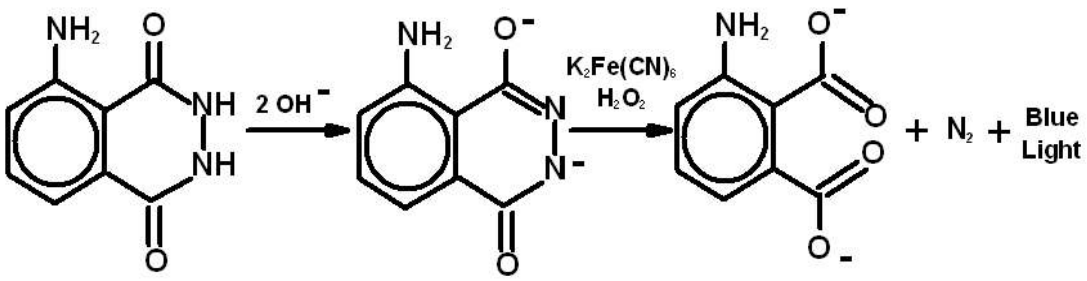
Chemiluminescence

Chemiluminescence of Luminol

Chemiluminescence is a chemical reaction that produces light with little or no heat. Usually, a chemiluminescent reaction generates a product molecule in a highly energized state. In order to dispose of this excess energy, the excited molecule emits a photon of light.

Luminol with structure **1** is an example of a molecule that exhibits chemiluminescence by the process shown below. An alkaline solution of luminol converts the molecule to the doubly ionized anion with structure **2**, which is oxidized by a combination of hydrogen peroxide and potassium ferricyanide to give nitrogen gas and the doubly ionized anion with structure **3**. However, the instant that structure **3** forms, it exists in what is called the *triplet excited state* in which the two electrons spin in the same direction. In less than a millionth of a second, this triplet state undergoes a slow spin-flip (known as inter-system crossing) to form a *singlet excited state*, which has two unpaired electrons with opposite spin directions. Again, in less than a millionth of a second, the singlet excited state undergoes subsequent decay to the normal or *ground state* accompanied by the emission of a photon as blue light. The basic reactions are given below.



1 **2** **3**

Luminol is widely used by forensic investigators to detect trace amounts of blood left at crime scenes as it reacts with iron in the hemoglobin of blood. It is also used by biologists in cellular assays for the detection of copper, iron, and cyanides.

Light Producing Procedure

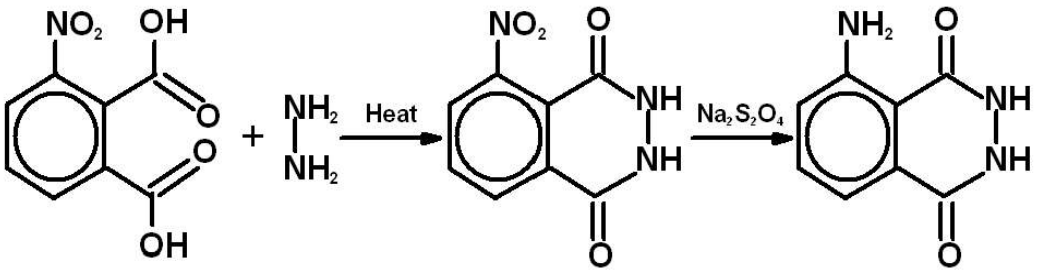
1. Dissolve 50 mg of luminol in 2 ml of 3M NaOH solution and 18 ml of water; this is stock solution A.

2. Prepare a second stock solution, B, by mixing 4 ml of 3% aqueous potassium ferricyanide, 4 ml of 3% hydrogen peroxide, and 32 ml of water.

3. Dilute 5 ml of solution A with 35 ml of water, and in a darkened room place, pour this solution and solution B simultaneously into an Erlenmeyer flask. Swirl the flask and to increase the brilliance, gradually add further small quantities of alkali and ferricyanide crystals.

Synthesis of Luminol

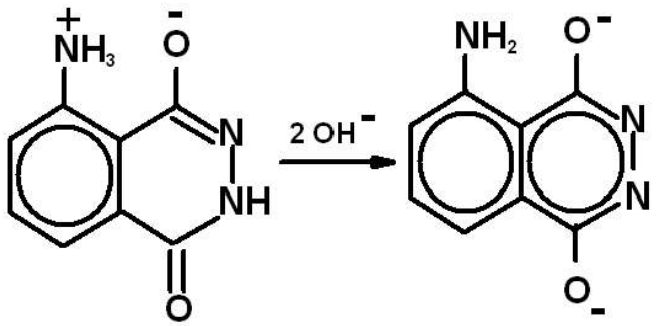
The synthesis of luminol begins with the reaction between 3-nitrophthalic acid (**4**) and hydrazine (**5**) in triethylene glycol. The temperature is raised to a point where excess water is distilled and dehydration to nitrophthalhydrazide (**6**) is complete within a few minutes. The nitro group of 6 is then reduced to an amino group using sodium hydrosulfite ($Na_2S_2O_4$) in alkaline solution to give the molecule luminol. The synthesis scheme is shown below.



