ISSN 0036-0244, Russian Journal of Physical Chemistry A, 2020, Vol. 94, No. 11, pp. 2369–2374. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2020, published in Zhurnal Fizicheskoi Khimii, 2020, Vol. 94, No. 11, pp. 1716–1722.

PHOTOCHEMISTRY AND MAGNETOCHEMISTRY

Chemiluminescence in the Auto-Oxidation of Luminol in Dimethyl Sulfoxide: Kinetic Effects of Alkalis, Quenching by Nitroblue Tetrazolium, and Elimination of Quenching by Hydrogen Peroxide

Yu. B. Tsaplev[a],* **and A. V. Trofimov**[a,b],**

[a]Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, 119334 Russia
[b]Moscow Institute of Physics and Technology, Dolgoprudnyi, Moscow oblast, 141701 Russia
*e-mail: tsap_04@mail.ru
**e-mail: avt_2003@mail.ru

Received February 28, 2020; revised February 28, 2020; accepted March 17, 2020

Abstract—Conditions and ways of inhibiting, quenching, and subsequently restoring the chemiluminescence of luminol in alkaline aerated dimethyl sulfoxide are determined. Data are obtained that testify to the key role of electron transfer from luminol dianions to oxygen in the auto-oxidation and chemiluminescence of luminol under these conditions.

Keywords: chemiluminescence, auto-oxidation, luminol, ion pairs, nitroblue tetrazolium, superoxide anion

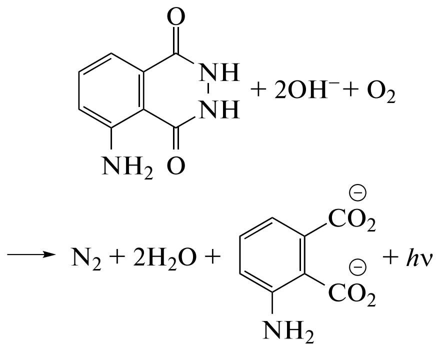
DOI: 10.1134/S0036024420110308

INTRODUCTION

Many chemiluminescent reactions in the liquid phase occur via auto-oxidation, and their initiation and occurrence are due to dissolved oxygen. However, the oxygen molecule (in the triplet state) interacts very slowly with the singlet state of a substrate (e.g., the bimolecular rate constant for the interaction between O_2 and luminol monoanions is 10^{-8} M^{-1} s^{-1} [1]).

When dimethyl sulfoxide (DMSO) was first used in organic chemistry, it was found that auto-oxidation processes proceed in alkaline DMSO much more rapidly than in water [2]. Alkaline DMSO was used as a test oxidation system in the search for chemiluminescence of many organic compounds (e.g., indoles [3]). However, the reason for fast auto-oxidation (and chemiluminescence) remains unknown to date.

The glow of luminol in alkaline DMSO was discovered by White [4]. This reaction, which we refer to below as the White reaction, was additionally studied by four research teams [5–12]. White et al. [5] noted that chemiluminescence was observed only in luminol solutions that contained just over one mole of base per mole of luminol. The oxidation of one mole of luminol consumes one mole of oxygen and two moles of alkali, and the products of the reaction are nitrogen, water, and 3-aminophthalate. It was shown that two oxygen atoms in the aminophthalate molecule are taken from the dissolved oxygen. The White reaction is written formally as



Independent variation of each of the initial concentrations of luminol, alkali, and oxygen showed that the reaction was of the first order in each of the reactants [5]. This was not confirmed (in either [5] or subsequent works) by primary kinetic data, but a kinetic scheme of reactions was proposed (Scheme 1), according to which the intensity of chemiluminescence in the quasi-stationary assumption is proportional to the concentrations of luminol dianion, alkali, and oxygen when $k_{-1}[H_2O] \gg k_2[O_2]$:

Scheme 1.

$$LH^- + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} L^{2-} + H_2O,$$

$$L^{2-} + O_2 \xrightarrow{k_2} AP^{2-} + N_2 + h\nu,$$

where LH^-, L^{2-}, and AP^{2-} are singly deprotonated luminol, doubly deprotonated luminol, and 3-aminophthalic acid, respectively.

