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# Luminol-Based Nitrogen Dioxide Detector

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**An instrument for the continuous detection of NO<sub>2</sub> in the sub-part-per-billion range is described. The instrument is based upon the chemiluminescent reaction between NO<sub>2</sub> in air and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) in alkaline solution. The present detector exhibits a 2-Hz response speed to changes of  $\pm 20$  ppb and a field detection limit of 30 ppt (parts per trillion). The instrumental technique has been expanded to measure NO by the catalytic oxidation of NO to NO<sub>2</sub> using CrO<sub>3</sub> on silica gel as the oxidizing agent; however, at low ambient NO concentrations some drift in the NO zero is observed. Interference from ambient O<sub>3</sub> is eliminated by modification of the inlet system and luminol solution.**

The nitrogen oxides, especially NO and NO<sub>2</sub> (NO<sub>x</sub>), are very important species in atmospheric chemistry. It has been shown that NO<sub>x</sub> is an important source of ozone in the troposphere (1) and that NO<sub>x</sub> is a reactant in tropospheric odd hydrogen chemistry (2). The NO<sub>x</sub> reactions produce a variety of inorganic and organic nitrates (3). Of these products, nitric acid and peroxyacetyl nitrate (PAN) are of prime interest with HNO<sub>3</sub> playing an important role in acid rain chemistry (4). To fully understand the atmospheric chemistry of NO<sub>x</sub>, a detector is needed which will measure NO<sub>x</sub> at the low parts-per-billion level or less for clean air studies (5).

Currently a number of methods are being used to monitor NO<sub>x</sub> concentrations in ambient air. By far the most common technique is based upon the chemiluminescent reaction between NO and O<sub>3</sub> (6, 7). While this reaction is specific for NO (8, 9), the technique can be expanded to measure NO<sub>2</sub> by first reducing NO<sub>2</sub> to NO (10) and then measuring total NO<sub>x</sub> as NO. Unfortunately, the reduction of NO<sub>2</sub> to NO is not specific for NO<sub>2</sub>, so that other nitrogen species (particularly HNO<sub>3</sub> and PAN) are also reduced to NO and act as interferences in the NO<sub>2</sub> measurements (11). Hence, NO<sub>2</sub> measurements are normally subject to some question. There exist two more direct means of NO<sub>2</sub> analysis for which field data have been reported. The first of these involves the photodissociation of NO<sub>2</sub> to form NO followed by chemiluminescent reaction with O<sub>3</sub> (12). This method is similar to those already mentioned but does not suffer from HNO<sub>3</sub> and PAN interference. The second direct NO<sub>2</sub> method uses the absorption of infrared radiation as its basis for detection. A tunable diode laser is used as the light source (13).

Recently, Maeda et al. described the development of an NO<sub>2</sub> detector based upon the chemiluminescent reaction of NO<sub>2</sub> with luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (14). Luminol has been under study since 1928 (15) and is known to chemiluminesce with a number of oxidizing agents in alkaline solution when metal catalysts are provided (16). This system has been used to measure H<sub>2</sub>O<sub>2</sub> as well as other oxi-

dizers produced by biological systems (17, 18) and to measure metal ion concentrations when excess H<sub>2</sub>O<sub>2</sub> (19) is added. Kok et al. (20, 21) devised a system for measuring H<sub>2</sub>O<sub>2</sub> at the 1 ppm level based upon the H<sub>2</sub>O<sub>2</sub>/luminol/metal ion reaction. Anderson et al. (22, 23) developed a system based on the luminol/H<sub>2</sub>O<sub>2</sub> chemiluminescence with the catalyst being a gas in equilibrium with the luminol solution instead of a metal ion. Anderson et al. claimed that this system can be used as a detector of NO<sub>2</sub>, O<sub>3</sub>, and SO<sub>2</sub> by using various inlet traps to distinguish between these gases. In our current work, we have developed a system which does not use H<sub>2</sub>O<sub>2</sub> and which detects NO<sub>2</sub> down to the 10 ppt (parts per 10<sup>12</sup> by volume) level. NO can also be monitored by oxidation to NO<sub>2</sub> with a proper oxidizing agent, although at low concentrations reliable quantitative conversion has yet to be demonstrated.

