Chemiluminescence Energy Transfer Reaction for the On-Line Preparation of Peroxymonocarbonate and Eu(II)−Dipicolinate Complex

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In this work, an on-line preparation of peroxymonocarbonate was formed innovatively, which offered a reliable intermediate for further investigation. The forming conditions of on-line peroxymonocarbonate ions were investigated in detail. Meanwhile, the energy transfer chemiluminescent reaction of peroxymonocarbonate and the Eu(II)−dipicolinate complex was studied. Through UV−visible absorption spectra, CL method, ESR spin-trapping technique, and mass spectrum experiments, it can be concluded that peroxymonocarbonate oxidizes Eu(II) to Eu(III), and simultaneously creates radicals. The bond rearrangement within radicals formed the singlet molecular oxygen. The energy originating from the singlet oxygen was accepted by the $(Eu(III)dipic)^-$ complex. The excited $(Eu(III)^*dipic)^-$ ions underwent radiative deactivation and emitted the chemiluminescence. The peroxymonocarbonate system was a simple, inexpensive, and relatively nontoxic alternative to other oxidants, and it can be used in a mild, neutral-pH environment.

1. Introduction

Carbonate or bicarbonate has often been used as buffer solution for the chemiluminescence (CL) medium. Much research[1−5] has indicated that carbonate or bicarbonate acted not only as a buffer reagent but also as the CL enhancer. It has been confirmed that CO_3^{2-} was a luminous species when it coexisted with a strong oxidant in basic solution. In our previous works,[3,5] a possible CL mechanism was suggested in which the CO_3^{2-}/HCO_3^- ions were attacked by superoxide radicals which generated carbonate free radicals ($CO_3^{\bullet-}$). Then, $CO_3^{\bullet-}$ recombined to generate excited triplet dimers of two CO_2 molecules $((CO_2)_2^*)$. With the decomposition of this unstable intermediate to CO_2, the energy was released and the CL was emitted. Recently, an interesting research work, the formation of a bicarbonate active species, peroxymonocarbonate (HCO_4^-), was reported.[6] It was discovered that the equilibrium of hydrogen peroxide and bicarbonate to form HCO_4^- occurred rapidly at 25 °C near neutral pH in aqueous solution or ethanol/water mixtures (eq 1).

$$HCO_3^- \text{ (solv)} + H_2O_2 \text{ (solv)} \rightleftharpoons$$

$$HCO_4^- \text{ (solv)} + H_2O \text{ (solv)} \quad K_1 = \frac{[HCO_4^-][H_2O]}{[H_2O_2][HCO_3^-]} \quad (1)$$

Such equilibrium had previously been observed by Richardson's group,[7] and HCO_4^- without metal ion was found to be a moderately reactive heterolytic oxidant that can be classified as an anionic peracid.[8] The effective equilibrium constant K_1 = 27 ± 1 at 25 °C was observed, and the observed equilibration rate constant was $(2.4 ± 0.1) × 10^{-3}$ s^{-1}.[6] The half-life for formation of HCO_4^- in ethanol/water (1.76:1, v/v) was about 300 s, and the electrode potential for the HCO_4^-/HCO_3^- couple was $1.8 ± 0.1$ V (vs NHE). From these data, it can be concluded

that it was suitable to select ethanol/water as the solvent, which shifted the equilibrium of eq 1 to the right, and favoring increased formation of peroxymonocarbonate ion. Recent experiments reported by Richardson et al.[6] showed that increases in catalytic efficiency of the bicarbonate−peroxide system could be achieved by using a more soluble source of ammonium bicarbonate and ethanol in place of $NaHCO_3$ and *tert*-butyl alcohol, respectively. Meanwhile, these two data points gave us an idea that the active species HCO_4^- has character similar to that of peroxymonosulfate (HSO_5^-), which had been used as a CL analytical reagent several years ago.[9]

