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TITLE Crime and Dye Lab Special Projects 1 Martin Enem LAB SECTION: BB2

INTRODUCTION Light is usually viewed as a result of the heating of a substance. The higher the temperature at which the substance is heated, the greater the vibrations that lead to certain light intensity given off by the molecule. It is this same theory that suggests why steel glows red hot when heated to high enough temperatures. The process of light emissions can also be induced through other means. 1 One of such means termed fluorescence occurs when a substance can be induced into giving off light is through absorption of a photon through light or other means of radiations. During this process, electrons are excited from their standard state. The electrons jump from their highest unoccupied orbital (HUMO) into a certain level of the unoccupied orbital (LUMO). As the electrons lose energy, they fall back to a lower orbital, thus emitting light. 1, 2 During the process leading to fluorescence, a certain change could occur during the excitation of the electron, changing the spin of the electron as it gets excited. This change has to be undone as the electron goes back to its standard state. The emitting of light in this process is known as phosphorescence. The process of fluorescence is much faster than phosphorescence. This is due to the fact that in phosphorescence, the electron has to undergo an extra step of undoing its current spin. This process is thermodynamically unfavorable compared to fluorescence. 1, 2 The last means by which light could be generated is through chemiluminescence. In such a case a chemical reaction occurs, causing an excitation of the product. As this product decays into its standard state, light is emitted. This form of luminescence is different from the previous two in the sense that no absorption of light is required to induce the glow. The glow results from the product of the chemical reaction having to

be in an excited state or of higher energy. 1 The process of photon absorption and emission is certain a phenomenon that plays a vital role in our society. Such importance is expressed during rescue operations, where victims can be easily found due to waving of a fluorescent material at night. It is evident that fluorescence becomes important when a thermally induced light emission is deemed impossible. 1, 2 Separation techniques were concepts that were important in the course of this experiment. One of such is through the means of UV Vis Spectrophotometer. The constituents of ink dyes can be determined using a spectrophotometer. By placing the dyes in the spec, various wavelength peaks will be obtained. These peaks can be used to find out the constituent colors making up the dye. Another important form of separation used during this experiment is chromatography. Chromatography utilizes the differences in polarity of substances as means of separation. Such is the case of a crime lab involving the finding of a pen used in writing on a piece of paper. If a pen out of four is used to write a note, the particular pen can be detected using chromatography. The dyes of all four inks and the unknown ink can be obtained. This ink can be run through a chromatograph column, allowing a solvent to run above the ink dyes, carrying them along. After a certain point the RF values of all the inks can be used to detect the particular ink. The RF value is the distance the ink travels divided by the distance travelled by the solvent. The ink with the same RF value as the unknown is the same ink used. Chromatography has a wide range of use. Its use can be stretched from this as it can also be used to indicate the component amino acids found in various proteins. The protein can be run through the column, resulting in the various amino acids that make the protein, to be separated. 1, 2 Materials and Methods (Summarized from Lab

Manual) 1 Procedure \* Phosphorescence Two different polyaromatic acids, 1-naphthoic acid and 4-biphenylcarboxylic acid, were obtained and drops were added onto two filter paper. The solvents heated to dryness for 11 minutes using a hot plate on low heat. The filter papers were then placed under long and short wavelength ultraviolet lamps. Observation of the intensity and duration of glow were recorded. \* Fluorescence Tonic water was poured into a beaker. Long and short wavelength UV lamp was shined above the beaker. Observations were recorded. Now, a beaker of water was taken to the UV lamp and was shined at the beaker. Observations were recorded. After, 3 drops of concentrated fluorescein solution was added into the beaker. The UV lamp was shined above the beaker and observations were recorded. \* Chemiluminescence 2 mL of Tekrakis-(dimethylaminoethylene) was added into a small test tube. This test tube was taken to the UV lamp and shined at. Observations about the intensity and duration of glow were recorded. After, 1 mL of luminol in DMSO was added into five test tubes. Now, in each test tube fluorescein, rhodamine, rubrene and perylene solutions were added in 4 four of the five test tubes. Observation of the color of each solution was recorded. At this point, 1M NaOH was added into each test tube and was then shined with a UV lamp. Further observations were recorded. Finally 1 g of Al<sub>2</sub>O<sub>3</sub> was added into four 250 mL Erlenmeyer flask. This was mixed with 1 mL of 3% H<sub>2</sub>O<sub>2</sub>. In each flask, perylene, tetracene (2, 3-benanthracene), 9, 10-Diphenylanthracene, and rubrene were added respectively. Now, 1 mL of oxalic chloride was added in each flask and swirled. Observations were then recorded. \* Crime Lab A water bath was heated and maintained at 65°C. Now, scribbles were made on papers from each of the four pens. The paper with each scribble was cut into small pieces and placed into a marked

