THE ROLE OF REACTIVE OXYGEN SPECIES IN THE CHEMILUMINESCENCE REACTION OF HYPOCHLORITE WITH LUMINOL

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ABSTRACT

The oxidation of luminol by hypochlorite to generate chemiluminescence (CL) has been studied by using a multipurpose photon counting system. The processes of light production , which invovle the reactive oxygen species (ROS), are discussed in terms of the NaOCl-Luminol reaction .

The addition of radical scavengers such as ascorbic acid and dimethylsulfoxide (DMSO) lead to decreases in the peak of CL reaction of both system NaOCl-Luminol and $FeSO_4$ – Luminol respectively, while the addition of persulfate lead to increase in the peak of CL. The results suggest that ROS radicals might be constant factors in CL reaction of NaOCl-Luminol system.

Key words:

Chemiluminescence , reactive oxygen species , luminol , hypochlorite.

INTRODUCTION

The oxidation of luminol by hypochlorite to generate chemiluminescences (CL) has been widely used as a detection method in many fields [1-4]. Dermination of hypochlorite in waters based on this reaction has been reported in several methods by many outhors [5 -7].

The mechanism and Kinetic of hypochlorite-luminol reaction has mainly been studied by means of the previously proposed of azaguinone intermediate formed when hypochlorite oxidizes

luminol [8-10].

In phagocytosis system the light emission reaction of hypochlorous acid is associated with the formation of reactive oxygen species (ROS), including superoxide radical **(O_2^-)** singlet oxygen (O_2^1), hydroxyl radical ($O^{\cdot}H$) and hydrogen peroxide (H_2O_2) [11].

Because of the importance used of hypochlorite in chlorination of water supplies and because the importance reactions of ROS radicals with luminol which have some diagnostic value in biological system, it has been considered necessary to use a method depending on the CL of luminol to investigate the role of reactive ROS radical in hypochlorite-luminol reaction system.

EXPERIMENTAL

Reagents and apparatus:

Luminol stock solution (5-amino-2-3-dihydrophthazine, -1,4-dione) in a concentration of $1.13x10^{-3}$M was used and prepared according to the modified method of Ewetz and Throe [12].

Standard hypochlorite solution was prepared according to the modified method of Marino and Ingle [6].

Other chemicals and reagents concentration used were as follows: persulfate (10^{-3} M), ascorbic acid (100 μM), H_2O_2(32.6×10^{-2}M), DMSO (1M) and $FeSO_4$ (1M).

Measurement of the emission light (CL) was made at room temperature in a multipurpose photon counting system type of 9635QB and CL signal was registered on the chart recorder as previously described by Al-Hashmi and Mohammed [13].

Procedure:

To maesure the CL signal of the NaOCl – Luminol system , 0.2 ml luminol in 10^{-1}M carbonate buffer at PH 10.2 was injected into the reaction vessel containing 10 µg/ml hypochlorite (fig.1a).

The effect of radical scavengers on CL signal were examined by addition of some scavengers compounds , such as ascorbic acid (0.2 ml) or DMSO (20µg/ml) to a reaction vessel containing 10µg/ml hypochlorite , thereafter, the CL signal was measured by an injection of 0.2 ml luminal (fig.1b-c).

To measure the CL signal of the $FeSO_4$- Luminol system , 0.2 ml luminol was injected in to a reaction vessel containing 1 ml $FeSO_4$(fig.2a) . 20µg/ml DMSO was added to 10µg/ml hypochlorite , thereafter , the CL signal was measured by an injection of 0.2ml luminol (fig.2b) .

Different peaks of CL signal were measured by injection 0.2 ml luminol to a reaction vessel containing series of different concentrations of NaOCl (fig.3).

In the experiment of NaOCl –Luminol –Persulfate system , the CL signal was made by injection 0.2 ml luminol in to a reaction vessel containing 10µg/ml hypochlorite and 0.5 ml prsulfate (fig.4) .

The injection effect of a series concentrations of luminol to 10 µg/ml hypochlorite were used to yield different CL peaks (fig.5) .

Different peaks of CL signal were measured by injection 0.2 luminol to a mixture reaction containing series of different concentrations of H_2O_2 (fig.6).

