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Fluorimetric Estimation of Riboflavin or Quinine

Practical 5 - Bench practical

Aim

To estimate riboflavin or quinine using a calibrated fluorescence response under controlled solution conditions.

Principle

Certain molecules emit light after excitation. Emission depends on analyte concentration, excitation and emission settings, solvent, pH and the chemical environment.

At suitable low concentrations, a calibration can relate fluorescence response to analyte concentration. Quenching, scattering and inner-filter effects can invalidate this simple relationship at higher concentrations.

Materials and Requirements

- Spectrofluorimeter and suitable clean cells
- Riboflavin or quinine reference material
- Method-specified solvent and blank
- Light-protective containers and volumetric glassware

Procedure

1. Record the selected analyte, solvent, pH, excitation and emission settings, slit widths and detector conditions from the adopted method.
2. Prepare a reference stock and dilute to a working series that is sufficiently dilute for a linear response. Protect light-sensitive preparations as required.
3. Scan or verify the reference excitation and emission behaviour before the quantitative run.
4. Measure the blank, calibrators, independent check and test solutions with consistent geometry and timing.
5. Inspect calibration residuals and assess whether dilution restores proportional response in a concentrated test preparation.
6. Calculate the test concentration from the blank-corrected calibration and apply all dilution factors.
7. Record solution age, light exposure and instrument settings so that another analyst can reproduce the measurement.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation	Concentration	Fluorescence response	Blank-corrected response
Blank	0	Record	0 reference
Reference levels	Record	Record	Record
Check preparation	Record	Record	Record
Test dilution	Record	Record	Record

Calculations

- $F_{corrected} = F_{measured} - F_{blank}$

• If F_corrected = mC + b, then C = (F_corrected - b) / m

Worked Example

Illustrative data: blank response 10 units, test response 90 units and calibration F_corrected = 40C.

C = (90 - 10)/40 = 2.00 micrograms/mL in the measured dilution.

A 25-fold dilution gives 50.0 micrograms/mL in the original preparation. Verify that the response lies within the calibrated range.

Result and Interpretation

• Report the concentration and the conditions under which the calibration was established.
• A decrease in response at high concentration can reflect optical or chemical interference rather than less analyte.
• Compare recovery in the relevant matrix before treating the method as suitable for a formulation.

Precautions

• Keep pH, solvent and temperature consistent.
• Avoid fluorescent contamination from detergents or damaged cells.
• Use the selected light-protection conditions.
• Dilute preparations when required instead of extrapolating beyond the working range.

Viva Questions and Answers

What is quenching?

A reduction in fluorescence caused by the molecular environment or interacting species.

Why protect riboflavin solutions?

Light exposure can change the analyte and its response.

Why record slit widths?

They influence signal intensity and spectral resolution.

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