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Chemiluminescence Studies Using Some Non Steroidal Anti-Inflammatory Drugs with Benzoyl Hydroperoxide

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Abstract: *The oxidation of luminol with benzoyl hydroperoxide (BzOOH) in alkaline medium was studied. The reaction occurring between luminol and benzoyl hydroperoxide in alkaline medium leads to the production of nitrogen gas with simultaneous emission of CL. The effect of non-steroidal anti-inflammatory drugs (NSAIDS) like diclofenac sodium, analgin & paracetamol has also been studied. The time dependence of the CL intensity of luminol and benzoyl hydroperoxide at different temperature and the CL emission spectra of the reactions have been recorded for better understanding of the reaction.*

Keywords: Chemiluminescence, Luminol, Benzoyl hydroperoxide, Non-steroidal anti-inflammatory drugs like Diclofenac sodium, Analgein & Paracetamol.

I. INTRODUCTION

Chemiluminescence (CL) is the simultaneous production of electromagnetic radiation (UV, Visible or IR) observed when a chemical reaction yield an electronically excited intermediate or product, which either luminesces or donates its energy to another molecule which then luminesces[1]. As one of the important approaches of light emission, chemiluminescence induced by chemical reactions has evoked considerable interest for its potential applications in chemical detection, bioanalysis, and cold light source[2].

Figure: Example showing Chemiluminescence

It has been suggested that breakdown of organic hydroperoxide generates $^1O_2$ (singlet oxygen). This singlet oxygen may be the source of the chemiluminescence (CL) observed during lipid peroxidation[3]. Decompositon of organic hydroperoxides by metals could yield alcohols, ketones, peroxyl and alkyl radicals which are capable of generating CL. The decomposition of CuOOH catalyzed by transition metal ions or haeme compounds have been found to exhibit CL. The use of luminol increase the sensitivity of such CL reaction.[4] In 1999, Tsaplev[5] proposed an alternative light-producing pathway for the oxidation of linear hydrazides with hypochlorite, in which the formation of an electronically excited state of molecular nitrogen was discussed. Reaction of t- Butyl hydroperoxide with phenyl hydrazine hydrochloride in presence and absence of luminol has been discussed by Khan and Chandel et al.[6]

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NSAIDS have been studied in term of their effect on the production of reactive oxygen species (ROS) since, in addition to having a widely accepted mechanism of action by inhibition of the enzymes involved in the production of pro-inflammatory lipid-derived mediators, these compounds may also interact with the oxidants produced by phagocytic cells.[7-10] Antwerpen et al.[11] studied the reactions of oxicam and sulfoanilide non steroidal anti-Inflammatory drugs with hypochlorous acid. In order to examine its reactivity with potential scavenging molecules from the non steroidal anti-inflammatory drugs family, a competition assay based on para-aminobenzoic acid (PABA) chlorination was developed. Earthwarmoorthy et al.[12] studied chemiluminescence detection of paracetamol by a luminol-permanganate system

Keeping in view of the above facts it has been thought to explore the reaction of luminol and BzOOH in presence of nsaids to understand the mechanism of the reaction. Therefore present paper deals with chemiluminescence studies using some nsaids like diclofenac sodium, analgin & paracetamol.

II. Experimental

The reagents used for present investigation were luminol, benzoyl hydroperoxide, buffer solution having pH 13 and nsaids like diclofenac sodium, analgein & paracetamol. All the chemical used in the present investigation were taken in solution form and the solutions were prepared by using AR grade material adopting standard method described in the textbook. An aqueous solution of all the chemical compounds were prepared in double distilled water. Solution of different concentration were prepared and tested. The concentration having most intense CL was determined and hence selected for further investigation.