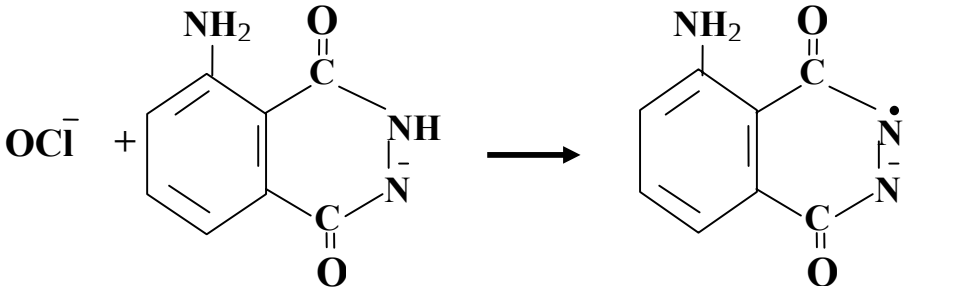
RESULTS AND DISCUSSION

Sodium hypochlorite-luminol CL:

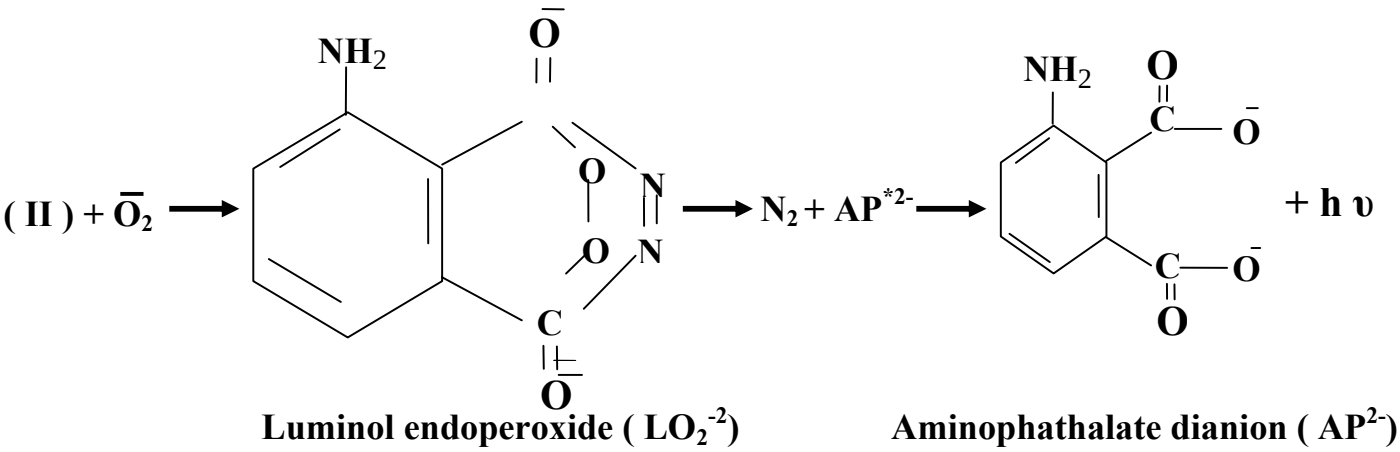
Figure (1a) shows the typical time course of CL generated in the oxidation of luminol by hypochlorite solution. As shown, the light emission increased rapidly to a maximum peak at about 3sec, thereafter light emission declined rapidly to the back ground level and remains constant over a span of 40 sec.

According to the scheme of Hodgson and Fridovich [14] it seems the mechanism of hypochlorite induced CL explained in term of both the azaquinone intermediate formed by two-electron oxidation of luminol and the radical formed by one-electron oxidation of luminol [10] or might be the hypochlorite acts as a secondary oxidant which serves to oxidize the luminol monoamine (LH^-) to a radical luminol ($L^{\bullet -}$) in subsequent reactions that involve the generation of superoxide radical (O_2^-), and in this case the initial of (O_2^-) is to react with luminol radical to produce an endoperoxide(LO_2^{2-}) which then decomposes to an electronically 3-amino-phthalate dianion (A^*P^{2-}) which then emits light through its return to ground state.

The intermediate reaction between hypochlorite and luminol can be accounted by the following reaction scheme :



Luminol monoanion (LH) (I) **Luminol radical (L•) (II)**



Luminol endoperoxide (LO_2^{-2}) **Aminophathalate dianion (AP^{2-})**

The effect of radical scavenger on NaOCl-Luminol CL reaction such as ascorbic acid which acts as an $^{\bullet}O_2^-$ - scavenging agent [2] and DMSO which acts as an $^{\bullet}OH$ scavenging agent [15]were observed in figures (1b) and (1c) respectively. As shown, the ascropic acid scavenging studied caused a strong inhibitory on light emission for CL reaction while DMSO agent resulted in little reduction in CL emission.

