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Chemiluminescence of Luminol: A Review

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Abstract
A chemiluminescent substance called luminol has attracted a lot of interest from scientists across a variety of disciplines because of its exceptional qualities and many uses. This review provides a comprehensive overview of luminol, covering its chemical structure, synthesis methods, and fundamental properties. The topic of discussion includes the chemical processes that go on during luminol chemiluminescence as well as the variables that affect the light emission. The paper also looks at luminol's uses in forensic science, where it has developed into a crucial tool for crime scene examinations. Exploration of luminol's use in latent fingerprint analysis, bloodstain detection, and arson investigations is done while stressing its sensitivity, selectivity, and potential drawbacks. The paper also discusses recent developments in luminol-based technology, including the creation of fresh luminol compounds and the incorporation of luminol with cutting-edge imaging methods. Finally, future perspectives and new study areas are highlighted, highlighting the necessity for ongoing investigation and optimization of luminol's properties to improve its efficiency in forensic analysis and broaden its utility in other scientific fields.

Keywords: Luminol, Chemiluminescence, Forensic Science

1. Introduction

Luminol is a compound with chemiluminescent qualities that is used in a variety of applications, the most notable of which being forensics. The most well-known and commonly used chemiluminescent reagent is Luminol ($C_8H_7N_3O_2$). It was originally synthesized around the turn of the twentieth century, but it wasn't until 26 years later that its remarkable CL qualities were found. This bicyclic chemical was given the moniker "Luminol" because of its unique CL properties [1]. Luminol In forensic, biological, and clinical sciences, Chemiluminescence is a useful technique. As Albrecht discovered in 1928, the oxidation of Luminol creates a brilliant blue light that combines with the iron in hemoglobin, allowing forensic experts to detect extremely minute quantities of blood [2]. It was recently revealed by Nenzel how to make luminol by making 3-nitrophthalic acid and hydrogen cyanide together in the presence of tri ethylene glycol (1995). It was heated with sodium dithionite, treated with acetic acid, and then cooled to make luminol, as shown in the Fig 1. The NO_2 group is converted to NH_2 group throughout the procedure. The procedure Similar to how it was briefly explained in 1934, 1949, and 1964 (Budavari, 1996) [3].

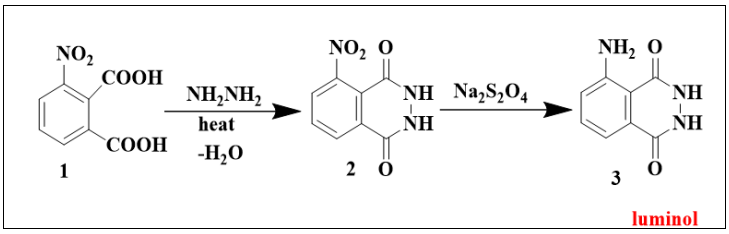

Fig 1: Synthesis of luminol

By oxidizing peroxide in the presence of a catalyst (peroxidases, Fe3+, HOCl), Luminol creates powerful chemiluminescence and the excited 3-aminophthalate anion, which has been identified as the light-emitting species [1]. In forensic sciences, Luminol is commonly used for immunoassays, nucleic acid assays, metabolic pathway monitoring, and reporter gene assays Luminol is also frequently utilized in cellular luminescence and as a sensitive approach for the identification of chemical

substances, including drugs and heavy metals [4]. Despite the fact that new uses continue to develop in the literature.

1.2 Luminol Chemistry

The reaction of a dicarboxylic acid anhydride with hydrazine at high temperature produces luminol and its derivatives [4] as showed in the Fig 2.

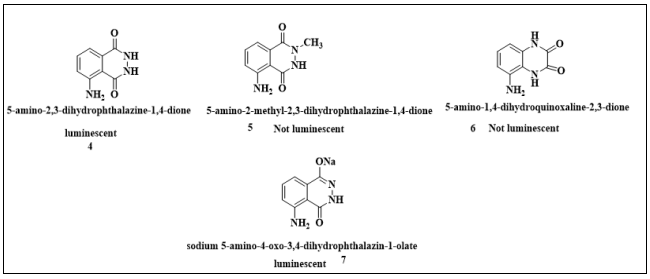

Fig 2: Structures of luminol derivatives

Differently substituted analogues at the aromatic ring, such as compound luminol, are easily accessible using this process. Variations in the non-aromatic ring, such as in the compounds 5-amino-2-methyl-2, 3-dihydrophthalazine-1, 4-dione and 5-amino-1, 4-dihydroquinoxaline-2, 3-dione, result in Chemiluminescence being completely lost. while the monosodium salt of Luminol sodium 5-amino-4-oxo-3,4-dihydrophthalazin-1-olate still shows luminescent properties [2].

