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CHEMILUMINESCENCE PRODUCED DURING REACTION BETWEEN LUMINOL-HEXACYANOFERRATE AND VMA

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Abstract

The Chemiluminescence properties of Luminol and Hexacyanoferrate in alkaline medium were studied in presence of Vanilmadelic acid. It was observed that the reduction of Vanilmadelic acid by potassium hexacyanoferrate(III) in alkaline luminol solution may be leads to increase the strong Chemiluminescence signal produced by the reaction between luminol and hexacyanoferrate (III). The increased Chemiluminescence intensity was proportional to Vanilmadelic acid concentration in the range of 5.0 x 10^{-6} g-L^{-1} to 2.0 x 10^{-4} g-L^{-1}. This can be used to determine Vanilmadelic acid.

Keywords:*Luminol-Hexacyanoferrate Chemiluminescence, Vanilmadelic acid*

1. Introduction

Chemiluminescence (CL) and bioluminescence (BL) are natural phenomena which have attracted the attention of mankind since the evolution of life on the earth. Chemiluminescence, the production of light during a chemical reaction, has proved to be a useful phenomenon in the laboratory and is finding ever increasing applications in analytical chemistry. Current researches on CL are focused in two general directions. One is the discovery of new CL reactions and investigation of their applicability for analysis of real samples and the other direction is the creation of CL detection systems for newly developed separation techniques. Researches on new CL reactions are very important since they open new horizons for the technique. However, sometimes the study of the mechanism of the reaction is bypassed and emphasis is given to the applications only. Nevertheless, a detailed study would help in studying and improving the analytical performance of the procedure.

CL based on the oxidation of luminol (LH_2) (5-amino-2,3-dihydro-1,4-phthalazinedione) is one of the most extensively studied and best known CL system [1-3]. The oxidation is usually carried out in an alkaline medium using an oxidant such as hydrogen peroxide [4], hypochlorite [5], permanganate [6], or hexacyanoferrate (III) [7]. The luminol-hexacyanoferrate system is one of the most efficient CL systems known to date. Luminol-CL in water is mostly applied for analytical purposes, in special forensic medicine (to detect trace amounts of blood); this is why luminol reaction in water has been intensely investigated. a,4-Dihydroxy-3-methoxybenzeneacetic acid (4-hydroxy-3-methoxymandelic acid, vanilmandelic acid, VMA) is the major urinary metabolite of the neurotransmitters epinephrine and norepinephrine. Determination of this species is important in the diagnosis of catecholamine-secreting tumours such as pheochromocytomas, paragangliomas and neuroblastomas [8]. Neuroblastoma is the most common solid tumour in childhood, and more than 90% of patients exhibit high urinary levels of VMA [9]. If diagnosed at an early age (less than one year) survival rates are excellent; however, children over one year old with advanced stage neuroblastoma have a poor prognosis, even with extensive treatment [10]. Due to the importance of early diagnosis, mass screening of infants for neuroblastoma by measurement of VMA levels is required by law in Japan. Screening trials have also been conducted in Australia, Austria, Brazil, Canada, France, Germany, Norway, the United Kingdom and the USA[10]. Therefore, In the present paper, the effect of VMA on the intensity of CL produced during reaction between luminol and hexacyanoferrate (III) is studied and reported.

2. Experimental details

2.1 Materials :

Luminol (from Thomas backer), NaOH, potassium hexacyanoferrate (III), (all from E merck, AR/GR grade), VMA (from Aldrich) used were of spectroscopic grade and were further distilled before use. Triple distilled water was used throughout during the studies.

2.2 Solutions preparation :

For the present investigation, 1.0 x 10^{-3} mol-L^{-1} Luminol and 4.0 x 10^{-2} mol-L^{-1} NaOH, were taken. This was the stock solution of Luminol. For the preparation of second solution, 2.0 x 10^{-5} mol-L^{-1} potassium hexacyanoferrate (III) and different amount of VMA were taken.

2.3 Instruments and Methods :

All the experiments were performed on a chemiluminometer setup connected to a X-Y recorder (Fig.1). Stock solution of luminol (1ml) was taken in reaction cell and potassium hexacyanoferrate (III) solution (1ml) was added through syringe.

