INDIAN INSTITUTE OF TECHNOLOGY, BOMBAY

COURSE	ROLL NO.	NAME	
CH117L	23B0912	Aditya Sanapala	
ASSIGNMENT NO.	DUE DATE	SUB. DATE	
2		10/10	

Experiment-2: Thin Layer Chromatography

Objectives:

1. To prepare a fluorescent dye in microscale using a one-pot sequential amide formation - Nucleophilic aromatic substitution reaction
2. To understand the principles and application of Thin Layer Chromatography.
3. Analysis of TLC using 'ImageJ'
4. Determination of molecular weight by using TLC-MS.

Introduction & Principles:

→ Chromatography is a technique used in the separation of components of a mixture of compounds.

→ Thin Layer Chromatography (TLC) is based on the selective adsorption of the solvent and solute on the stationary phase.

→ The technique consists of applying the mixture on a stationary phase (a thin layer of silica gel, SiO_2, on aluminium) and allowing a mobile phase (toluene) to climb up the adsorbent.

→ The most important criterion in qualitative identification is the R_f value (called retention value), which is defined as

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

The dye:

→ Fluorophores are molecules which can be excited and relax efficiently in a radiative pathway.

→ On absorption of a photon, a molecule in the singlet ground state (S_0) gets excited to the first excited state (S_1).

→ It can relax back to the ground state (S_0) by emission of a photon of lower energy than the incident one.

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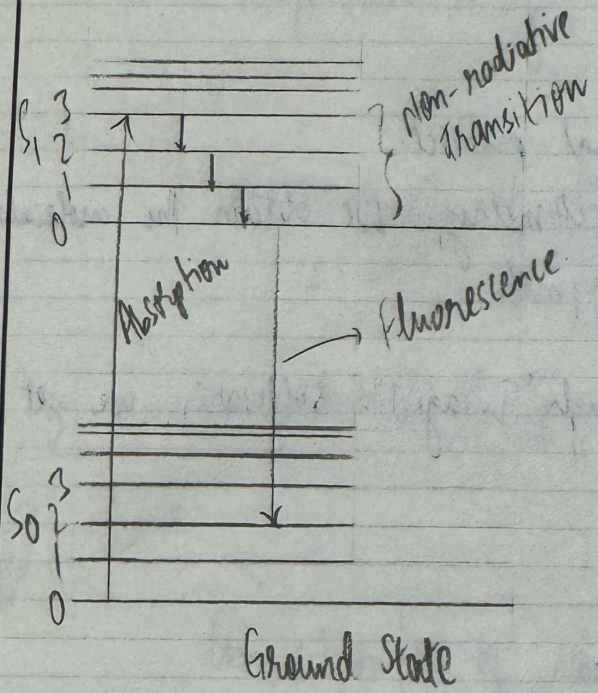
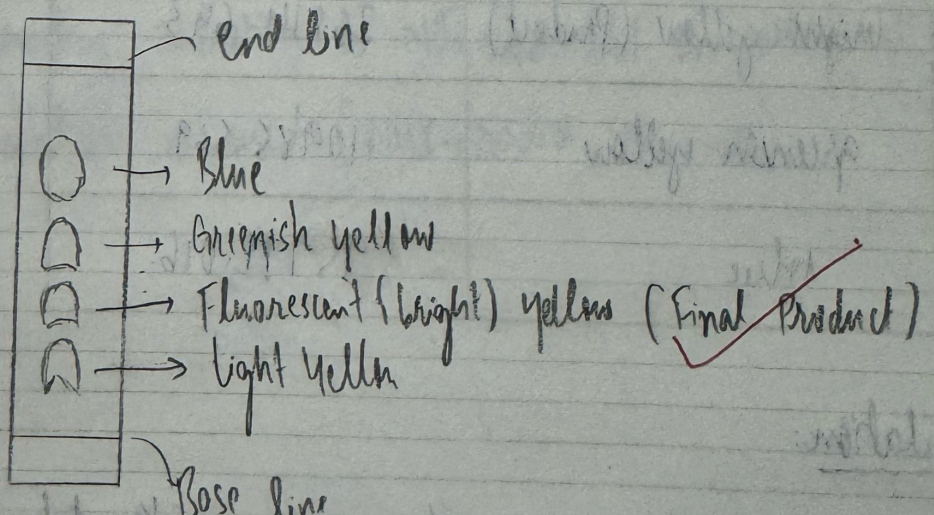


Energy diagram.

Observation:

(under long UV)



TLC

→ 4 spots are visible on the TLC when observed in long UV.

→ From bottom, the spots are in:

Light yellow
fluorescent (bright) yellow → This is our product.
greenish-yellow
and blue.

→ The second spot from the bottom is our final product.

→ After running the TLC through mass spectometry, we obtain the molecular weight of the final product as 381.5 g/mol.

→ After running the image of the TLC through "ImageJ" software, we get the area of the spots as follows:

Spot Color	Area
Light yellow	21005.915
bright yellow (Product)	26814.643
greenish-yellow	10985.569
blue	15771.016

Calculation:

Composition % of a particular spot = $\frac{\text{integrated area of spot}}{\Sigma \text{ integrated area of all spots}} \times 100$

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So, Composition % of pale yellow spot = $\frac{21005.915}{74517.143} \times 100 = 28.18\%$

Composition % of bright yellow spot = $\frac{26814.643}{74517.143} \times 100 = 35.98\%$

Composition % of greenish-yellow spot = $\frac{10985.569}{74517.143} \times 100 = 14.74\%$

Composition % of blue spot = $\frac{15711.016}{74517.143} \times 100 = 21.08\%$.

→ In mass spectrometry, radiation is NOT used but in NMR & IR spectroscopy, radiation is used (NMR → radio, IR → IR).