

CHEMILUMINESCENCE IN DOSIMETRY

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An application of a sensitive apparatus with a photomultiplier (lumino-photometer) for the measurement of chemical luminescence has been described. The reaction of luminol oxidation has been modified for the detection of trace amounts of H_2O_2 using haemin as catalyst; The stabilizing effect of the admixture of a complex salt of bivalent copper with triethanolamine on the haemin solution has been observed. The lower sensitivity limit has been found to be the concentration of 2×10^{-8} M H_2O_2 in the reacting medium. In conformity with previous studies the oxidation effects of the lowest concentrations of H_2O_2 cause a linear increase of the total intensity of the light emitted.

Determination of the trace concentrations of H_2O_2 in water and aqueous solutions (e. g. after the radiolytic decomposition) can be performed by chemiluminescence reactions. The luminescence of luminol (3-aminophthalic acid hydrazide) can be particularly recommended for this purpose. When oxidized, alkaline solution of luminol produces intense blue-violet luminescence. Even oxygen alone causes a slight luminescence of the luminol solution while luminescence of considerable intensity occurs if oxidizing reagents and consequently hydrogen peroxide are added to the solution, especially in the presence of some substances which increase the rate of luminol oxidation. Their effect is not purely catalytic because they are fully inactivated during the reaction. *Weber* (1) therefore suggested to define this effects as promotional. The effect of these reagents is however similar to the catalytic effect and is commonly called so. Complex ions of iron and copper, and a number of organic compounds such as the peroxidases present in plants, as well as haemoglobin, haemin etc. belong to this group.

SOME PROPERTIES OF LUMINOL

Luminol is only slightly soluble in water ($0,8 \times 10^{-3}$ M/l) (2) and neutral solutions of luminol show a slight bluish fluorescence in the short-wave light. This effect can be attributed to the molecular form of luminol. Luminol is soluble in an alkaline medium, where it dissociates and reacts in this form with oxidizing agents evolving light energy.

All factors influencing the emission of the luminol reaction have been experimentally investigated in detail by *Weber* et al. (3). On the basis of these experiments, quantitative methods have been developed for the determination of trace amounts of blood (1), ferricyanides, copper compounds (4, 5) and a number of organic substances (6, 7), e. g. highly toxic organophosphorus compounds.

Dependence of the luminescence intensity on the concentration of components during the luminol oxidation with hydrogen peroxide has also been studied by *Weber* (3e) who used higher concentrations (0,01 — 0,5 M). The determination of minute amounts of H_2O_2 and other oxidation products formed by the radiolysis of water is the subject of the present work.

Besides luminol and the catalyst 4 components are present in the reaction medium, whose concentrations may be varied. The luminol concentration is chosen according to the reaction conditions so that it may be higher than the concentrations of the catalyst and hydrogen peroxide which in turn may vary within a wide range. The reaction solution is very sensitive to the addition of these components and the concentration of each of them may be lowered to about 10^{-8} M/l.

When the maximum emission is reached the chemical luminescence time measured is minimal; the quantum of light emitted is constant but is spread over a long time interval at low light intensities. A number of inhibitors also exist. Some of them have been more thoroughly studied by *Weber* (KCN, aniline, hydroquinon and others) (3f).

METHOD OF MEASUREMENT

The intensity of luminol chemiluminescence may be quantitatively measured by means of a photoelectric device — a luminophotometer. Its main part is a radiation detector a photoelectric cell or — in a more sensitive device — a photomultiplier. The block diagram of the apparatus used in our experiments is shown in Fig. 1, the total view of the device is given in Fig. 2, while Fig. 3 presents the details of the photomultiplier with a cooled reaction vessel. In our assembly the detector was the Soviet photomultiplier FEU 19 M, with a spectral sensitivity (350–600 nm) corresponding to the emission of luminol. High voltage is supplied to the photomultiplier electrodes from a high-voltage generator with an inbuilt stabiliser (the Soviet stabiliser BC 16). The photocurrent from the photo-

multiplier is supplied to a recorder (the Czechoslovak electronic recorder EZ 2), which is a millivoltmeter recording the deviation of its pointer. The speed of automatic displacement of the recording paper is 360

