

## DETERMINATION OF HYDROGEN PEROXIDE IN RIVER WATER BASED ON CATALYTIC EFFECT OF TiO<sub>2</sub> NANO PARTICLE ON PEROXY OXALATE CHEMILUMINESCENC OF TRIAZIN DYE DERIVATIVE

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### ABSTRACT

This work is first report showed that supported TiO<sub>2</sub> NPs on alumina could act catalytic activity on peroxyoxalate chemiluminescence system. In this work the catalytic effect of supported Titanium dioxide nanoparticles (TiO<sub>2</sub> NPs) on the trichloroperoxy oxalate – hydrogen peroxide (PO-CL) reaction is investigated. Supported TiO<sub>2</sub> nanoparticles exhibited the better CL catalysis activity than SnO and Carbon nanotubes. Supported TiO<sub>2</sub> nanoparticles catalyze the chemiluminescence reaction between TCPO (bis-(2,4,6-trichlorophenyl) oxalate) and H<sub>2</sub>O<sub>2</sub> and produce a strong CL signal in the presence of triazinyl dye derivative as fluorophore. The CL signal intensity was linear with the hydrogen peroxide concentration in the range of 5 to 1000 nmol ml<sup>-1</sup> with a detection limit of 1.2 nmol ml<sup>-1</sup> and regression coefficient was 0.992 (n=4). The relative standard deviations for measurement of 300 and 500 nmol ml<sup>-1</sup> hydrogen peroxide were 2.2 and 3.4%, respectively(n=4). Hydrogen peroxide concentration in river water was 8.7 nmol ml<sup>-1</sup> with RSD=5.4%(n=3).

**KEYWORDS:** Peroxyoxalate Chemiluminescence; Hydrogen peroxide ; TiO<sub>2</sub> nanoparticles.

### INTRODUCTION

The determination of hydrogen peroxide is of great importance in biochemistry, environmental fields and clinical control (Guilbault, 1970; Tijssen, 1985). Many biological substances produce H<sub>2</sub>O<sub>2</sub> in biochemical reactions catalyzed by various enzymes, so they can be determined indirectly by the determination of H<sub>2</sub>O<sub>2</sub> (Ci and Wang, 1991). A lot of methods have been proposed for its determination including spectrophotometry (Possanzini and Di Palo, 1995; Tanner and Wong, 1998), fluorimetry (Chen *et al.*, 1999; Qin *et al.*, 1998), high performance liquid chromatography (Wang and Glaze, 1998), and amperometry (Madsen and Kromis, 1984). Chemiluminescence method is commonly used in the determination of hydrogen peroxide because of its low detection limit and wide dynamic range that can be achieved with relatively simple instrumentation (Robards and Worsfold, 1992).

However, established systems for analytical purposes fall into one of a limited number of types, such as luminol (Zhou *et al.*, 1999), dimethylbisacridinium nitrate (lucigenin) (Kamidate *et al.*, 1995), bis-(2,4,6-trichlorophenyl)oxalate (TCPO) (Zhi *et al.*, 1998) and pyrogallol (Nakano *et al.*, 1993). Furthermore, these systems lack selectivity due to the possible interferences from catalyzing actions of many metal ions (Co<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>3+</sup>, Cr<sup>3+</sup> and Ni<sup>2+</sup> for luminol-H<sub>2</sub>O<sub>2</sub> system).

In recent years there has been much interest in using nanoparticles in many branches of science and technology (Duan *et al.*, 2008; Zhang *et al.*, 2005). This could be attributed to the unique chemical and physical properties of nanoparticles. In chemical studies, the role of these species is to catalyze reactions between reactants. In fact the nanoparticles play the nanocatalyst role in some reactions such as peroxyoxalate chemiluminescence. In this work we used supported TiO<sub>2</sub> NPs that was patented in Iran (Taghavinia and Bazaz, 2009).

