

Fluorescence Quenching

Experiment 9 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare an assigned fluorophore response with and without a quencher.

Principle

Quenching reduces observed fluorescence through specified physical or chemical mechanisms. Absorption or matrix changes can also alter the measured signal.

Requirements

- Assigned fluorophore/quencher system, fluorimeter, blanks and supplied or measured comparison data.

Procedure

1. Prepare the comparison

Keep fluorophore concentration and optical conditions matched across the assigned quencher levels.

2. Read signals

Record blank-corrected unquenched F_0 and quenched F values.

3. Plot

Plot the assigned quenching relation; inspect whether it is linear over the chosen range.

4. Assess mechanism limits

Discuss inner-filter or other instrumental effects and avoid assigning a mechanism from one ratio alone.

Pharmaceutical Analysis

Observations and Record

Quencher concentration	F	F ₀ /F
0	_____	_____
_____	_____	_____
_____	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- For the applicable Stern-Volmer model: $F_0/F = 1 + K_{sv}[Q]$.
- Example: $F_0 = 100$, $F = 80$ and $[Q] = 0.01$ mol/L give $K_{sv} = (1.25 - 1)/0.01 = 25$ L/mol, if that model is valid.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use matching blanks and inspect absorption-related interference.
- Model fit alone does not establish one quenching mechanism.

Viva Questions and Answers

1. What does F₀ mean?

The fluorescence without quencher under the matched conditions.

2. Why can a quencher appear effective for the wrong reason?

It may alter absorption or optical transmission rather than only the fluorophore process.

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