Spectro-photometry Method

Sep 02, 2019

Spectrophotometry method for the detection of Biochemical parameters

Xiaohua Du[1], Xia Liu[2], Mawolo James Blackar[2], Yingjie Zhou[3], Haifeng Wang[2], Fayang Liu[2], Zhiqing He[3]

[1]Faculty of Veterinary Medicine, Gansu Agricultural University, Lanzhou City, Gansu Province, People's Republic of China, [2]College of Life Science and Technology, Gansu Agricultural University, Lanzhou City, Gansu Province, People's Republic of China, [3]Gansu Endangered Animal Protection Center of State Forestry Administration, Wuwei City, Gansu Province, People's Republic of China

1 Works for me dx.doi.org/10.17504/protocols.io.6wchfaw

Du Xiaohua

ABSTRACT

Definition of key terms

Spectrophotometry is an experimental technique that is used to measure the concentration of solutes in a specific solution by calculating the amount of light absorbed by those solutes.[1] This technique is powerful because certain compounds will absorb different wavelengths of light at different intensities. By analyzing the light that passes through the solution, you can identify particular dissolved substances in solution and how concentrated those substances are. A automatic analyser (7180) is the device used to analyze solutions in a laboratory research setting.

Desecription of Automatic analyser

The Automatic analyser (7180) is a medium-sized biochemistry automatic analyzer.The minimum required amount of reaction solution is smaller due to a new optical design, and running cost is lower and working efficiency higher because the operating unit is usable in the same way as a general-purpose personal computer. Further, this analyzer features a wide range of applications including routine, stat and special analyses.

Benefit of Spectrophotometry method

The usage of Automatic analyser (Hitachi Ltd 7180 Serial, Tokyo, Japan) enabled us to obtained more samples result in less time and this prevented other factors from influencing our tested results.

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GUIDELINES

Operating Unit

The analyzer is now easier to operate than before because of its user interface featuring an operability unique to a general purpose OS (Windows®) and the touch screen.

Analytical Unit

After deliberation on how best to make opera-tions comfortable and analysis smooth, the Model 7180 was developed. Its operator-friendly design concept is visible everywhere from the action warning lamp to the transparent top cover which allows checking of the disks at any time. With this, we started the manchine and its ran the entire tests and we obtained our sample results.

Routine Test

The 7180 has a high capability of simultaneous 86 channels and 800 tests/hour adequate as a routine analyzer and has a flexibility in

application to new tests and new assays.The enriched flexible random access function wider in degree of freedom has raised the potentiality of clinical tests to an even higher level.

Basically, there are three (3) major parts involve in using this method with series of steps. The detailed steps of the procedures can be found in the step section.

PART I: Sample preparation
PART II: Running the experiment
PART III: Analyzing the Absorbance Data

References:

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MATERIALS TEXT

Materials Required:

1. Automatic analyser
2. Cuvette
3. Blank solution
4. Glass
5. Wash bottle
6. Lint free cloth
7. blank

Reagents:

1. Cobalt (II) chloride
2. Hexaaquacobalt (II) ion
3. Ferrocene
4. Crystal violet
5. Rose bengal
6. Coumarin

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for safety warnings and hazard information.

Safety methods to follow during our experiment at the laboratory:

1. Wear lab coat and gloves while in the lab. When you enter the lab, switch on exhausted fans and make sure that all the chemicals and reagents required for the experiment are available.
2. Clean all working apparatus with chromic acid and distilled water and ensure that all the apparatus are free from water droplets while performing the experiment.
3. Calibrated the electronic weigh balance before taking the measurements.
4. Ensure that the spectrophotometer working properly was done.
5. Make sure the cuvette is handled with tissue paper and dodn't touch it with your hands.
6. Wipe the cuvette with tissue paper before placing in the spectrophotometer.
7. Clean all apparatus with soap and distilled water. Upon completion of the experiment, recap the reagent bottles. Switch off the light and exhaust fan before leaving the lab.

8. Discarded the used gloves in a waste bin.

BEFORE STARTING

Safety methods to follow during our experiment at the laboratory:

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PART I: Sample preparation

1 **Turn on the automatic analyser:** Turn the **automatic analyser** on to warm up to give an accurate reading.

The warm up process will take **00:15:00** before the samples can be run.

While the **automatic analyser** was warming up, prepare the samples to be run.

