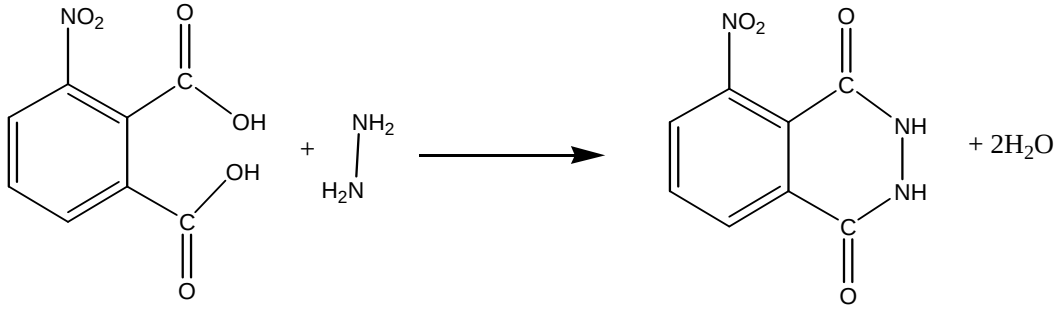
The production of visible light as a result of a low-heat-producing chemical reaction is called chemiluminescence. A chemiluminescent reaction generally produces one of the product molecules in an electronically excited state. The excited state relaxes to a lower energy state and in the process emits a photon of visible light:

A* (excited) → A (relaxed) + hν

The light produced by fireflies and other bioluminescent organisms has fascinated observers for many years. There are many different organisms that have developed the ability to emit light. They include bacteria, fungi, protozoans, hydras, marine worms, sponges, corals, jellyfishes, crustaceans, clams, snails, squids, fishes, and insects. Curiously, other than fish, this list does not include many higher forms of life. The chemiluminescent compound in fireflies is called luciferin. The enzyme, which induces it to emit light, is called luciferase.

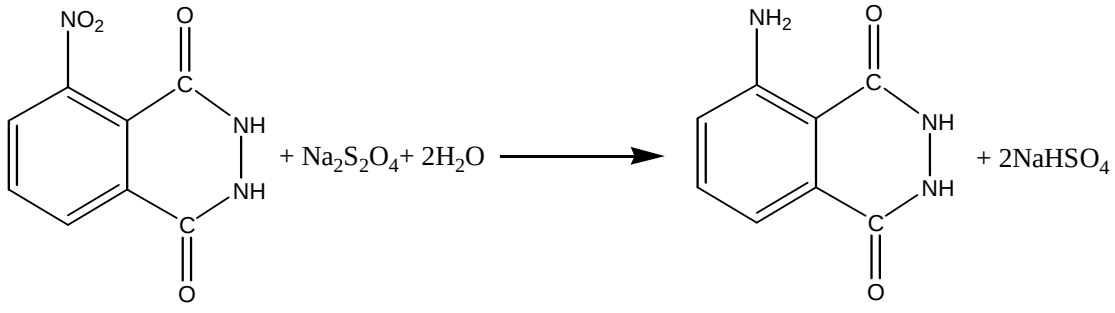
In this experiment, the chemiluminescent compound luminol, or 3-aminophthalhydrazide, will be synthesized from 3-nitrophthalic acid.

The first step of the synthesis is the simple formation of a cyclic diamide, 3-nitrophthalhydrazide, by reaction of 3-nitrophthalic acid with hydrazine. Reduction of the nitro group with sodium dithionite affords luminol.



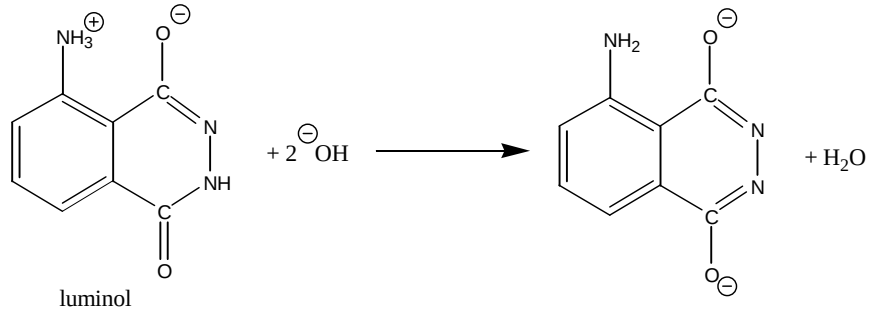
3-nitrophthalic acid

3-nitrophthalhydrazide



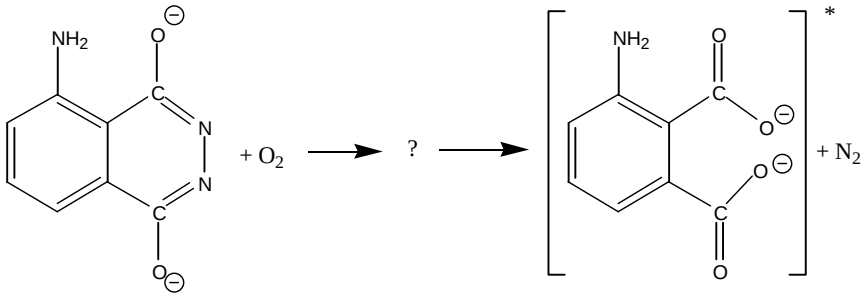
luminol

In neutral solution, luminol exists largely as a dipolar ion (zwitterion). This dipolar ion exhibits a blue fluorescence after being exposed to light. However, in alkaline solution, luminol is converted into its dianion.