The triazin dyes have been proven to be very useful in the study of environmental and biological samples. This triazin dye was reported as optical brightener (Um *et al.*, 2005). Several analytical and clinical applications of triazin dye as optical brightener and fluorophore have also been reported (Fujino *et al.*, 2000). A new triazin dye has been synthesized (Safaei-Ghomi *et al.*, 2003) (Figure 1). We report first study of chemiluminescence arising from the reaction of bis(2,4,6-trichlorophenyl)oxalate (TCPO) with hydrogen peroxide in the presence of this triazin dye (Shamsipur *et al.*, 2007a; 2007b). In this work we report first study for determination of hydrogen peroxide in river water based on this new POCL system and catalytic effect of TiO<sub>2</sub> NPs.

The chemiluminescence arising from the reaction of bis(2,4,6-trichlorophenyl)oxalate (TCPO) with hydrogen peroxide in the presence of triazin dye have been studied. Also the optimum concentrations of all reagents were achieved from previous studies and then effect of TiO<sub>2</sub> NPs concentrations on POCL was investigated. (Shamsipur *et al.*, 2007b).

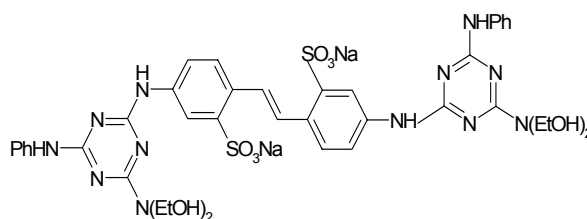


Figure 1. Structure of triazin dye

In this work based on the catalytic effect of supported TiO<sub>2</sub> NPs a new, rapid, simple, sensitive and inexpensive method was proposed for the determination of hydrogen peroxide. The proposed CL system was applied for the determination of hydrogen peroxide in river water samples.

## EXPERIMENTAL

### Apparatus

The CL and fluorescence signal intensities were measured using a Varian Cary Eclipse fluorescence spectrofluorimeter equipped with a xenon-arc source and standard quartz cells with 10 mm path length. All measurements were performed at the room temperature.

### Materials

Analytical reagent-grade chemicals were used without further purification. TCPO was purchased from Acros Organics Co. Hydrogen peroxide, ethyl acetate; ethanol and all other chemicals were purchased from Merck. NPs TiO<sub>2</sub> (P-25) was purchased from Degussa.

An aqueous TiO<sub>2</sub> suspension was prepared by adding TiO<sub>2</sub> nanoparticulates to a 60 ml acidic solution (pH=3.5) and 190 ml ethanol was added and then provided alumina support wash coated with this slush. These segments were taken out and dried under room conditions at 30 °C constant temperature during 24 hour. Homogenous powder was prepared from these segments then a suspension solution of this powder was used directly. When we transferred all reagents in cuvette except triazin dye, there are not chemiluminescence and fluorescence emission.

A  $1.0 \times 10^{-2}$  M stock solution of TCPO (bis-(2,4,6-trichlorophenyl) oxalate) was prepared by dissolving sufficiency of it in ethyl acetate. Working solutions of TCPO were prepared by diluting the stock solution with appropriate amounts of ethyl acetate solution. Stock solution of hydrogen peroxide ( $1.0 \times 10^{-2}$  mol L<sup>-1</sup>) was prepared by dissolving appropriate amount of it in ethanol and stored at 4 °C in the refrigerator. Working standard solutions of H<sub>2</sub>O<sub>2</sub> were freshly prepared from the stock solution.

This study was done with using a Varian Cary Eclipse fluorescence spectrofluorimeter. In chemiluminescence mode xenon-arc lamp was off automatically. This was done in all spectrofluorimeter systems automatically. Slit band width was 2 and 10 nm at fluorescence and chemiluminescence mode respectively. The fluorescence spectrum of triazin dye was recorded in the absence of hydrogen peroxide, TCPO and TiO<sub>2</sub> NPs. Solution A was made with adequate TCPO, Triazin and TiO<sub>2</sub> NPs. Solution B contained adequate hydrogen peroxide. Solution A was transferred into the instrument quartz cuvette via polypropylene syringes. Then solution B was injected in the quartz cuvette and the chemiluminescence spectrum was recorded immediately.