The rapid increase in CL peak height (fig.1a) and the inhibitory effect of O_2^- radical scavenger on CL signal (fig.1b) , explained the resultant increase in the production of $^{\bullet}O_2^-$ generated in this process .

In all of the process discussed above , the secondary oxidant , hypochlorite ,may play strong role in the interpretation of the mechanism of $^{\bullet}O_2^-$ generation in CL of NaOCl- Luminol reaction .

Hydroxyl radical-luminal CL:

The CL signal from $FeSO_4$-luminol system is shown in figure 2. As shown, the CL signal was inhibited by DMSO which act as an OH scavenging agent. The results presented above suggest that the importance of the system $FeSO_4$-luminol as a source of CL by hydroxy radical.

The results in Fig. (Ic) and Fig. (2) indicate that OH radical may not be involved in NaOCL-luminol CL reaction.

Effect of hypochlorite concentration on peak CL:

Figure 3 shows that the peak of CL reaction as a function of hypochlorite concentrations.

As shown , the CL peak responses were linearly proportional to the hypochlorite concentration in range of 0.1-10 μg/ml .The wide linear dynamic range of this method is may be satisfactory for the determination of hypochlorite in some waters.

Hypochlorite-luminol-Persulfate system:

The role of the study singlet oxygen $^1O_2$ for luminol-CL reaction on a mixture of persulfate-hypochlorite is illustrated in figure 4. The light emission was observed from this reaction upon the injection of luminol to a mixture solution.

It is well known that the chemical reactivities of persulfate permit the accumulation of H_2O_2 in persulfate-luminol reaction [14] and also the well known artificial H_2O_2-NaOCl system was used to generate the $^1O_2$ [11], according to these facts the CL signal generation from this reaction is in some way may be due to the reaction between $^1O_2$ and luminol radical.

The influence of luminol concentration:

The initial rate of luminol consumption (maximum light intensity) in the oxidation of hypochlorite was measured in the presence of various concentrations of luminol (Fig. 5). The consumption of luminol was found to follow saturation kinetics and maximum consumption reache levels above 1×10^{-3}M and below this level the activity was essentially directly proportional to the concentration of luminol.

The influence of H_2O_2 concentration:

The CL signal of hypochlorite-luminol reaction in the presence of various concentration of H_2O_2 was investigated (Fig.6). As shown, the CL peak signal in the oxidation of luminol by hypochlorite was found to reach a maximum at about 30×10^{-2}M H_2O_2 and below this level the activity was essentially directly proportional to the concentration of H_2O_2.

It seemed according to Violet *et al* [11] the amount of CL signal might be proportional to the concentration of $^1O_2$ formed by the H_2O_2-NaOCl reaction.

Finally, in all of the processes discussed above I reports on some observations of CL emitted in the oxidation of luminol by hypochlorite which involve ROS radicals and I consider that the hypochlorite oxidant might be acts to oxidize the luminol monoanion to the luminol radical, and in all cases the initial role of ROS radicals are to react with the luminol radical to produce the endoperoxide dianion.

Conclusions

The CL emitted during the oxidation of luminol by hypochlorite is important process in the determination of hypochlorite in water. The mechanisims of light production are studied in

terms of ROS – CL reaction by using some radical scavengers.

Further work is needed to study other luminol derivatives and other radical scavengers in addition to other CL reagents to find whether there is another more sensitive CL indicator that can be successfully used to detect more about the role of ROS radical in CL hypochlorite.

دور الجذور الأوكسيجينية الحرة في استثارة التألق الكيميائي المحدث من أكسدة الهايبوكلورايد بواسطة محلول اللومينول

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الخلاصة

تم دراسة التألق الكيميائي المحتث من أكسدة اللومينول بواسطة محلول الهايبوكلورايد باستخدام العداد الفوتوني المتعدد الأغراض البحثية. حيث تم مناقشة العمليات الحادثة في تفاعل اللومينول-هايبوكلورايد والتي تشمل تفاعلات جذور الأوكسي الحرة.

أحدثت إضافة قانصات الجذور الحرة مثل حامض الاسكوربيك ومحلول ثنائي مثيل سلفوكسايد نقصان في قمة التألق الكيميائي في كل من تفاعل اللومينول-هايبوكلورايد وتفاعل اللومينول-كبريتات الحديدوز على التوالي بينما كان تأثير إضافة البرسلفات على تفاعل اللومينول-هايبوكلورايد زيادة في التألق الكيميائي. النتائج المقترحة هنا تشير على إن الجذور الحرة ربما تعتبر عوامل أساسية ثابتة في تفسير تفاعل اللومينول-هايبوكلورايد.

