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# Direct evidence of singlet molecular oxygen [ $O_2 (^1\Delta_g)$ ] production in the reaction of acetonitrile with hydrogen peroxide in alkaline solutions

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## Abstract

In this work, we report direct evidence of the formation of singlet molecular oxygen [ $O_2 (^1\Delta_g)$ ] in the reaction of hydrogen peroxide ( $H_2O_2$ ) with acetonitrile in alkaline solutions. The formation of  $O_2 (^1\Delta_g)$  was characterized by: (i) the dimol light emission in the red spectral region ( $>610$  nm) using a red-sensitive photomultiplier tube (PMT); (ii) the monomol light emission in the near-infrared region (1270 nm) with a newly available tube coupled to a monochromator; and (iii) the quenching effect of sodium azide. Direct spectral characterization of the near-infrared emission that attributes the emission to the transition of  $O_2 (^1\Delta_g)$  to the triplet ground state ( $O_2 (^3\Sigma_g^-)$ ) was done to unequivocally demonstrate the presence of  $O_2 (^1\Delta_g)$ . For comparison,  $O_2 (^1\Delta_g)$  derived from the thermolysis of the endoperoxide of 1,4-dimethylnaphthalene or from the  $H_2O_2$ /hypochlorite system were also monitored. The product of the reaction (acetamide) was also detected by mass spectrometry (MS). This evidence clearly demonstrates that the reaction of  $H_2O_2$  with acetonitrile in alkaline solutions generates  $O_2 (^1\Delta_g)$ , confirming the mechanism proposed by McKeown and Waters.

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**Keywords:** Singlet oxygen; Hydrogen peroxide; Acetonitrile; Red light emission; Near-infrared emission

## 1. Introduction

According to the mechanism proposed by McKeown and Waters [1], singlet molecular oxygen ( $O_2 (^1\Delta_g)$ ) could be generated by the reaction of alkaline hydrogen peroxide ( $H_2O_2$ ) with nitriles. They could measure a low-level chemiluminescence (CL) with a small red component using a photomultiplier tube (PMT) and a red filter. Recently, Lu et al. [2] described in this journal a method for the determination of mela-

tonin by the weak CL emitted when mixed with alkaline aqueous  $H_2O_2$  and acetonitrile. According to their proposal,  $O_2 (^1\Delta_g)$  could be generated by the reaction of the nitrile with alkaline  $H_2O_2$ , which in turn reacted with melatonin causing CL emission. In an earlier paper, Lu's group [3] also postulated the generation of  $O_2 (^1\Delta_g)$  from the reaction of alkaline  $H_2O_2$  with acetonitrile, despite in both works there was no spectroscopic evidence to support the proposal.

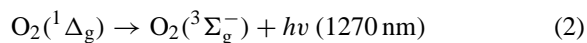
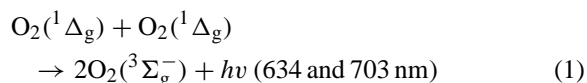
The involvement of  $O_2 (^1\Delta_g)$  with the red luminescence was elucidated by spectroscopic investigations using the reaction between  $H_2O_2$  and hypochlorite by Khan and Kasha [4,5]. They observed the presence of

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two bands at 634 and 703 nm that were attributed to the simultaneous transitions of two molecules of oxygen in the  $^1\Delta_g$  state by Arnold et al. [6]. This reaction was studied in detail by Cahill and Taube [7], which showed that the atoms forming  $O_2$  ( $^1\Delta_g$ ) came from  $H_2O_2$ , and it was demonstrated that in alkaline solutions the yield of  $O_2$  ( $^1\Delta_g$ ) of this reaction is essentially 100% [8]. The transition of a single molecule of  $O_2$  ( $^1\Delta_g$ ) to the ground state occurs in the infrared region of the spectra at 1270 nm, and was investigated by Browne and Ogrysló [9] using the  $H_2O_2$ /hypochlorite system. The lifetime of  $O_2$  ( $^1\Delta_g$ ) in solution is dependent of the nature of the solvent and is longer in a deuterated medium which is reflected directly in the intensity of luminescence [10,11]. Also, some substances are able to reduce the lifetime and CL of  $O_2$  ( $^1\Delta_g$ ), such as sodium azide [12] and histidine [13]. These effects together are commonly used to demonstrate the participation of  $O_2$  ( $^1\Delta_g$ ) in different systems [14].