Intensity *i* of chemiluminescence is related to reaction rate w as

$$i \sim w = \frac{k_1k_2}{k_{-1}[H_2O]}[LH^-][OH^-][O_2].$$

White and Burcey [6] assumed that the emitter of the reaction was 3-aminophthalate producing light from the electron-excited singlet state. Lee and Seliger [7–9] determined the quantum yield and recorded the chemiluminescence spectra for several reactions. These included the White reaction, for which the quantum yield was 0.012 E/mol. It was established that the emitter is 3-aminophthalate.

Lee and Seliger [9] also characterized the dependence of luminol chemiluminescence quantum yield Φ_{cl} on the concentration of water in alkaline DMSO. This proved that Φ_{cl} grows as the concentration of water decreases, and it reaches a maximum at a concentration of 0.63 mol/L, below which water does not affect Φ_{cl}. However, it does affect the kinetics of chemiluminescence; e.g., as the concentration of water rises from 0 to 0.14 mol/L, the maximum intensity of chemiluminescence falls by a factor of 3 [10, 11].

Beck and Joó [12] studied the effect the concentration of oxygen has on the intensity of chemiluminescence in DMSO–EtOH and showed that instead of a linear dependence (which would be expected from Scheme 1), the dependence plateaus. Beck and Joó assumed that the stage of interaction between the luminol dianions and oxygen is the equilibrium formation of the adduct (L^{2-} O_2):

$$L^{2-} + O_2 \leftrightarrow (L^{2-}O_2) \rightarrow AP^{2-} + N_2 + h\nu.$$

Neither inhibitors nor quenchers of chemiluminescence were found for the White reaction. It is known only that the quenchers of triplets (including oxygen, diphenyl ketone, acetophenone, diacetyl, pyrene, anthracene, and iodide) and free radical scavengers do not affect chemiluminescence at concentrations of up to 1 mM [9]. The White reaction is the simplest and most easily obtained chemiluminescent reaction, but it has not found practical applications yet, likely due to the lack of known ways to influence it. The aim of this work was to find such ways.

EXPERIMENTAL

The following reagents were used in this work: dimethyl sulfoxide (spectroscopy grade); LiOH, NaOH, KOH, luminol, and hydrogen peroxide (30%) (all of chemically pure grade); and nitroblue tetrazolium chloride (analytically pure grade).

The kinetics of chemiluminescence was investigated with a two-channel chemiluminometer comprising two photon-counting heads, H7360-1 and H7360-2 (Hamamatsu, Japan); a CNT-202 counter (Spetspribor, Belarus); and a computer. The chemiluminometer detected light in two spectral ranges, 430–490 and 480–560 nm. The design of the chemiluminometer was described in [13].

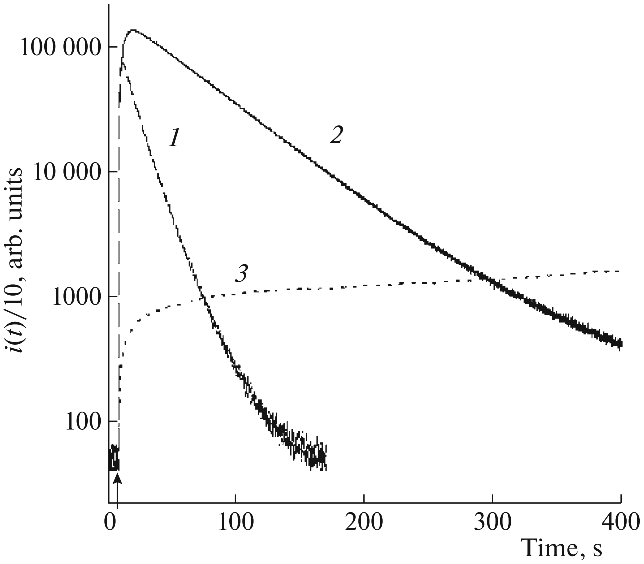


Fig. 1. Kinetics of luminol chemiluminescence in alkaline DMSO in the presence of (*1*) 4.6 mM KOH, (*2*) 4.6 mM NaOH, or (*3*) 7.7 mM LiOH and (*1*) 0.77, (*2*) 7.7, or (*3*) 77 nM luminol.