Figure 1. The typical time course for luminol-dependent CL induced by hypochlorite as a control curve (a). The effect of addition radical scavengers ascorbic acid curve (b) and DMSO curve (c) on a control curve (a). The conditions and components were as described in procedure.

Figure 2. The typical time course for luminol-dependent CL induced by $FeSO_4$ as a control curve (a). Curve (b) represents the effect of addition DMSO on a control curve (a). The conditions and components were as described in procedure.

Figure 3. The proportionality between the light emission (CL peak) and a various concentrations of hypochlorite solutions. All reactions were done in triplicate and reported values represent the mean values ± S.E.M. The conditions and components were as described in procedure.

Figure 4. The typical time course for luminol-dependent CL induced by a reaction mixture of persulfate-hypochlorite curve (a) as compared to a reaction containing only mixture solution curve (b). The conditions and components were described in procedure.

Figure 5. Influence of a various concentration of luminol on CL peak of hypochlorite luminol CL reaction. The conditions and components were as described in procedure.

Figure 6. The influence of various concentrations of H_2O_2 on CL peak of hypochlorite-luminal reaction. The conditions and components were as described in procedure.

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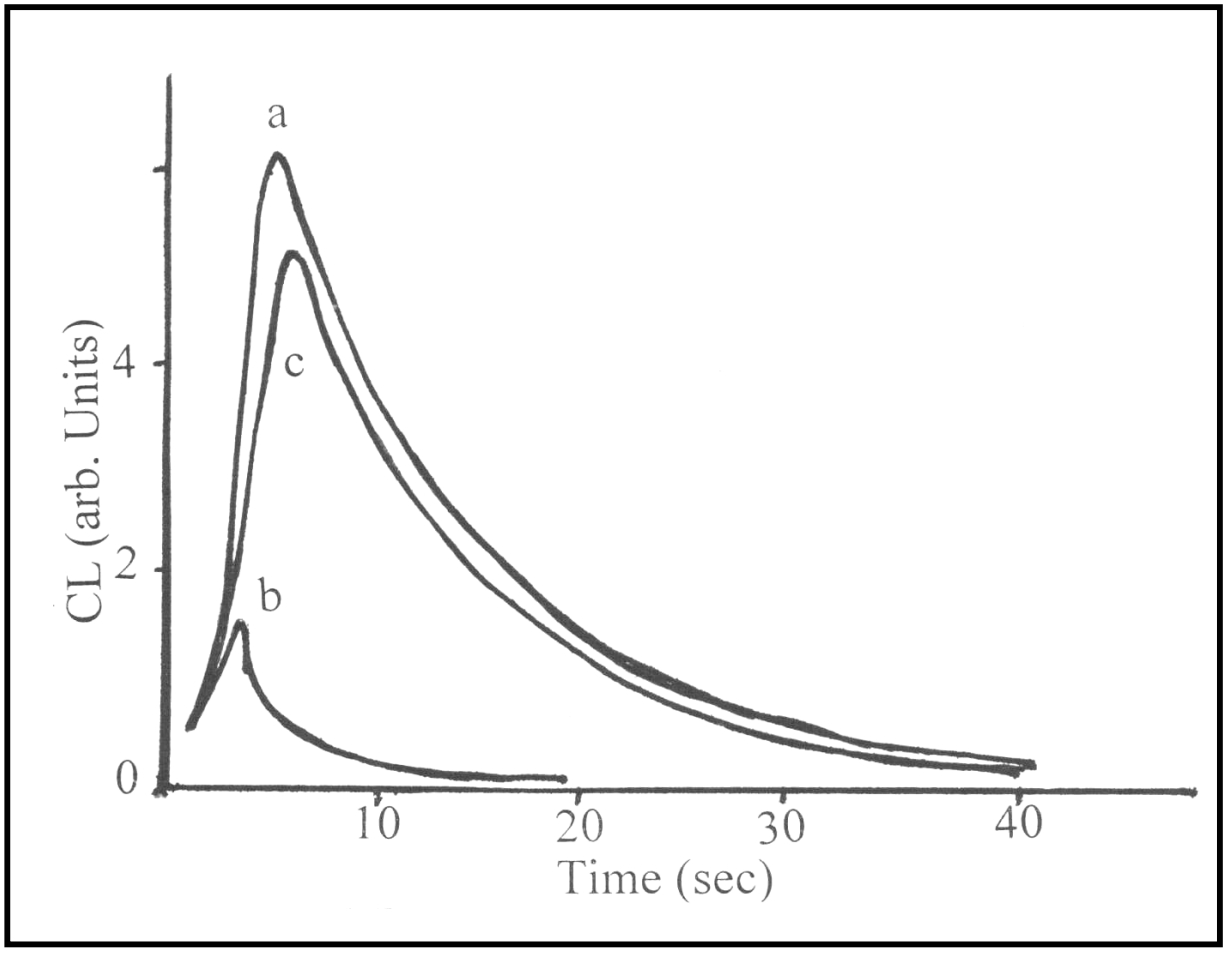