## EXPERIMENTAL SECTION

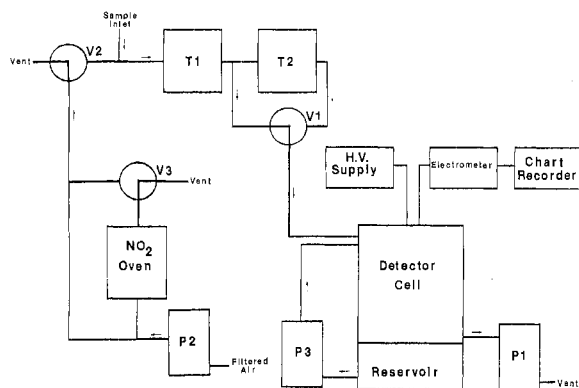
**Apparatus.** The basic scheme for the chemiluminescence detector is shown in Figure 1. The three main sections are the gas delivery system, reaction chamber, and measuring electronics. Sample gas is pulled through the system by pump P1. Trap T1 is filled with 500 mL of cotton to remove O<sub>3</sub> and yet let NO<sub>x</sub> pass. Trap T2, which is switched into and out of the stream by valve V1, contains 10% by weight CrO<sub>3</sub> on 6-12 mesh silica gel for NO to NO<sub>2</sub> conversion. The intake is exposed to a calibration and zero gas by the activation of valve V2. Auxiliary pump P2 supplies filtered diluent air from which NO<sub>2</sub> has been removed to V2. The same filtered air is also passed through an NO<sub>2</sub> permeation tube oven and can be added for calibration via valve V3. The reaction chamber will be described in detail later in this paper. The light output is detected with an RCA-1P28 photomultiplier tube whose photocurrent is amplified by a Kiethley 414S picoammeter and recorded on a Heath/Schlumberger strip chart recorder. The photomultiplier tube is operated at -1000 V. The luminol is supplied by liquid pump P3 at 1.0 mL/min. The cell is designed so that the luminol supply is recycled for extended use.

**Reagents.** Luminol was obtained from Aldrich Chemical Co. and used without further purification. NaOH, Na<sub>2</sub>SO<sub>3</sub>, and methanol were all obtained from the J. T. Baker Chemical Co., CrO<sub>3</sub> from Mallinckrodt, and Silica Gel (6-12 mesh) from Davison Chemical. Deionized water was obtained by passing distilled water through a Millipore Milli-Q deionizing system.

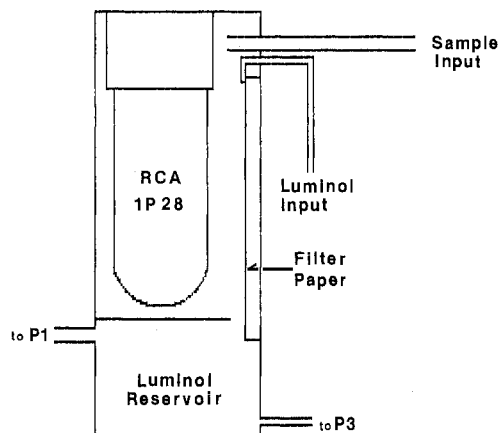
## RESULTS AND DISCUSSION

**Parameter Optimization.** The response of the system to changes in a number of parameters was investigated. These parameters include concentration of NaOH, Na<sub>2</sub>SO<sub>3</sub>, luminol, and methanol, shelf life of the prepared solution, sample air flow rate, and cell geometry. The initial tests on effects of concentration were done with a cell similar to the one described by Maeda et al. The effect of concentration of the reagents on chemiluminescence intensity was studied by varying the concentration of one component over a range while holding all of the other components constant. As was reported by Maeda et al. (14), the maximum chemiluminescence output was obtained when the luminol concentration was between  $1 \times 10^{-3}$  to  $1 \times 10^{-4}$  M and the output was essentially independent of luminol concentration for this range. We found that the signal was at a maximum for an NaOH concentration of  $5 \times 10^{-2}$  M decreasing by a factor of 4 for a factor of 2

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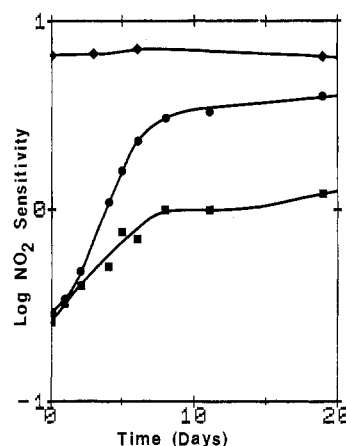


**Figure 1.** Block diagram of luminol  $\text{NO}_x$  detector: (P1) KNF Neuberger Model N79 MNP air pump; (P2) Thomas Industries Model 107CA18-1 air pump; (P3) Paragon Model 4465-01 liquid pump; (T1, T2) traps; (V1, V2, V3) Nacom Model M223W1A three-way solenoid valves.



