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A chemiluminescence method for the detection of electrochemically generated H_2O_2 and ferryl porphyrin

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Abstract

Electrochemical formation of H_2O_2 and the subsequent ferryl porphyrin were examined by measuring luminol chemiluminescence and absorption spectrum using flow-injection method. Emission was observed under the cathodic potential (0.05 V at pH 2.0 and −0.3V at pH 11.0) by the electrochemical reduction of buffer electrolytes solution but no emission was observed at anodic potentials. Fe^{III}TMPyP solution was added at the down stream of the working electrode and was essential for the emission. Removal of dissolved O_2 resulted in the decrease of emission intensity by more than 70%. In order to examine the lifetime of reduced active species, delay tubes were used in between working electrode and Fe^{III}TMPyP inlet. Experimental results suggested the active species were stable for quite long. The emission was quenched considerably (>90%) when hydroperoxy catalase was added at the down stream of the working electrode whereas SOD had little effect. Significant inhibition of the emission by the addition of alkene at the down stream of the Fe^{III}TMPyP inlet was considered as evidence of oxo-ferryl formation. The spectra at reduction potential under aerated condition were shifted to the longer wavelength (>430 nm) compared to the original spectrum of Fe^{III}TMPyP (422 nm). All the spectra were perfectly reproduced by a combination of Fe^{III}TMPyP and $O{=}Fe^{IV}$TMPyP (438 nm) spectra. These observations lead to the conclusion that H_2O_2 was produced first by electrochemical reduction of O_2, which then converted Fe^{III}TMPyP into $O{=}Fe^{IV}$TMPyP to activate luminol. The current efficiencies for the formation of H_2O_2 were estimated as about 30–65% in all over the pH.

Keywords: Iron porphyrin; H_2O_2 formation; Luminol chemiluminescence; Electrochemical reduction

1. Introduction

High-valent oxo-iron porphyrins are commonly proposed as an intermediate in the catalytic mechanisms of peroxidase, cytochrome *P*-450 and cytochrome oxidase enzymes [1,2]. In general, ferryl porphyrins could be achieved either by the oxygenation of ferrous porphyrins [3] or by the chemical oxidation of ferric porphyrins [4]. It has been suggested that the reactions of iron(III) porphyrin complexes with hydrogen peroxide or alkyl hydroperoxides form oxo-iron(IV) porphyrin species via a O–O bond homolysis [5,6] and an iron(IV) radical cation by the bond heterolysis [7]. Recently the reaction mechanism of water soluble Fe^{III}TMPyP with H_2O_2 and the catalytic activity of the reaction intermediates on chemiluminescent peroxidation of luminol has been studied by a group of our laboratory [8]. It can also be generated by electrochemical oxidation of the corresponding hydroxy-iron(III) porphyrins [9,10]. We have been working on the formation of ferryl porphyrin by both electrochemical oxidation and reduction of Fe^{III}TMPyP and it was detected successfully by measuring luminol chemiluminescence [11]. But chemiluminescence was also observed while only buffer electrolytes solution had undergone electrochemical reduction. We assume the electrochemical reduction of dissolved molecular oxygen to hydrogen peroxide followed by its reaction with Fe^{III}TMPyP to produce ferryl porphyrin was responsible for the emission.

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Electrochemical reduction of molecular oxygen to hydrogen peroxide has been extensively studied in aqueous solutions using carbon electrode [12–14]. However, the electrochemical process of hydrogen peroxide formation was limited to high pH where the HO_2^- anion has good stability [15] but recently the methods have also been successfully investigated in aqueous acidic solutions [16]. The same period has seen extensive efforts to develop electrochemical processes for the destruction of organic molecules in effluents and waters [17]. Hydrogen peroxide is however a much more attractive mediator since the reactant can be oxygen from air and any excess hydrogen peroxide eventually decomposes only to oxygen and water, therefore leaving no residual chemicals in the environment. The idea of electrosynthesis of hydrogen peroxide in aqueous medium can be considered as a supplement to those existing washing machines requiring no chemical detergents (Sanyo Electronics, Japan). In this work, we report a systematic investigation on hydrogen peroxide production by electrochemical reduction of molecular oxygen using simple carbon electrode in all possible acidic and ionic solutions. The purpose of this report is to provide timely information and ideas to stimulate thoughtful actions focusing on the resolution of waste management and environmental restoration problems. The simultaneous formation of hydrogen peroxide and their reaction product, oxo-ferryl porphyrin from iron porphyrin, was estimated by a simple and sensitive luminol chemiluminescence method. Ferryl porphyrin was also identified by measuring absorption spectrum. Carboxylic alkene solutions were also employed to estimate the oxo-ferryl formation.