Luminol is a powerful chemiluminescent substance that emits blue light under oxidative circumstances (the energy produced as a photon originates straight from a strong exothermic reaction) [5].

In and of itself, the approach is highly useful and intriguing, especially for analytical reasons. In addition to heavy metal quantification, *in vivo* analytical chemistry, and biosensors, Chemiluminescence analysis has the advantages of a simple apparatus, low detection limits, a large calibration range, and a quick analysis time. Chemiluminescence is now widely employed in a variety of sectors, including medicines, environmental studies, and even life sciences [6].

1.3 Derivatives Containing Luminol

Hideyuki Yoshida *et al.* (2000) [7] were reacting a sequence of aromatic aldehydes with 7-amino-6-sulfanylphthalazine-1, 4(2H, 3H)-dione, a novel class of luminol-related compounds with a 2-arylbenzothiazole moiety were created. These compounds were evaluated to exhibit chemiluminescence that was 1–40 times more luminous than luminol [7].

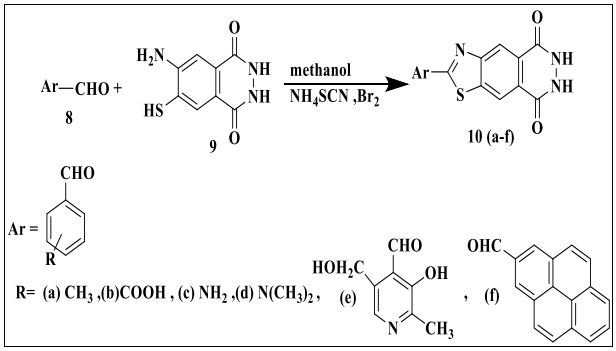

Fig 3: A new compound that is similar to luminol and has a 2-arylbenzothiazole

Banan H. *et al.* (2017) [8] were used The Japp-Klingemann reaction to make a novel N-(phthalazin-5-yl)hydrazonoyl chloride derivative 11 accessible from luminol, and amidrazones 14a-e were discovered to have weak to moderate activity against breast cancer cell line (MCF-7) with MIC50 25-100 M/mL as shown in Fig 4.

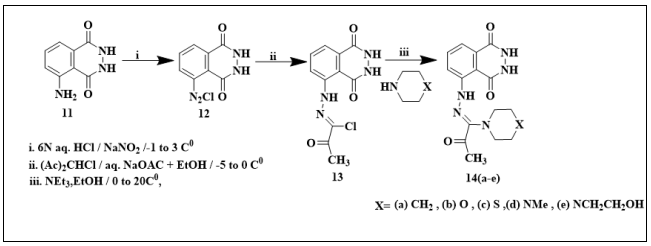

Fig 4: Synthesis of amidrazones

R. Aswathyl *et al.* (2017) [9] synthesized by combining diazotized 5-aminophthalhydrazide with 2-naphthol, a new heterocyclic luminol derivative has been created. Under microwave assisted solvent free circumstances, this chemical, Phthalhydrazide-5-azo-2- naphthol, is flexible in building stable metal complexes with cobalt(II), nickel(II), copper(II), and zinc(II) ions as showed in the Fig 5.

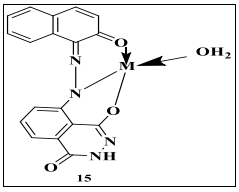

Fig 5: Structure of the metal (II) complex, where M=Co(II), Ni(II), Cu(II), Zn(II)

2. Applications

2.1 Luminol Chemiluminescence

Chemiluminescence is a phenomena in which light is created during a chemical process through chemiexcitation. In a nutshell, oxidation activates specific molecules including reactants, intermediates, and fluorophores to generate an oxidized high-energy intermediate, which will breakdown or transmit its energy to surrounding fluorophores before returning to its ground states and generating luminescence [10].

Chemiluminescence may be classified into two forms based on the distinct chemical energy conversion mechanisms: direct chemiluminescence and indirect chemiluminescence [10].