Though there were a lot of established chemiluminescence (CL) systems for analysis applications, they suffered from one or more disadvantages, such as toxicity or release of some byproducts which were unfriendly to the environment. The peroxymonocarbonate system, bicarbonate-activated peroxide, offered a simple, inexpensive, and relatively nontoxic alternative to other oxidants. This feature made it widely used as an oxidant in many reactions,[10] such as methionine,[11] alkenes,[8,12,13] and sulfide.[6,14] However, the oxidant was unstable and hydrolyzed rapidly, forming HCO_3^- and H_2O_2. It was obviously difficult to store the HCO_4^- ions. Hence, on-line preparation of HCO_4^- was likely to be the preferable method in most applications. Furthermore, the CL systems with HCO_4^- as oxidant were very few. To the best of our knowledge, no CL system which used HCO_4^- as oxidant has been reported. Thus, it is desirable to exploit the CL system. In this work, the flow analysis technique was used to introduce a continuous flowing stream without a gap into the channel. At the same time, by using the peristaltic pump, it was easy to go along a multichanneled stream and control the flow rate for the appropriate on-line formation time. Through a three-way piece, the two streams (H_2O_2 in ethanol/water (1.76:1, v:v) binary solvents and HCO_3^-) can mix sufficiently to form on-line HCO_4^- ions. The presence of this active carbon oxygen intermediate can be observed using NMR[7,15] during mixing H_2O_2 and HCO_3^-. The higher ratio of ethanol/water corresponded to an increasing amount of HCO_4^-.

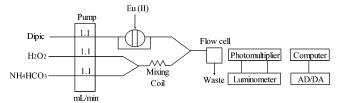* To whom correspondence should be addressed. FAX: (+86)-010-62841953. E-mail: jmlin@mail.rcees.ac.cn.

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Figure 1. Schematic diagram of flow-injection CL detection system for (A) the on-line preparation of HCO_4^- solution; (B) the investigations of energy transfer CL.

However, using only ethanol leads to phase separation or insolubility of the salt. In our work, the ratio 1.76:1 was employed, and the concentration of NH_4HCO_3 was sufficiently low such that solubility saturation effects were not observed.

In view of the short lifetime and high activity of this active carbon oxygen intermediate, and unique luminescence properties of Eu(II) ion complexes, i.e., long luminescence decay time and narrow emission bands, Eu(II) complexes were particularly well-suited for an energy transfer study in HCO_4^- CL reaction. In 1983, a series of studies on CL emitted during oxidation of Eu-(II) ions in complexes with organic ligands by hydrogen peroxide had begun.[16] This reaction, involving radicals, led to the appearance of excited Eu(III) ions, and the CL intensity significantly increased after complexation of Eu(II) ions. In the present work, we found that an ultraweak CL was observed after injecting Eu(II) into the HCO_4^- solution prepared on-line. Introduction of a ligand caused an increase in the CL intensity of the system. The kind of ligand was also selected carefully. It showed that the CL intensity was the strongest when dipicolinate was used as the ligand of Eu(II). The goal of the present study was to study the mechanism of the CL energy transfer reaction with HCO_4^- as the oxidant, which was explored through UV−visible absorption spectral analysis, chemiluminescent (CL) method, mass spectra, and ESR spin-trapping techniques, and the results suggested that radical reactions played a dominant role in the HCO_4^- energy transfer CL system. These new phenomena would further enable people to exploit more applications of the peroxymonocarbonate as a potentially useful analytical reagent.

2. Experimental Section

2.1. Reagents and Materials. Hydrogen peroxide (30%, Alfa Aesar, U.S.A.), ammonium bicarbonate (AR), and ethanol (AR) were used without further purification. Hydrogen peroxide solution (2.0 mol/L) was prepared by diluting 20 mL of 30% (v/v) hydrogen peroxide to 100 mL with water and was standardized by potassium permanganate standard solution. Sodium bicarbonate, sodium carbonate, potassium bicarbonate, and potassium carbonate, analytical reagent, were used as received. The Eu(II) solutions used were obtained by reduction according to McCoy's method.[17] The degree of reduction of europium was determined by iodometric titration immediately prior to measurement. For ^{13}C NMR studies, 98% ^{13}C-enriched sodium bicarbonate (Alfa Aesar, U.S.A.) and 99.95% D_2O (Alfa Aesar, U.S.A.) were used. 2,2,6,6-Tetramethylpiperidone (98%) and 99% 9,10-diphenylanthrathene (Acrás Organics, Belgium) were used.