2 **Load the proper volume of the sample into the cuvette:** The automatic analyser contains a volume of 1 milliliter (mL) and test tubes with a volume of 5 mL.

Use different pipettes for each sample to prevent contamination. [3]

3 **Prepare of a control solution:** Known as a blank, the control solution has chemical solvent in which the solute to be analyzed is dissolved in. The blank should have the same volume as the solution to be analyzed and kept in the same kind of container.

4 **Wipe the outside of the cuvette:** Before placing the cuvette into the automatic analyser, make sure that the cuvette is cleaned to avoid interference from dirt or dust particles.

We used a lint free cloth, and remove water droplets or dust that were on the outside of the cuvette.[4]

PART II: Running the experiment

5 **Select and set the wavelength of light to analyze the sample with:** Use a single wavelength of light (monochromatic color) to make the testing more effective. The color of the light to choose is the one known to be absorbed by one of the chemicals thought to be in the test solute. Set the desired wavelength according to the specifications of your spectrophotometer.

6 **Calibrate the machine with the blank:** Place the blank into the cuvette holder and shuted the lid.

On an analog automatic analyser, there was a screen with a needle that moves based on the intensity of light detection. When the blank got in, we saw the needle moved to the right.

Record the values, while the blank still in the machine, move the needle to zero using the adjustment knob.

- When we removed the blank, the calibration was still in place. During measuring the rest of our samples, the absorbance from the blank was automatically subtracted out.
- A single blank was use per session so that each sample is calibrated to the same blank.

7 **Remove the blank and test calibration:** Remove the blank and the needle should stay at 0 (zero). Put the blank back into the machine to ensured the needle readout doesn't change.

If the machine is properly calibrated with your blank, everything should stay at 0.

8 **Measurement of the absorbance of experimental sample:** Removed the blank and place the experimental sample into the machine. Slide the cuvette into the designated groove and ensure it stond upright. Wait about **00:00:10** until the needle is steady or until the digital numbers changed. Recorded the values of % transmittance and/or absorbance.

- The absorbance is also known as the optical density (OD).
- The more light that is transmitted, the less light the sample absorbs. Generally, we wanted to record the absorbance values which will usually be given as a decimal, for example, 0.43.

9 **Repeat the test with successive wavelengths of light:** The samples have multiple unknown compounds that vary in their absorbance depending on wavelength. To eliminate uncertainty, repeat your readings at 25 nm intervals across the spectrum. This allow you to detect other chemicals suspected to be in the solute.

PART III: Analyzing the Absorbance Data

10 **Calculation of the transmittance and absorbance of the sample:** Transmittance is how much of the light that passed through the sample reached the automatic analyser. Absorbance is how much of the light has been absorbed by one of the chemicals in the solute. Many modernautomatic analyser have an output of transmittance and absorbance, when you recordthe intensity, calculate these values.[5]

- The transmittance (T) is found by dividing the intensity of the light that passed through the sample solution with the amount that passed through the blank. It is normally expressed as a decimal or percentage. $T = I/I_0$ where I is the intensity of the sample and I_0 is the intensity of the blank.
- The absorbance (A) is expressed as the negative of the base-10 logarithm (exponent) of the transmittance value: $A = -\log_{10}T$.[6] For a T value of 0.1, the value of A is 1 (0.1 is 10 to the -1 power), meaning 10% of the light is transmitted and 90% is absorbed. For a T value of 0.01, the value of A is 2 (0.01 is 10 to the -2 power), meaning 1% of the light is transmitted.

11 **Plotting the absorbance values versus the wavelengths on a graph.** Plot the absorbance value on the vertical y-axis against the wavelength of light used for a given test plotted on the horizontal x-axis. Plotting the maximum absorbance values for each wavelength of light tested, produces the sample's absorbance spectrum and identifies the compounds making up the test substance and their proportions.

An absorbance spectrum usually has peaks at certain wavelengths that can allow you to identify specific compounds.

12 **Comparing our absorbance spectrum plot to known plots of specific compounds:** Compounds have unique absorbance spectrum and always produced a peak at the same wavelength every time they are measured. Comparing your plots of unknown compounds to those of known compounds, identify the solutes that compose of your solution.

This method can also to identify contaminants in sample. If you are expecting 1 clear peak at a specific wavelength and you get 2 peaks at separate wavelengths, you know something is not right in your sample.