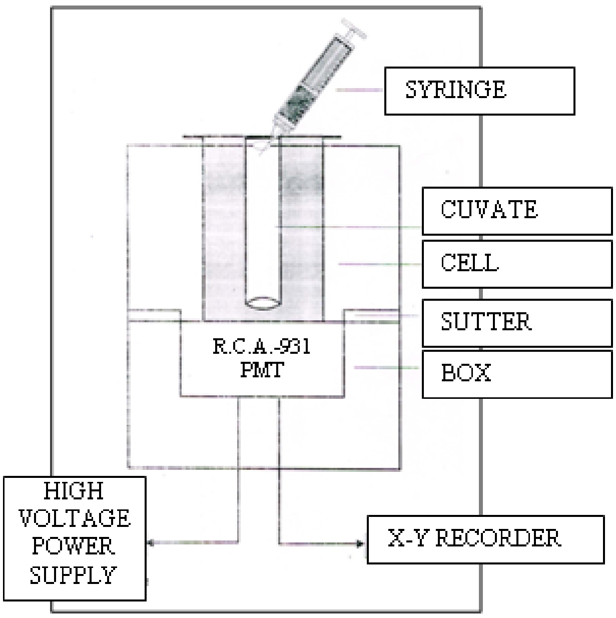


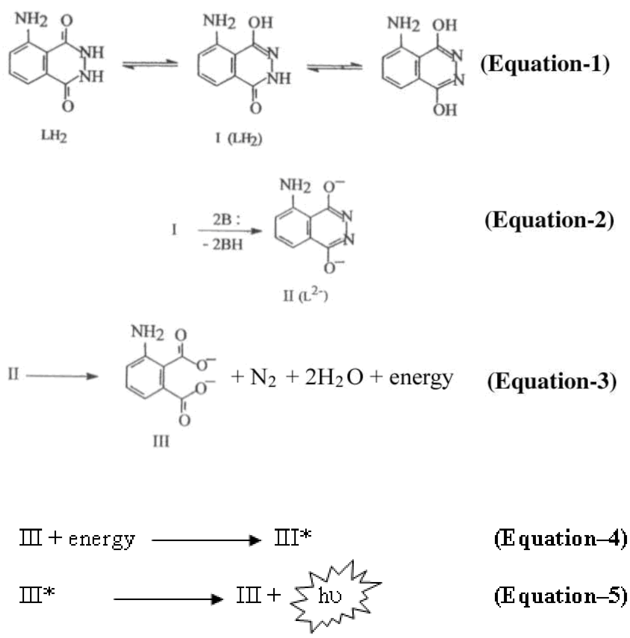
Figure – 1 : Experiment Setup of Chemiluminometer

3. Results and Discussion

The CL signal produced by the reaction between luminol and potassium hexacyanoferrate (III) in alkaline medium were studied in presence of VMA. It was observed that VMA could greatly increase the strong CL signal produced by the reaction between luminol and hexacyanoferrate (III) in alkaline medium. The increased CL intensity was linear with VMA concentration in the range of 5.0×10^{-6} g-L^{-1} to 2.0×10^{-4} g-L^{-1}, and the limit of detection was 4.0×10^{-6} g-L^{-1} (3σ) with a relative standard deviation of 2.6~4.2% (n = 5).

The structure of luminol (Eq.1) confers acidic properties, so in presence of a base there results the dianionic species L^{2-} is formed (Eq.2), which on oxidation with hexacyanoferrate (III) yields the 3-Aminophthalate dianion (III) along with release of reaction energy (Eq.3). This energy is absorbed by species III and which forms the excited state (Eq.4) and then returns to ground state with CL emission (Eq.5). Thus 3-aminophthalate is the luminophor of the luminol CL system, and that the emission maximum of the CL reaction is at 425 nm [11, 12].

The reduction of VMA by potassium hexacyanoferrate(III) in alkaline luminol solution may be leads to increase the strong CL signal produced by the reaction between luminol and hexacyanoferrate (III). Under the conditions selected as described above a calibration graph showed that relative increase in CL intensity was proportional to VMA concentration in the range of 5.0×10^{-6} g-L^{-1} to 2.0×10^{-4} g-L^{-1}. This can be used to determine VMA. A series of standard solutions of VMA were determined under the optimized conditions to test the linearity of the calibration graph. The results are summarized in **Table-1**.



Reaction scheme I

4. Conclusions

From the study on luminol- potassium hexacyanoferrate(III) CL behavior involving the effect of VMA, the important conclusions drawn are:

(i) Luminol CL depends largely on VMA concentration.

(ii) The increased CL intensity was linear with VMA concentration in the range of 5.0×10^{-6} g-L^{-1} to 2.0×10^{-4} g-L^{-1}.

(iii) The limit of detection of VMA was 4.0×10^{-6} g-L^{-1} (3σ) with a relative standard deviation of 2.6~4.2% (n = 5).

(iv) Effect of VMA on luminol- potassium hexacyanoferrate(III) CL can be applied for determination of hydrazine in different samples.

5. Acknowledgement

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6. References

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Table – 1 : Calibration Graph of VMA

VMA concentration (in ng/mL)	**Equation** Y = aC + b	**Correlation coefficient**	**RSD** (n = 5)%	**LOD** (in ng/mL)
5~20	Y=1687C–7.67	0.9966	3.9	4.0 (3ó)
20~50	Y=293.4C+11.6	0.9987	4.2	
50~100	Y=87.9C+12.3	0.9994	2.7	
100~150	Y=34.5C+126.7	0.9996	2.6	
150~200	Y=7.6C+243.6	0.9998	3.1	