Water was purified using a Barnstead Nanopure system (18.3 MΩ cm^{-1}, Barnstead, Iowa, U.S.A.).

2.2. Procedure for CL Detection. The schematic diagram of the flow system employed in this work is shown in Figure 1. A multichannel peristaltic pump was used to deliver all flow streams in this system. PTFE tubing (1.0 mm i.d.) was used as connection material in the flow system. The on-line HCO_4^-

solution was prepared by the mixing H_2O_2 (in ethanol/water (1.76:1, v/v) mixture) and bicarbonate through a three-way piece. A 35 cm mixing coil was used for efficient mixing of H_2O_2 and bicarbonate. A Eu(II) solution was injected into the carrier stream (dipic) using a six-way injection valve with a 150 μL sample loop, and then, it was merged just prior to reaching a flow cell with a stream of HCO_4^- solution. The flow cell was a flat spiral-coiled colorless glass tube (1.0 mm i.d.; total diameter of the flow cell, 3 cm, without gaps between loops) and located directly facing the window of the CR-105 photomultiplier tube (Hammamatsu, Tokyo, Japan) operated at −800 V. The CL signal produced in the flow cell was detected and recorded with a computerized ultraweak luminescence analyzer (type LF-800, manufactured at Microtec NITI-ON, Funabashi, Japan). Data acquisition and treatment were performed with LUM-2000 software running under Windows XP.

2.3. Spectra Measurements. The CL spectra of the proposed systems were examined by a series of high-energy optical filters (425, 440, 460, 515, 535, 575, 595, 620, 640 nm).

UV−visible absorption spectra, electron spin resonance (ESR) spin-trapping spectra, and mass spectra were measured on a model UV-2401 spectrophotometer (Shimadzu, Japan), X-band ESP-300E spectrometer (Bruker, Germany), and a Platform II mass spectrometer (LSMS-2010, Shimadzu, Japan), respectively.

3. Results and Discussion

3.1. On-line Preparation of HCO_4^- Ions. Taking into account the relative difficulty of storing the unstable HCO_4^-, on-line preparation of HCO_4^- solution was likely to be the preferred method. The flow analysis method was a fast, continuous method. On the basis of discrete instant sampling by injection into a carrier stream, the system allowed continuous on-line analysis to be performed in a fast, greatly simplified way. As the continuous flowing stream was characterized by a turbulent rather than a laminar flow, the discrete instant sampling created geometrically well-defined segments of sample solution within the flowing stream. It introduced a continuous flowing stream without a gap into the channel, which makes the on-line mixing true. At the same time, by using the peristaltic pump, it was easy to go along a multichanneled stream and control the flow rate for the appropriate on-line formation time. These merits guaranteed sufficient reaction time. It provided the room for on-line preparation of HCO_4^- ions.

The on-line HCO_4^- solution was obtained by mixing H_2O_2 (in elthanol/water, 1.76:1, v/v) and bicarbonate through a three-way piece. The flow system used in this work was shown in Figure 1. It consists of a peristaltic pump with two lines. The flow streams H_2O_2 in 1.76:1 ethanol/water solution and bicarbonate were used to prepare the on-line HCO_4^- ions. The CL emission intensity was used to quantify HCO_4^- ions. Catalysis by sodium bicarbonate, potassium bicarbonate, and ammonium bicarbonate was carefully studied in ethanol/water, and the source of bicarbonate had a significant effect on the CL intensity. It was shown that ammonium bicarbonate was the best catalyst. The concentration of NH_4HCO_3 was optimized over the range of 0−0.2 mol/L in a FIA-CL mode described in the Experimental Section. The results showed that the CL intensity increased along with the increased concentration of NH_4HCO_3. However, if the concentration of NH_4HCO_3 was more than 0.05 mol/L, the CL intensity increased instead, and high concentrations of bicarbonate easily aroused solubility saturation effects. Thus, 0.05 mol/L ammonium bicarbonate was selected as the optimum concentration of NH_4HCO_3 throughout the study.