We studied here the reaction of  $H_2O_2$  with acetonitrile, using: (i) CL measurement of the dimol light emission in the red spectral region ( $\lambda > 610$  nm) using a red-sensitive photomultiplier tube (reaction 1); (ii) CL measurement of the monomol light emission in the near-infrared region (1270 nm) with a new photomultiplier tube coupled to a monochromator (reaction 2); and (iii) the quenching effect of sodium azide. Also, the detection of the reaction product acetamide by mass spectrometry was performed to provide additional evidence of the mechanism for the formation of  $O_2$  ( $^1\Delta_g$ ) in this system.



## 2. Experimental

### 2.1. Dimol light emission of singlet oxygen

Low-level CL was measured with a single-photon counting system as described elsewhere [15,16], equipped with a red-sensitive photomultiplier tube cooled to  $-20^\circ\text{C}$  by a thermoelectric cooler. The potential applied to the photomultiplier tube was

$-1.2$  kV. The phototube output was connected to an amplifier discriminator (Model 1121, Princeton Instruments, NJ) and to the computer for data acquisition. Selective light emission at wavelengths  $>610$  nm was obtained using a cut-off filter (Melles Griot visible filters 03FCG101) placed between the cuvette and the PMT. Sample solutions were poured into a thermostat-equipped glass cuvette ( $35 \text{ mm} \times 6 \text{ mm} \times 55 \text{ mm}$ ) with mirrored walls, with temperature adjusted to  $40^\circ\text{C}$ . Typically, 2 ml of 4 or 10 M  $H_2O_2$  was injected into the solution contained in the cuvette (2 ml of pure acetonitrile, 1.8 ml of MilliQ water, and 0.2 ml of 1 M sodium carbonate) using a syringe injection pump (Syringe Pump Model 22, Harvard Apparatus, MA) at a flow rate of  $0.7 \text{ ml min}^{-1}$ .

### 2.2. Monomol light emission and spectral measurements of singlet oxygen in the near-infrared region

The  $O_2$  ( $^1\Delta_g$ ) monomol light emission spectrum was measured with a special photocounting apparatus developed in our laboratory, equipped with a monochromator capable of selecting emissions in the near-infrared region (800–1400 nm). The apparatus consists of a photomultiplier tube (RSS09 PMT, Hamamatsu Photonics KK, Shizuoka, Japan) cooled to  $-80^\circ\text{C}$  with liquid nitrogen (S600 PHOTOCOOL<sup>TM</sup>, PC176TSCE005 cooler, Products for Research Inc., MA) to reduce the dark current. The power was provided by a high voltage dc power supply (Model C3360, Hamamatsu) and the applied potential was set to  $-1.5$  kV. The light emitted from the sample was processed through a monochromator (M300, Edinburgh Analytical Instruments, Livingston) equipped with a diffraction grating capable of selecting wavelengths in the infrared region. A silicon filter was used (Spectrogon UK Ltd., Glenrothes, UK). The PMT output was connected to the computer and the signal acquired. The monochromator was controlled and the data acquired using the F-900 ver. 6.22 software program (Edinburgh Analytical Instruments). Typically, 3–5 scans in the range 1200–1350 nm were recorded and averaged to yield the spectrum. The assay was conducted in a thermostated quartz cuvette ( $10 \text{ mm} \times 10 \text{ mm} \times 30 \text{ mm}$ ) with continuous stirring (CUV-O-STIR<sup>®</sup>, model 333, Hellma).

Monomol light emissions were also measured using the monochromator fixed at 1270 nm, using the equipment described. The experiments were typically carried out by adding 1 ml of acetonitrile, 0.9 ml of water and 0.1 ml of 1 M NaCO<sub>3</sub> or NaOH into a cuvette, and injecting 1 ml of 4 M H<sub>2</sub>O<sub>2</sub> (1.33 M final concentration) after 50 s recording light out, put by means of a syringe injection pump at a flow rate of 0.7 ml min<sup>-1</sup>.