Fig.1

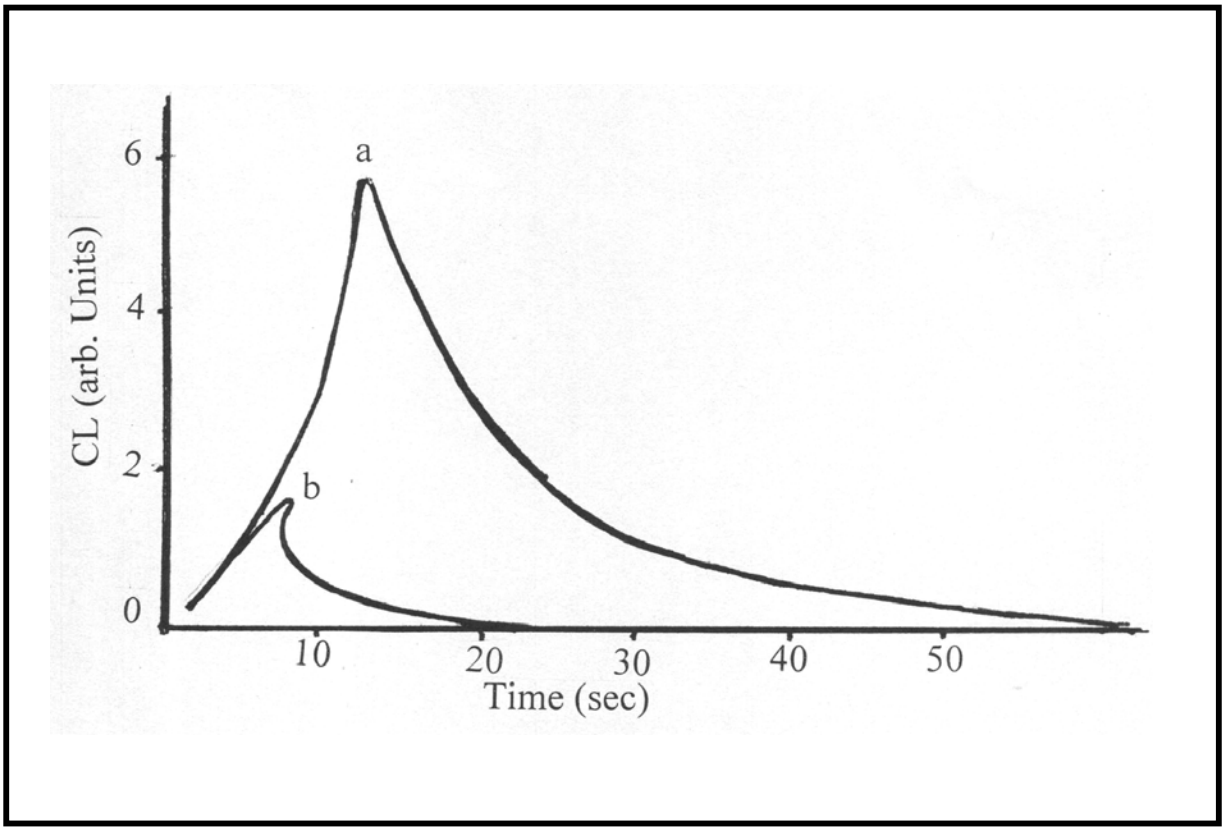

Fig 2

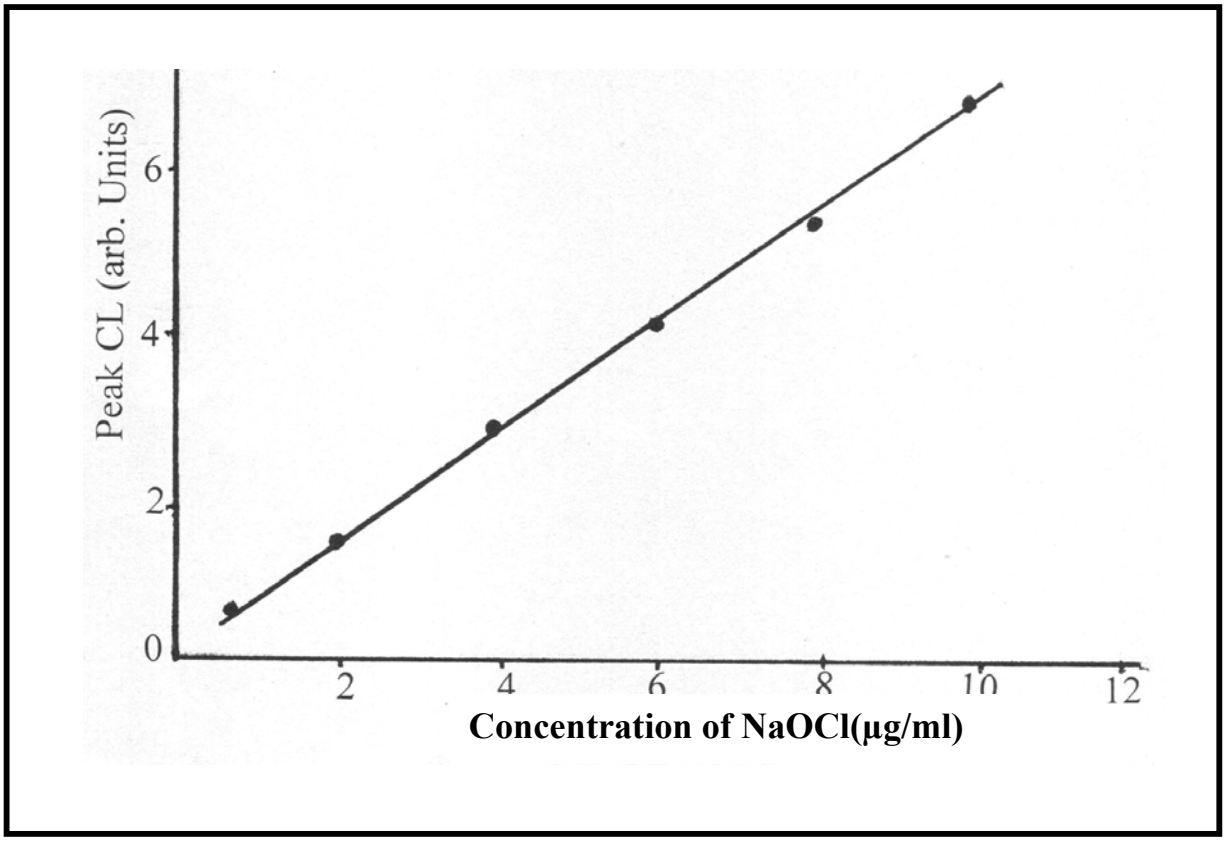
Peak CL (arb. Units)
0
2
4
6
2
4
6
8
10
12
Concentration of NaOCl(µg/ml)