Second, the concentration of H_2O_2 optimized over the range 0−2 mol/L in ethanol/water (1.76:1, v/v) was examined.
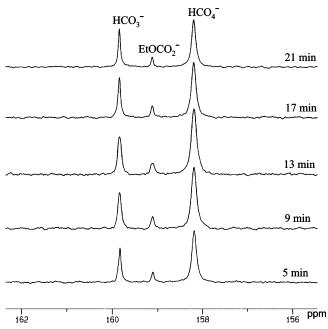
Figure 2. ^{13}C NMR spectra for a solution at 25 °C of NaH$^{13}CO_3$ (0.05 mol/L) in 1.76:1 (v/v) ethanol/water with $[H_2O_2] = 2.0$ mol/L. Times shown are for the completion of acquisition from the time of mixing.

Figure 3. The CL kinetic curve of the system. Conditon: injection 50 μL 0.01 mol/L Eu(II)−dipicolinate complex into HCO$_4^-$ solution (2 mol/L H$_2$O$_2$ and 0.05 mol/L HCO$_3^-$ were prepared 10 min prior to ensure the preequilibration of peroxymonocarbonate formation).

Figure 4. Chemiluminescence profiles in batch system. (a) Injection of 50 μL of 0.01 mol/L Eu(III) into the HCO$_4^-$ solution; (b) injection of 50 μL 0.01 mol/L Eu(II) into the HCO$_4^-$ solution; (c) injection of 50 μL 0.01 mol/L Eu(II)−dipicolinate complex into the HCO$_4^-$ solution.

Excessively high H$_2$O$_2$ concentration can give rise to air bubbles in the flow line. Excessively low H$_2$O$_2$ concentration cannot produce enough HCO$_4^-$ ions. So, after a series of experiments, 2 mol/L H$_2$O$_2$ was selected as the optimum.

Third, the mixing time interval and the flow rates of H$_2$O$_2$ and bicarbonate played key roles in the on-line preparation of HCO$_4^-$ solution. Both too-long and too-short times cannot support formation of enough HCO$_4^-$ ions to induce CL emission. In addition, the lifetime of the active carbon oxygen intermediate was a dominant factor in the flow CL system because transient light emission was monitored. Since the lifetime of the HCO$_4^-$ ions was very short, the point of mixing was reasonably designed. The experiments of the different mixing coil length and the effect of flow rates showed that when a 35 cm mixing coil and 1.1 mL/min flow rate were used, the maximum amount of HCO$_4^-$ ions were obtained. A mixing coil that is either too long or too short could not produce the most HCO$_4^-$ ions.

To examine the HCO$_4^-$ ions of on-line preparation, we used 98% ^{13}C-enriched NaHCO$_3$ to examine bicarbonate−peroxide in ethanol/water solvents by ^{13}C NMR at 25 °C. Hydrogen peroxide concentration of 2.0 mol/L was employed, and $[H^{13}CO_3^-] = 0.05$ mol/L for all studies. Ethanol/water (1.76:1, v/v) solutions of hydrogen peroxide with bicarbonate catalyst were mixed 10 min prior to the experiment to ensure the preequilibration of peroxymonocarbonate formation for the whole experiment. ^{13}C NMR spectral measurements were carried out on an Avance DPX 400 MHz NMR instrument (Bruker, Germany). H$_2$O$_2$ (10%) was replaced by D$_2$O. D$_2$O was injected into a NMR tube immediately before measurements. The spectra were recorded periodically for 4 min. The ^{13}C NMR spectroscopic results strongly supported the on-line formation of HCO$_4^-$ in this flow system. Besides the peak for bicarbonate at 159.8 ppm, a single additional peak at 158.2 ppm assigned to HCO$_4^-$ was observed (chemical shifts relative to TMS). The peaks assigned to HCO$_3^-$, HCO$_4^-$, and EtOCO$_2^-$ in ethanol/water solution are shown in Figure 2. It was shown that the quantity of HCO$_4^-$ with the time change remained nearly steady
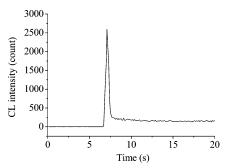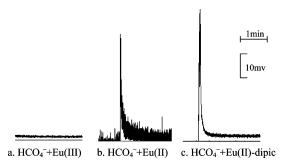
within 20 min. Thus, on-line preparation of HCO$_4^-$ ions offered a reliable intermediate for further investigation.