**Figure 2.** Schematic drawing of luminol detector cell showing the relative positioning of the photomultiplier tube and the filter paper support; P1 and P3 are same as given in Figure 1.

change in NaOH concentration about this maximum. This is in good agreement with the value reported by Kok et al. (20) but differs from the maximum reported by Maeda et al. The next parameter to be examined was the geometry of the detector cell. Maeda et al. (14) described a cell which consisted of a pool of luminol which was constantly being replaced by a pump, over which the air sample was drawn. A photomultiplier tube was placed close to the surface of this pool and monitored the chemiluminescence output. This cell design was found to have two serious drawbacks. It was very sensitive to both movement during sampling and positioning of the cell to keep it level. In addition, it exhibited a slow response to changes in the concentration of  $\text{NO}_2$ . We observed that the  $\text{NO}_2$ /luminol reaction occurs primarily at the gas/liquid interface but the bulk liquid also exhibited some chemiluminescence which continued long after the  $\text{NO}_2$  was removed. This is undesirable since it results in an increased response time to changes in  $\text{NO}_2$  concentration. In our new design, shown in Figure 2, these problems are eliminated. The luminol solution was supported on a 1 in. by 3 in. strip of cellulose fiber filter paper (Whatman-1 qualitative) in front of the photomultiplier tube window. Luminol is continuously supplied at the top of the paper and the excess drips off the bottom into the luminol reservoir which is outside the photomultiplier tube viewing region. The photomultiplier tube is surrounded on three sides by a nylon insert which forces the sample air to pass between the front of the photomultiplier tube and the filter paper reaction region. This ensures ample contact between the sample air and the luminol solution. The result is a cell which is insensitive to movement and placement as well as exhibiting a faster response time. The response time



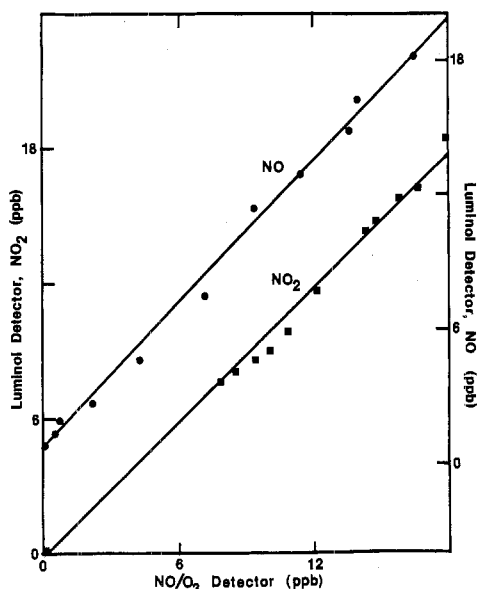
**Figure 3.** The effect on sensitivity of the storage time of the luminol solution under several conditions: (■) luminol,  $1 \times 10^{-4}$  M; (●) luminol,  $1 \times 10^{-3}$  M; (◆) luminol,  $1 \times 10^{-3}$  M,  $\text{Na}_2\text{SO}_3$  added to  $1 \times 10^{-2}$  M; All solutions had  $[\text{NaOH}] = 5 \times 10^{-2}$  M.

was measured by cycling valve V3 in the inlet system to turn the  $\text{NO}_2$  on and off at a known frequency. It was found that with a sampling rate greater than 0.5 L/min the detector could follow up to a 2 Hz modulation of 20 ppb of  $\text{NO}_2$  with no loss of signal. The effect of the flow rate of the sample gas on detector sensitivity was also examined. The detector output increased with air flow rate to 0.5 L/min and was insensitive to variations in the flow rate above this. These results are in agreement with the results reported by Maeda et al. for a similar study.