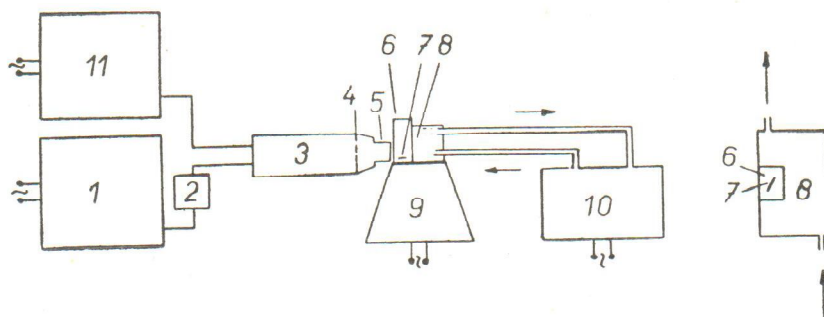


Fig. 1. Diagram of measuring system

1 high voltage generator, 2 voltmeter, 3 photomultiplier, 4 shutter, 5 light guide, 6 cuvette, 7 stirrer, 8 thermostatic vessel, 9 electromagnetic stirrer, 10 thermostat Höppler, 11 electronic recorder

mm/hr. The whole assembly is placed in a dark-room. Shortly before measuring a photographic shutter which shuts a protective cylinder containing the photomultiplier, opens and the solutions in the cuvette, heated on a waterbath to a desired temperature, are mixed. The maximum deviation recorded at the beginning of the reaction is considered to be a measure of the maximum intensity of luminescence, I_{\max} . After reaching its maximum height, the point of the recorder is falling with time drawing a kinetic curve which represents the integral of the light intensity over the total time of emission t , until the luminescence dies out. Thus the area below the curve is a measure of the total quantum of light emitted during the reaction. The area can be expressed by the relation:

$$\int_0^t f(t) dt = \int_0^t I dt.$$

Devices of similar design have been described in several modifications and they have been used for the determination and registration of very low chemiluminescence intensities. *Babko and Lukovskaja* (8) have used a similar procedure for the determination of small amounts of copper compounds (2×10^{-8} g Cu in 1 ml). Two procedures have been described for the determination of trace amounts of H_2O_2 in irradiated water: by *Mayneord et al.* (9) and by *Armstrong and Humphreys* (10); the latter authors have suggested the determination of H_2O_2 as a chemical dosimeter independent of the specific energetic loss of radiation used. In both works copper salts have been used as catalysts of luminol oxidation.

The total sensitivity of our luminophotometer depends on the following factors: the distance of the photomultiplier cathode from the reaction cuvette, the diameter of the photographic shutter aperture, the voltage supplied to the photomultiplier electrodes and the adjustment of the recorder sensitivity which may be varied in the ratio of 1 : 50.

EXPERIMENTAL

Each chemiluminescence reaction is abnormally sensitive to foreign admixtures and impurities, which catalyse the reaction positively or negatively. The results are consequently very distorted. The reaction vessels were therefore washed in chromosulphuric acid and were then exposed to hot steam for 20 minutes. The water used for the preparation of solutions was distilled 3 times, the third time in a quartz apparatus.

Luminol produced by Merck was recrystallized from acetic acid and the product was used for the preparation of a stock solution according to the procedure suggested by *Weber* (1) : 0,354 g luminol was dissolved in 62,5 ml of 0,4 N NaOH and the volume was made up to 500 ml with distilled water. The solution cannot be used immediately but must be kept standing for at least a week, when it reaches the equilibrium, is stable for about a month and gives reproducible results.