3.2. The Energy Transfer CL Reaction in the Peroxymonocarbonate System. As one of the rare earth elements, complexes of europium (II) ion have received much attention due to its unique luminescence properties, such as long luminescence decay time and narrow emission bands which can form highly luminescent complexes with several groups of ligands.[18−20] Of particular concern to its applications is the study of coordination compounds in solution[21,22] and luminescent assays for biochemistry.[23,24] After many experiments, the Eu-(II)−dipicolinate complex was employed to investigate the energy transfer mechanism for the HCO$_4^-$ system. As shown in Figure 3, it was found that, by injecting Eu(III) (50 μL) into HCO$_4^-$ solution, no CL emission was observed with our apparatus. However, when a Eu(II) solution (50 μL) was injected into HCO$_4^-$ solution, bright CL can be observed. When injecting Eu(II)−dipicolinate complex (50 μL) into HCO$_4^-$ solution, the CL was enhanced greatly. At the same conditions, when the Eu(II)−dipicolinate complex (50 μL) was also injected into H$_2$O$_2$ solution, no CL was observed. This sufficiently showed that HCO$_4^-$ played a key role as an oxidant rather than H$_2$O$_2$ in the system. Because the metal ions can induce CL emission, it was suspected that the Zn^{2+} and its byproducts induced CL emission during the reduction process of Eu^{3+} to Eu^{2+}. Therefore, the effects of Zn^{2+} and its byproducts on the system were also investigated. When injecting Zn^{2+} and its byproducts into the on-line preparation HCO$_4^-$ solution, no CL was observed. This sufficiently showed that the HCO$_4^-$−Eu(II)−dipicolinate system induced CL emission. The kinetic profiles of CL for the system HCO$_4^-$−Eu(II)−dipicolinate were shown in Figure 4, from which it was evident that a maximum was reached within several seconds when the Eu(II)−dipicolinate complex (50 μL) was also injected into HCO$_4^-$ solution. The
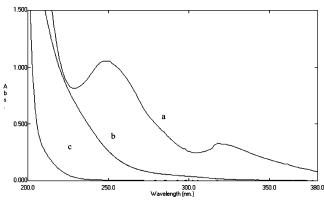
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Figure 5. Absorption spectra of Eu(II) (a), Eu(II) after reaction (b), Eu(III) (c); concentration of all reagents: 1×10^{-3} mol/L.

Figure 6. The CL spectral distribution for the system $HCO_4^- - $Eu-(II)$-$dipicolinate with cutoff method.

intensity of emission of lanthanide ions depended on the stability of the complex formed with the ligand and the La(III) hydration number.[25,26] Experiments showed that the CL emission reached a maximum when the molar ratio of Eu(II) to dipicolinate was 1:1. The strong CL phenomenon provided the favorable condition for the mechanism study of the HCO_4^- CL phenomenon.

The optimum conditions for the $HCO_4^- - $Eu(II)$-$dipicolinate reaction were investigated in detail, containing the effects of the molar ratio of ligand/metal, the flow rate, and sample volume on the CL intensity.

3.3. Investigation of the Mechanism in the $HCO_4^- - $Eu-(II)$-$Dipicolinate Reaction. In the present work, we employed the equilibrium of bicarbonate$-$peroxide to form on-line HCO_4^- ions. Many investigations have indicated that some of the lanthanide ions (mainly Eu(II)) showing strong luminescence were used as luminescent probes, activators, and energy transfers (donors or acceptors). HCO_4^-, as an active carbon oxygen intermediate, had high activity, and it was easy to oxidize Eu-(II) to Eu(III).

$$HCO_4^- + Eu(II) \rightarrow CO_3^{\bullet -} + \bullet OH + Eu(III) \qquad (2)$$