In fluorescence mode, steady-state fluorescence spectra were recorded. The excitation and emission monochromators were set at 390 and 430 nm, respectively. A spectral bandwidth of 2 nm was used. In chemiluminescence mode time-resolved luminescence measurements were carried out. The temperature was set at 25.0° C. Solution A was made with 1.0 ml of TCPO (0.01 M), 0.5 ml of Triazinyl (0.001 M) and 0.5 ml of catalyst (0.015 g L<sup>-1</sup>). Solution B contained hydrogen peroxide 3.0 M. Solution A was transferred into the instrument quartz cuvette via polypropylene syringes. Then 100 µl of B stock solution was injected in the quartz cuvette and the chemiluminescence spectrum was recorded. In the case of hydrogen peroxide determination, river water that has hydrogen peroxide was used as solution B and was injected in the quartz cuvette finally and the chemiluminescence was recorded. Based on previous chemiluminescence study on POCL of Triazin dye, optimized concentration of TCPO, H<sub>2</sub>O<sub>2</sub> and Triazin were acquired.

## RESULTS AND DISCUSSION

Since the first discovery by Chandross of the development of a strong chemiluminescence from the reaction between oxalylchloride, hydrogen peroxide and various fluorescent compounds (Chandross, 1963), the so-called peroxyoxalate-chemiluminescence (PO-CL) has received an increasing attention (Shamsipur and Chaichi, 2003). In this type of chemiluminescence, the reaction between hydrogen peroxide and an activated derivative of oxalic acid can produce chemiluminescence in the presence of a fluorophore (Stigbrand *et al.*, 1996). This reaction has successfully been used as a highly sensitive detection technique in several procedures developed for low level determinations of a wide variety of analytes including a range of different fluorophores (Bowie *et al.*, 1996), fluorophore labeled amino acids as well as different quencher species (Nakamura *et al.*, 2000).

Decomposition of hydrogen peroxide on TiO<sub>2</sub> surfaces under visible irradiation ( $\lambda > 420$  nm) was investigated, and an interesting result was found that small organic compounds such as salicylic acid were degraded in the presence of TiO<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> under visible irradiation (Li *et al.*, 2001).

The supported TiO<sub>2</sub> nanoparticles- can be enhance CL intensity, so we could ascribe that TiO<sub>2</sub> nanoparticle could catalyze the decomposition of H<sub>2</sub>O<sub>2</sub> to produce some reactive intermediates such as hydroxyl radical and superoxide anion. These species could react with TCPO to produce radical TCPO and enhance the CL signal. In the solution cantoning of supported TiO<sub>2</sub> and TCPO without fluorophore the fluorescence and chemiluminescenc intensity are very low and negligible. In the optimum concentration of reagents without supported TiO<sub>2</sub> nanoparticles, in POCL system with TRIAZ as fluorophore, chemiluminescenc intensity was shown but in the presence of TiO<sub>2</sub> NPs, this signal can be enhance and rise up to 3 folds.

Our preliminary experiments revealed that the addition of excess amount of hydrogen peroxide to an ethyl acetate solution containing 2.0×10<sup>-4</sup>M TCPO and 1.5×10<sup>-4</sup>M TRIAZ (green-yellow) resulted in the emission of a very intense blue light (Shamsipur *et al.*, 2007b). The chemiluminescence and fluorescence emission spectrum of the TCPO-TRIAZ system was recorded in the ethyl acetate solution. As shown in Figure 2, the fluorescence and chemiluminescence spectrum have good correspondence. It is finding that the fluorescence emission and chemiluminescence spectra have a peak in 429.0 nm.

The CL emission spectrum of the TCPO-TRIAZ-H<sub>2</sub>O<sub>2</sub> system was recorded in the absence and presence of supported TiO<sub>2</sub> NPs. As can be seen from Figure 3, the CL intensity in the absence of supported TiO<sub>2</sub> NPs was 375.0 a.u. but in the presence of different concentrations of supported TiO<sub>2</sub> NPs, the CL intensity increased. This increase is depending on concentration of embedded supported TiO<sub>2</sub> NPs (Figure 3).