Since the instrument was being designed for ambient air measurements, the response to  $\text{O}_3$  which was reported by Maeda et al. (14) and confirmed in our lab was considered undesirable. Hence,  $\text{Na}_2\text{SO}_3$  was added to the system to reduce the sensitivity to  $\text{O}_3$  and enhance the sensitivity to  $\text{NO}_2$  as was suggested by Maeda et al. It was found that the maximum effect on  $\text{NO}_2/\text{O}_3$  response ratio was at an  $\text{Na}_2\text{SO}_3$  concentration of  $1 \times 10^{-2}$  M and decreased by only 25% for an order of magnitude change in concentration in either direction. It was noted in our original studies using solutions containing only luminol and NaOH that the chemiluminescence response increased with time after the solution had been prepared. Figure 3 shows the chemiluminescence increasing for the first 10–12 days and then remaining constant with time. Further, it was found that the introduction of  $\text{Na}_2\text{SO}_3$  to the system reduces this induction period to less than 1 h. The reason for this induction period and its shortening by  $\text{Na}_2\text{SO}_3$  is still unclear. The prepared solutions were stored in a dark cabinet until aliquots were removed for testing; however, continued work with luminol solutions has shown that this precaution is unnecessary.

A final addition to the solution composition was made after we discovered that addition of methanol further increases the sensitivity and specificity for  $\text{NO}_2$ . The signal doubled when the solution was made up to 0.05% (v/v) in methanol and then decreased to the level of the no added methanol solution as more methanol was added. In addition, the ratio of the  $\text{NO}_2$  response to the  $\text{O}_3$  response also increased to a maximum at 0.05% added methanol then decreased with increasing methanol.

**Gas Inlet System.** The  $\text{NO}_2$ /luminol detector is preceded by a gas inlet system which was designed to serve four functions: remove the  $\text{O}_3$  interference, oxidize NO to  $\text{NO}_2$ , calibrate, and zero the detector. As mentioned previously, the luminol has an inherent response to  $\text{O}_3$  which is undesirable. While this interference was reduced by the addition of  $\text{Na}_2\text{SO}_3$  and methanol, it can still be a large interference in some applications. The detector exhibits an  $\text{NO}_2$  response



**Figure 4.** Correlation between the luminol  $\text{NO}_x$  detector and conventional  $\text{NO}/\text{O}_3$  detector (26) for both  $\text{NO}$  and  $\text{NO}_2$ . The line through the origin (■) represents the  $\text{NO}_2$  response correlation (slope = 1.0, intercept = 0.5 ppb,  $r = 0.993$ ); the upper line (●) showing the  $\text{NO}$  correlation is displaced 5 ppb upward for clarity and is referenced to the right axis (slope = 1.0, intercept = 0.76 ppb,  $r = 0.994$ ). Luminol,  $1 \times 10^{-4}$  M;  $\text{Na}_2\text{SO}_3$ ,  $1 \times 10^{-2}$  M;  $\text{NaOH}$ ,  $5 \times 10^{-2}$  M; methanol, 0.05%.

which is only 30 times greater than the  $\text{O}_3$  response at the maximum. This means that in air which has 90 ppb  $\text{O}_3$ , the detector would report an  $\text{NO}_2$  concentration 3 ppb above the actual value. Hence, a trap, T1 (Figure 1), was needed at the inlet of the instrument to remove  $\text{O}_3$  without removing  $\text{NO}$  or  $\text{NO}_2$  and without changing the  $\text{NO}/\text{NO}_2$  ratio. A trap filled with 500 mL of bulk cotton (24) was tested. It was found that the  $\text{O}_3$  removal was essentially 100% (as checked with a Dasibi Model 1003-AH ozone detector) while the  $\text{NO}_x$  was passed with only a 5% loss and no change in  $\text{NO}/\text{NO}_2$  ratio. The conversion of  $\text{NO}$  to  $\text{NO}_2$  was accomplished by inserting into the gas stream a trap, T2, filled with 10% by weight  $\text{CrO}_3$  on 6–12 mesh silica gel as described by Levaggi et al. (25). The trap is an aluminum cylinder 25 cm long and 3.5 cm in diameter with glass wool plugs at both ends to confine the oxidizer.  $\text{NO}$  is oxidized to  $\text{NO}_2$  by the  $\text{CrO}_3$  and measured as  $\text{NO}_2$ .

