CH243 Lab 7b: Luminol
Chemiluminescence

I. 3-Aminophthalhydrazide (“Luminol”)

II. Chemiluminescence

All chemical reactions proceed with the evolution or absorption of energy. In most instances the energy evolved or absorbed is in the form of heat, although many experimenters from time to time have noticed that visible radiation was produced when various chemical reactions were conducted in a darkened room. In most instances of this kind the intensity of the light emitted is low. The oxidation of 3-aminophthalhydrazide in alkaline solution, however, gives one of the most brilliant displays of chemiluminescence on record and has the added advantages that, in contrast to certain other compounds which exhibit this phenomenon, the materials are easily obtained, are safe to handle, and are used in very dilute solutions.

Today’s experiment is a small-scale adaptation of the procedure given by Huntress, Stanley, and Parker. These authors not only describe the preparation of 3-aminophthalhydrazide but also present a discussion of chemiluminescence that will be of interest.

PROCEDURE:

MATERIALS

3-Nitrophthalic anhydride  Sodium hydroxide, 10% solution
Hydrazine sulfate  Potassium ferricyanide, K₃Fe(CN)₆
Sodium acetate, NaC₂H₃O₂ 3H₂O  Hydrogen peroxide, 3% solution
Glycerol  Hydrochloric acid
Sodium dithionite, Na₂S₂O₄

"Luminol"
I. 3-Aminophthalhydrazide (“Luminol”)

Into a 25 x 200-ml test tube introduce 1 g of 3-nitrophthalic anhydride, 0.7 g of hydrazine sulfate, 1 g of hydrated sodium acetate, and 4 ml of water. Support the tube at an angle of about 45° on an iron stand by means of a clamp, and heat the mixture to boiling. Note that the escaping steam carries with it the vapors of acetic acid.

The hot suspension now contains hydrazine 3-nitrophthalate. The composition of the salt formed by hydrazine and 3-nitrophthalic acid apparently is not known with certainty. The structure shown in the equation is one possibility.

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{O} \\
\text{O} & \quad \text{O} & \quad \text{C} \\
\text{O} & \quad \text{N} & \quad \text{H}_3 \\
\text{N} & \quad \text{H}_3 & \quad \text{SO}_4^{2-} + 2 \text{CH}_3\text{C-O-Na}^{\oplus} \rightarrow \text{O} & \quad \text{N} & \quad \text{O} \\
\text{NH}_3 & \quad & \text{H}_2\text{O} & \quad \text{O} & \quad \text{C} & \quad \text{N} & \quad \text{H}_3 \\
\text{O} & \quad \text{N} & \quad \text{H}_3 & \quad \text{Na}_2\text{SO}_4 + 2 \text{CH}_3\text{C-OH}
\end{align*}
\]

In order to convert the salt (hydrazine 3-nitrophthalate) to 3-nitrophthalhydrazide (belonging to the amide family of compounds), a temperature considerably above the boiling point of water is required. To attain this temperature introduce 5 ml of glycerol and a boiling chip, insert a thermometer whose bulb rests on the bottom of the tube, and heat the tube to remove water. No stopper is used; the mouth of the tube is left open to facilitate the escape of steam.

Boil the contents of the tube rather vigorously but do not apply heat so rapidly that excessive bumping occurs. Stir the contents of the tube with a stirring rod and note the rise in temperature. After a temperature of 120° is attained, the thermometer reading rises quickly to 200°. At this point heat the tube gently and intermittently so that a temperature of 200°-220° is maintained for 3-4 minutes. During this period the contents of the tube acquire an orange-yellow color.

Allow the tube to cool to about 100° and add 40 ml of water. Warm the tube over the bunsen burner to accelerate the coagulation of the light yellow 3-nitrophthalhydrazide and set the tube in an ice bath for a few minutes.