Cyclic hydrazides may emit light in both protic and aprotic environments. A base, hydrogen peroxide [11], and an oxidizing agent are required for light emission in water and aqueous mixes of alcohols or organic solvents. As oxidants, ferricyanide or hypochlorite salts are often utilized, although chelated metals such as iron and copper may also cause light emission. Only oxygen and a strong base are required for luminescence in aprotic media. The scheme depicts a general process of Luminol-based light emission as showed in Fig 6.

The di anion formed by base-promoted deprotonation of Luminol (with negative charges on the nitrogen atoms) is in equilibrium with its equivalent "enolic" form. When the latter is exposed to molecular oxygen or hydrogen peroxide (depending on the circumstances), an unstable endo

peroxide develops, which decomposes to release an electrically excited aminophtalate di anion and a molecule of N_2. The excited states (T1, S1) of the freshly created di anion relax to the ground state (S_0), producing light. Most authors3 agree on a stepwise single electron transfer process in which radical species are involved under particular circumstances (typically when peroxides and metals are utilized). However, the creation of electrically excited di-anion intermediates is also a critical step for light emission [2].

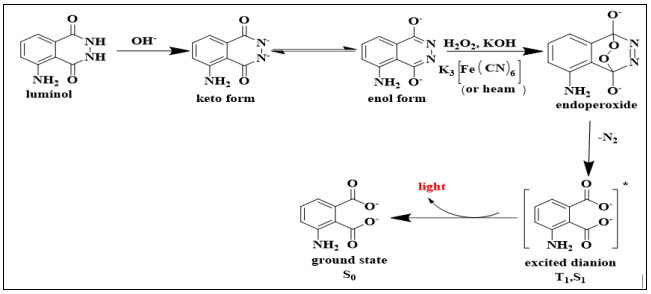

Fig 6: General Mechanism of Luminol light emission

Xiaohua *Li et al.* (2013) [12] created a novel form of H_2O_2 sensor array. As shown in Fig 7, the membranes of the distinctive CL sensor array were made of CeO_2 nanoparticles, which have outstanding catalytic effects on the luminol-H_2O_2 CL reaction in an alkaline medium.

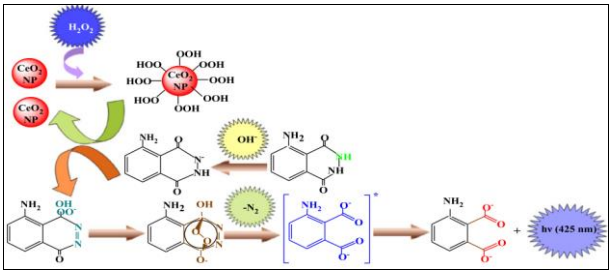

Fig 7: The possible response mechanism of the CL sensor

Na Lia and Shubiao Ni (2014) [13] utilized the presence of nucleophiles Ag NPS might be utilized as reductants in chemiluminescence (CL) to create the CL emission of luminol, a typical CL reagent used in forensic inquiry to detect small amounts of blood.

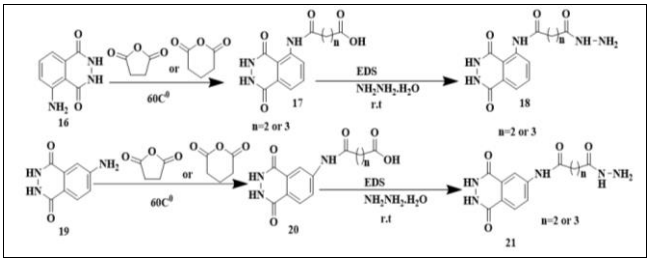

Fig 8: Luminol and isoluminol derivatives with a hydrazide group: synthetic pathway

Aldo Roda *et al.* (2016) [14] introduced A critical assessment of progress in chemical luminescence-based biosensors that detect chemical luminescence (including Chemiluminescence, bioluminescence, electro generated Chemiluminescence, and thermo Chemiluminescence).

Takayuki Shibata *et al.* (2019) [15] synthesised luminol and isoluminol derivatives and they investigated labelling efficiency of 18 and 21 as shown in the design, utilizing horseradish peroxidase (HRP) as a model target molecule as showed in the Fig 8.

Zhao Zhang *et al.*(2018) [16] used Peroxidases in the most widely used luminol-H_2O_2 CL system were evaluated from two perspectives of horseradish peroxidase (HRP) and several anionic peroxidases, which were utilized in many different CL systems for the detection of hydrogen peroxidase (H_2O_2) as showed in the Fig 9.