### 2.3. LC/electrospray ionization mass spectrometry analysis of the acetamide product

Acetamide was determined by liquid chromatography (LC) electrospray ionization (ESI) mass spectrometry (MS) in the positive ion mode using a Platform II mass spectrometer (Micromass, Altrincham, UK). The source temperature of the mass spectrometer was kept at 100 °C, and the flow rates of drying and nebulizing gas were optimized at 300 and 15 l h<sup>-1</sup>, respectively. The cone voltage was set to 20 V. The capillary potential and high electrode potential were set to 3.0 and 0.5 kV, respectively. Full scan data were acquired over a mass range of 40–100 *m/z*. The data were pro-

cessed by means of the Mass Lynx NT data system, version 3.20 (Micromass).

## 3. Results and discussion

### 3.1. Light emission detection of singlet oxygen

The measurement of CL originating from radiative transition of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>) to its ground state is an important method for the detection and characterization of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>). Two types of CL derive from O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>): dimol emission and monomol emission. In the experiment, the mixture of H<sub>2</sub>O<sub>2</sub> and acetonitrile in alkaline solutions produced a rapid increase in light emission in the wavelength range corresponding to monomol and dimol emission of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>) (see reactions 1 and 2). As illustrated in Fig. 1, trace b, the injection of 2 ml of 10 M H<sub>2</sub>O<sub>2</sub> into a 4 ml alkaline solution of 50% acetonitrile produced a strong emission in the red region (λ > 610 nm). Using the special photocounting apparatus, the monomol light emission at 1270 nm was also observed in the reaction of alkaline H<sub>2</sub>O<sub>2</sub> with acetonitrile, as shown in Fig. 2, trace b.

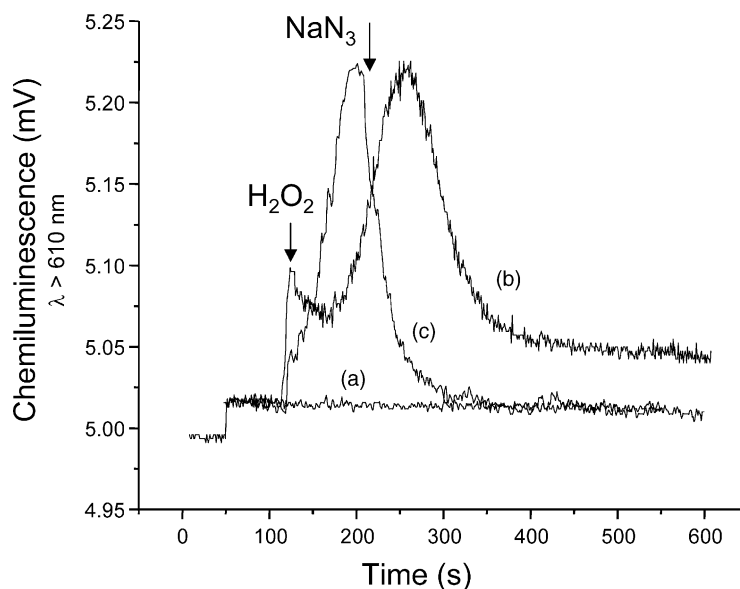


Fig. 1. Dimol light emission of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>) generated in the reaction of alkaline H<sub>2</sub>O<sub>2</sub> and acetonitrile: (a) control without H<sub>2</sub>O<sub>2</sub>; (b) injection of 2 ml of 10 M H<sub>2</sub>O<sub>2</sub> (after 100 s) into 4 ml of 50% acetonitrile in water containing 0.2 ml of 1 M Na<sub>2</sub>CO<sub>3</sub>; (c) injection of sodium azide (to give a 71 mM final concentration into the cuvette) 100 s after the injection of the H<sub>2</sub>O<sub>2</sub>, at the maximum signal as in (b). Injection rate was set to 0.7 ml min<sup>-1</sup>.

