

A Quantitative Study of Chemiluminescence from the Iodine-Luminol Reaction

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Abstract: The reaction of iodine with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in basic aqueous solution generates chemiluminescence. The intensity of chemiluminescence is proportional to $[I_2]$, $[I_2]^2$, $[I_2]^3$, or a combination thereof, depending on the pH of the solution and the iodine concentration. If the reaction pH is properly adjusted, chemiluminescence is linearly proportional to iodine concentration from 5×10^{-10} to 3×10^{-7} M. The first-order process requires oxygen while the second- and third-order processes occur when no oxygen is present. The triiodide ion does not react with luminol to generate chemiluminescence. It is proposed that luminol reacts with OI^- to form an azaquinone intermediate. The azaquinone can react with O_2 , OI^- , and IO_2^- to generate the first-, second-, and third-order processes, respectively.

Several oxidants react with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in basic aqueous solution to generate chemiluminescence. For example, hydrogen peroxide,¹ hypochlorite,² permanganate,³ and iodine⁴ all cause light emission. The luminol-peroxide system has been extensively studied^{5,6} and is one of the most efficient chemiluminescent systems known. The other systems have not been investigated as thoroughly.

The iodine-luminol system was first studied by Babko, *et al.*⁴ They found it could be used to measure iodine concentration, achieving a detection limit of 10^{-5} M; a linear relationship between iodine and chemiluminescence intensity was observed up to 5×10^{-5} M iodine. Because iodine is a versatile reagent in chemical analysis, we chose to do a quantitative study of the iodine-luminol system, to define its analytical utility. Sensitive analytical methods for iodine are important to investigators in biomedical fields (protein-bound iodine), environmental science (SO_2 in air), and agriculture (arsenic).

The nature of the intermediate formed when luminol is oxidized by iodine is also of interest. Both the azaquinone formed by two-electron oxidation of luminol and the radical formed by one-electron oxidation of luminol have been proposed as intermediates in the aqueous oxidation of luminol.^{5,7-11} It is possible that either intermediate can occur depending on what oxidizing agent is reacted with luminol. Since iodine oxidizes by two electrons, the azaquinone intermediate seems most likely for the iodine-luminol reaction.

In the present paper we report a quantitative study of the iodine-luminol system with special emphasis on its

analytical utility. We have achieved a detection limit of 5×10^{-10} M iodine and a linear first-order response between 5×10^{-10} and 3×10^{-7} M iodine. We have found that the relationship between chemiluminescence intensity and iodine concentration can be expressed by

$$I_{CL} = b_1[I_2] + b_2[I_2]^2 + b_3[I_2]^3 \quad (1)$$

the coefficients varying with pH. The first-order process requires oxygen in addition to iodine while the second- and third-order processes are oxygen independent. The results are consistent with an azaquinone intermediate.

Experimental Section

Apparatus. The apparatus for chemiluminescence analysis has been described elsewhere.^{12,13}

Chemicals. Luminol from Eastman Organic Chemicals was converted to the sodium salt and was purified by recrystallization from basic aqueous solution.

The purified sodium luminol was dissolved in 0.1 M H_3BO_3 -KOH buffer to control the pH in the reaction cell. The H_3BO_3 concentration was maintained constant while the amount of KOH was varied to achieve the desired pH.

All reagents were prepared using a Continental Water Conditioning Co. deionization system.

A 2×10^{-3} M iodine solution was prepared by weighing out reagent grade iodine. Other standards were prepared by dilution.

Procedures. A 500-ml sample volume was used in this study. Such a large sample is convenient because the amount of sample consumed for one measurement (2.5 ml) is small relative to the total sample volume. Consecutive measurements on a sample can be made without having to correct for volume changes.

A glass sample bottle was used in this study. Plastic adsorbs and desorbs iodine readily. Dilute I_2 solutions in glass showed decreasing activity as a function of time. In one experiment, the peak from 2×10^{-7} M I_2 in 10^{-2} M, pH 7, phosphate buffer decreased by 10% after 30 min. This loss is believed to be due to causes other than adsorption because desorption of iodine from the glass surface was not observed when a concentrated iodine solution was replaced by a solution containing no iodine. Most experiments used short time durations, so that I_2 loss would be negligible. If this was not possible, a correction factor was included. The sample bottle was shaken manually to mix the contents because Teflon stirring bars also adsorb and desorb iodine readily.