Fig .3

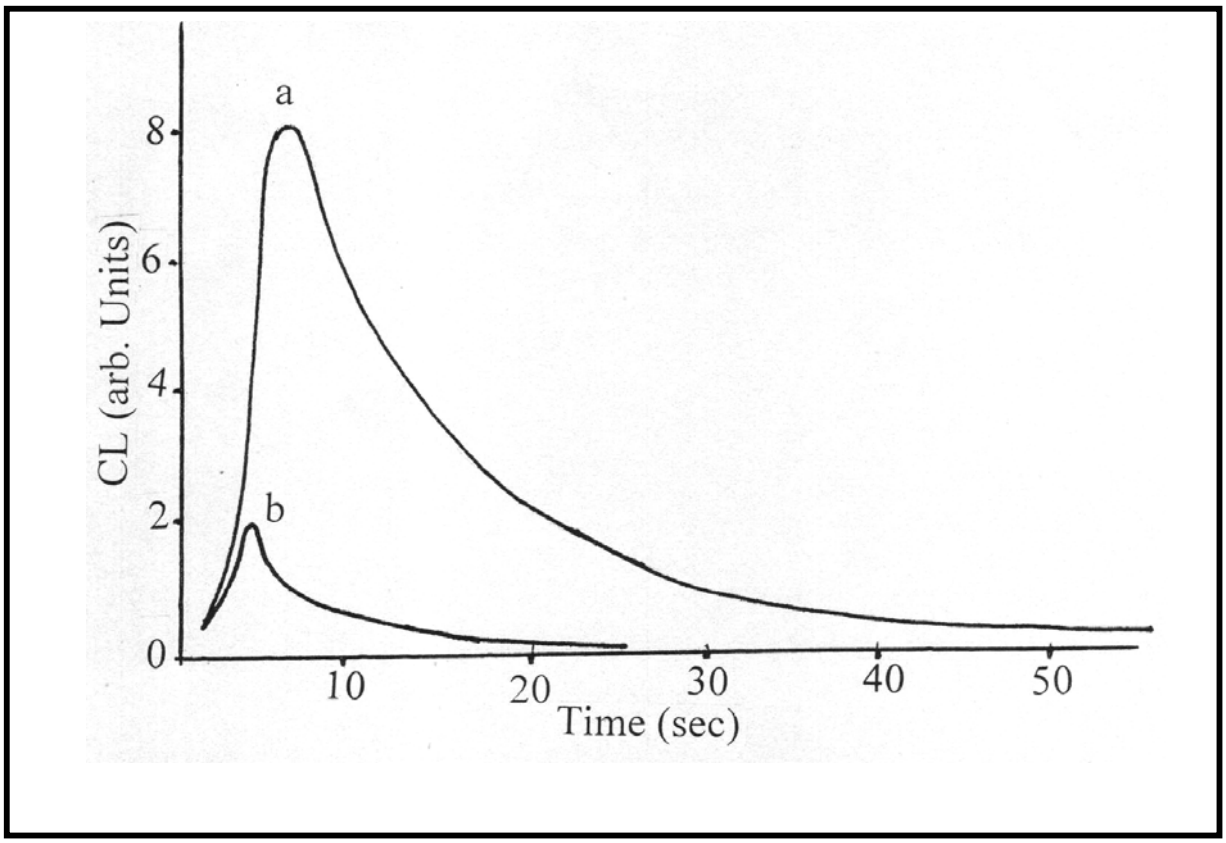

Fig .4

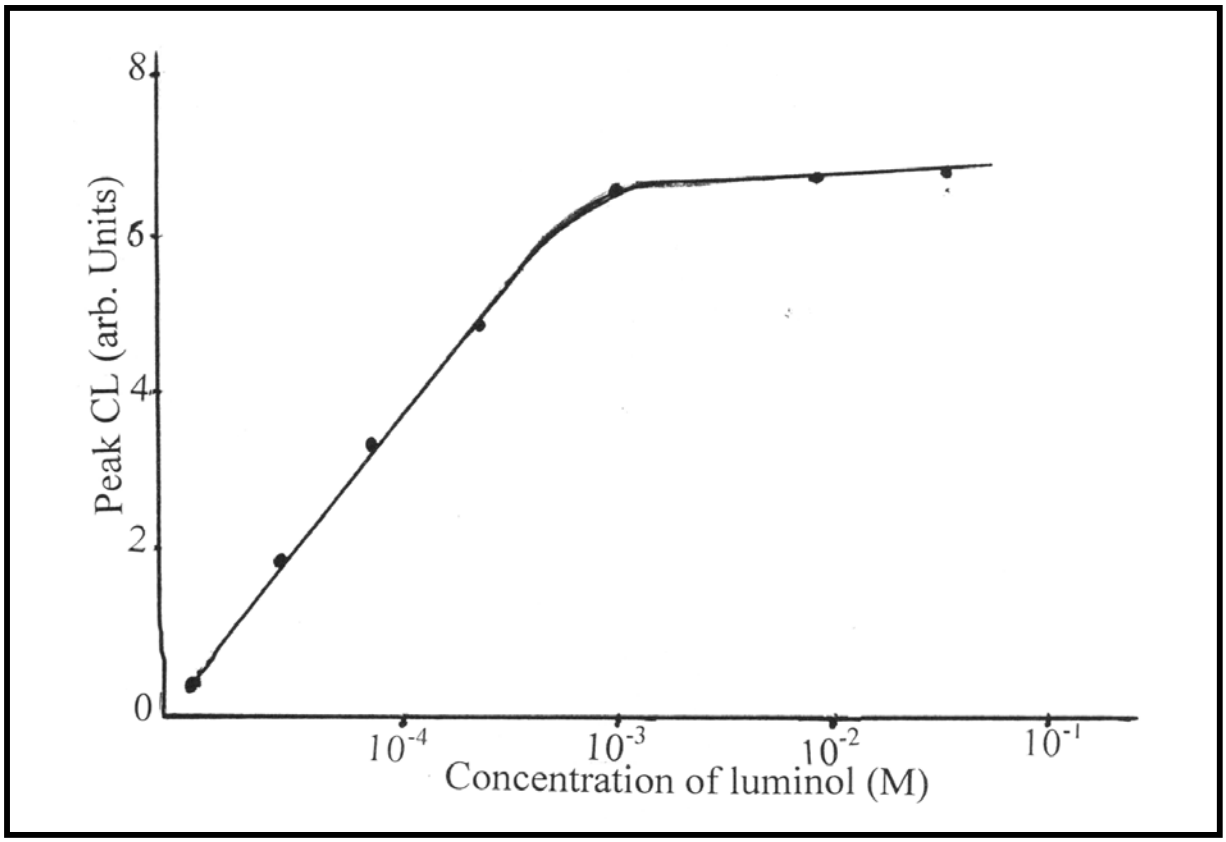
Peak CL (arb. Units)
8
6
4
2
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
Concentration of luminol (M)