test tube. Also, a scribble of an unknown pen was obtained. The paper was also cut into small pieces and placed in a test tube. 2 mL of methanol was added into each test tube and was placed in the hot bath for 5 minutes. The test tubes were allowed to cool, the color of the inks were recorded and poured into five cuvettes. Using a UV-Vis spectrophotometer, the wavelength peaks and absorbance of each ink solution were recorded. Four of the five dyes were then poured into new marked test tubes. The solutions were then boiled for 14 minutes until there were less than 1 mL. The solutions were allowed to cool. Now, a 250 mL beaker was obtained. In it was added a small amount of methanol (less than half a centimeter in height). A filter paper was then obtained and cut towards the end (B. 1). This was placed at the side of the beaker. Finally, an alumina plate was cut in the dimension of 8 x 4 cm. A line was drawn at a 1 cm height using a pencil. In this line, drops of the four inks were spotted. This plate was then leaned inside the end of the beaker (B. 2). The plate was left in the solvent until it reached  $\frac{3}{4}$  of the way up. The height the inks and solvent reached up the plate was recorded. Result Table 1: Polyaromatic acids under UV Lamp

Compound	Color Without UV Lamp	Color in UV Lamp	Intensity	Duration
1-naphthoic acid	Colorless	Blue	Bright	Fades quickly
4-biphenylcarboxylic acid	Colorless	Purple	Not very Bright	Fades slightly slower

Table 2: Florescence compounds under UV Lamp

Compound	Color without UV Lamp	Color in UV Lamp	Intensity	Duration
Quinine	Colorless	Blue	Bright	Fades quickly
Fluorescein	Green	Yellow-Green	Bright	Remains dimly lit for 30 sec

Table 3: Mix of Quinine and Fluorescein Compound

Compound	Color in UV Lamp	Intensity	Duration
Quinine and Fluorescein mix	Bright blue-yellow color	Bright	Remains dimly lit in yellow color

Table 4: Tetrakis-(dimethylaminoethylene) (TKDE)

under UV Lamp Compound | Color without UV Lamp | Color in UV Lamp | Intensity | Duration | TKDE | Green | Green | Bright | Instantaneous | Table 5: TKDE in Dyes Compound in TKDE | Color of Compound | Color in TKDE | 1M NaOH added | Blank( Only TKDE) | Light green | Light green | Intense Green | Fluorescein | Green | Yellow | Intense Green | Rhodamine | Pink | Pink | Intense Pink | Rubrene | Orange | Orange | Intense Orange | Perylene | Green | Green | Dark intense green | Table 6: Al<sub>2</sub>O<sub>3</sub>, H<sub>2</sub>O<sub>2</sub>, C<sub>2</sub>H<sub>2</sub>Cl<sub>2</sub> mixed with Dyes Compound | Color after mix | Addition of Luminol | Perylene | Yellow | Colors Are Brighter | Tetracene | Green | | 9, 10 Dipehenylanthracene | Blue | | Rubrene | Orange | | Table 7: Color of Extracted Ink Pen Number | Color | 1 | Purple | 2 | Purple | 3 | Purple | 4 | Clear | Unknown | Purple | Table 8: UV Vis Measurements of Ink Dyes (Wavelength Peak) Pen Number | Wavelength/Absorbance (1) | Wavelength/Absorbance (2) | Wavelength/Absorbance (3) | 1 | 301. 8/0. 829 | 449. 4/0. 574 | 563. 7/1. 266 | 2 | 304. 8/1. 750 | 419. 2/1. 542 | 524/1. 97 | 3 | 302. 9/1. 56 | 417. 2/1. 274 | 527. 2/1. 9 | 4 | No Peaks | Unknown | 301. 9/0. 766 | 418. 4/0. 551 | 565. 8/1. 5 | Table 9: RF values of Ink Pen Number | Distance traveled by Solvent | Distance traveled by ink | Rf value | 1 | 3. 4 | 2. 2 | 0. 65 | 2 | 3. 4 | 2. 55 | 0. 75 | 3 | 3. 4 | 2. 5 | 0. 74 | 4 | No ink obtained | Unknown | 3. 4 | 2. 5 | 0. 74 |