The catalysts investigated were solutions of Fe^{3+} and Cu^{2+} salts and haemin. The Fe^{3+} salts are unsuitable because they emit light intensely after addition of luminol even in the absence of H_2O_2 . Addition of Cu^{2+} salts causes only a very short luminescence which cannot be reliably registered by means of our device having a relatively long response time. It has been known from earlier works (9, 10) that haemoglobin and haemin give a light of an enormously high intensity when luminol is oxidized in the presence of H_2O_2 ; the luminescence time is as long as 60 minutes and more, what depends on the reaction conditions.

The stock solution of haemin was prepared as follows: 0,05 g haemin was dissolved in 5 ml 0,1 N NaOH and the volume was made up to 100 ml with distilled water. By diluting samples of the stock solution in the ratio 1 : 10 0,005% haemin solutions were obtained which could be used as admixtures. The haemin solutions produced a strong catalytic effect upon the luminol luminescence; unfortunately the results were not fully reproducible. The catalytic effect might be caused by the aggregatory state of this dye in the solution which need not always be the same; thus the deviations can be explained. The effect of haemin can be stabilized and the results become reproducible, if a complex catalyst is used: this consist of haemin in a solution of cupric complex salt of triethanolamine. The solution of cupric complex salt was prepared by adding 1 ml triethanolamine $\text{N}(\text{C}_2\text{H}_4\text{OH})_3$ to 24 ml 0,1 N CuSO_4 . The resulting solution is intensely blue.