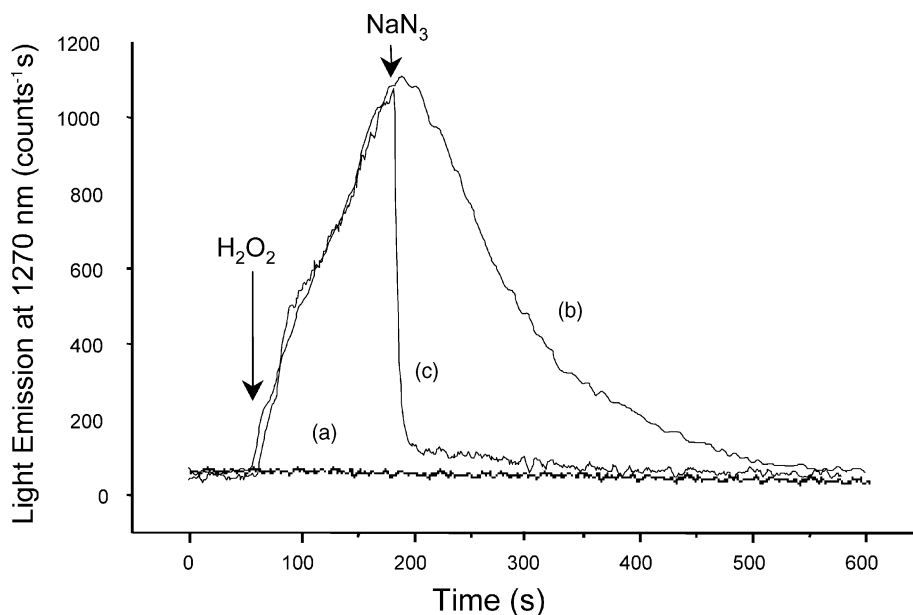


Fig. 2. Monomol light emission of  $O_2$  ( $^1\Delta_g$ ) generated in the reaction of alkaline  $H_2O_2$  and acetonitrile (a); control without  $H_2O_2$ ; (b) injection of 1 ml of 4 M  $H_2O_2$  after 50 s into 2 ml of 50% acetonitrile in water containing 0.1 ml of 1 M  $Na_2CO_3$ ; (c) injection of sodium azide (to give a 1.7 mM final concentration in the cuvette) 100 s after of the  $H_2O_2$  injection, at the maximum signal as in (b). Injection rate was set to  $0.7 \text{ ml min}^{-1}$ .