Discussion The purpose of the first experiment was to compare the intensity and duration of glow of two aromatic acids. This test was done in order to further understand the concept of phosphorescence. In theory, this form of luminescence is slower due to the fact that the excited electron must undergo a change in its spin before it can go back to its standard state. This process is thermodynamically unfavorable, thus leading to a slower time before glow appears. 1-naphthoic acid and 4-biphenylcarboxylic acid were

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stained on filter paper. Upon shining UV lamp on the two filter papers it was observed that the 1-naphthoic acid had a brighter glow than 4-biphenylcarboxylic acid. On the other hand, it had a shorter afterglow duration than 4-biphenyl acid. In general, the rate of glow was slower compared to the fluorescence test, consistent with the theory. Fluorescence is another form of luminescence that is much faster than phosphorescence. This phenomenon is as a result of the excited electron not having to change its spin upon returning to its ground state. It usually has a bright luminescence that is fast to induce using a UV lamp. During this test, UV lamp was shined over tonic water, inducing a bright blue color. This glow fades away very fast, consistent with the theory. As another test, fluorescein was shown over a UV lamp, inducing a bright green yellow glow. During this analysis, the glow remained for 30 seconds upon removing the solution from the lamp. Finally tonic water was mixed with fluorescein and shined using a UV lamp. This induced a blue yellow color which became a dimly lit yellow color after the light was off. Chemiluminescence is the last form of luminescence that was tested during the experiment. This form of luminescence is formed as a result of a reaction. The product formed contains a substance that is of higher energy, resulting in a glow as the electron gives off the wavelength of light viewed. This form of luminescence is different as it does not require a UV lamp to induce the glow. The first part of this test involved placing tetrakis-(dimethylaminoethylene) solution in a UV lamp. This induced a bright green glow that was instantaneous. A glow can also be obtained without the use of a UV lamp. TKDE oxidizes in air, emitting a blue-green glow. The next experiment involved the mix of luminol with four different dyes. Upon the addition of NaOH, oxidation of the solutions was initiated,

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leading to the intense colors of each dye. The chemiluminescence test involved the mixture of alumina, hydrogen peroxide and oxalyl chloride with four dyes. A reaction began upon addition of luminol, leading to brighter solutions. The next section of the lab experiment involved the use of separation techniques. One of four pens was used to write a note. The purpose of this experiment was to find which pen was used. After cutting the paper with writings into the various test tubes, methanol was used in order to dissolve the ink. The ink for each solution was placed in cuvettes and readings done using a UV Vis spectrophotometer. UV Vic is used as it gives the peaks of the wavelength of light absorbed by each ink solution. This peak can be compared to literature sources to find the particular colors that make up the ink solution. This can be a useful way of finding the particular ink used. In this experiment, the ink from pen 4 could not be extracted by methanol. As a result, it could be inferred that pen 4 was not used to write the note. For further test, chromatography was then used to find the pen used to write the note. The three known inks and unknown were placed on an alumina plate. This was placed in a solution of methanol, inducing capillary movement. As the solvent climbed up the plate, the inks are also carried along, based upon their polarity. When the solvent has travelled a certain distance, its length of travel is measured, including the length traveled by each ink. By dividing the length of travel of the ink by the length of travel of the solvent, an Rf value is obtained (A. 1). The ink with a matching Rf value with the unknown ink is the ink of the pen used.

APPENDIX Appendix A (A. 1) RF Value  $R_f = \frac{\text{Distance travelled by Ink}}{\text{Distance travelled by Solvent}}$  Appendix B (B. 1) Filter Paper to be cut (B. 2)

Chromatography Apparatus Appendix C (C. 1) Calculation of Rf Values (Eq. A.



1) Ink 1: 2. 23. 4= 0. 65 Ink 2: 2. 553. 4= 0. 75 Ink 3: 2. 53. 4= 0. 74 Ink 4:

No ink obtained Ink 5: 2. 53. 4= 0. 74