Standard additions were made with 50 or 100 μ l Grunbaum pipets.

In some experiments the background solution (*ca.* 70 ml) was taken directly from the sample bottle, leaving *ca.* 430 ml in the sample bottle. This was done to ensure that the blank level of light

(1) H. O. Albrecht, *Z. Phys. Chem.*, **136**, 321 (1928).

(2) H. H. Seliger in "A Symposium on Light and Life," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1961, p 204.

(3) A. K. Babko and N. M. Lukovskaya, *Dopov. Akad. Nauk. Ukr. SSR*, 619 (1962).

(4) A. K. Babko, L. V. Markova, and N. M. Lukovskaya, *Zh. Anal. Khim.*, **23**, 401 (1968).

(5) E. H. White, *ref 2*, p 183.

(6) F. McCapra, *Quart. Rev., Chem. Soc.*, **20**, 485 (1966).

(7) K. D. Gundermann, "Chemilumineszenz Organischer Verbindungen," Springer-Verlag, New York, N. Y., 1968, pp 83-87.

(8) W. R. Seitz, *J. Phys. Chem.*, submitted for publication.

(9) M. M. Rauhut, A. M. Semsel, and B. G. Roberts, *J. Org. Chem.*, **31**, 2431 (1966).

(10) B. Epstein and T. Kuwana, *Photochem. Photobiol.*, **4**, 1157 (1965).

(11) P. B. Shevlin and H. A. Newfeld, *J. Org. Chem.*, **35**, 2178 (1970).

(12) W. R. Seitz, W. W. Suydam, and D. M. Hercules, *Anal. Chem.*, **44**, 957 (1972).

(13) W. R. Seitz and D. M. Hercules, *Anal. Chem.*, **44**, 2143 (1972).

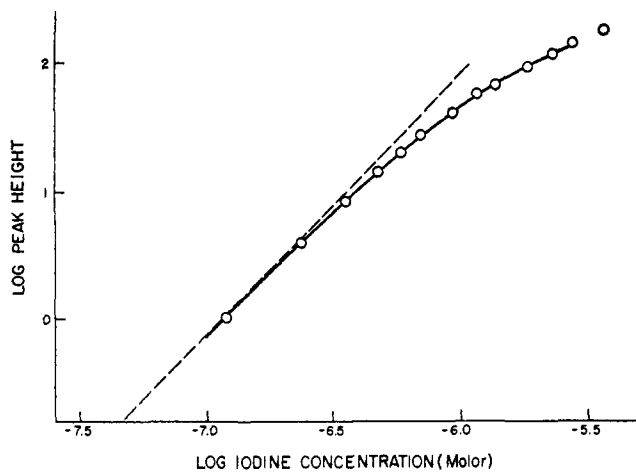


Figure 1. Log peak height vs. log iodine concentration at pH 10.02 (80 cc O_2 /min, 1.5×10^{-4} M luminol): (O) experimental data, (---) second-order response.

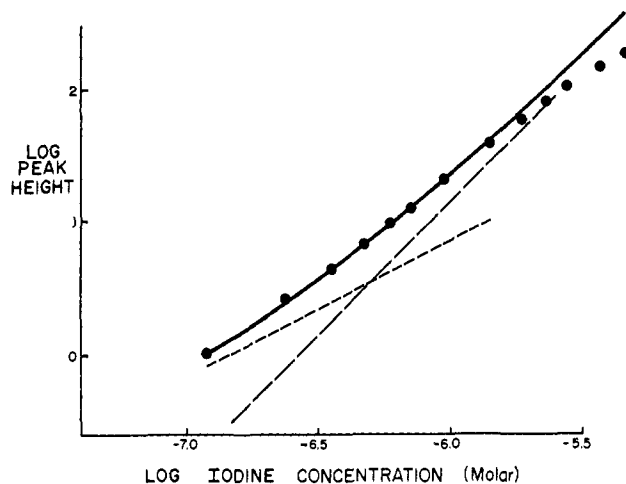


Figure 2. Log peak height vs. log iodine concentration at pH 10.60 (80 cc O_2 /min, 1.5×10^{-4} M luminol): (●) experimental data, (—) computer calculated least-squares fit, (---) first-order component of response, (-·-·) second-order component of response.

emission was the same as the reference level. The iodine concentrations were corrected for the reduction in sample volume.