The reaction vessels were 2-mL polypropylene microtubes in which solutions of the reagents were mixed. We typically added 50 μL of a DMSO solution of luminol or luminol with nitroblue tetrazolium chloride using a light-isolated injector to 600 μL of DMSO supplemented with an alkali (LiOH, NaOH, or KOH) to a concentration of 1–5 mM and (if required by the experiment) hydrogen peroxide. The reaction mixture in a microtube had small area of contact with air and a relatively large volume, which complicated neutralization of the alkali by atmospheric carbon dioxide and preserved the stability of the alkali concentration for 10–20 min.

In recording the light yield in the reaction of luminol with the LiOH solution, the reagents were mixed outside the cuvette compartment of the chemiluminometer, after which the reaction vessel was closed with a stopper and placed in the cuvette compartment.

The electronic absorption spectra were recorded with a Lambda 25 spectrophotometer (Perkin-Elmer, United States). The solutions in alkaline DMSO were handled in a quartz cuvette with an optical path length of 1 cm; the cuvette was closed tightly with a ground stopper.

The concentrations of the reagent are indicated below as the initial concentrations in the reaction mixture.

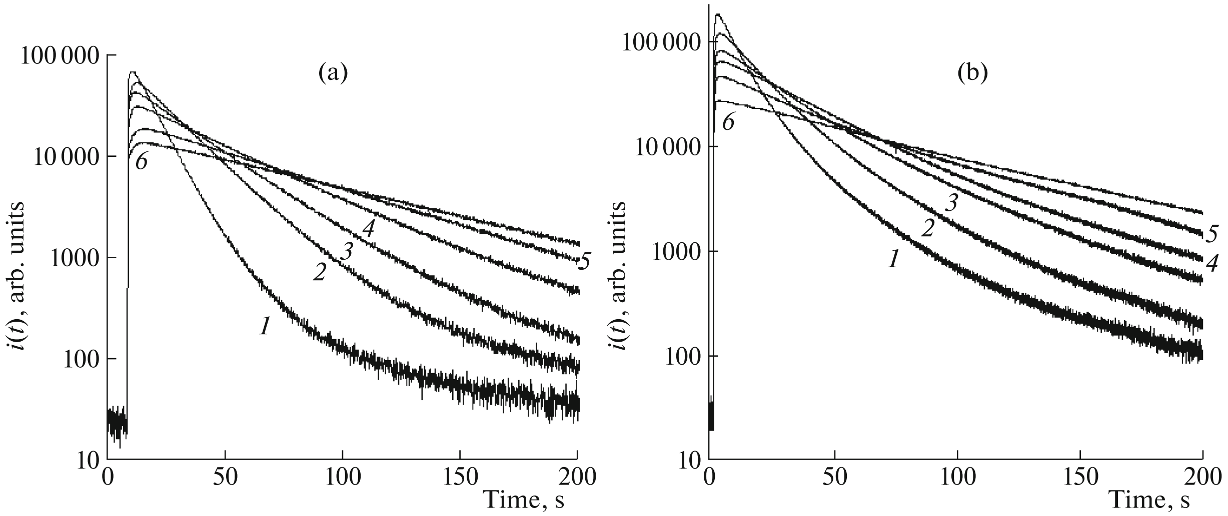


Fig. 2. Inhibition of luminol chemiluminescence (a) in the presence of KOH with additions of KI and (b) in the presence of NaOH with additions of NaI. The initial luminol concentration was 0.77 nM in the KOH solution and 7.7 nM in the NaOH solution. The concentration of alkali was 3 mM. The concentrations of KI were (*1*) 0, (*2*) 1.5, (*3*) 3, (*4*) 6, (*5*) 12, and (*6*) 24 mM. The concentrations of NaI were (*1*) 0, (*2*) 0.38, (*3*) 0.77, (*4*) 1.5, (*5*) 3.1, and (*6*) 6.2 mM. The concentration of H_2O in DMSO was 0.17 M.