A series of UV$-$visible absorption experiments were carried out to confirm the change of Eu(II) before and after the reaction. The absorption spectrum of Eu(II) ions in water environment revealed two bands with maxima at $\lambda = 248$ and 318 nm (Figure 5a), ascribed to the electronic transitions $4f \rightarrow 4f^6 5d$.[27] In the concentration range $10^{-4}-10^{-2}$ mol/L, for $\lambda = 248$ nm, the Eu^{2+} solutions satisfied the Lambert$-$Beer law. The other components of the system studied, H_2O_2, HCO_3^-, and HCO_4^-, did not give significant signals for $\lambda = 248$ and 318 nm. This fact was used for the Eu^{2+} study before and after the reaction. Two bands (248 and 318 nm) disappeared in the absorption spectrum after reaction (Figure 5b). Moreover, this absorption spectrum did not overlap with the Eu(III) absorption spectrum (Figure 5c). However, both shapes were very similar. On the basis of these, we concluded that Eu(II) was oxidized to Eu-(III) after the $HCO_4^- - $Eu(II)$-$dipicolinate reaction.

The CL spectral distribution was measured with the cutoff filter method, as shown in Figure 6. The strong maximum at about 620 nm indicated that the observed CL was emitted by the Eu^{3+} ions. The $E°$ value was 1.81 V for HCO_4^-/HCO_3^- and -0.43 V for Eu^{3+}/Eu^{2+}.[28] Taking into account the CL spectrum as well as the electrode potential of redox, we could conclude that the excitation of Eu^{3+} to the luminescent level $^5D_0$ (2.10 eV) was possible. In the redox system of Ru^{2+}/Ru^{3+} with 2,2′-bipyridine, the excited product was identified as a metal (Ru^{2+}) to ligand charge-transfer triplet.[29] In addition to these, Elbanowsli et al.[30] has found that the $(Eu(II)-N_3)^{2+}$
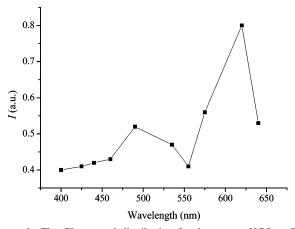
complex can easily accept energy from the singlet oxygen dimers. So, we deduced that HCO_4^- ions could oxidize Eu(II) to Eu(III), and in this process, singlet oxygen was produced, which transferred its radical energy to the Eu(III).

The thermodynamic analysis estimates suggested the O$-$O bond homolysis as the activation step[31] during the process of oxidation for Eu(II). Simultaneously, the process brought the $CO_3^{\bullet -}$ radical and the hydroxide radical. Radical recombination involved the perhydroxyl radical which transfered to the superoxide ion radical.

$$H_2O_2 + CO_3^{\bullet -} \rightarrow HCO_3^- + \bullet HO_2 \quad [32] \qquad (3)$$

$$\bullet HO_2 \rightleftharpoons H^+ + O_2^{\bullet} \quad [33] \qquad (4)$$

Reaction 4 was only the first step, which was followed by the interaction of perhydroxyl radical with superoxide ions and the interaction of two perhydroxyl radicals.[34] The oxygen ion radical thus formed can take part in the following subsequent reactions leading to the formation of singlet oxygen molecules:

$$O_2^{\bullet -} + \bullet OH \rightarrow {}^1O_2 + HO^- \qquad (5)$$

$$HO_2^{\bullet} + O_2^{\bullet -} \rightarrow {}^1O_2 + HO_2^- \qquad (6)$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow {}^1O_2 + H_2O_2 \qquad (7)$$

Excess of H_2O_2 resulted in an increased yield of $O_2^{\bullet -}$, as well as the formation of singlet oxygen. The presence of singlet oxygen ($^1O_2$) was also studied using various methods, such as enhancing and quenching CL method as follows.

