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Spectrophotometer

Hello,,,,,,,,,, Welcome to the lecture series on Food Science and Technology. In todays lecture deals on the topic entitled “Spectrophotometer”
The present episode consist of following five sections they are

1. Introduction
2. Basic Principle
3. Theory
4. Instrumentation
5. Applications with respect to food analysis

Let us see one by one

1. Introduction

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. Spectrophotometry 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 concept of spectrometry 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.

There are two kinds of spectrophotometers: single beam and double beam. A double beam spectrophotometer compares the light intensity between two light paths,one path containing a reference sample and the other the test sample. A single beam spectrophotometer measures the relative light intensity of the beam before and after a test sample is inserted. A double beam machine makes comparison readings easier and more stable. But a single beam machine can have measure a wider range of light frequencies, have simple optical systems and more compact. Spectrophotometer instruments measure the spectralreflectance or transmittance of visible light wavelengths 400 nm to 700 nm.

Over the last decade, spectrophotometers have beenutilized in the food industries for colorstandardization and Quality control inspection of ingredients priorto use, particularlyin jams, jellies, preserves, beverages, etc

2. Theory

Spectrophotometric law conducts the qualitative and quantitative analysis of the material by measuring the absorbance when the measured material in specific wavelengths. The frequently-used wavelength range: Ultraviolet region of 200~400nm (2) Visible region of 400~760nm, (3) Infrared region of 2.5~25 micro meter. The instruments are ultraviolet spectrophotometer, UV-Vis spectrophotometer, infrared spectrophotometer or atomic absorption spectrophotometer. In order to ensure the measurement accuracy and precision, all instruments should get regular correction verification in accordance with the national metro logical verification regulations.One instrument cannot be used to measure absorbance at all wavelengths because a given energy source and energy detector is suitable for use over only a limited range of wavelengths.

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) = $\log (I_0/ I)= \varepsilon bc$

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

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"
- "ε" is a constant called molar extinction coefficient

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.

3. The Spectrophotometer Instrument

All spectrophotometer instruments are designed to measure the absorption of radiantenergy 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 ormonochromator
- 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.

The energy source is to provide a stable source of light radiation, whereas thewavelength selector permits separation of radiation of the desired wavelength fromother radiation. Light radiation passes through a glass container with sample. Thedetector measures the energy after it has passed through the sample. The readoutdevice calculates the amount of light absorbed by the sample displays the signal from the detector as absorbance or transmission.

Both filters and mono-chromators are used to restrict the radiation wavelength.Photometers make use of filters, which function by absorbing large portions of thespectrum while transmitting relatively limited wavelength regions.

Monochromators that permit the continuous variation and selection of wavelength, the effective bandwidth of a monochromatorabout from 1 to 5 nm is satisfactory for most applications.

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 2μm

Accurate spectrophotometric analysis requires the use of good quality, matched cells. These should be regularly checked against one another to detect differences that can arise from scratches. The most common cell path for UV-Visible region is 1cm. Care must be taken to duplicate the position of such cells with respect to the light path; otherwise, variations in path length and in reflection losses will cause errors.

General Measurement Procedures

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.

4. Application of Spectrophotometer in Food Industry

Identifying Food Dyes with Spectrophotometers

The term spectrophotometric analyzer of food is used for various spectrophotometric sensors, devices, instruments, probes and testers dedicated to measuring physical and chemical parameters characterizing ingredients in food products and beverages. Non Infra Red (NIR) spectrophotometry is of particular usefulness for food analysis because the spectra of organic samples comprise broad bands arising from overlapping absorption peaks corresponding to C-H, O-H and N-H chemical bonds. The main advantage of NIR spectrophotometry in routine food analysis is simplicity and speed: usually no sample preparation is necessary and the time of analysis is not greater than 1 minute. Another advantage of NIR spectrophotometry is that it allows several constituents to be identified concurrently. But the disadvantage of this techniques is relatively weak absorption due to high-moisture food products and ingredients.

There are several reasons for using spectrophotometric analyzer of food in food manufacturing industries are as follows:

- checking the quality of food;
- monitoring of the food production process;
- providing data necessary for production control;
- specification of food products necessary for their labelling;
- precise classification of food products enabling their better pricing

There are more-or-less evident economic benefits behind each of them.

As I mentioned earlier, in spectrophotometers the reflected light from the sample is then split to its various components within the visible spectrum. The resulting data can be analyzed to give a quantifiable measure of the sample's color. This can be especially important in the food industry because foods have specific dyes and therefore any deviation can cause Sevier problem on food quality. The manufacturer is required to

strictly adhere to the product label with regards to the ingredients, including food dyes that make up the recipe for each food product. Thus, food producers tend to be very careful when analyzing food colors, and there are three primary methods for doing so.

Before production food producers tend to collect resources and ingredients from many places before they produce the actual end product. These ingredients, raw materials, can be analyzed using spectrophotometers to ensure that no nonstandard ingredients reach the production line, and this cuts down on waste and saves time by making sure that only approvedingredients are used for the production.

During production the food manufacturing process can sometimes involve many steps and it's possible that food colorings could become mixed or diluted at any stage of this process. Because of this, food producers often scan small test batches of products during the production process. This is done to ensure the quality of the end product and can identify any production-line level problems before they cause major issues down the line.

The manufacturer can also look into checking the color of the product as it is being produced. Color information can also be obtained, in real time, and can report to process control indicating whether the product color is within prescribed tolerances.

After production is completed,there are many factors that can cause food dyes to change color. If they are exposed to either oxygen or light for too long or accidentally mixed with other dyes, even small deviations in dye color can cause big changes in the color of end products. Because of this, food manufactures generally test a number of product samples before the finished product is shipped to retailers or distributors.