**Interferences.** The response of the system to species other than  $\text{NO}_2$  and  $\text{NO}$  was investigated. No response was seen when the detector was exposed to 20 ppb each of  $\text{HNO}_3$ ,  $\text{NH}_3$ , or  $\text{HCN}$ . PAN is seen as an  $\text{NO}_2$  interferent with a response equal to that for  $\text{NO}_2$ .  $\text{CH}_3\text{ONO}$  is not measured on the  $\text{NO}_2$  channel but is converted to  $\text{NO}_2$  by the  $\text{CrO}_3$ /silica gel trap and therefore would be measured as  $\text{NO}_2$ . The  $\text{O}_3$  interference has been discussed above.

Two tests of the system have been performed to date. The first of these was a laboratory intercomparison between the luminol detector and a conventional  $\text{NO}/\text{O}_3$  chemiluminescence detector. Both detectors ran for several days sampling lab air which showed enough variation in the  $\text{NO}$  and  $\text{NO}_2$  concentrations to constitute a useful sample. Figure 4 shows a correlation between the output of the two detectors separated into  $\text{NO}_2$  and  $\text{NO}$ . The data show that the luminol detector compares very favorably with the conventional detector for both  $\text{NO}_2$  and  $\text{NO}$ . The nonzero intercept of the  $\text{NO}$  correlation can be interpreted as a problem in zeroing the  $\text{NO}_x$  channel of the luminol detector.

The second test of the system was a field study of  $\text{NO}$  and  $\text{NO}_2$  in clean air off the ocean. The detector was housed in

a trailer on a cliff overlooking the ocean along the southwest edge of the island of Bermuda. Measurements were made from Aug 6 to Aug 27, 1982, without interruption aside from daily calibration.  $\text{NO}_2$  concentrations measured ranged from a high of 8 ppb to a low of 65 ppt with the average value being 400–500 ppt. While the detector exhibited a detection limit of 10 ppt when tested in the lab without the inlet system in place, during the study the detection limit was calculated to be 30 ppt with a signal to noise ratio of 2. In addition, it was found that a 500-mL aliquot of luminol solution can be used, and recycled, at the rate of 1.0 mL/min for up to 48 h with only a 20% loss of signal by the end of the sampling period. There is slight increase in system sensitivity during the first 12 h of use of a batch of luminol which may arise from flushing the old solution out of the cell. Some variation of the sensitivity among several luminol batches arises from the condition of the filter paper which supports the luminol solution in the detector cell. Experience has shown that the filter paper gets clogged with debris after extended use presumably by evaporation. A decrease in system sensitivity is taken as a signal to replace the filter paper with a fresh sheet. Both the ambient temperature and humidity varied widely during the study with no apparent effect on the response of the detector to  $\text{NO}_2$ . The conversion efficiency of  $\text{NO}$  to  $\text{NO}_2$  by the  $\text{CrO}_3$ /silica gel was 60–70% initially and decreased by 10% over a period of 9 days. The conversion efficiency was found to fluctuate by up to 5% with time which may be attributed to the effect of sample gas humidity on conversion efficiency discussed by Levaggi et al.

## CONCLUSION

Our work shows that the chemiluminescence of luminol with  $\text{NO}_2$  can be the basis of a continuous monitor for ambient  $\text{NO}_2$ . The system currently has a detection limit of 30 ppt and this limit may be improved on by the selection of a better photomultiplier tube and associated electronics as well as further optimization of the cell design. The detector may also be adapted to measure  $\text{NO}$  by the addition of an oxidizer, although the effect of humidity on the oxidizer must be reduced. In addition, it has proven to be difficult to zero the detector for no  $\text{NO}$ . While this is not a problem while measurements are being made on a 30 ppb scale, it can prove to be very troublesome on the 300 ppt scale which was used in the Bermuda field trip. While we have attempted to make corrections for this zeroing problem on a low scale, it has proven to be difficult and not unequivocal and thus introduces a large amount of uncertainty into our sub-part-per-billion  $\text{NO}$  data.

## ACKNOWLEDGMENT

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**Registry No.**  $\text{NO}_2$ , 10102-44-0;  $\text{NO}$ , 10102-43-9; luminol, 521-31-3.