Several different media, 10^{-2} M HCl, 10^{-3} M $H_2PO_4^-$, and 10^{-2} M phosphate buffer, were used in the sample bottle and reference syringe. The iodine-luminol reaction behaved the same in all sample-bottle media.

Reaction pH's were obtained by measuring the cell effluent.

Results

Chemiluminescence vs. Iodine Concentration. Figures 1-4 show the intensity of chemiluminescence as a function of iodine concentration at four different reaction pH's. These data were obtained by running calibration curves using the method of standard additions with overlapping ranges. Each calibration set included five data points and was run at least twice. For example, to obtain the data in Figure 1, duplicate calibrations were run over the ranges $1.2-6 \times 10^{-7}$ M I_2 , $2.4-12 \times 10^{-7}$ M I_2 , etc.

The peak height for 1.20×10^{-7} M iodine was arbitrarily assigned a value of 1.00. The peak height for 2.40×10^{-7} M iodine was calculated from its average ratio to the peak height for 1.20×10^{-7} M iodine in the calibrations over the $1.2-6 \times 10^{-7}$ M iodine range.

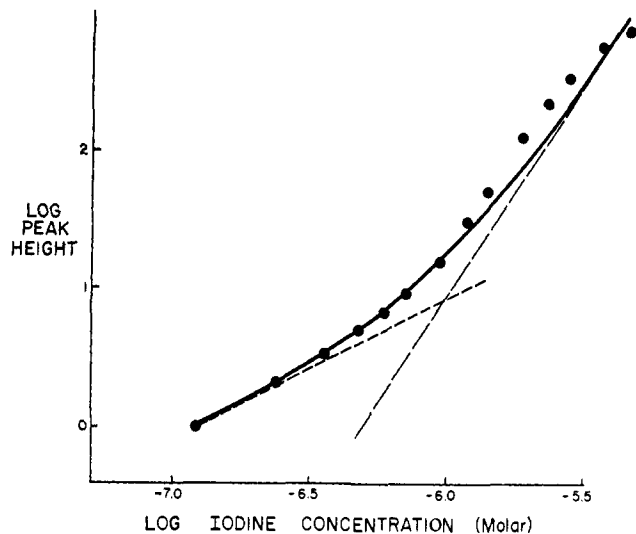


Figure 3. Log peak height vs. log iodine concentration at pH 11.60 (80 cc O_2 /min, 1.5×10^{-4} M luminol): (●) experimental data, (—) computer calculated least-squares fit, (---) first-order component of response, (-·-·) third-order component of response.

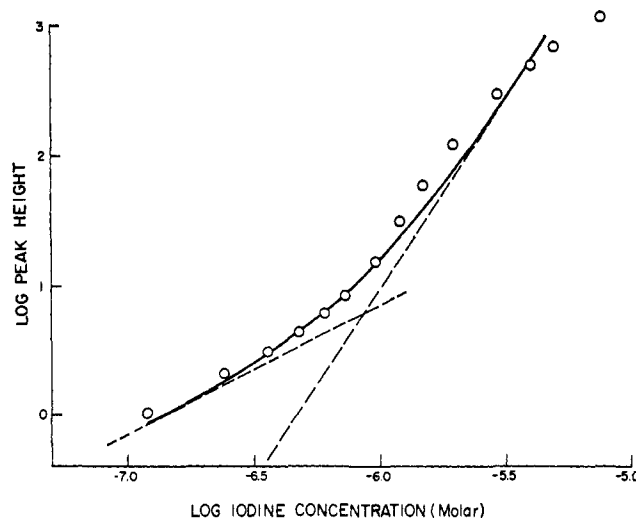


Figure 4. Log peak height vs. log iodine concentration at pH 12.20 (80 cc O_2 /min, 1.5×10^{-4} M luminol): (●) experimental data, (—) computer calculated least-squares fit, (---) first-order component of response, (-·-·) third-order component of response.

The peak height determined for 2.40×10^{-7} M iodine was then used as a standard for calculating the peak height for 3.60×10^{-7} M iodine by the same ratio method, and this procedure was continued up to the highest I_2 concentration. The above procedure was adopted to eliminate effects due to gradually changing sensitivity (e.g., deterioration of the luminol-buffer solution), to smooth out the calibration curve, and to normalize the calibrations over the entire range covered.