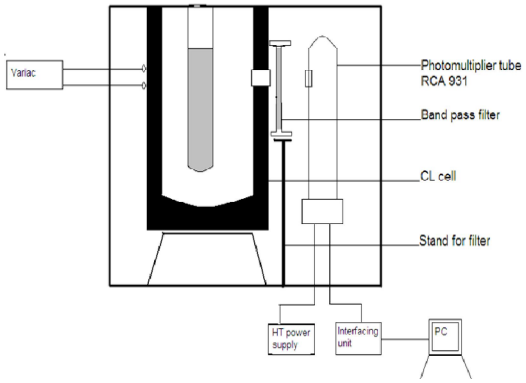

Figure: Experimental setup of chemiluminescence

Assembly for CL measurements essentially consisted of a chemiluminescent cell, high voltage power supply, light detector and a PC linked through interface. The kinetics of chemiluminescence was recorded with a RCA 931 photomultiplier tube (PMT); the photomultiplier was interfaced with a PC. The chemiluminescence cell and photomultiplier tube (PMT) were placed in a light box. Two circular holes made on the top surface of the box. One for placing syring to inject BzOOH in the reactor and other for placing thermocouple in the CL cell. The reactor was fitted inside the top surface of the light tight box and it rested just below the circular hole in which the syringe was placed. The reactors ware highly transparent glass tube of 1.0 cm diameter and 5 cm length made by IMX machine (USA) situated in front of the entrance window of a photoreceiving device. The box was covered with black cloth and syringe was placed on the hole.

III. Results and Discussion

In order to study Chemiluminescence of luminol and BzOOH in presence of nsaids, we tried to detect CL by adding BzOOH in the aqueous solution of different concentration of luminol in a buffer solution having pH 13 as shown in figure 1. It has been observed that CL intensity increases with time, attains a maximum value, then decreases gradually.

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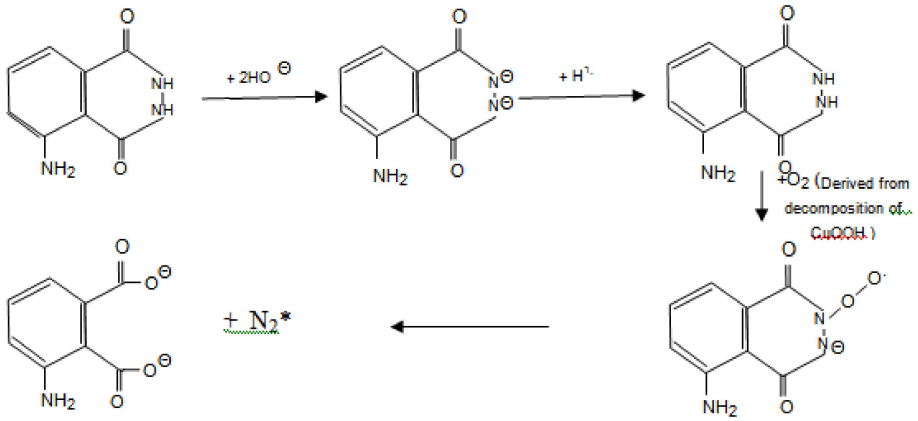
During the present investigation it has been observed that the reaction of luminol and BzOOH shows CL. The time dependence of CL intensity of the reaction is shown in figure 1, which clearly indicates that the reaction leading to light emission is quite rapid. It was also observed that by increasing the concentration of luminol while keeping the concentration of peroxide constant, the CL intensity increases, which may be due to the greater production of light emitting species during the chemical reaction. The probable mechanism explaning the above facts can be summarized as follows,

$$BZOOH \rightarrow BzO\cdot + \cdot OH$$

$$BzO\cdot + BzOOH \rightarrow BzOH + BzOO\cdot$$

$$2BzOO\cdot \rightarrow O_2{}^* + 2BzO\cdot$$



The mechanism suggest that BzOOH decomposes to give O_2* which reacts with luminol in alkaline medium to produce aminophthalate ion (excited state) which ultimately decomposes to singlet nitrogen, singlet nitrogen then produces luminescence.