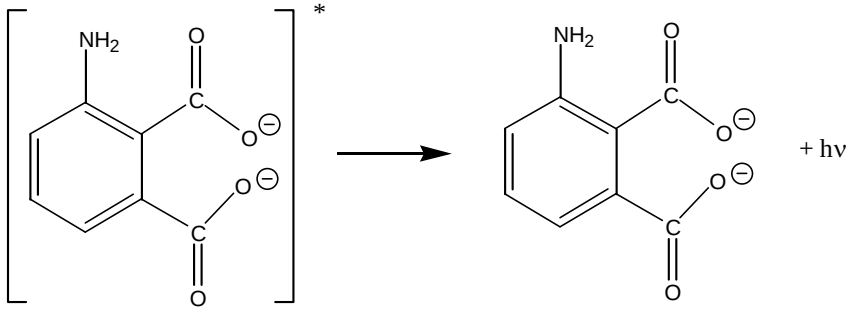


luminol

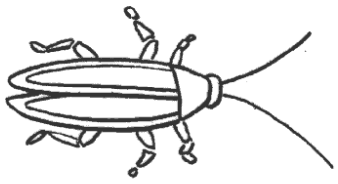
The dianion of luminol undergoes a reaction with molecular oxygen to form peroxide of unknown structure. This peroxide is unstable and decomposes with the evolution of nitrogen gas, producing the 3-aminophthalate dianion in an electronically excited state.

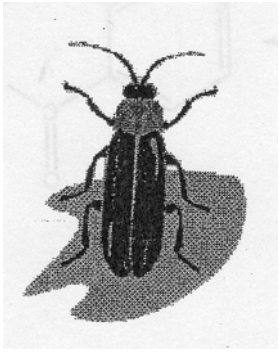


The excited dianion emits a photon, which is observed as visible light.



FIREFLY





http://www.metnet.state.mt.us/Special/Natives%20Are%20Nice/HTM/gifs/firefly.gif

Procedure: **Part I**

3-nitrophthalhydrazide:

Place 1.3 g of 3-nitrophthalic acid and 2 mL of a 10% aqueous solution of hydrazine in a large test tube. At the same time, heat at least 20 mL of water in a beaker. Clamp the test tube to your ring stand. Heat the test tube over a microburner until the solid dissolves. Add 4 ml of triethylene glycol to the test tube. Add a boiling stone to the test tube.

Monitor the heat by placing your 250° C thermometer in the test tube held in place by a split stopper and/or a clamp. The bulb of the thermometer should be immersed in the solution without touching the glass. Heat the solution with a micro burner (the small one) until the liquid boils vigorously (the temperature will rise to about 120° C). Continue heating and allow the temperature to increase rapidly until it rises above 200° C. About five minutes will be required for this heating. Remove the burner briefly when the temperature 200° C has been achieved, and then resume gentle heating in order to maintain a fairly constant temperature of 210° to 220° for about two minutes. Be careful, this tends to boil over!

Allow the test tube to cool to about 100°. Add the 20 mL of hot water which was prepared previously. Cool the test tube to room temperature. Collect the crystals of 3-nitrophthalhydrazide by vacuum filtration using a Hirsch (small porcelain) funnel. Use a piece of filter paper that covers only the bottom of the Hirsch funnel. It is not necessary to dry the product thoroughly in order to proceed with the next reaction step.

Part II.

Luminol:

Transfer the 3-nitrophthalhydrazide back to the large side-arm test tube. Add 6.5 mL of a 10% sodium hydroxide solution, and stir the mixture until the hydrazide dissolves. Add 4 g of sodium dithionite (sodium hydrosulfite). Using a pipet, add about 10 mL of water to wash the solid from the walls of the test tube. Heat the test tube with a microburner until the solution boils. Stir the solution with a long glass rod. (Do not stir with a thermometer.) Maintain the boiling, with stirring, for 5 minutes. Add 2.6 mL of glacial acetic acid.

Cool the test tube to room temperature. Stir the mixture during this cooling step. Finally, cool the solution in an ice bath. Collect the crystals of luminol by vacuum filtration using a Hirsch funnel. The luminol may be used without thorough drying for the chemiluminescence experiments.

Adapted from Experiment 48: Luminol, in Pavia, Lampman and Kriz, “Introduction to Organic Laboratory Techniques: A Contemporary Approach” (1976) Saunders

Part III.

Chemiluminescence experiments:

Cover the bottom of a 250 mL Erlenmeyer flask with a layer of KOH pellets. Add about 25 mL of dimethylsulfoxide (DMSO) and your luminol to the flask. Stopper it with a rubber stopper and shake it vigorously to mix air into the solution. In a dark room a faint glow of light will be observed. The intensity of the glow will increase with continued shaking of the flask and occasional removal of the stopper to admit more air.