Figure 1 shows log peak height vs. log iodine concentration at a reaction cell pH of 10.02. The dashed line corresponds to perfect second-order response. It can be seen that below *ca.* 1.2×10^{-7} M I_2 the peak height shows a squared dependence on iodine concentration. As iodine concentration increases, the deviations from the squared response progressively increase. There is no indication of a first-order process occurring over this concentration range at pH 10.02. The detection limit at this pH is 5×10^{-8} M I_2 .

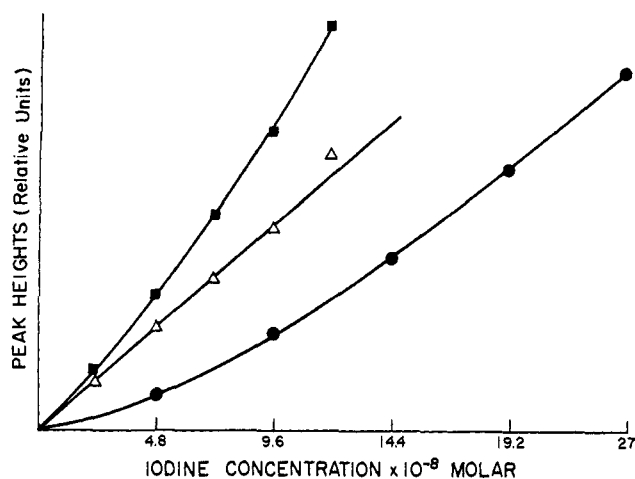


Figure 5. Effect of deoxygenating reagents on peak height vs. iodine concentration (pH 10.9, 1.5×10^{-4} M luminol): (●) oxygen present, (○) reagents deoxygenated, (Δ) difference between response in the presence and absence of oxygen.

Figures 2, 3, and 4 show peak height vs. iodine concentration at reaction cell pH's 10.60, 11.60, and 12.20, respectively. At these pH's the observed response is not purely first, second, or third order. Instead, the data at pH 10.60 can be resolved into first- and second-order components while the data at pH 11.60 and 12.20 can be resolved into first- and third-order components.

The least-squares fit for resolving these data into different order components was determined using a computer program for regression analysis.¹⁴ The data were fitted to the equation

$$I_{CL} = b_0 + b_1[I_2] + b_2[I_2]^2 + \dots + b_n[I_2]^n$$

where I_{CL} is the peak height, $[I_2]$ is the iodine concentration/ 1.2×10^{-7} M, and $b_0, b_1, b_2, \dots, b_n$ are the coefficients for the various order processes. The computer calculates the least-squares values of the "b" coefficients and the variance of the fit.

For the data at pH 10.60 the fit considering only first- and second-order processes was so good that no other terms were necessary. Figure 2 includes the peak heights calculated from the least-squares "b" values, along with the experimental peak heights. The separate contributions of the first- and second-order terms are also shown. The calculation was based on the seven lowest iodine concentrations. At higher iodine concentrations, response falls off with increasing iodine.

For cell pH's 11.60 and 12.20 a good fit was obtained when only first- and third-order terms were considered for the seven lowest iodine concentrations (1.2 – 6.0 and 9.6×10^{-7} M I_2). The peak heights calculated from the least squares fit are included in Figures 3 and 4. The separate contributions of the first- and third-order terms are also shown. Above 9.6×10^{-7} M I_2 , peak heights are greater than the calculated peak heights up to 48 and 38.8×10^{-7} M iodine at pH's 11.60 and 12.20, respectively. Other combinations of different order processes including zeroth-, second-, and fourth-order processes as well as first and third were considered in unsuccessful attempts to find a better fit covering a wider iodine concentration range. For all combinations of various order processes, the calculated fit varied significantly with the number of points considered.

(14) W. Nonidez, private communication.

When the variance of the fit was small, it corresponded to conditions unlikely to occur physically (e.g., high zeroth-order coefficients, negative coefficients, etc.). At higher concentration, response falls off with increasing iodine. At reaction cell pH's 10.60, 11.60, and 12.20, the data for concentrations below 1.2×10^{-7} M agree with peak heights calculated from the least-squares fit.