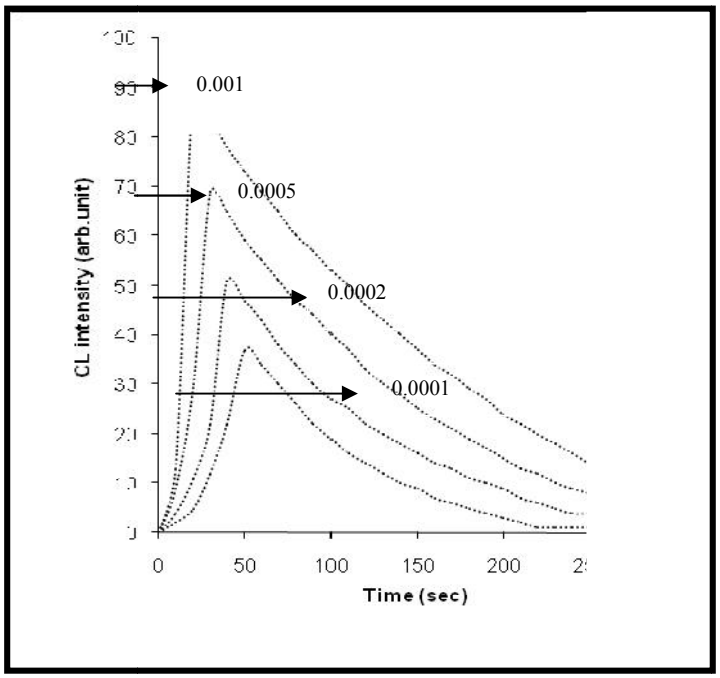


Figure (1): Time dependence of CL intensity of reaction between luminal and BzOOH for different concentration of luminol.

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The reaction of luminol and BzOOH in presence of nsaids like diclofenac sodium, analgin & paracetamol showed decrease in CL intensity which is indicated in the figure 2,3 and 4, showing large area under the CL intensity time curve with slower rise and decay kinetics. Therefore, the mechanism of CL emission is different in presence of nsaids compounds as compared to that of luminol and BzOOH reaction. Many anti-inflammatory agent (including some NSAIDS like, diclofenac sodium, analgin & paracetamol etc) act as scavenger of active oxygen species such as hydroxyl radical, superoxide anion radical etc.[13-20]

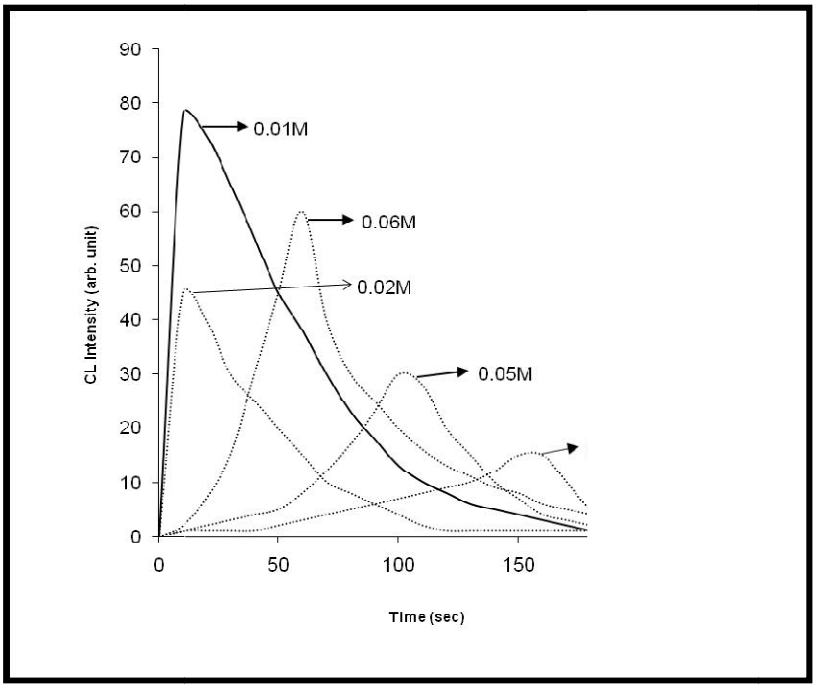

Figure (2): Time dependence of CL intensity for different concentration of diclofenac sodium under the reaction condition of 0.001M luminol and BzOOH 13 pH buffer

