Fluorescence Quenching

Practical 05 | Instrumental Methods of Analysis and Novel Drug Delivery Systems

Aim

To study Fluorescence Quenching, and interpret the recorded observations.

Principle

- A quencher can reduce fluorescence through different mechanisms. A Stern-Volmer relationship is appropriate for the stated simple dynamic-quenching model; static association and optical inner-filter effects require separate interpretation.
- An optical response is related to analyte concentration over the validated working range.
- A blank, suitable standards and consistent optical conditions are necessary for interpreting the response.

Materials and Apparatus

- Prepared fluorescent standard and quencher series, fluorimeter and completed comparative records.
- Assigned analyte, suitable reference standard, solvent/reagents and volumetric glassware.
- Specified optical instrument and compatible cells or sample-introduction system.

Method

1. Select the optical method and settings appropriate to the assigned analyte.
2. Prepare a method blank and a series of independently documented calibration concentrations.
3. Check the instrument response and measure the blank and standards using consistent settings.
4. Prepare the test solution and apply documented dilution where necessary.
5. Read the test response within the established calibration range.
6. Calculate concentration, correct for dilution and report the original-material basis.

Observation Record

Complete this record using actual laboratory findings or the provided teaching data.

Observation / parameter	Record / finding
Quencher concentration	_____
Unquenched F0	_____
Observed F	_____
F0/F	_____
Model fit	_____

Calculations and Interpretation

- Simple dynamic Stern-Volmer model: F0/F = 1 + KSV[Q].
- Plot F0/F against quencher concentration; the slope is KSV within the valid model.
- State concentration units because they determine the units of KSV.

Worked Illustration

Illustration: F0 = 100 and F = 50 give F0/F = 2.0. Under the simple dynamic model at [Q] = 0.010 mol/L, KSV = 100 L/mol.

The values in this illustration are for calculation practice.

Result

Record the actual finding, calculated value with units where relevant, and a conclusion supported by the observations.

Finding: _____

Conclusion: _____

Precautions

- Use clean compatible cells and consistent orientation where cells are used.
- Keep readings within the calibration range and examine the calibration fit.
- Avoid assuming that another analyte or solvent has the same response factor.

Viva Questions

1. Does reduced intensity alone prove dynamic quenching?

No. Static association, absorption of excitation/emission light and instrumental effects can also reduce the observed signal.

2. What is the role of a blank?

It corrects the contribution of the solvent, reagents and measurement background.

3. Why use several calibration levels?

They establish the usable response-concentration relationship and help reveal nonlinearity.

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