RESULTS AND DISCUSSION

Alkaline DMSO and Luminol

In water, anions with localized charge (HO^-, t-BuO^-) form strong hydrogen bonds. In bipolar aprotic solvents, they are not stabilized by hydrogen bonds and readily participate in reactions of nucleophilic substitution, addition, and deprotonation [14]. The deprotonation of luminol is an important step in the considered luminescent reaction. The first ionization constant of luminol is such that $pK_{a1} = 6.7$, and luminol participates as a monoanion in most chemiluminescent reactions in aqueous solutions. The second ionization constant of luminol is such that $pK_{a2} = 15.1$ (at an ionic strength of 5 [15]), and the transition of luminol to the doubly deprotonated form requires a superbasic medium (e.g., alkaline DMSO).

The chemical activity of alkalis in DMSO depends on the hydration with water occurring in the solution, and on the association into ion pairs caused by the alkali metal cations. The constants of association of HO^- anions and ions of K^+, Na^+, and Li^+ in DMSO are 1400, 50000, and 790000 M^{-1}, respectively [14], because of which the effect of the formation of ion pairs is apparent even in diluted solutions.

Let us compare the efficiency of the excitation of the luminescence of luminol in DMSO, depending on the type of alkali. Figure 1 presents the semi-log kinetic curves of the glow after adding a portion of the luminol solution to DMSO containing KOH, NaOH, or LiOH. We can see that the reaction is fastest in the presence of KOH, is noticeably slower in the presence of NaOH, and is very slow in the presence of LiOH. In the presence of KOH, almost all of the light is emitted within 100 s; in the presence of NaOH, it is emitted within 500 s. More than 24 h were needed to record the light yield in the presence of LiOH. At the same time, the yield of luminol chemiluminescence quanta in the presence of LiOH was ~40% of those in the presence of KOH or NaOH, for which the yields were equal. This suggests there were no differences in the efficiency of exciting the luminescence of luminol in DMSO in the presence of KOH or NaOH, and in the presence of LiOH, they were not as great as might be expected. However, there were clear differences in the light yield kinetics even between reactions in the KOH and NaOH solutions. These differences could be due to differences between the constants of association between the HO^- anions and the cations.

The formation of an ion pair from the HO^- anion and the M^+ cation is described by the equilibrium $HO^- + M^+ \leftrightarrow HO^-M^+$. Concentration $[HO^-]_f$ of the free hydroxide ions[1] is easy to find if constant of association K and total concentration of hydroxide B in the solution are known:

$$[\mathrm{HO^-}]_f = 0.5[-K^{-1} + (K^{-2} + 4BK^{-1})^{0.5}]. \qquad (1)$$

Concentration $[HO^-]_f$ changes not only when the hydroxide concentration is changed, but when the salt MX is added as well. Concentration $[HO^-]_f$ in a solution containing MOH and salt MX at concentration S is given by the formula

$$[\mathrm{HO^-}]_f = 0.5\{-(K^{-1} + S) + [(K^{-1} + S)^2 + 4BK^{-1}]^{0.5}\}. \qquad (2)$$

---

[1] Since the constant of hydration of the hydroxide ion exceeds 1600 M^{-1}, a water molecule also accompanies the free (not participating in an ion pair) hydroxide ion.

According to formula (1), concentrations $[HO^-]_f$ at $B = 4.6$ mM in the KOH and NaOH solutions were 1.5 and 0.3 mM, respectively. So, the rate of luminol deprotonation in KOH is five times higher than in NaOH.