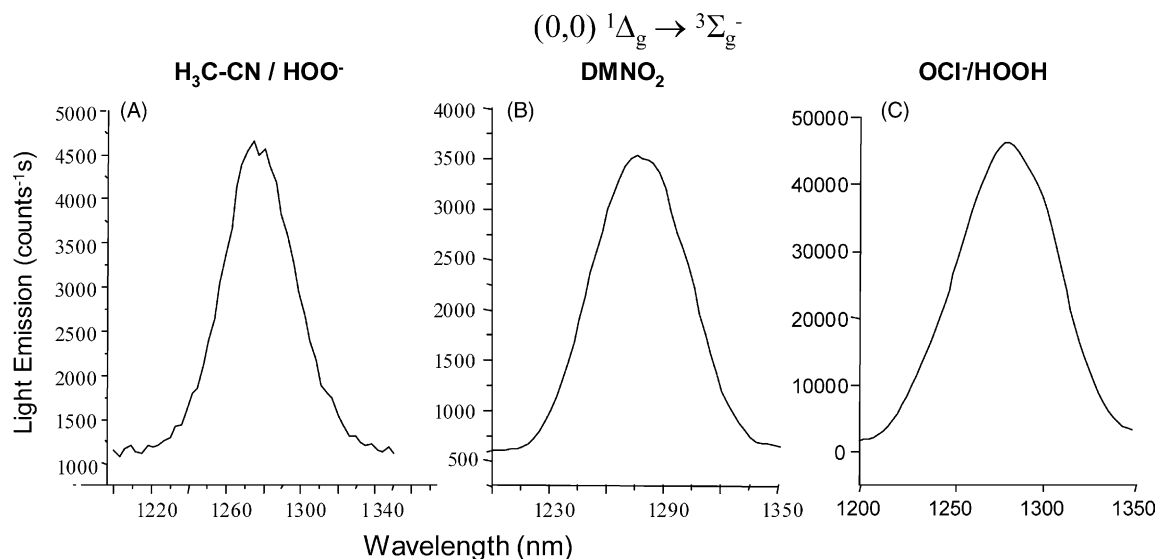


Fig. 3. Monomol light emission spectrum of  $O_2$  ( $^1\Delta_g$ ) generated in the reaction of alkaline  $H_2O_2$  and acetonitrile recorded in the near-infrared region 1200–1350 nm. (A), alkaline  $H_2O_2$ /acetonitrile: 1 ml of 4 M  $H_2O_2$  was injected into 2 ml of 50% acetonitrile in water containing 0.1 ml of 1 M  $Na_2CO_3$  at a flow rate of  $0.7 \text{ ml min}^{-1}$ ; (B), thermodissociation of  $DMNO_2$  (15 mM in chloroform) at  $40^\circ\text{C}$ ; (C),  $H_2O_2$ /hypochlorite, 2 ml of hypochlorite (0.3 M) was injected into 1 ml of  $H_2O_2$  (3 M) at a flow rate of  $1.4 \text{ ml min}^{-1}$ .

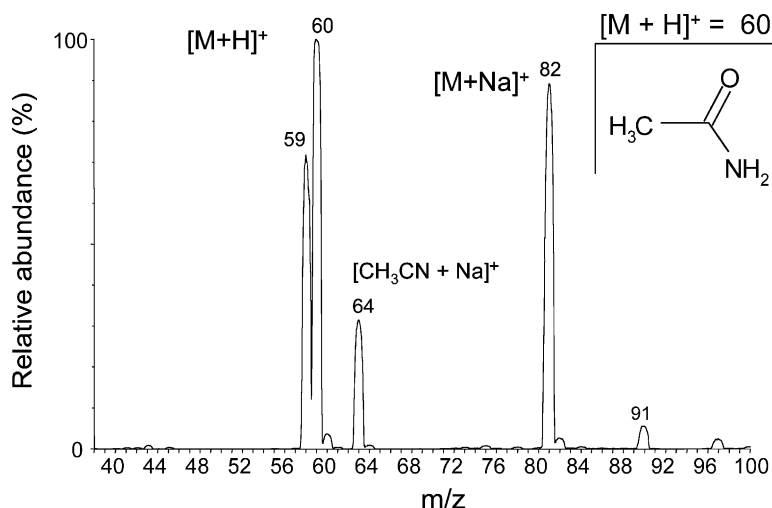
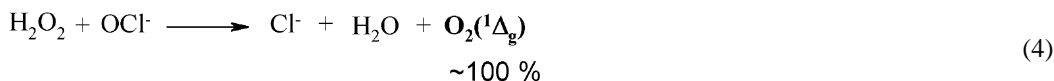
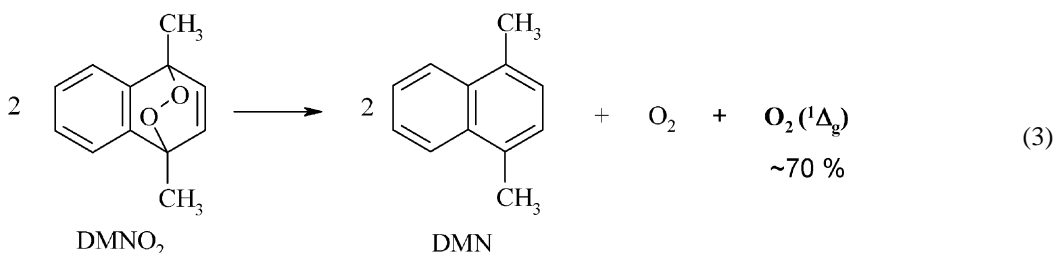


Fig. 4. Mass spectrum after the reaction of  $\text{H}_2\text{O}_2$  and acetonitrile in alkaline solution.