It is well-known that the luminescent reagents, such as luminol,[35] lucigenin,[36] and p-methoxyphenyl cypridina luciferin analogue (MCLA),[37] can react with reactive oxygen species to generate bright CL emission. In particular, MCLA has been used as a CL probe for the determination of $O_2^{\bullet -}$ and $^1O_2$. Furthermore, it has been shown that other reactive oxygen species such as H_2O_2 and HO$\bullet$ are not able to elicit the luciferin analogue CL emission.[38] Thus, the effect of the luminescent reagents on the HCO_4^- system CL was investigated. At the same concentration (1×10^{-5} mol/L), it was seen that these luminescent reagents enhanced greatly the CL intensity, and MCLA enhanced CL intensity 75 times, lucigenin about 30 times, and luminol 20 times. These showed that the $^1O_2$ was generated in this CL system. The generation of $^1O_2$ in the examined system was also confirmed by the observed quenching effect.[39] The CL signal intensity was effectively quenched (about

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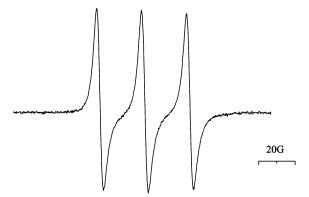*J. Phys. Chem. A, Vol. 110, No. 23, 2006* **7513**

Figure 7. ESR spectrum of nitroxide radicals generated by reaction of the TEMP probe in HCO_4^-−Eu(II)−dipicolinate system. Conditions: receiver gain, 8.00e + 04; mod amplitude, 1 G; sweep width, 100.00 G; microwave power, 3.17e + 00 mW.
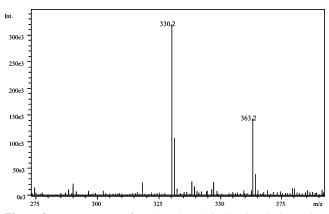

Figure 8. Mass spectra of DPA and $DPAO_2$ by chemical trapping with the DPA. 60 mmol/L DPA. Conditions: The reacted solution was placed for several minutes at room temperature, in a biphasic system composed of chloroform and H_2O (1:1, v/v) and protected from light.

13 times) by NaN_3, which was a strong indication that $^1O_2$ was the emitter. On the basis of these experiments data, we further speculated that $^1O_2$ participates in the energy-transfer CL reaction. To further characterize the generation of $^1O_2$ in this CL reaction, the ESR spin-trapping technique and mass spectra were explored.

Room-temperature ESR spectroscopy was used to detect and identify free radical intermediates formed during reactions. Since some of the free radicals in these experiments were too short-lived to be detected directly, the spin-trapping technique was used. 2,2,6,6-Tetramethylpiperidone (TEMP)-specific target molecule of $^1O_2$, was used as the spin-trapping reagent. The reaction of $^1O_2$ with TEMP results in the formation of 2,2,6,6-tetramethylpiperidone-*N*-oxide (TEMPO),[40,41] with the product being a stable nitroxide radical with a characteristic spectrum. Reagents were dissolved in appropriate amounts in alcohol. The choice of alcohol as a cosolvent was supported by several arguments: TEMP was soluble in alcohols, the lifetime of $^1O_2$ was increased six times in comparison to H_2O, and an interaction of ·OH with TEMPO was suppressed. The sample in a flat quartz cell was measured in the cavity of the ESR spectrometer. No signal was detected in the TEMP background. The original free radical intermediate was then identified by the resulting hyperfine coupling constants of its TEMP adduct. ESR spectra were recorded with a Bruker ESP-300E spectrometer operating in the X-band at room temperature. The microwave frequency was 9.83 GHz, and the modulation amplitude was 2.035 G.

Spectra were recorded on a computer interfaced to the spectrometer. Figure 7 presented the production of $^1O_2$ in the HCO_4^-−Eu(II)−dipicolinate CL system.