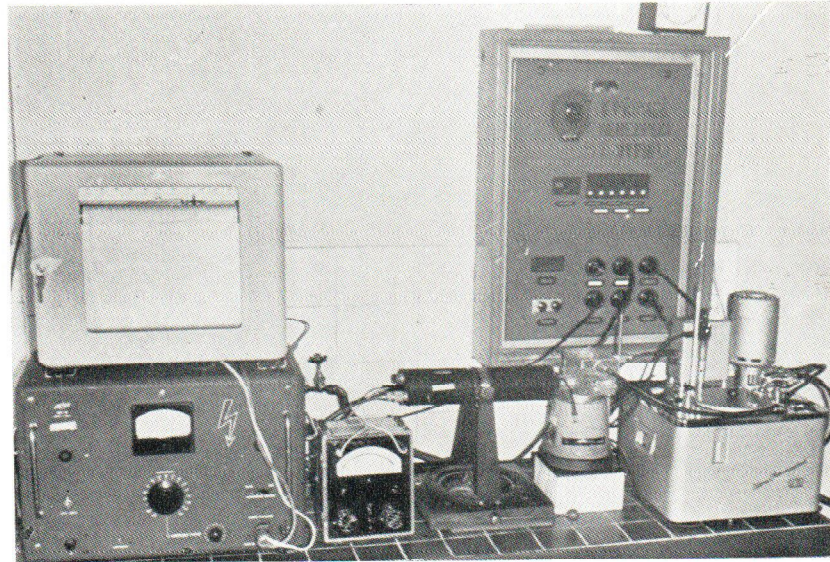


Fig. 2. *Luminophotometer*

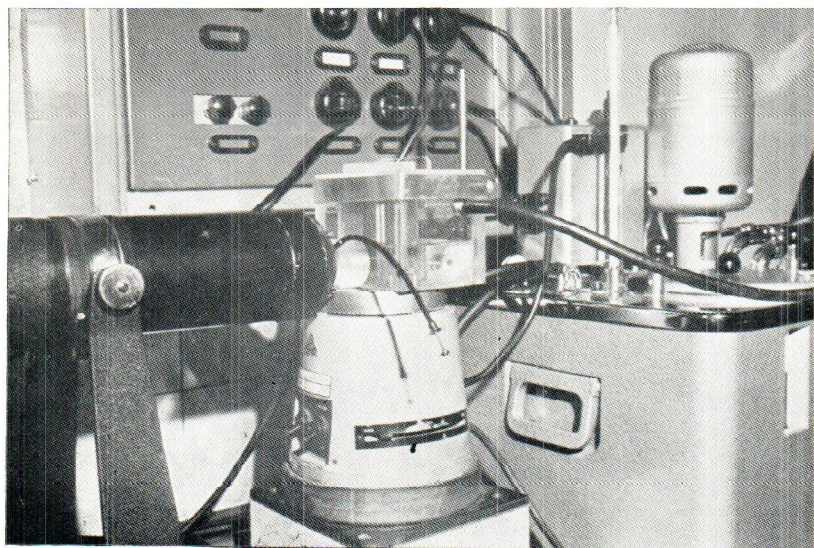


Fig. 3. *Photomultiplier with cooled reaction vessel*

Standard solutions of hydrogen peroxide were prepared by diluting perhydrol containing 30–33% H_2O_2 (Lachema, R. G., Czecho-Slovakia). The stability of very diluted solutions is of course poor; a solution diluted to a concentration of 10^{-4} M is still relatively stable. From such a solution desired concentrations could be prepared shortly before use.

The luminescence reaction was at first accomplished in small reaction volumes; to avoid the errors in dosing small volumes a reaction volume of 100 ml was used and the solutions were mixed in special cuvettes of Lange's colorimeter.

The stock solutions:

- A. 10 ml luminol solution, 60 ml water,
- B. 1 ml haemin solution (0,005%), 0,5 ml complex cupric solution, 8,5 ml water,
- C. 20 ml H_2O_2 solution.

The A and B solutions were mixed first; owing to the dissolved aerial oxygen, the resulting solution exhibits spontaneous luminescence. This

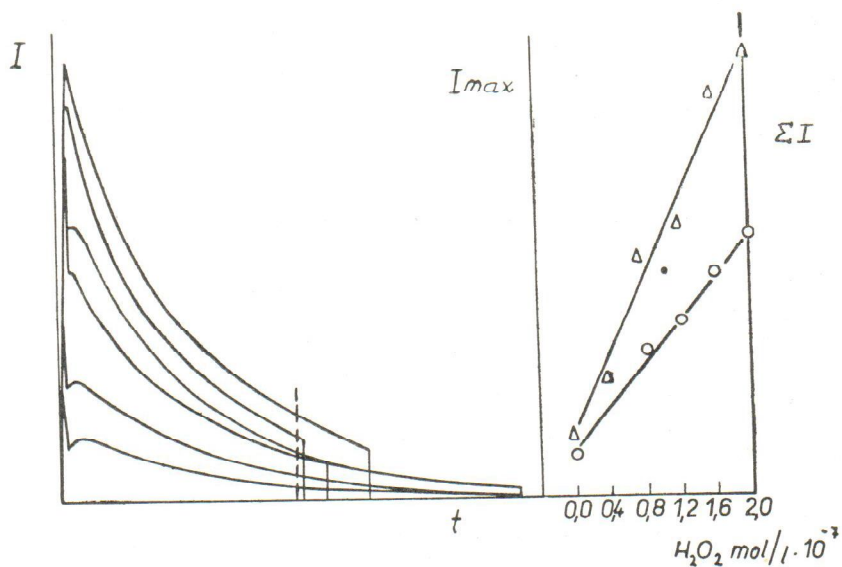


Fig. 4.

On the left side: dependence of I_{max} on t (kinetic curves)

On the right side: dependence of I_{max} on c (concentration) (Δ), dependence of I on c (\circ)

incidental luminescence disappears within half an hour and then the solution may be used for the measurement of light energy emitted after addition of solution C. The cuvette containing the solution (A + B) is

put into a thermostated vessel and, after thermal equilibration, solution C is quickly added. The mixing of the solutions is accomplished by a small magnetic stirrer.

The concentration of individual components in the reaction solution is as follows: 4×10^{-4} M luminol, 5×10^{-2} M NaOH, $2,4 \times 10^{-4}$ M Cu^{2+} and 50 $\mu\text{g}/100$ ml haemin.