4 **5** **6** **1**

3-Nitrophthalic Acid **Hydrazine** **3-Nitrophthalhydrazide** **Luminol**

In dilute, weakly acidic or neutral solution luminol exists mainly as the dipolar ion (**7**), which exhibits beautiful blue fluorescence.



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Synthetic Procedure:

1. Heat a flask containing 15 ml of water on a steam bath.

2. Then heat a mixture of 1g of 3-nitrophthalic acid and 2 ml of an 8% aqueous solution of hydrazine (caution) in a 20 x 150-mm test tube over a sand bath until the solid is dissolved.

3. Add 3 ml of triethylene glycol and clamp the tube in a vertical position in a hot sand bath.

4. Insert a thermometer in the sand bath, add a boiling chip, and an aspirator tube connected to an aspirator. Boil the solution vigorously to distill the excess water (110-130 ^{o}C). Let the temperature rise rapidly (3 - 4 minutes) until it reaches 215 ^{o}C..

5. Remove heat, note the time, and by intermittent gentle heating, maintain a temperature of 215-220 °C for 2 minutes.

6. Remove the tube, cool to about 100 °C (crystals of the product often appear), add 15 ml of hot water, cool under tap water, and collect the light yellow granular nitro compound.

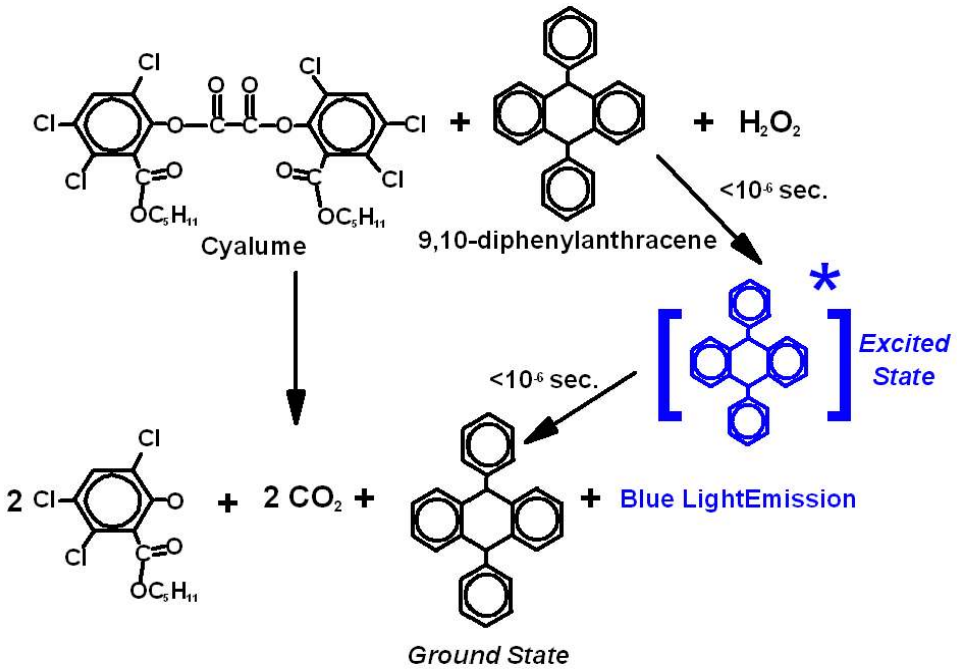
7. Transfer the nitro compound at once to the uncleaned test tube in which it was prepared. Add 5 ml of 3 M NaOH solution, stir with a rod, and add 3g of fresh sodium hydrosulfite dihydrate. to the resulting deep brown-red solution. Wash the solid down the walls with a little water.

8. Heat to the boiling point, stir, and keep the mixture hot for 5 minutes, during which time some of the reduction product may separate.

9. Add 2 ml of glacial acetic acid, cool under tap water, and stir. Collect the resulting precipitate of light-yellow luminol. On standing, the filtrate usually deposits a further crop of luminol.

Other Examples of Chemiluminescence

Chemiluminescent reactions have been studied extensively and the chemical industry has developed them for glowing light sticks. Light sticks work due to a reaction of hydrogen peroxide with a molecule called Cyalume, which was developed by the American Cyanamid Company. The chemical name for Cyalume is bis(2,4,5-trichlorophenyl-6-carbopentoxy)oxalate and it is used in conjunction with a fluorescent dye such as 9,10-diphenylanthracene (DPA). The Cyalume is converted to 2,4,5-trichloro-6-carbopentoxyphenol and carbon dioxide while the energy generated from the reaction is transferred to the DPA. This enables the DPA to go from its normal or ground state to an excited state, which rapidly decays back to the ground state in less than a millionth of a second with the emission of blue light. The chemical reaction is shown below.



If light is the product of a reaction that is biochemical in nature, the process is often referred to as bioluminescence. Numerous examples of bioluminescence exist in nature, most notably the flashes of light emitted by the male firefly. Other examples include various marine organisms such as sponges, coral, jellyfish, clams, and a few other types of fish. In these organisms, a compound called luciferin reacts with the enzyme luciferase, which has already formed a complex with adenosine triphosphate (ATP). The actual reaction involves several steps, but the end result is the production of an excited molecule that subsequently emits light. It is important to note that the names luciferin and luciferase are generic terms for the active agents in bioluminescent organisms and that the actual molecular structures may differ significantly from organism to organism.