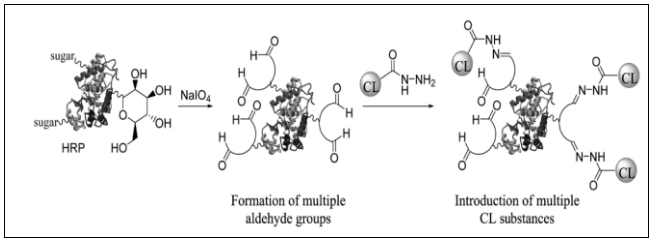

Fig 9: Incorporation of Synthesized Hydrazide Compounds into HRP Schematic Illustration

Mingwang Yang *et al.* (2020) [10] introduced tutorial review of Chemiluminescence for bioimaging and therapeutics that they focused on the recent advancements of chemiluminescent platforms based on luminophore substrates including luminol and its derivatives.

Virendra V. Singh *et al.* (2021) [17] Luminol serves as the receptor and a signaling element, and the ionic liquid (1-ethyl-3-methylimidazolium dicyanamide) provides the necessary and polarizing medium to realize the detection at room temperature, as shown in Fig 10. This combination of luminol and an ionic liquid in water exhibits fluorescence detection of sulfur mustard (SM) within seconds, making it a quick and inexpensive chemo sensing method.

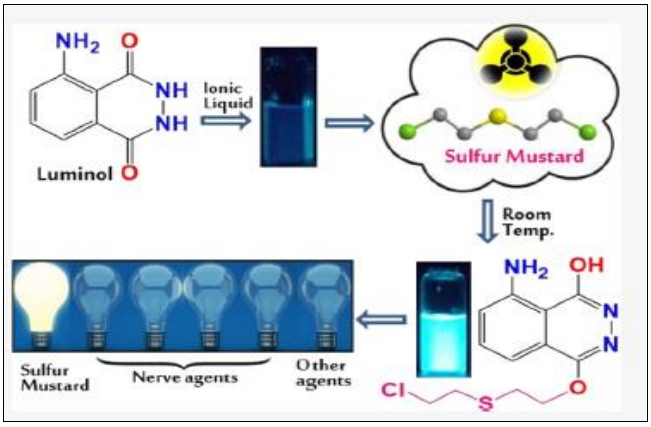

Fig 10: Schematic presentation of SM detection using luminol

Li, Hongdan *et al* (2021) [18] Black phosphorus quantum dots (BP QDs) were made using a solvothermal exfoliation method in alkaline N-methyl-2-pyrrolidinone. This method increases the production of active oxygen species, which oxidize luminol and lead to intense chemiluminescence emission at 425nm. The reaction of luminol with BP QDs is specifically catalyzed by cobalt (II) ion.

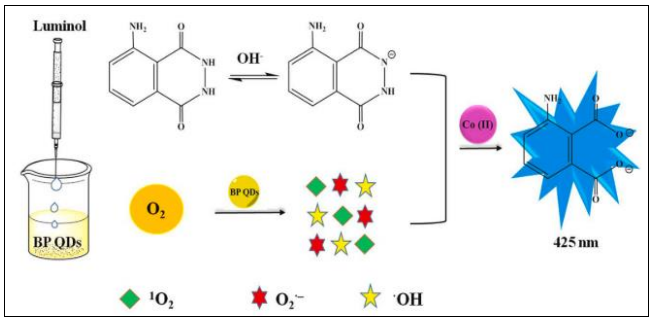

Fig 11: Schematic of a luminol CL device based on BP QDs for cobalt (II) ion assay

3. Conclusion

A fascinating phenomena with significant applications is luminol's Chemiluminescence. Its singular capacity to produce light through chemical reactions has transformed forensic procedures and scientific research, advancing our knowledge of biological processes and assisting in criminal investigations. Future advancements in this field of study should lead to even more fascinating discoveries and uses for luminol Chemiluminescence. Numerous benefits are provided by the chemiluminescent features of luminol, including great sensitivity, little background noise, and ease of detection. These features make it an ideal tool for both observing biological processes in living organisms and for identifying and quantifying reactive oxygen species. The discipline of bioluminescence imaging has undergone a revolution thanks to luminol's capacity to produce light without the aid of outside light sources, enabling scientists to observe dynamic biological activities in real-time.

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