In Fig. 4 the results of a series of luminescence measurements are shown for H_2O_2 concentration range from 2×10^{-8} — 2×10^{-6} M. The kinetic curves in the left part of the diagram represent continuous changes of the luminescence intensity I with time t . The curves have 2 maxima, the first of which is sharp, sloping down steeply especially in case of diluted H_2O_2 solutions; this may be caused by oxygen which is always present in the solutions. The second maximum is lower and corresponds to the emission of a product of the reaction between luminol and H_2O_2 .

In the right part of the diagram the dependence of I_{max} (the second maximum) on the H_2O_2 concentration (points Δ) and of the integral of the light emitted (ΣI) on the H_2O_2 concentration (points \circ) is plotted. The dependence is linear in the given concentration range; the linearity is good especially with the total luminescence intensity. The minimum determinable concentration of 2×10^{-8} M H_2O_2 corresponds to the determination sensitivity of 0,0007 $\mu\text{g}/\text{ml}$ (the error of the determination is 10%).

MECHANISM OF CHEMILUMINESCENCE

The mechanism of luminol oxidation is not quite precisely known. The oxidation agents considerably affect the composition of the reaction products and every scheme suggested is therefore questionable to a great extent. Various schemes have been suggested (14–18). In a new approach the luminescence has been explained by the mutual effect of free radicals, created from H_2O_2 , which play the main role. The reaction proceeds in 3 principal stages:

1. the ionisation of luminol
2. the oxidation of the luminol ion formed, yielding an anion of semiquinone
3. the reaction of the ionic radical of semiquinone with the HO_2 radical; this process gives rise to the luminescence.

In further text the following symbols will be used to avoid the structural formulae:

In alkaline solutions dissociation of luminol produces the univalent LH^- ion:



The luminol ion is oxidized by an oxidation agent (1) always present in the solution (these may be trace amounts of metallic ions, oxygen, free

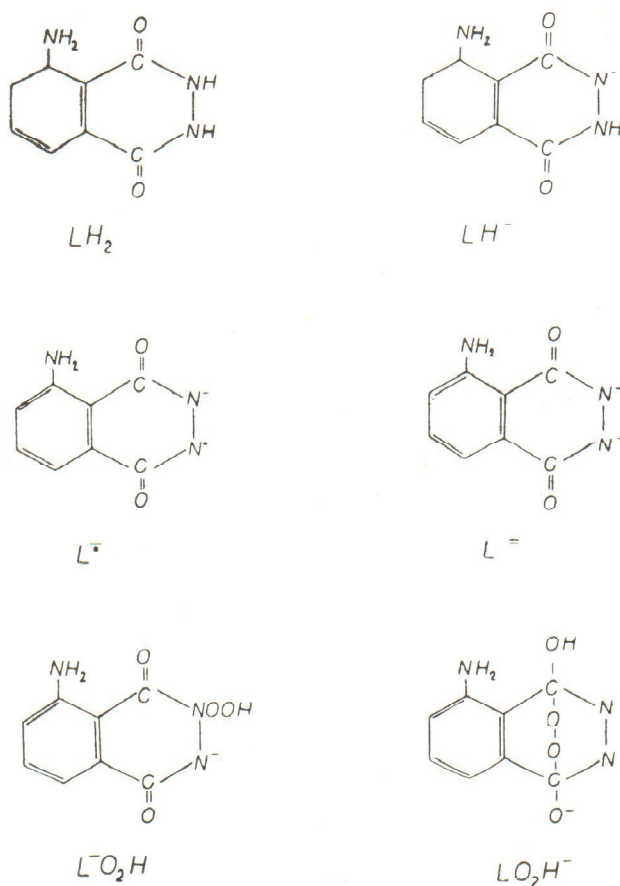
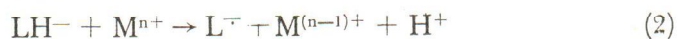


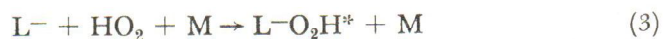
Fig. 5. Symbols used in equations

LH_2 = luminol, LH^- = mono- and divalent ions of luminol, L^\bullet = radical of semiquinone, $L-O_2H$ = reaction product of semiquinone with HO_2 , LO_2H^- = transformed structure of reaction product

radicals, generally denoted by M^{n+}) and as a result, an ionic radical of semiquinone structure L^\bullet is formed:



The ionic radical reacts with the present HO_2 radicals and this reaction leads to the emission of luminescence:





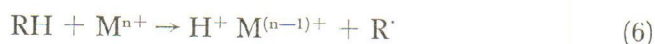
This principal scheme makes it possible to interpret all known properties of the chemiluminescence of phthalic-acid hydrazides. The occurrence of the so-called spontaneous luminescence i. e. the luminescence in the absence of H_2O_2 is the formation of the semiquinone ion promoted by the presence of metallic ions (Cu^{2+} , Fe^{3+}) according to equation (2). Simultaneously the oxidation of luminol by dissolved oxygen proceeds with the formation of the HO_2 radical



It is seen that both radicals necessary for luminescence can also appear in the absence of H_2O_2 .

On the other hand L^- radicals may be consumed by various non-luminescence processes such as autooxidation, dismutation and oxidation of the semiquinone ion; all these processes decrease the efficiency of reaction and consequently the emission of light.

Finally, the increase in the reaction efficiency by means of certain admixtures of a general formula RH can also be explained. To these compounds belong substances capable of autooxidation (benzaldehyde, glucose) which yield very reactive R^{\cdot} radicals in solutions:



These substances can therefore contribute to an increase in the concentration of HO_2 radicals and also to an increase in the number of semiquinone ions according to the reaction:



by which their activation effect on the spontaneous luminescence can be easily explained. Generally it is possible to say that the addition of oxidizing agents brings about an increase in the concentration of L^- according to equation (2). The effect of these agents on the luminescence is restricted by a sufficient concentration of HO_2 formed by several reactions (5). Some oxidizing agents such as NaOCl , KMnO_4 and others cause a decomposition of luminol converting it into L or into phthalic acid evolving N_2 . In the presence of H_2O_2 the concentration of HO_2 is high and the luminescence is therefore very intense. The OH radicals can also react with the luminol ions:



and thus increase the concentration of semiquinone radicals.

The addition of reducing agents intensifies the luminescence effect if the necessary free radicals occur as a result of reactions (8, 9, 10).

From the survey presented it is seen that the emission of light during the luminescence of luminol is caused by association of free radicals. One of the radicals is always the semiquinone radical, while the other is most frequently HO_2 or another radical which indirectly promotes the formation of HO_2 . Therefore the oxidation of luminol can serve as a good detecting method for the study of the formation and the reactions of radicals formed in water after irradiation.

In the water irradiated with gamma or X rays various free radicals appear from which some (especially HO_2) interact with the semiquinone radical producing chemical luminescence.

The oxidizing products formed by radiolysis of water can thus be well traced with the help of the reaction of luminol. In Fig. 6 a series of curves