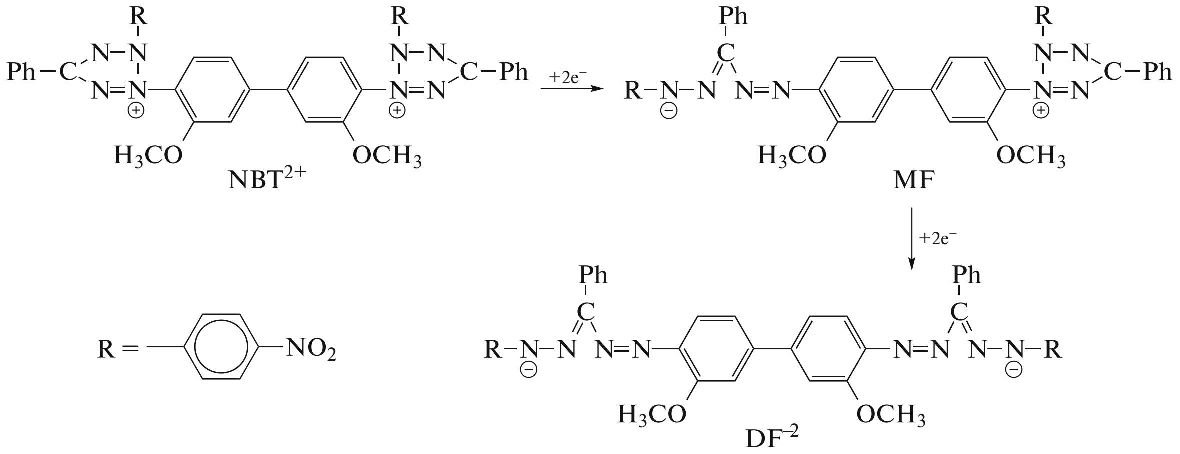
Figure 2 illustrates the effect additions of NaI and KI to the KOH and NaOH solutions have on the kinetics of chemiluminescence. It is seen that increases in the concentration of NaI in the NaOH solution and the concentration of KI in the KOH solution are accompanied by a deceleration of the reaction and a reduction in the maximum intensity of chemiluminescence. However, NaI and KI are not quenchers; they are inhibitors, since the intensity of emission in the reaction remains virtually constant. The kinetic curves show that the intensity of chemiluminescence after passing through the maximum falls exponentially. The measured parameter was decay constant k_d of kinetic curve.

Varying the concentration of KI in KOH and the concentration of NaI in NaOH leads to a symbate change in k_d and concentration $[HO^-]_f$ calculated according to formula (2). In Fig. 3, points *1* obtained by varying the concentration of NaI in NaOH and points *2* obtained by varying the concentration of KI in KOH lie on the same line, so k_d is proportional to concentration $[HO^-]_f$. This is formally consistent with Scheme 1. The k_d value is independent of the type of alkali, but it is determined by $[HO^-]_f$. It is easy to see from formula (1) that the first order of the reaction with respect to alkali, which was identified by White et al. [5], is possible only at $4BK \ll 1$.

The above description concerns the effect of salts containing the same cation as the alkali. However, adding lithium salts to alkaline DMSO containing, e.g., KOH slows the reaction and lowers the maximum intensity of chemiluminescence. This effect can be used for determining lithium in nonaqueous solutions.

Nitroblue Tetrazolium and Luminol

Nitroblue tetrazolium (NBT^{2+}) is a well-known chromogenic reagent for the superoxide anion [16, 17]. The structural formulas of NBT and the stable products of its reduction (monoformazan (MF) and diformazan (DF^{2-})) are shown below:



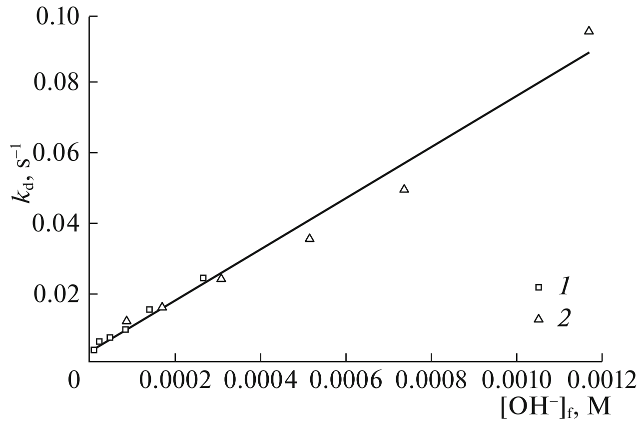


Fig. 3. Dependence of k_d on the concentration of free hydroxide ions, as determined by varying (*1*) the concentration of NaI in NaOH and (*2*) the concentration of KI in KOH.