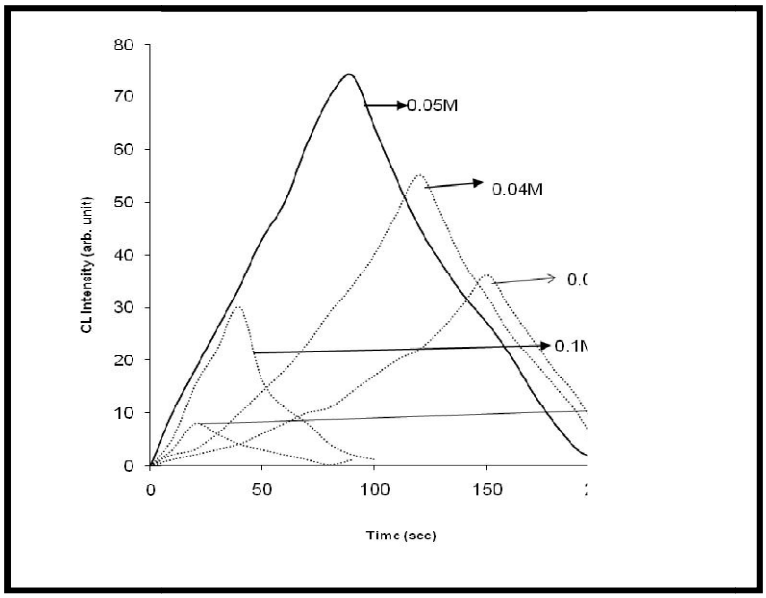

Figure (3): Time dependence of CL intensity for different concentration of analgin under the reaction condition of 0.001M luminol and BzOOH at 13 pH buffer

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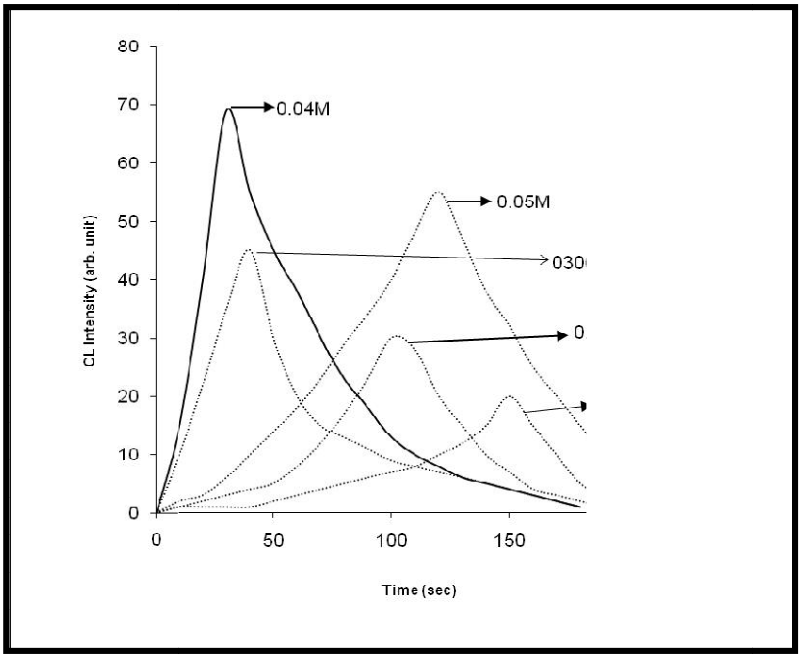

Figure (4): Time dependence of CL intensity for different concentration of Paracetamol under the reaction condition of 0.001M luminol and BzOOH at 13 pH buffer

The temperature dependence CL intensity of luminol and BzOOH substantially increase as temperature attains an optimum value then further decrease with increase in temperature as shown in figure 5.