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## Bis(2,4-dinitrophenyl) Oxalate as a Chemiluminescence Reagent in Determination of Fluorescent Compounds by Flow Injection Analysis

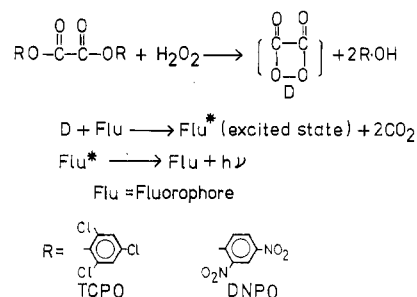
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The chemiluminescence (CL) reaction of bis(2,4-dinitrophenyl) oxalate (DNPO) or bis(2,4,6-trichlorophenyl) oxalate (TCPO) with hydrogen peroxide was used for the detection of the fluorescent compound dansylalanine (DNS-Ala) in buffer solution. Factors affecting the CL intensities, such as water, halogen salts, and phenols, 2,4-dinitrophenol (DNP) and 2,4,6-trichlorophenol (TCP), which are reaction products, were studied. Halogen salts greatly quenched both CL intensities, and the water content in the reaction medium also acted to reduce them. While the effect of TCP on the CL intensity in the TCPO-CL reaction was large, that of DNP in the DNPO-CL reaction was small. The DNPO-CL reaction was superior to that of TCPO-CL in sensitivity in the flow injection analysis of DNS-Ala. The detection limit of DNS-Ala in the former reaction was 5 fmol.

In the conventional fluorescence detection system, a part of the stray radiation or Raman scattering derived from the light source, which raises the background level, often interferes with improvement of the system's sensitivity. To solve this problem, a chemiluminescence (CL) detection method, without light source, is a plausible candidate for the detection of fluorescent compounds. The reaction of bis(2,4,6-trichlorophenyl) oxalate (TCPO) and hydrogen peroxide ( $H_2O_2$ ) (1) (Scheme I) was applied to the detection of fluorescent compounds, such as dansyl amino acids, on thin-layer chromatograms (2, 3) and aromatic hydrocarbons (4). In previous papers (5, 6), we applied the CL reaction using TCPO and  $H_2O_2$  to a fluorescence detection system for high-performance liquid chromatography (HPLC). With this method, dansyl amino acids and fluorescamine labeled catecholamines were separated and detected at the 10 fmol and 25 fmol levels, respectively. For the quantification of a trace amount of biogenic substances, however, one or two orders of magnitude higher sensitivity is required. According to the results by Rauhut et al. (1, 7), among the oxalates, bis(2,4-dinitrophenyl) oxalate (DNPO) gave one of the highest CL quantum yields. Therefore, in this work we took DNPO and studied the various

Scheme I



factors affecting the CL intensity in comparison with the TCPO reaction. The application of the DNPO-CL reaction to flow injection analysis will be reported.

### EXPERIMENTAL SECTION

**Reagents.** TCPO was prepared by following the method of Mohan and Turro (8) and recrystallizing from ethyl acetate. DNPO was prepared by following the method of Rauhut et al. (7) and recrystallizing from acetonitrile. Dansylalanine (DNS-Ala, cyclohexylamine salt) and  $H_2O_2$  were purchased from Sigma Chemical Co. (St. Louis, MO) and Mitsubishi Gas Kagaku Co. (Tokyo, Japan), respectively. All other chemicals were of reagent grade.

**Experiments in the Static System.** Ethyl acetate and acetonitrile were selected as the solvent for oxalates (DNPO and TCPO) and  $H_2O_2$ , respectively. As a representative of fluorescent compounds, DNS-Ala was used. Two-hundred-fifty microliters of 0.05 M  $H_2O_2$  solution and 40  $\mu$ L of 100 nM DNS-Ala solution ( $1.0 \times 10^{-2}$  M phosphate buffer,  $Na^+$ ) were premixed in a borosilicate glass tube (6  $\times$  50 mm, Fisher Co., Boston, MA) for 10 s, and 100  $\mu$ L of 1.0 mM oxalate solution was added instantaneously with a microsyringe and mixed vigorously with a mixer (type TM-100, Thermonics Co., Tokyo, Japan) for a few seconds. The tube was immediately set inside a CHEM-GLOW photometer (Aminco Co., Baltimore, MD) and the CL intensity was measured. The time course of the relative CL intensity was recorded after the addition of oxalate solution.

Net CL intensity of DNS-Ala was obtained by subtracting the CL intensity of the reaction solution without DNS-Ala (blank) from that with DNS-Ala. Fluorescence (FL) intensity was also measured under almost the same conditions where the CL reaction