Li et al. [18] proposed a procedure for the spectrophotometric determination of superoxide using an NBT solution in DMSO. NBT is converted to MF at a concentrations of superoxide lower than or equal to the doubled concentration of NBT. The maximum of the absorption of MF in DMSO is at ~680 nm. At a concentration of superoxide exceeding a concentration of NBT four times greater, all of the NBT is converted to DF^{2-}, the maximum of whose absorption is ~730 nm.

It is known, however, that nitroblue tetrazolium in DMSO in the presence of NaOH or KOH is converted to monoformazan, and not diformazan [19, 20]. Figure 4 shows the spectra of NBT in alkaline DMSO without and with luminol at different concentrations. We can see the evolution of the spectra from the one characteristic of MF with no luminol to the one char-

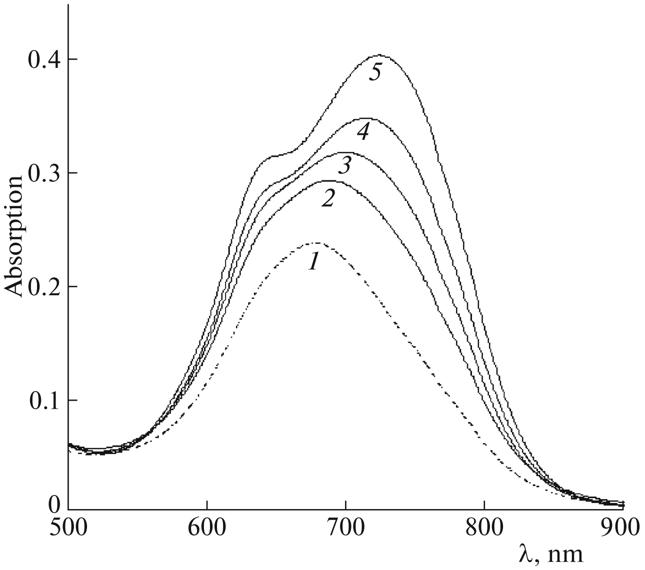


Fig. 4. Spectra of NBT in alkaline DMSO (*1*) without and (*2–4*) with luminol. The NBT concentration was 4 μM. The luminol : NBT ratios of concentrations were (*2*) 0.5, (*3*) 1, (*4*) 2, and (*5*) 4. The concentration of NaOH was 1 mM.

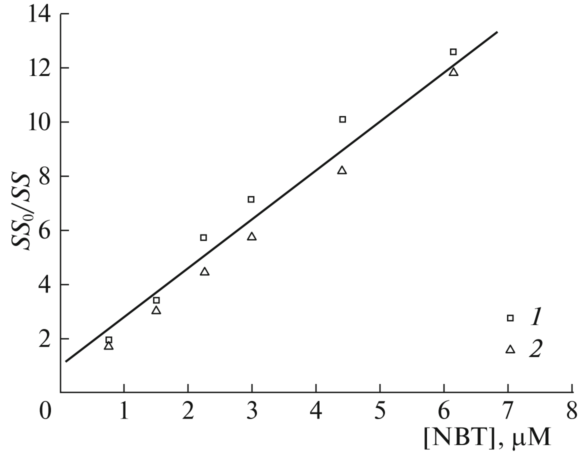


Fig. 5. Dependence of the quenching of luminol chemiluminescence in alkaline DMSO on the concentration of NBT. SS_0/SS is the ratio of the light yields in the reaction without and with NBT. The initial luminol concentrations were (*1*) 7.7 and (*2*) 31 nM. The concentration of NaOH in DMSO was 3 mM.

acteristic of DF as the ratio of the luminol : NBT concentrations grows. The formation of diformazan in the presence of luminol suggests that in the luminol dianion–oxygen adduct (L^{2-} O_2) postulated by Beck and Joó, electrons are transferred to oxygen to form superoxide anions and luminol radical anions $L^{-\bullet}$.