Clean Up: Dispose of any waste in a properly labeled containers in the fume hood.

Checklist for completing the "Prelab" section:
(refer to Laboratory Syllabus for complete directions)

____ *Title and date*
____ *Purpose*.

____ *Physical constants*. Create a table of physical constants and safety data for all chemicals and solutions referred to in the lab handout.
http://domin.dom.edu/faculty/jbfriese/chem254/chemical.htm

Structures and equations:

____ Write the balanced equation (with structural formlas) for the overall reaction of 3-nitrophthalic acid to luminol.

____ Draw the structures of triethylene glycol, sodium dithionite and DMSO

___ *Flowchart.* Refer to "Procedure" Reference any sources you use.

Calculations.

1.) How many moles are in 1.3 g of 3-nitrophthalic acid?

2.) If 3-nitrophthalic acid is the limiting reagent, what is the theoretical yield of luminol?

3.) Calculate the atom economy of the balanced reaction of 3-nitrophthalic acid to luminol. Show your calculations please.

Safety Question:
What are the four ways a poison can enter your body?
Give one example how each of these might (accidentally) happen in a chemistry lab.

Experimental Observations and Data:

Hand in a copy of your experimental observations and data before you leave lab.

Experimental Observations: Refer to Laboratory Syllabus for guidelines.

Raw Data: Refer to Laboratory Syllabus for guidelines.

Lab Report Checklist:

Discussion and Conclusion.

_____ Name two other organisms besides the firefly (*Pyractomena borealis)* that produce light. Give common names and Latin binomial names of species. Reference your source(s).

_____ Define and differentiate between a) luminescence, b) chemiluminescence, c) fluorescence, and d) phosphorescence. Reference any sources you use.

_____ How does luminol detect the presence of blood? What component or property of blood does luminol detect?

_____ What is (or would be) a practical application of chemiluminescence besides "glow sticks" and blood detection?

> The form of the living creatures was like the appearance of burning coals of fire and torches. Fire was moving back and forth between the living creatures; it was bright, with lightning coming out of it. The creatures were darting back and forth like flashes of lightning.
>
> Ezekiel 1:13,14

Name	Formula	M.W. g/mole	m.p. ^{o}C	b.p. ^{o}C	Density g/mL
3-nitrophthalic acid	$C_8H_5NO_6$	211.13	213-216		
hydrazine	N_2H_4	32.05	2.0	113.5	1.0036
triethylene glycol	$C_6H_{14}O_4$	150.17	-7.2	285	1.1274
10% sodium hydroxide	NaOH	40.0	1M sol ~4	1M sol. ~102	1 M sol. ~1.0
sodium dithionite (Sodium Hydrosulfite)	$Na_2O_4S_2$	174.11			
glacial acetic acid	$C_2H_4O_2$	60.05	16.7	118	1.049
potassium hydroxide	KOH	56.11	About 360		
Dimethyl Sulfoxide (DMSO)	C_2H_6OS	78.14	18.45	189	1.100
luminol	$C_8H_7N_3O_2$	177.16	319-320		

Name	Solubility	Safety Information
3-nitrophthalic acid	soluble in water, slightly soluble in alcohol insoluble in nonpolar solvents	Warning: irritating to skin, eyes and mucous membranes. Avoid skin contact
hydrazine	Miscible with water Miscible with alcohol Slightly miscible in nonpolar	Warning: Corrosive. Warning: Irritant Warning Explosive Danger: Carcinogen.
triethylene glycol	Miscible with water Miscible with alcohol Slightly miscible in nonpolar	Poison by intravenous route. Mild toxicity. Combustible.
10% sodium hydroxide	soluble in water, soluble in alc, insoluble in nonpolar solvents	Warning: Corrosive Warning: irritating to skin, eyes and mucous membranes. Avoid skin contact
sodium dithionite Sodium Hydrosulfite	Very soluble in water Slightly soluble in alcohol Insoluble in nonpolar solvents	Caution: Flammable Solid
glacial acetic acid	Miscible with water, Miscible with alcohol, some miscible with nonpolar solvents.	Corrosive! Moderately toxic by various routes. Moderate fire and explosion hazard.
potassium hydroxide	Soluble in water Slighly soluble in alcohol Insoluble in nonpolar sovents	Danger: corrosive, Danger: caustic, hygroscopic, Poison, Irritant and corrosive to membranes.
Dimethyl sulfoxide	Miscible with water, Miscible with alcohol, some miscible with nonpolar solvents.	Skin and Eye Irritant. Teratogen. Moderately toxic.
Luminol	soluble in water, slightly soluble in alcohol insoluble in nonpolar solvents	Warning: irritating to skin, eyes and mucous membranes. Avoid skin contact

References: 1) Merck Index, 11th ed.
2) www.chemfinder.com
3) Hazardous Chemical Desk Reference, Lewis and Sax, 1987
4) Aldrich catalog online