To further characterize the generation of  $\text{O}_2 (^1\Delta_g)$  in this reaction, the effect of sodium azide on CL intensity was examined using both the red PMT detector (Fig. 1, line c compared with line b) and the special monochromator coupled to a highly sensitive PMT (Fig. 2, line c compared with line b). The CL signal intensity was effectively quenched by sodium azide, which is a strong indication that  $\text{O}_2 (^1\Delta_g)$  is the emitter. Additionally, neither acetonitrile (Fig. 1a) nor  $\text{H}_2\text{O}_2$  elicited CL when present alone.

the infrared emission spectrum of  $\text{O}_2 (^1\Delta_g)$  produced by the reaction of alkaline  $\text{H}_2\text{O}_2$  with acetonitrile (Fig. 3A). The emission spectra of  $\text{O}_2 (^1\Delta_g)$  generated in the thermodissociation of  $\text{DMNO}_2$  [17] (reaction 3, Fig. 3B) and the  $\text{H}_2\text{O}_2$ /hypochlorite system [8] (reaction 4, Fig. 3C) were also recorded for comparative purposes. As expected, an emission maximum at 1270 nm, characteristic of  $\text{O}_2 (^1\Delta_g)$ , was observed in all the systems tested, confirming the generation of  $\text{O}_2 (^1\Delta_g)$  in the reaction of  $\text{H}_2\text{O}_2$  and acetonitrile in alkaline solutions.

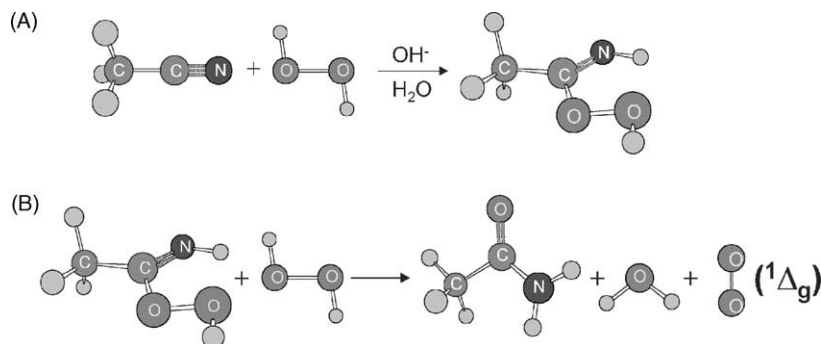


### 3.2. Singlet oxygen spectrum

Besides the direct kinetic detection of the monomol emission of  $\text{O}_2 (^1\Delta_g)$  at 1270 nm, we also recorded

### 3.3. Detection of the reaction product acetamide by mass spectrometry

The incubation of  $\text{H}_2\text{O}_2$  and acetonitrile in alkaline media resulted in the formation of acetamide. The



Scheme 1. Reaction of acetonitrile with hydrogen peroxide in alkaline media generating acetamide, water and singlet molecular oxygen.

reaction mixture was analyzed by electrospray ionization MS. The mass spectrum of the product acetamide recorded in the positive mode exhibits a major  $[\text{M} + \text{H}]^+$  ion at  $m/z = 60$ , corresponding to the positively charged molecular ion (Fig. 4). The spectrum also displays an intense  $[\text{M} + \text{Na}]^+$  ion at  $m/z = 82$  and  $[\text{CH}_3\text{CN} + \text{Na}]^+$  ion at  $m/z = 64$  (Fig. 4).

#### 4. Conclusions

Spectroscopic and chemical evidence demonstrate singlet oxygen formation during the reaction of  $\text{H}_2\text{O}_2$  with acetonitrile in alkaline solution. According to the mechanism proposed by Wiberg in 1953 for oxygen production, we can expect first a nucleophilic attack by the peroxide anion ( $\text{OOH}^-$ ) on the carbon of the nitrile (Scheme 1A), followed by a reaction with a second molecule of  $\text{H}_2\text{O}_2$  generating acetamide, water and  $\text{O}_2(^1\Delta_g)$  (Scheme 1B). Taken together, these novel observations serve as important evidence of  $\text{O}_2(^1\Delta_g)$  production in the reaction of  $\text{H}_2\text{O}_2$  and acetonitrile in alkaline solution, as initially proposed by McKeown and Waters [1].

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