The interaction between $L^{-\bullet}$ and $O_2^{-\bullet}$ was studied in [1, 21]; it is characterized by a bimolecular rate constant of 2.3 × 10^8 M^{-1} s^{-1} and produces peroxide (LO_2H^-, LO_2^{2-}), which plays a key role in luminol's chemiluminescence. We may assume that the removal of superoxide anions by NBT should quench the chemiluminescence in the White reaction, and this is what happems. Figure 5 shows the dependence of the yield of light in the reaction on the NBT concentration in the Stern–Volmer coordinates. It is seen that NBT is an efficient quencher that has a notable effect even at concentrations of less than 1 μM, and this is the only (currently known) quencher in the White reaction that acts at such a concentration.

When superoxide is removed from the reaction (in this case, by NBT), the path of luminol consumption changes. The luminol radical anions rapidly disproportionate into diazaquinone L and L^{2-} [21], and the diazaquinone is then consumed in the reaction with hydroxide ions [21]. Diazaquinone can be returned to the light path of conversions using hydrogen peroxide, the reaction with which gives the peroxide LO_2H^-, LO_2^{2-} again. These reactions are presented below with the rate constants [21]:

$$2L^{-\bullet} \rightarrow L + L^{2-} \quad (5\times10^8\ M^{-1}\ s^{-1}),$$

$$L + HO^- \rightarrow \text{products} \quad (4\times10^6\ M^{-1}\ s^{-1}),$$

$$L + (HO_2^-, O_2^{2-}) \rightarrow LO_2H^-,\ LO_2^{2-} \quad (5\times10^7\ M^{-1}\ s^{-1}).$$

It thus follows that the return of the half of the formed diazaquinone to the light path at HO^- concentrations of ~3 mM requires hydrogen peroxide at a concentration of 0.24 mM. Figure 6 presents data on the restoration of the light yield in the reaction with NBT using hydrogen peroxide. We can see that at a sufficiently high concentration of hydrogen peroxide, the quenching caused by NBT is completely elimanated. The light yield is restored to 50% at a concentration of hydrogen peroxide of ~0.22 mM, which is close to the calculated estimate.

Scheme of Reactions

The scheme of reactions that includes Beck and Joó's refinement and evidence of the formation of superoxide and luminol radical anions that was obtained in this work can be written as

$$LH^- + HO_f^- \leftrightarrow L^{2-} + H_2O, \tag{3}$$

$$L^{2-} + O_2 \leftrightarrow (L^{2-}O_2), \tag{4}$$

$$(L^{2-}O_2) \leftrightarrow (L^{-\bullet}O_2^{-\bullet}), \tag{5}$$

$$(L^{-\bullet}O_2^{-\bullet}) \rightarrow LO_2^{2-}, \tag{6}$$

$$LO_2^{2-} \rightarrow AP^{2-} + N_2 + h\nu, \tag{7}$$

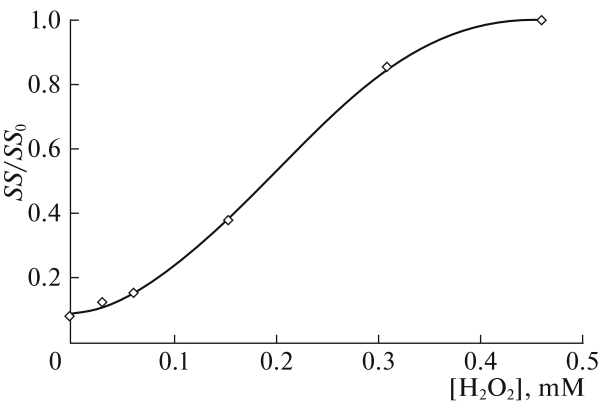


Fig. 6. Restoration of chemiluminescence in the presence of NBT by hydrogen peroxide. SS_0 is the light yield without NBT or hydrogen peroxide. The concentration of NBT was 6 μM, the concentration of luminol was 7.7 nM, and the concentration NaOH was 3 mM.

where HO_f^- is a hydroxide anion that does not participate in an ion pair. The product of reaction (5) is in parentheses because we assume that it (along with $(L^{2-}O_2)$) is an adduct. In DMSO, the separation of the charge in adduct $(L^{2-}O_2)$ to form $(L^{-\bullet}O_2^{-\bullet})$ is an energetically advantageous process. Once the energy advantage disappears when DMSO contains a sufficiently high concentration of a solvent that forms strong hydrogen bonds with anions (water, alcohol), there is no charge separation in reaction (5), and there is no chemiluminescence along path (3)–(7).