Effect of Flow Rate. One factor that could modify peak shape is loss of light in the exit tube of the cell if some of the chemiluminescence is occurring after the sample leaves the cell. If a different percentage of light is lost at different I_2 concentrations, then the peak height ratios for the different concentrations would be modified. To check if this was occurring calibration curves were obtained over the concentration range 4 – 20×10^{-7} M I_2 at cell pH 9.9 (second-order process) and 4 – 20×10^6 M I_2 at cell pH 11.8 (third-order process) at flow rates of 4.41, 2.25, and 1.15 ml/min/syringe. In both cases peak heights were proportional to flow rate and peak height ratios were independent of flow rate. These data show that under the conditions used all the iodine is reacted within the cell and no light is lost in the exit tube. For a more detailed discussion of the effect of varying flow rate see ref 12.

Conditions for Analysis. First-order response is most useful for analytical applications. The widest range of perfect first-order response is observed at higher pH's where first- and third-order response occur. This is because third-order response becomes significant only at high iodine concentrations. Response is first order down to the detection limit of 5×10^{-10} M I_2 . Greatest sensitivity for the first-order process was observed at cell pH ca. 11.5. Above that pH sensitivity decreased while at lower pH's the second-order process interfered. At pH's above 12.2 instead of first-order response, chemiluminescence per standard addition of iodine falls off with increasing iodine in the concentration range between 10^{-8} and 3×10^{-7} M. On occasion this effect was observed at cell pH's below 12.2.

Sensitivity is greater with a freshly prepared luminol solution. Sodium luminol dissolved in water lost most of its activity on standing for a few days, whereas sodium luminol in KOH– H_3BO_3 buffer at pH 12 was usable for several months. This indicates that sodium luminol decomposes into products that quench the iodine–luminol chemiluminescing reaction.

Effect of Oxygen. The three processes, first, second, and third order, were tested to see if they required oxygen. Figure 5 shows peak height as a function of iodine concentration in the presence and absence of oxygen at pH 10.9. At this pH, both first- and second-order processes occur. Exclusion of oxygen causes the peak height to be reduced by an amount linearly proportional to the concentration of iodine. This means that the first-order process requires oxygen while the second-order process does not. The failure of the calibration in the absence of oxygen to show exactly second-order response is attributed to residual oxygen still reaching the cell.

Exclusion of oxygen did not affect calibration curves over the range 4 – 20×10^{-7} M I_2 at pH 11.8 where third-order response predominates. This shows that the third-order process also does not require oxygen.

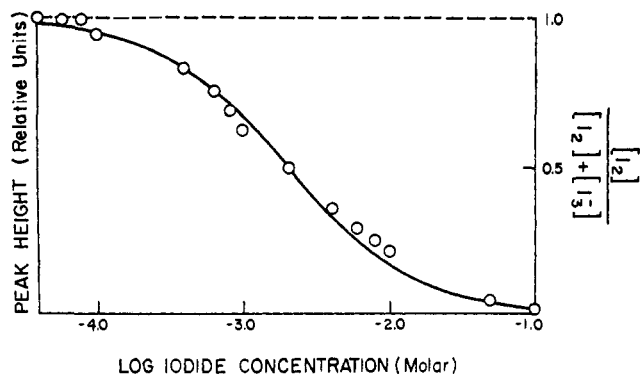


Figure 6. Effect of adding I^- on I_2 peak height: (O) experimental data, (—) calculated value for $[I_2]/([I_2] + [I_3^-])$ based on $K_f = 10^{2.7}$ for the triiodide ion, (---) peak height in the absence of added I^- (100% I_2).

Effect of Iodide. Standard iodine solutions are normally prepared in the presence of excess I^- to form the triiodide, I_3^- , ion. This increases the solubility of iodine and keeps the iodine from volatilizing. Triiodide, however, does not react with luminol to generate chemiluminescence. Figure 6 shows iodine peak height as a function of added I^- under conditions where first-order response predominates. The calculated percentage of iodine in the form of I_2 rather than triiodide as a function of iodide concentration exactly matches the observed decrease in chemiluminescence peak height. The calculated values were based on the equilibrium according to the value

$$\frac{[I_3^-]}{[I_2][I^-]} = 10^{2.7}$$

reported in Sillen and Martell.¹⁵ This experiment was run at cell pH's 10.5 and 11.85 to see if the concentration of I^- needed to quench chemiluminescence was a function of pH. A slight shift was observed. Approximately twice as much iodide was needed to reduce chemiluminescence by 50% at pH 11.85.

The second- and third-order processes are similarly quenched by added iodide.