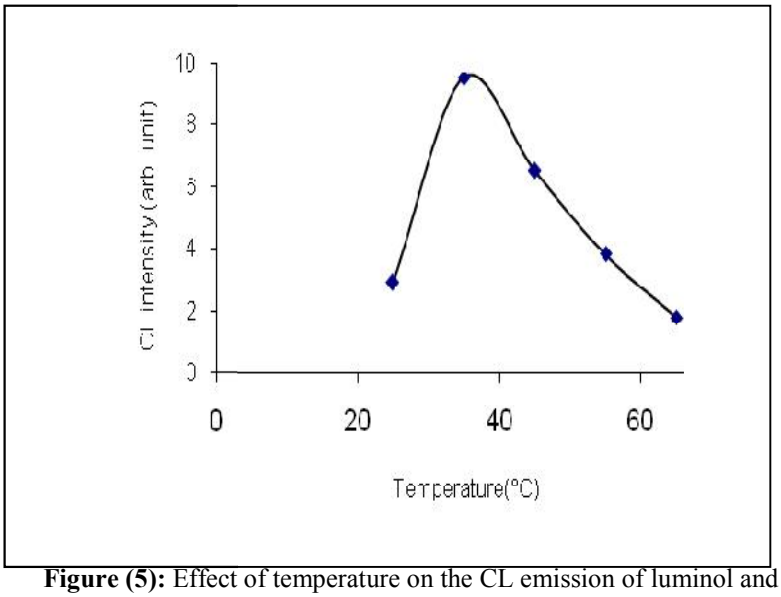

Figure (5): Effect of temperature on the CL emission of luminol and BzOOH

The chemiluminescence spectra of luminol and BzOOH system show a broad emission band with maximum (λ_{max}) at 485nm figure 6.

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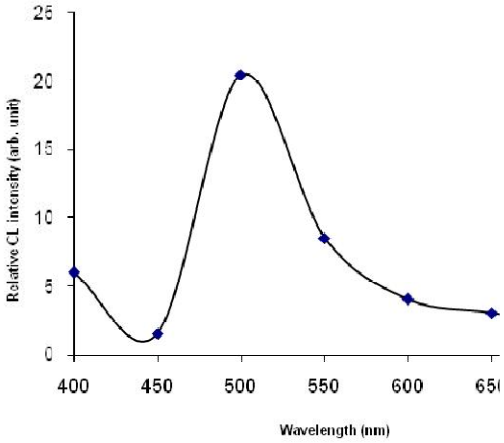

Figure (6): CL emission spectra for the reaction of luminol and BzOOH

The probable mechanism of the diminished CL intensity, effect of temperature and the CL spectra can be explained by suggesting following mechanistic pathway of the reaction.

BzOOH → BzO· + ·OH

Diclofenac sodium + ·OH → Diclofenac sodium - OH

(Diclofenac sodium spin adduct of ·OH)

Analgin + ·OH → Analgein - OH

(Analgin spin adduct of ·OH)

Paracetamol + ·OH → Paracetamol - OH

(Paracetamol spin adduct of ·OH)

Decrease in the concentration of ·OH radical directly hampers the production of singlet oxygen which caused enhancement of CL intensity when it reacted with luminol as previously mentioned.

IV. Conclusion

The Chemiluminescence behaviour of luminol with BzOOH and the subsequent decrease by nsaids (diclofenac sodium, analgin & paracetamol) is reported. The proposed study undoubtedly could be applied for the detection of numerous analytes in addition to the nsaid studies. It is hoped this study will stimulate further investigation in this field.

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