CONCLUSIONS

(1) The strong-base hydroxide anion is responsible for the deprotonation of luminol in alkaline DMSO. Alkali metal cations form ion pairs with hydroxide anions and act as inhibitors of chemiluminescence in the White reaction. The kinetics of the chemiluminescence in the reaction is determined by the concentration of the hydroxide ions, which do not participate in ion pairs.

(2) The interaction between luminol dianions and dissolved oxygen produces an adduct in which electrons are transferred to oxygen to form luminol radical anions and superoxide radical anions. The superoxide radical anions were detected through the reaction with nitroblue tetrazolium. The chemiluminescence in the White reaction was efficiently quenched by nitroblue tetrazolium.

(3) The quenching of chemiluminescence by nitroblue tetrazolium is relieved by hydrogen peroxide, confirming that the luminol radical anions form because they disproportionate into diazaquinione, which chemiluminesces in the reaction with hydrogen peroxide.

FUNDING

This work was supported by the Russian Foundation for Basic Research, project no. 19-53-18019_Bolg_a.

REFERENCES

1. G. Merényi, J. Lind, X. Shen, and T. E. Eriksen, J. Phys. Chem. **94**, 748 (1990).
2. A. Parker, in *Advances in Organic Chemistry: Methods and Results,* Ed. by R. A. Raphael, E. C. Taylor, and H. Wynberg (Inerscience, New York, London, Sydney, 1965), Vol. 5.
3. R. F. Vasil'ev, A. V. Trofimov, and Yu. B. Tsaplev, Russ. Chem. Rev. **79**, 77 (2010). https://doi.org/10.1070/RC2010v079n02ABEH004064
4. E. H. White, J. Chem. Educ. **34**, 275 (1957).
5. E. H. White, O. Zafiriou, H. Kagi, and J. Hill, J. Am. Chem. Soc. **86**, 940 (1964).
6. E. H. White and M. M. Burcey, J. Am. Chem. Soc. **86**, 941 (1964).
7. J. Lee and H. H. Seliger, Photochem. Photobiol. **4**, 1015 (1965).
8. J. Lee and H. H. Seliger, Photochem. Photobiol. **11**, 247 (1970).
9. J. Lee and H. H. Seliger, Photochem. Photobiol. **15**, 227 (1972).
10. J. D. Gorsuch, Ph. D. Thesis (MIT Press, Boston, MA, 1969). https://dspace.mit.edu/handle/1721.1/52931.
11. J. D. Gorsuch and D. M. Hercules, Photochem. Photobiol. **15**, 567 (1972).
12. M. T. Beck and F. Joó, Photochem. Photobiol. **16**, 491 (1972).
13. Yu. B. Tsaplev, R. F. Vasil'ev, and A. V. Trofimov, Russ. J. Phys. Chem. B **14**, 431 (2020).
14. B. A. Trofimov, A. M. Vasil'tsov, and S. V. Amosova, Russ. Chem. Bull. **35**, 682 (1986).
15. K. E. Haapakka, J. J. Kankare, and J. A. Linke, Anal. Chim. Acta **139**, 379 (1982).
16. C. Auclair and E. Voisin, *Handbook of Methods for Oxygen Radical Research,* Ed. by R. F. Greenwald (CRC, Boca Raton, FL, 1985), p. 123.
17. M. Hayyan, M. A. Hashim, and I. M. AlNashef, Chem. Rev. **116**, 3029 (2016).
18. R. H. Liu, S. Y. Fu, H. Y. Zhan, and L. A. Lucia, Ind. Eng. Chem. Res. **48**, 9331 (2009).
19. K. Umemoto and N. Okamura, Bull. Chem. Soc. Jpn. **59**, 3047 (1986).
20. K. Umemoto, Y. Nagase, and Y. Sasaki, Bull. Chem. Soc. Jpn. **67**, 3245 (1994).
21. G. Merényi, J. Lind, and T. E. Eriksen, J. Biolumin. Chemilumin. **5**, 53 (1990).

Translated by V. Glyanchenko