Fig.5

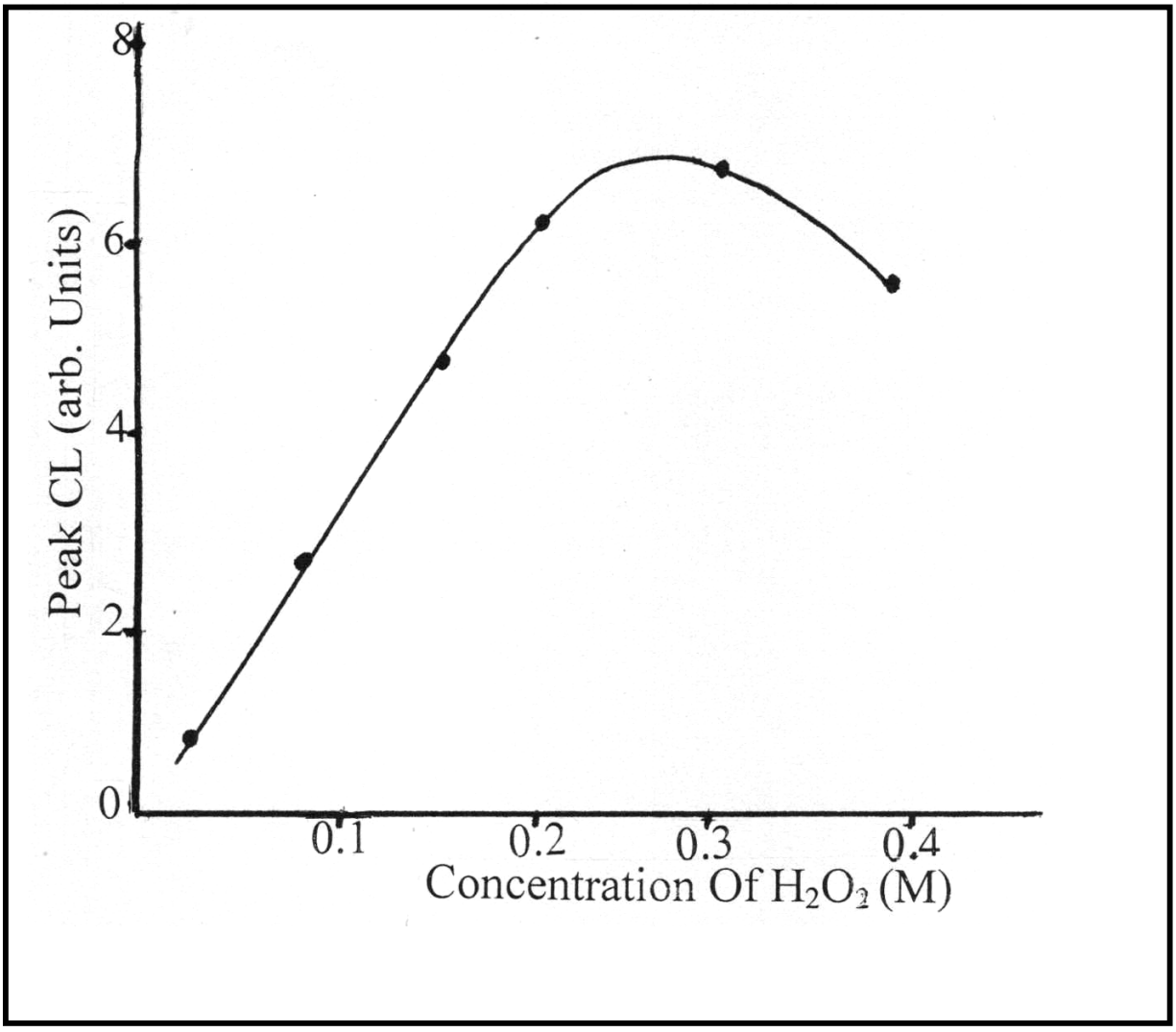

Fig.6