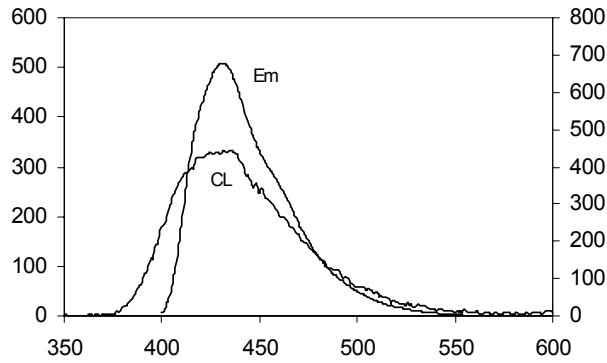


Figure 2. Fluorescence and chemiluminescence spectra of TRIAZ.  $\lambda_{\max}$  of fluorescence emission and chemiluminescence spectrum is 429.0 nm

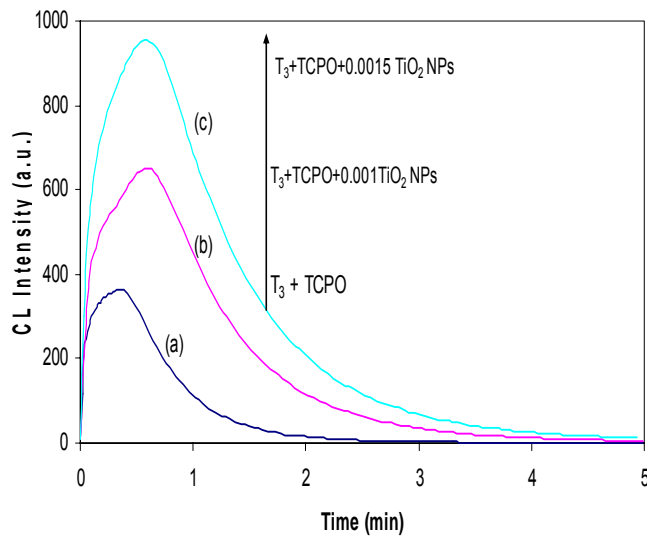


Figure 3. Chemiluminescence kinetic profiles of TCPO-TRIAZ- $\text{H}_2\text{O}_2$  system mixed with various concentrations of supported  $\text{TiO}_2$  NPs. The concentrations of supported  $\text{TiO}_2$  NPs are: (a) 0.0, (b)  $0.001 \text{ g L}^{-1}$ , (c)  $0.0015 \text{ g L}^{-1}$

Thus we can determine hydrogen peroxide in real samples with this proposed chemiluminescence method. The CL spectrum of TCPO-TRIAZ- supported  $\text{TiO}_2$  NPs system in the presence of different amounts of  $\text{H}_2\text{O}_2$  is presented in Figure 4.

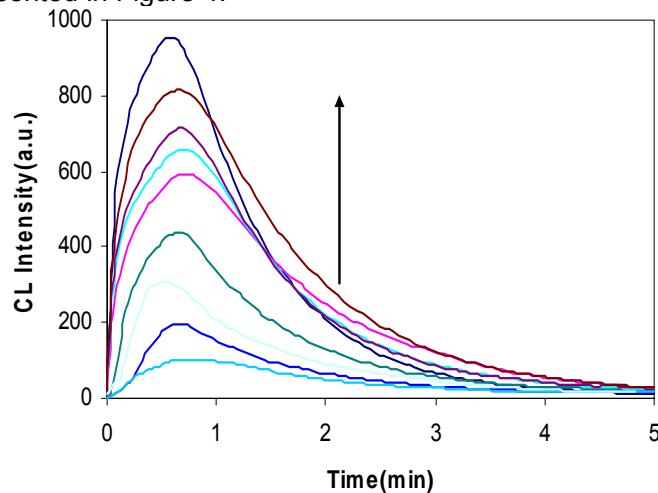


Figure 4. Intensity-time profiles of the TCPO light reaction with different concentrations of hydrogen peroxide catalyzed by supported  $\text{TiO}_2$  NPs. TRIAZ  $1.5 \times 10^{-4}$ , TCPO  $2.0 \times 10^{-4} \text{ mol L}^{-1}$ .  $\text{TiO}_2$  NPs is  $0.0015 \text{ g L}^{-1}$ . (Regression coefficient was 0.992 in calibration curve)