Regardless of the color being used in food, any federally approved dye will have a known quantifiable value. Because of this, food manufacturers are usually able to quickly find and identify color deviations before they can become a problem for retailers or consumers.

EXAMPLE: SPECTROPHOTOMETRIC ANALYSIS OF WINE

Wine, like many elite types of alcohol, is judged very specifically on the basis of taste. Wine enthusiasts begin evaluating the quality of wine from its color, before even sipping it. However, the flavor for most wines begins in the vineyard with the harvesting of certain grapes. The grapes used will set the tone for the type of wine and the specific processes used for pressing and fermentation will determine the final taste.

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

Some of the factors that are evaluated are the hue and color intensity of the wine. The CIE scale is a standard used internationally to relay values of color in relation to lightness in combination with values of red, green, blue, and yellow. Factors such as hue and intensity can provide information about the quality of the wine.

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.

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 spectrophotometeris able to determine: ethanol,glucose and fructose, maleic acid, volatile acid, total acid and pH in finished wine or under fermentation.

Estimation of Protein by spectrophotometric Analysis

Since proteins absorb light at a specific wavelength, a spectrophotometer 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.

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.In addition, the structure of your protein of interest may also affect the UV absorbance of aromatic side chains. As such, the temperature, pH, ionic strength, and the presence of detergents can affect the ability of aromatic residues to absorb light at 280 nm, and change the value of the protein's extinction coefficient.

Procedure

1. Warm up the UV lamp (about 15 min.)
2. Adjust wavelength to 280 nm
3. Calibrate to zero absorbance with buffer solution only
4. Measure absorbance of the protein solution
5. Adjust wavelength to 260 nm
6. Calibrate to zero absorbance with buffer solution only
7. Measure absorbance of the protein solution

Also ASD's portable, bench top and online Vis/NIR instrumentation provides the flexibility to measure where and when it is most advantageous to you. Gone are the days of collecting samples, sending them to a laboratory, and waiting for the results. ASD's Vis/NIR measurement technology offers the cost effective solution you need to:Easily analyze product in the field, on the receiving dock, in the warehouse or manufacturing facilities, even through glass and most clear plastic bags and bottles

- Monitor in-process and finished product to ensure quality and consistency

- Minimize risk of product and material waste

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

Scientists are also utilizing Vis/NIR spectroscopy to develop specific aspects of ingredients to enhance the nutritional quality of foods and beverages.

The quantitative determination of caffeine in beverages and soft drinks using UV spectroscopy

Caffeine is a naturally occurring alkaloid which is found in the leaves, seeds or fruits of over 63 plants species worldwide. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. The worldwide consumption of products derived from these natural materials means that caffeine is one of the most popular and commonly consumed drugs in the world. Caffeine's gain more popularity because of its biological significance and also proved as a mild central nervous system stimulant.

It is generally agreed that there is little risk of harm when a person consumes less than 300 mg of caffeine a day. However at times of anxiety or stress, or during pregnancy, recommends consumption of less than 200 mg a day. While there are no regulatory requirements to control or label food products with their caffeine content, numerous studies have been carried out to determine the typical caffeine content of commonly consumed beverages. A wide variety of methods have been employed with High Pressure Liquid Chromatography being the method of choice in many analytical studies as it commonly is subject to fewer interferences than alternative methods.

HPLC is an expensive and laborious technique that is not typically found in the scientific teaching labs of schools and colleges. Therefore HPLC methods of application note will investigate an alternative analytical method that uses UV spectroscopy to analyse and quantify the caffeine content of some common beverages and soda drinks.

Caffeine can be extracted from aqueous solutions with chlorinated solvents such as dichlormethane and chloroform, a technique commonly employed commercially to de-caffeinate coffee and tea. After the caffeine is extracted it can be analysed directly by measuring the absorbance of the solvent solution at 260nm.

Let us see the methodand procedure for the determination of caffeine

Standard Caffeine, Dichloromethane, Purified water are required reagents in this technique

In the first step Standard Preparation:-

A 1000ppm stock standard of caffeine was prepared by dissolving 198.2mg of caffeine in 200.0ml purified water. Working standards were prepared by pipetting 25, 12.5, 10, 7.5, 5 and 2.5ml aliquots of the stock standard solution into separate 50.0ml volumetric flasks and diluting to volume with purified water.

Sample Preparation:-

Take 200ml aliquots of boiling purified water to each of two 250ml beakers containing 2g of instant coffee or tea. The coffee and tea preparations were stirred for 30 seconds using a magnetic stirrer at 500rpm and allowed to cool to room temperature. The soft drink samples were used as supplied by the manufacturer.

Caffeine Extraction Procedure:-

A 50ml aliquot was taken from each working standard or sample solution. This aliquot was placed into a separating funnel and 25ml of dichloromethane was added. The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion.

The dichloromethane layer was removed to a clean flask and the extraction procedure wasrepeated twice more and the solvent layers combined.

Sample Measurement:-

Aliquots of the extracted standards were placed into quartz cuvettesand analysed using a spectrophotometer. The concentration mode was then used to quantify the caffeine concentration of the sample solutions with measurements performed against a dichloromethane blank.

Conclusion

Spectrophotometer is aninstrumental technique, which is used to measure the reflectance or transmittance light of the object across the full spectrumof visible light wavelengths, 400 nm to 700 nm. The Spectrophotometer isworking on the basis Beer-Lambort's law, it offergreaterspecificity,making them the instruments of choice for food product color formulation, specificationof standards and tolerances, inter-plant colorcommunication and color quality control in processingoperations.

Thank you