Effect of Luminol Concentration. The effect of varying the luminol concentration on each of the three processes is shown in Figure 7. Each process has its own characteristic dependence.

Discussion

Table I lists the reported reactions of iodine in alkaline

Table I. Iodine Reactions in Base

- | | |
|-----|---|
| (1) | $I_2 + OH^- \rightarrow HOI + I^-$ |
| (2) | $HOI \leftrightarrow H^+ + OI^- \quad K_a = 10^{-11}$ |
| (3) | $3OI^- \rightarrow 2I^- + IO_3^-$ |
| (4) | (a) $2IO^- \rightarrow I_2O_2^{2-}$ |
| | (b) $2IO^- \rightarrow IO_2^- + I^-$ |
| (5) | $I_3^- + OH^- \rightarrow I_2OH^- + I^-$ |

solution. Iodine dissociates to hypoiodous acid and iodide (reactions 1 and 2). The ionization constant for hypoiodous acid is approximately 10^{-11} .¹⁰ Hypoiodite disproportionates to iodide and iodate (reaction

(15) L. G. Sillen and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. 17 (1964).

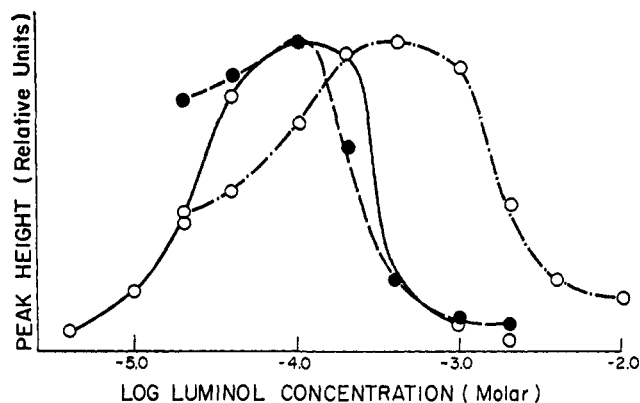


Figure 7. Peak height vs. luminol concentration for first (---), second (—), and third (-·-) order process. Conditions: first-order process, $2 \times 10^{-7} M I_2$, pH 11.4; second-order process, $4 \times 10^{-8} M I_2$, pH 9.8; third-order process, $8 \times 10^{-7} M I_2$, pH 12.7.

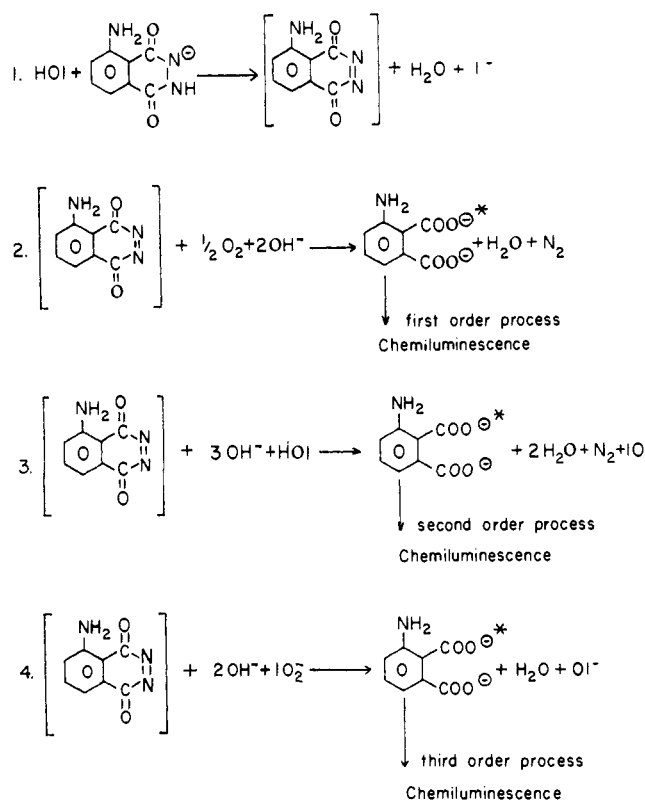


Figure 8. Proposed mechanism of the iodine-luminol reaction.