### Tolerance of foreign substances

The water of this river was product from snow melting. There are not industrial pollutant factory in the river area. So the river water is free from common pollution. We did a operation research on the chemical and microbiological quality of this river water for Hamedan Province Water and Waste water Company (2011). We obtained that the concentration of metal ions such as iron and manganese were very low. The mean value of iron and manganese were determined 0.166 and 0.367 ppm respectively with spectrophotometric standard methods. The mean electrical conductance of water was obtained 81.55  $\mu\text{s cm}^{-1}$ . All measurements were performed in a one year period at 2009-2010 years. As the results has shown concentration of metal ions in this river water were very low, therefore we recognize that, determination of metal ions interferences are not necessary for this environmental sample. Application possibility of reported method for determination of hydrogen peroxide in biological samples was investigated with some compound interference reported.

The influence of various compounds and amino acids such as cholesterol, cystein, hystidine, imidazole, lactose, starch, sucrose, albumin and citric acid were tested. The results are listed in Table 1. Cystein, imidazole, lactose and starch are shown an obviously effect with this assay, but cholesterol, sucrose, albumin and citric acid have a little effect. The all of these compounds in the tested concentration, have negative effect on chemiluminescence intensity except sucrose.

Table 1. Effect of interference substances

Interference	Concentration	$\Delta\text{Int} = \text{Int} - \text{Int}_0$	$(\Delta \text{Int} / \text{Int}_0) \times 100$
Cholesterol	$1.0 \times 10^{-4}$	-54	-7.53
Cystein	$1.0 \times 10^{-4}$	-150	-23.54
Hystidine	$1.0 \times 10^{-4}$	-67	-8.59
Imidazole	$1.0 \times 10^{-4}$	-198	-25.24
Lactose	$1.0 \times 10^{-4}$	-93	-14.17
Starch	$1.0 \times 10^{-4}$	-176	-21.62
Sucrose	$1.0 \times 10^{-4}$	11	1.61
Albumin	$1.0 \times 10^{-4}$	-3	-0.48
Citric Acid	$1.0 \times 10^{-4}$	-21	-2.85

Int : chemiluminescence intensity in the presence of interference

Int<sub>0</sub> : chemiluminescence intensity in the absence of interference

### Determination of H<sub>2</sub>O<sub>2</sub> in river water samples

The method was applied for the determination of H<sub>2</sub>O<sub>2</sub> in river water samples. We add a small amount of H<sub>2</sub>O<sub>2</sub> to the water and determine the amount of hydrogen peroxide in real samples. Hydrogen peroxide in river water samples determined 8.7 nmol ml<sup>-1</sup> with RSD=5.4 %(n=3). Recovery test results are shown in table 2. By the result of this table we can conclude that the present method seems useful for the determination of H<sub>2</sub>O<sub>2</sub> in similar real environmental samples.

Table 2. Recovery test of hydrogen peroxide in river water samples

Sample no.	Added (nmol ml <sup>-1</sup> )	Found (nmol ml <sup>-1</sup> )	Recovery (%)	RSD (n=4, %)
1	300	301	100.3	3.2
2	320	323	101.3	2.3
3	430	428	99.5	2.9
4	540	537	99.4	3.4
5	550	556	101.0	2.2

### CONCLUSION

This work is first report that showed supported TiO<sub>2</sub> NPs on alumina porous could act as a nanocatalyst on the TCPO-TRIAZ-H<sub>2</sub>O<sub>2</sub> system to generate CL. It was suggested that the chemiluminescence of the TCPO-TRIAZ-H<sub>2</sub>O<sub>2</sub> system was due to catalytic ability of supported TiO<sub>2</sub> NPs to decompose of hydrogen peroxide to reactive species such as to hydroxyl radical. The Abas

Abad river water was used as a real environmental sample. This method can be used successfully for other similar real environmental samples.

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