Introduction to Laboratory Work, Safety Rules, Photometry

Practical Lesson on

Medical Chemistry and Biochemistry

General Medicine

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2026/27

Task 1 – Identification of an unknown substance using its absorption spectrum

Determine the absorption maximum of the standard solutions and an unknown sample using spectrophotometry. Subsequently, identify the unknown substance in the sample by comparing the results. This type of analysis is feasible only if the sample contains a single substance – in this case, a dye – whose absorption spectrum does not significantly overlap with that of another substance. If the sample contains multiple substances, a necessary step prior to qualitative analysis (e.g., using spectrophotometry) is the separation of individual analytes into distinct fractions. Chromatography is a very common separation method in such cases.

Reagents

Aqueous solutions of blue food dyes E131, E132, and E133 – 7.5 μg/mL

Procedure:

1. On the spectrophotometer, select the "wave scan" measurement from the "applications" menu and confirm the selection.
2. Set the wavelength range from a minimum of 400 nm to a maximum of 700 nm.
3. Using an automatic pipette, fill the cuvette with 1 mL of distilled water and place it into the spectrophotometer.
4. Zero the spectrophotometer by pressing the "0T" button.
5. Empty all the water (blank sample) from the cuvette and fill it with 1 mL of the beverage to be analysed.
6. Measure the sample spectrum and record the wavelength corresponding to the absorption maximum:

   λ Amax = …… nm

   Use the > < arrow keys on the spectrophotometer's control panel to position the indicator line at the wavelength corresponding to the absorption maximum.
7. Empty the sample from the cuvette, rinse it several times with distilled water, and use an automatic pipette to fill it with 1 mL of the E131 dye standard. Measure the sample spectrum and record the wavelength corresponding to the absorption maximum. Analyse the remaining dye standards (E132 and E133) in the same manner.
8. Identify the dye present in the sample by comparing the spectrophotometric analysis results of the unknown sample with those of the standards.
9. Using information from a review article in the PubMed database, evaluate the potential risks associated with the use of the identified synthetic dye in food.

Task 2 – Quantification of a substance in solution using photometry

For a known substance with a defined absorption spectrum, the fact that absorbance is directly proportional to concentration can be used for quantification in the case of true solutions. A calibration curve is constructed from a set of calibration solutions of known concentrations, allowing the concentration of an unknown sample to be accurately determined.

Reagents:

Aqueous stock solution of the blue dye at a concentration of 7.5 µg/mL.

Procedure:

1. Using automatic pipettes, prepare a set of working solutions (A, B, C, D, and a blank) from the stock solution of the blue dye and distilled water in clean glass test tubes, following the instructions in the table. Prepare each sample – except for the blank – in triplicate. Briefly vortex each solution before use.

Sample	***Blank***	***A***	***B***	***C***	***D***
Volume of stock solution	*0 mL*	*2 mL*	*1 mL of solution A*	*1 mL of solution B*	*1 mL of solution C*
Volume of distilled water	*1 mL*	*0 mL*	*1 mL*	*1 mL*	*1 mL*

2. Fill in the table in protocol sheet with the concentrations of the respective working solutions (A, B, C, and D) and their dilution factors (how many times the solution is diluted) relative to the original stock solution.
3. Set the spectrophotometer to the optimal wavelength for quantifying the substance identified in Task 1.
4. Zero the spectrophotometer using a blank.
5. Sequentially measure the absorbances of the prepared working solutions A, B, C, and D. Record the results in Table 2.
6. Construct a calibration curve showing the dependence of absorbance on concentration based on the measured results.
7. Measure the absorbance of the beverage sample.
8. Determine the concentration of the blue dye in the analysed beverage from the constructed calibration curve.
9. Calculate the total amount of the blue dye in 0.5 L of the beverage.