As soon as the 3-nitrophthalhydrazide has settled well, pour off the supernatant solution and shake the residual yellow powder with another 40-ml portion of water. Warm the mixture, if necessary, to increase the rate of sedimentation, and, after chilling in ice, decant the clear upper solution from the 3-nitrophthalhydrazide which remains in the bottom of the tube.
Reduction of 3-Nitrophthalhydrazide to 3-Aminophthalhydrazide. The nitro compound is now contained in the test tube along with a little water. Add to the contents of the tube 5 ml of a 10% solution of sodium hydroxide. To the red solution thus obtained add 3 g of sodium di thionite, Na₂S₂O₄ (also called sodium hydrosulfite), as a reducing agent and heat the contents of the tube to boiling.

Maintain a temperature at or near the boiling point for 3-4 minutes. The red color of the solution quickly gives way to the yellow color of the 3-aminophthalhydrazide, some of which usually precipitates almost immediately. The quantity of this precipitated amino compound increases when the contents of the tube are cooled and acidified with glacial acetic acid (about 2 ml of the acid will be required).

Collect the 3-aminophthalhydrazide (“Luminol”) on a small Büchner funnel and wash it twice with 25-ml portions of water. Remove the filter paper with the “Luminol,” and place both the paper and moist product on a watch glass. Use part of the moist “Luminol” for Part II and place the watch glass (or Petri dish) containing the remainder in an oven to dry. The recorded melting point of 3-aminophthalhydrazide is about 310°.

II. Chemiluminescence
Prepare the following solutions:

A. Dissolve a quantity of the moist “Luminol” estimated to contain 0.1 g of the dry material in 10 ml of 10% sodium hydroxide solution and dilute the solution to 1 liter.

B. Dissolve 0.5 g of potassium ferricyanide [K₃Fe(CN)₆] in 75-100 ml of water, add 15 ml of ordinary 3% hydrogen peroxide solution, and dilute the solution to 1 liter.

Take these solutions to a room that can be darkened where you have at hand a reagent bottle of hydrochloric acid and one of sodium hydroxide solution. Place a 6-inch funnel in the mouth of a large flask (3-5 liters), turn off the lights and mix solutions A and B by pouring them through the funnel into the large flask. The chemiluminescence appears as soon as the solutions are mixed. The light emitted will illuminate the room so that you can now easily locate your bottles of reagents.

Add to the mixed solutions in the large flask a little sodium hydroxide and note that the chemiluminescence is intensified. Then acidify a portion of the luminescent solution with hydrochloric acid and note that the emission of light ceases and that it reappears if the solution is again quickly made alkaline.

LABORATORY REPORT AND QUESTIONS

Instructor________________________________ Name_____________________________
Date____________________________________ Lab. Sec____________________________
Desk No.________________________________

State the procedure followed in this experiment briefly but in enough detail to permit your repetition of the experiment without consulting a reference book. Write the equations for the chemical reactions involved.

SPECIAL REAGENTS

Ammoniacal Cuprous Chloride  Dissolve 10 g of cuprous chloride in 100 ml of concentrated ammonium hydroxide and dilute the solution to 500 ml.

Ammoniacal Silver Nitrate Solution.  See Tollens’ Reagent.

Benedict’s Qualitative Reagent  Prepare solution A by dissolving 173 g of sodium citrate and 100 g of anhydrous sodium carbonate in about 600 ml of water and dilute the solution of 850 ml. Prepare solution B by dissolving 17.3 g of crystalline copper sulfate (CuSO₄ · 5H₂O) in 100 ml of water and diluting the solution to 150 ml. Add solution B to solution A and mix well for the preparation of Benedict’s solution.

Ceric Nitrate Reagent  Dissolve 20 g of ceric ammonium nitrate [(NH₄)₂Ce(NO₃)₆] in 50 ml of water containing 7 ml of concentrated nitric acid (D = 1.42). If necessary, warm the mixture to hasten the dissolving process. Allow the solution to cool and keep it in a tightly stoppered bottle as a stock reagent.

2,4-Dinitrophenylhydrazine Reagent  Dissolve 3 g of 2,4-dinitrophenylhydrazine in 15 ml of concentrated sulfuric acid, and then add this solution, with stirring, to a solution of 20 ml of water in 70 ml of 95% ethanol. Stir the mixture thoroughly and filter it. The filtrate is used as the test reagent.