**INSTRUMENTAL ANALYSIS LABORATORY**

**NAME OF THE EXPERIMENT:** FL

**DATE OF THE EXPERIMENT:** 17.04.2023 – 18.04.2023

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**INSTRUMENTAL AND EXPERIMENTAL PART**

**INSTRUMENTATION:**

The device used in the FLUORESCENCE experiment is called F-2500 Fluorescence, Spectrophotometer HITACHI. The source used is a Xe lamp, and our detector is Photomultiplier Tube.

In this experiment, our energy source is a high-intensity.lamp .The excitation wavelength, selector is used to specify the light coming. from the source at specific wavelengths. In contrast, the emission wavelength selector determines the light emitted by passing through the sample. The detector used to measure the intensity of the light is called the photomultiplier tube. A. reference light beam attenuator is used to reduce the density of the beam. The device converts the radiation into an electrical signal with precision transducers, and then the computer is used as a read-out device.

**CALCULATION PART**

**1)Draw excitation and emission spectra on the same graph and compare fluorescence.**

**Graph 1. Emission Spectrum**

**Graph 2.** **The emission spectrum of fluorescein between 470 and 550 nm and set excitation MC to 440 nm 450 nm**.

**CALCULATION PART**

**Graph 3. Bandwidth changes with 2,5 to 20 nm**

**Graph 4. Via varying Concentrations Intensity Graph**

**Part C:**

**Graph 5. Calibration Curve**

**Part D:**

**Graph 6.** **Emission and Excitation Spectrum at pH=2**

From the Calibration Curve Graph (Graph 5)

Equation curve is equal to,

y=(1x109) c - 188,99  
R² = 0,9827

Fluorescence intensity of our unknown is **2577,6 nm.**

Put y = 2577,6 then x equal to

2577,6 =(1x109) c -188,99

c=2.38 x 10-6 M

**Calculating Detection Limit:**

LOD = (3\*s\*C) / Iavg

Iavg: Average fluorescence intensities of 10 measurements

s: Standard deviation of ten measurements

C: Minimum standard concentration that is detected

For “I” Solution, fluorescence intensities,

**Table 1. Ten measurement fluorescence intensities for I Solution**

|  |  |
| --- | --- |
| **Measurement** | **Intensity** |
| 1 | 632,4 |
| 2 | 648,2 |
| 3 | 648,5 |
| 4 | 649,7 |
| 5 | 643,2 |
| 6 | 635,9 |
| 7 | 642,2 |
| 8 | 646,3 |
| 9 | 642,8 |
| 10 | 638,8 |

Iavg = 648,2

Standard Deviation of ten measurement ”s” = = 7.731

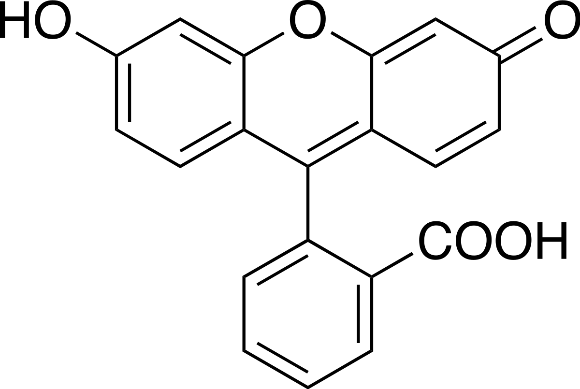
LOD= = 8.52x10-8

**E)** The HCl added to “e” solution pH become 2, then the emission intensity become 111,2 before added to HCl the intensity of solution “e” was 5204. Intensity decreased.

**POST LAB QUESTIONS**

1. While monochromators are displaced,. in the emission spectrum, in the excitation spectrum, the emission monochromator is tuned in the splitting, monochromator according to the wavelength of radiation detected .and emitted at different wavelengths. An excitation spectrum can be the same as an absorption spectrum, but radiation, absorption can be achieved. On the other hand, if the same conditions are met, the excited states emit a fluorescent emission. When looking at Graph 5, it will be seen that the concentration and the Relative Fluorescent Intensity are directly proportional.
2. The intensity of the light transmitted, by the spectrophotometer is measured according to the intensity transmitted from the blank side. On the other hand, the intensity of the transmitted light is measured by making it more sensitive by spectrofluorometer [2].
3. pH changes in the solution medium affect the fluorescent properties of fluorescein. If the pH exceeds 7, the highest absorption can be observed at 490 nm. If the temperature increases, the intensity of the fluorescein fluorescence decreases; in addition, the fluorescein intensity of the fluorescein may be affected by the polarity of the solvent. In addition, if the lifetime of fluorescein, fluorescence is desired to be extended may increase the viscosity of the solution. It was mentioned above that fluorescein, fluorescence intensity can be affected by the polarity of the solvent. It is a poorly soluble compound in water. As a result, it becomes difficult to observe the fluorescent signal. A diluted NaOH solution is used to see a suitable observable fluorescent signal. As a result of this process, the fluorescence dissolves well in water, and this provides a detectable fluorescence signal.[2]

**Figure 1. Structure of fluorescein**



1. Higher source density gives rise to higher absorbance readings; spectroscopy is absorbance spectroscopy. On the other hand, in fluorescence spectroscopy, one can increase the fluorescence intensity up to a point with a higher source density, but if the source density is increased too much, the fluorescence may fade and decrease the intensity.[1]
2. A higher fluorescence intensity can be observed with a wider slit. A lower slit is observed at a lower fluorescence intensity.[3]
3. An increase in pH causes an increase in fluorescence intensity. With an increase in pH, the possibilities of resonance forms that the molecule can form increase and positively affect the observation of the resonance fluorescence intensity. Looking at Graph 1 and Graph 6 of this experiment, it was observed that the emission spectrum decreased when the pH decreased to 2. In general, to say that the emission, that is, the fluorescence intensity, increases with the increase in pH, it is necessary to look at the studies carried out to observe the best fluorescence intensity at which the pH value of the molecules is studied.
4. F which is the power of the fluorescence emission when it has the constant power of the beam incident (P0) on the solution we can realize that formula:

c is the concentrations of solutions and K is the constant.

At low concentrations from that formula, the graph of the calibration curve will be linear but for solutions that have high concentrations linearity of the calibration curve will be gone.[1]

**REFERENCES**

1) Hinterdorfer, P., & Dufrêne, Y. F. (2006). Detection and localization of single molecular recognition events using Atomic Force Microscopy. *Nature Methods*, *3*(5), 347–355. <https://doi.org/10.1038/nmeth871>

2) Skoog, D. A., & Crouch, S. R. (2018). Chapter 26 An Introduction to Chromatographic Separations. In F. J. Holler (Ed.), *Principles of Instrumental Analysis Seventh Edition* (7th ed). Cengage Learning Cengage Learning.

3) Tanaka, T. (2012). *Experimental methods in polymer science: Modern methods in polymer research and technology*. Academic Press.