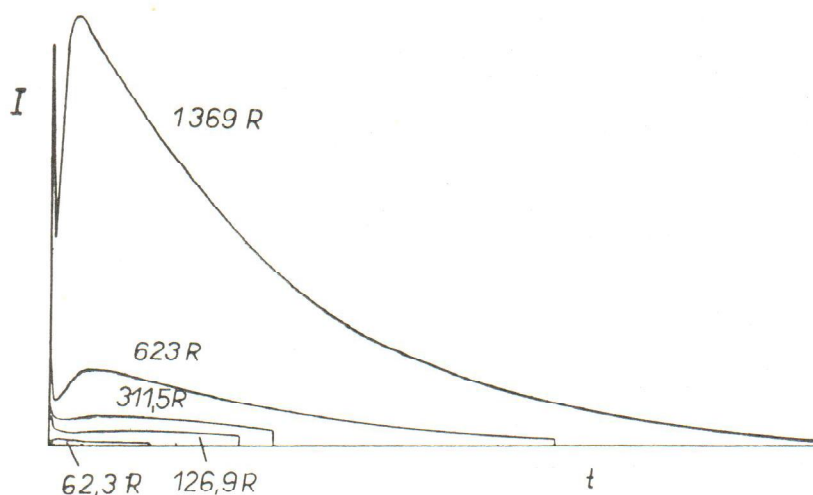


Fig. 6. Kinetic curves of chemiluminescence of irradiated water

is plotted showing the concentration differences between the products formed during the irradiation of water with gamma rays. A dose as low as 60 R can be easily read out (the source ^{60}Co) what is a relatively good result for a chemical dosimeter. In this case the errors of the measurements are greater - 20%. The experiments carried out so far have shown that the sensitivity as well as the accuracy of the method can be increased. When this is achieved, it will be possible to use the results of these determinations in dosimetry and radiation chemistry.

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Sadržaj

KEMILUMINESCENCIJA U DOZIMETRIJI

Izvršeni su temeljni pokusi za primjenu kemiluminescencije luminola (3-aminoftalhidrazida) u kemijskoj dozimetriji radioaktivnog zračenja. Princip metode se sastoji u tome da se čista voda obasjava određeno vrijeme zrakama nekog izvora radioaktivnog zračenja, pa se nakon toga luminolskom kemiluminescencijom kvantitativno odredi vodikov peroksid koji je nastao u toj vodi djelovanjem γ -zraka.

Reagens za kemiluminescenciju sadrži u lužnatoj otopini u određenoj koncentraciji luminol, vodikov peroksid i prikladan katalizator oksidacione reakcije. Kao katalizator u pokusu opisanom u ovoj radnji upotrijebljen je hemin u lužnatoj otopini uz dodatak otopine bakrenog sulfata u trietanolaminu. Tai dodatak stabilizira heminsku otopinu, koja uslijed toga djeluje katalitički uvijek u istoj mjeri. Koncentracije pojedinih komponenata u reakcionoj smjesi bile su uvijek ove: luminol 4×10^{-4} M, NaOH 5×10^{-2} M, bakreni (II)-ion 2.4×10^{-4} M i hemin 50 $\mu\text{g}/100$ ml. Ovoj reakcionoj smjesi dodana je određena količina obasjavane vode, pri čemu je ukupni volumen reakcione smjese bio uvijek 100 ml.

Konstruirana je fotoelektrična mjerna aparatura za registraciju intenziteta takvih reakcionih otopina ovisno o reakcionom vremenu. Slika 1 prikazuje shemu ove aparature. Kiveta s reakcionom otopinom (6) nalazi se u termostatu (8 i 10), a miješa se

elektromagnetski (7 i 9). Intenzitet kemiluminescencije mjeri se fotomultiplikatorom (3) preko pisaa (11), koji automatski daje krivulje kemiluminescencije (intenzitet kao funkciju vremena). Samu mjernu aparaturu prikazuju slike 2 i 3.

Slika 4 prikazuje niz krivulja kemiluminescencije za rastuće koncentracije vodikova peroksida (2×10^{-8} — 2×10^{-6} M). Krivulje prikazuju djelomično dva maksimuma, od kojih jedan očito pripada djelovanju elementarnog kisika u reakcionoj otopini, a drugi nastaje utjecajem vodikova peroksida. Na istoj slici prikazana je još i ovisnost maksimalnog intenziteta luminescencije (I), kao i ΣI (zbroj svjetala) o koncentraciji vodikova peroksida. Vidi se da postoji linearna ovisnost.

Rezultati mjerenja s vodom koja je obasjavana γ -zrakama prikazani su na slici 6. Vidi se da postoji pravilan odnos između upotrijebljene doze zračenja (R) i intenziteta, odnosno zbroja svjetala kemiluminescencije (I). Čak i doza od svega 60 R (izvor ^{60}Co) daje efekt kemiluminescencije koji ova aparatura registrira. To je dobar rezultat za kemijsku dozimetriju, koja se redovito upotrebljava za određivanje znatno većih doza.

Prikazana je ukratko teorija mehanizma kemiluminescencije luminola.

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