ppm range. The reaction is

$$3bipy + Fe^{2+} <===> Fe(bipy)_3^{2+}$$

The complex conforms to an octahedral geometry with coordinate covalent bonds being formed between the adjacent sp^3d^2 hybrid orbitals of the Fe^{2+}. The complex forms rapidly, is stable over the pH range 3 to 9, and may be used to determine iron(II) concentrations in the range of 0.5 to 8 ppm.

If the sample contains ferric forms, then it must be reduced to iron(II) to produce the colored species. A suitable reagent for this purpose is hydroxylamine hydrochloride.

The concentration of iron in the sample could be calculated from Beer's Law however in this procedure we employ a different method. We will prepare a standard solution and compare absorbance readings of the sample and the standard solution. This technique minimizes the effects of instrument and solution variation.

Let us see the experimental procedure,

<u>Preparation of the Original Fe Solution.</u>

To an accuracy of ± 0.1 mg weigh out enough ferrous ammonium sulfate, to prepare 250 mL of a solution which is 0.00200 M with respect to that compound. Quantitatively transfer the salt into a 250 mL volumetric flask, add sufficient water to dissolve the salt, add 8 mL of 3 M H_2SO_4, dilute to the mark with distilled water and mix well. We shall call this the Stock Fe Solution. Pipet 10 mL of this solution into a 100 mL volumetric flask, add 4 mL of 3 M H_2SO_4 and dilute to the mark with distilled water and mix well. Label this solution as Original Fe Solution and calculate the concentration of Fe, in ppm, in this solution.

<u>Determination of the Absorbance of the Standard Fe Solution.</u>

Before beginning this part of the procedure be sure to record the number of the colorimeter that you are using for this part of the analysis. The number of the colorimeter is found on a small blue tag on the front of the colorimeter. Further absorbance measurements must be made with the same colorimeter and the same cuvettes in order for this method to work. Label the solution containing the Fe as Standard Fe Solution and calculate its Fe concentration in ppm. Determine the absorbance of this solution at the wavelength of maximum absorbance previously determined. For a blank use the solution which does not contain Fe. Make at least three measurements. In each case reset the zero and the 100% transmission. Record both the percent transmission and

absorbance values. Empty your cuvette and refill it with another portion of the same solution and again determine the absorbance value. Calculate the average of all six absorbance values.

Analysis of the Fe Unknown

Clean a 100 mL volumetric flask, pipette 10 mL of unknown solution into it and then add 4 mL of 3 M H_2SO_4, mix well and then make up to the calibration mark with distilled water. Mix well by inverting and shaking the stoppered flask. Pipet 10 mL of this solution into a 50 mL volumetric flask and then in this exact order add 1 mL of 10% hydroxylamine hydrochloride solution, 10 mL of 0.1% bipyridyl solution and 4 mL of 10% sodium acetate solution. Be sure to mix well after the addition of each reagent, by gently shaking or swirling, but not inverting, the flask. After all reagents have been added fill the flask to the mark with distilled water and mix well by inverting and shaking. Determine the absorbance of this solution using the previous blank solution as the reference and the wavelength of maximum absorbance determined earlier. Measure the absorbance at least three times. Empty the cuvette and refill it with another portion of solution and again determine the absorbance

5.Conclusion

A colorimeter is generally any tool that characterizes color samples to provide an objective measure of color characteristics. The colorimeter is an apparatus that allows the absorbance of a solution at a particular frequency of visual light to be determined. Colorimeters hence make it possible to ascertain the concentration of a known solute, since it is proportional to the absorbance.

The difference in the amount of monochromatic light transmitted through a colorless sample and the amount of monochromatic light transmitted through a test sample is a measurement of the amount of monochromatic light absorbed by the sample.

In most colorimetric tests the amount of monochromatic light absorbed is directly proportional to the concentration of analyte. However, for a few tests the relationship is reversed and the amount of monochromatic light absorbed is inversely proportional to the concentration of the test factor.

Colorimeter extensively used in the food and beverage industries to maintain required minerals like Fe, Phosphate, dyes in food materials.

Thank you