To chemically detect $^1O_2$, we used 9,10-diphenylanthracene (DPA), which was suitable for reactions in organic phases.[42] This method was based on the rapid and specific reaction of $^1O_2$ with DPA ($k_r = 1.3 \times 10^6 \ M^{-1} \ s^{-1}$) forming a stable DPA endoperoxide ($DPAO_2$).[42] After the reaction, the solution was diluted 1:100. DPA and its endoperoxide ($DPAO_2$) were analyzed by electrospray ionization mass spectrometry analyses. Full-scan data were acquired over a mass range 100−500 *m/z*. The mass spectrum of DPA recorded in the positive mode exhibits a major $[M]^+$ ion at *m/z* = 330, corresponding to the positively charged molecular ion. The spectrum of $DPAO_2$ displayed an intense $[M + H]^+$ ion at *m/z* = 363. The reaction of HCO_4^- and Eu(II) in the presence of 60 mM DPA resulted in the formation of $DPAO_2$. Detection of the anthracene endoperoxide ($DPAO_2$) provided further evidence of the formation of $^1O_2$ in this HCO_4^-−Eu(II)−dipicolinate CL system (Figure 8). For the sake of comparison, we also detected the

$^1O_2$ deriving from the H_2O_2/molybdate[43] and H_2O_2/hypochlorite[44] systems by mass spectrometry. Their results were coincident. These novel observations identified the generation of $^1O_2$ in the reaction of HCO_4^- with Eu(II)/Eu(III)−dipicolinate complex, suggesting a potential $^1O_2$-dependent mechanism.

According to the literature,[45] it can be concluded that $^1O_2$ generated during the reaction included O_2 ($^1\Delta_g$) and O_2 ($^1\Sigma_g^+$), being of higher energy than the gound-state triplet oxygen, which can transfer its excess energy to acceptor (Eu(III)−dipic), thereby engendering CL emission.

$$O_2 \ (^1\Delta_g) + O_2 \ (^1\Delta_g) \rightarrow O_2 \ (^1\Sigma_g^+) + O_2 \ (^3\Sigma_g^-)$$
$$K = 1.3 \times 10^3 \quad (8)$$

$$O_2 \ (^1\Sigma_g^+) + O_2 \ (^1\Delta_g) + [Eu(III)(dipic)]^- \rightarrow$$
$$2O_2 \ (^3\Sigma_g^-) + [Eu(III)(dipic)^*]^- \quad (9)$$

The product of Eu(II) ion oxidized by peroxymonocarbonate is Eu(III) ions. The Eu(III)−dipicolinate complex accepted energy from the singlet oxygen to obtain the excited Eu(III)−dipicolinate complex which, on returning to the ground state, emits radiation of wavelength $\lambda = 615$ nm corresponding to the transitions $^5D_0$−$^7F_2$.[16]

$$[Eu(III)(dipic)^*]^- \rightarrow [Eu(III)^*(dipic)]^- \quad (10)$$

$$[Eu(III)^*(dipic)]^- \rightarrow [Eu(III)(dipic)]^- + h\nu \quad (11)$$

Besides the dominant band at $\lambda = 615$ nm, the CL spectra include a low-intensity band with a maximum at about 490 nm, which can be interpreted as arising from the emission of excited carbonyl groups.[46,47]

4. Conclusions

A simple, rapid, and convenient method to prepare peroxymonocarbonate ions based on the continuous flowing stream was developed. The kinetic and spectroscopic results strongly supported the formation of peroxymonocarbonate ions. The reaction of hydrogen peroxide and bicarbonate to form peroxymonocarbonate proceeded rapidly near neutral pH in ehthanol/water mixtures. Solubility of the bicarbonate catalyst was enhanced by use of NH_4HCO_3 rather than group I salts, which tended to have low solubility in the mixed solvents and could lead to phase separation.

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A high electrode potential for the HCO_4^-/HCO_3^- couple (1.8 ± 0.1 V vs NHE) can induce an ultraweak chemiluminescence in the HCO_4^-−Eu(II) system. When adding dipicolinate into this system, the chemiluminescent emission intensity was efficiently enhanced. The CL mechanism of HCO_4^-−Eu(II)−dipicolinate system was investigated in detail by various methods. Briefly, the peroxymonocarbonate oxidation system was an inexpensive and relatively nontoxic alternative to other oxidants, and our experiments indicated that it can be used in the systems where a mild, neutral pH was required. The potential role of peroxymonocarbonate ions as a reactive oxygen species is worthy of further investigation in view of the kinetic and mechanistic results. Further studies of this type are underway in our laboratory.

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