3).¹⁶ The rate is second order in hypoiodite, and the rate-determining step is believed to be either reaction 4a or 4b.^{17,18}

Figure 8 shows our proposed explanation of the experimental observations on the iodine-luminol system. The first step is the reaction of hypoiodous acid/hypoiodite with the luminol anion to form an azaquinone intermediate. This azaquinone can react with oxygen, HOI or IO_2^- ($I_2O_2^{2-}$), to generate chemiluminescence that is first, second, and third order in iodine concentration, respectively. A quantitative expression for steady state chemiluminescence as a function of hypo-

(16) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," Interscience, New York, N. Y., 1962, p 446.

(17) K. J. Morgan, *Quart. Rev., Chem. Soc.*, 8, 123 (1954).

(18) O. Haimovich and A. Treinin, *J. Phys. Chem.*, 71, 1941 (1967).

halogenite concentration has been derived for the reaction between hypochlorite and luminol.⁸ This reaction is similar to the iodine–luminol reaction except that no third-order process is observed since hypochlorite disproportionation is not significant at room temperature.¹⁶

This mechanism satisfactorily explains several features of the iodine–luminol system. The observation that the second- or third-order processes do not require oxygen is satisfactorily explained because the two oxygen atoms that are added to luminol as it reacts to the aminophthalate dianion can come from hypiodite and H₂O as shown in reactions 3 and 4 of Figure 8.

The positive deviation from calculated first- and third-order response in Figures 3 and 4 is consistent with reaction 4 of Figure 8 in which hypiodite is one of the products. This hypiodite can then react again leading to greater chemiluminescence intensity than if all three hypiodites required by reaction 4 were consumed.

The occurrence of the second-order process at pH's below 11 can be correlated with known behavior of iodine.¹⁹ Below pH 11, As(III) can be accurately titrated with iodine. The hypiodous acid formed by iodine dissociation reacts completely with As(III). Above pH 11, analytical errors occur because disproportionation becomes rapid enough to compete with the OI⁻–As^{III} reaction. Similar behavior is proposed here for the iodine–luminol reaction. Below pH 11, hypiodite reacts with the azaquinone intermediate faster than it reacts with itself. Above pH 11 it reacts with itself more rapidly than it reacts with the azaquinone.

The reaction of hypiodite and luminol to generate the azaquinone (reaction 1 of Figure 8) must be fast enough to compete with hypiodite disproportionation. The efficiency of the first-order process is about the same below and above pH 11.

The pH dividing the second- and third-order responses is the same pH at which hypiodous acid ionizes. The separation between the two processes is quite sharp. At pH's as low as 11.2 pure third-order re-

(19) R. K. McAlpine, *J. Chem. Educ.*, **26**, 362 (1949).

sponse was observed. A combination of second- and third-order processes was never observed. This can be explained if the rate of hypiodite disproportionation is proportional to [OI⁻]² while the rate of the second-order process, reaction 3 of Figure 8, is proportional to [HOI].

The fact that I₃⁻ does not react with luminol to generate chemiluminescence can be correlated with the formation of I₂OH⁻ in basic solution as shown in reaction 5 of Table I.²⁰ I₂OH⁻ presumably does not react with luminol to form the intermediate necessary for chemiluminescence.

There is no direct evidence that the azaquinone intermediate is formed. However, azaquinones have previously been proposed as intermediates in phthalhydrazide chemiluminescence. Azaquinones have been prepared that produce the same chemiluminescence upon oxidation as the corresponding phthalhydrazide.²¹

The radical formed by one-electron oxidation of luminol has also been proposed as an intermediate in luminol chemiluminescence.^{5,7,10,11} However, a one-electron oxidation involving hypiodite would require the formation of iodine atoms or some other thermodynamically unlikely iodine species. Also, reactions for the first, second, and third processes involving a radical intermediate similarly require unusual iodine species.

This study confirms that, at least with certain oxidizing agents, an azaquinone intermediate can occur when luminol is oxidized. Since with other oxidants the evidence favors one-electron oxidation of luminol to a radical, it appears that the luminol reaction can proceed by more than one path. This is important since changing hydrazide structure may affect the CL efficiency differently for different reaction pathways.

Acknowledgment. We wish to thank W. Hardy for some preliminary measurements. This work was supported by the National Institute of General Medical Sciences under Grant GM 17913.

(20) J. Sigalla, *Nature (London)*, **183**, 178 (1959).

(21) K. D. Gundermann in "Chemiluminescence and Bioluminescence," M. J. Cormier, D. M. Hercules, and J. Lee, Ed., Plenum Press, New York, N. Y., 1973, pp 209–231.