2. Experimental

2.1. Materials

5,10,15,20-Tetrakis (*N*-methyl-4-pyridyl) porphyrin (H_2TMPyP) as sodium salt was purchased from Mid-Century Laboratories and was used without further purification. Hydrogen peroxide (30%, Ishizu Seiyaku Ltd.), luminol (Ishizu Seiyaku Ltd.), cyclopent-2-ene-1-acetic acid (Aldrich Chemical Co.), hydroperoxy catalase (Sigma Chemical Co.), superoxide dismutase (SOD) (Sigma Chemical Co.) and mannitol (Wako Chemical Ind.) were used as received. The other reagents used were of the highest purity available. Deoxygenating was achieved by rigorous bubbling with highly pure N_2 gas.

2.2. Sample preparation

The iron(III) derivative of H_2TMPyP was prepared and the purity of the product was checked according to the standard procedures [15]. The solutions of $Fe^{III}TMPyP$ were prepared in 0.1M Na_2SO_4 aqueous solutions. Buffer solutions (20 mM) were prepared from $HClO_4$, phosphate, carbonate and NaOH for the pH range of 0–14. Solutions of 0.2 mM luminol were prepared in 0.1M Na_2CO_3 aqueous buffer. Supporting electrolytes include 0.1M Na_2SO_4 or $NaClO_4$. The pH values were measured with a TOA HM-20S pH meter.

2.3. Experiments

Chemiluminescence and spectrophotometric experiments were performed with a laboratory constructed flow-injection apparatus, the diagram of which is shown in Fig. 1. Buffer electrolytes solutions of different pH were passed through channel 1 (ch1) for electrochemical oxidation and reduction at working electrode. The electro-generated species traveled through suitable length of delay tubes (0–70 cm) before mixing with $Fe^{III}TMPyP$ solutions, supplied through channel 2 (ch2). Luminol solutions passed through the following channel (ch3), immediately mixed with the reaction products of $Fe^{III}TMPyP$ to give emission. All experiments were carried out at a continuous flow rate of 2.7 ml/min, resulting to the delay time at a maximum of 90 s. The flow of solutions was controlled by a 4-channel Ismatec® Reglo-Digital pump, Model ISM-834.

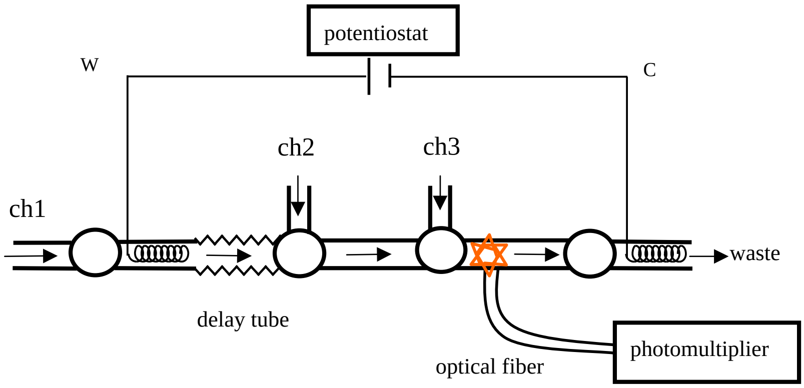

Fig. 1. Schematic diagram of the flow-injection apparatus.

2.3.1. Measurement of chemiluminescence

The intensity of luminol chemiluminescence was detected with a photomultiplier via an optical fiber mounted with an observation cell. Cyclic voltammetry was performed by using a three-electrode cell in which a glassy carbon rod was used as working electrode. A platinum wire was used as the counter electrode and a home-made Ag/AgCl/KCl(sat) electrode as the reference electrode. Cyclic voltammogram and the emission intensity were recorded simultaneously by a computer-supported instrument.

2.3.2. Absorption spectra measurements

Absorption spectra, as a function of potentials were measured with a Hewlett Packard Model 8450A diode array spectrophotometer. The sample was introduced into the spectrophotometric cell through a narrow tube from the down stream of the Fe^{III}TMPyP solutions inlet (ch2 of the flow-injection apparatus in Fig. 1). The sets of spectra were analyzed using software DPR, developed in our laboratory. The number of components in optical spectra was chosen on the basis of the assumed model of acid–base equilibrium system. The contribution of each component in the spectra, as a function of pH and potential, was determined by DPR.

3. Results and discussion

3.1. Luminol chemiluminescence as a function of potential for buffer electrolytes

Luminol chemiluminescence was observed when only buffer electrolytes solution was electrochemically reduced and the iron porphyrin solution was supplied through the down stream of the working electrode (ch2 in Fig. 1). The addition of iron solution was essentially needed for launching emission. Emission observed only when potentials were applied and the intensity was directly proportional to the current production. Cyclic voltammogram showed the reduction potential shifted towards more negative region compared to that of Fe^{III}TMPyP reduction (data not presented). Fig. 2 has shown such an emission pattern for 10 μM Fe^{III}TMPyP in aqueous buffer solution of pH 2.5 as a result of electrochemical reduction of 20 mM buffer solutions. Emission observed by the direct electrochemical reduction of Fe^{III}TMPyP under same conditions was also represented in order to compare the nature of redox potentials and emission intensity.

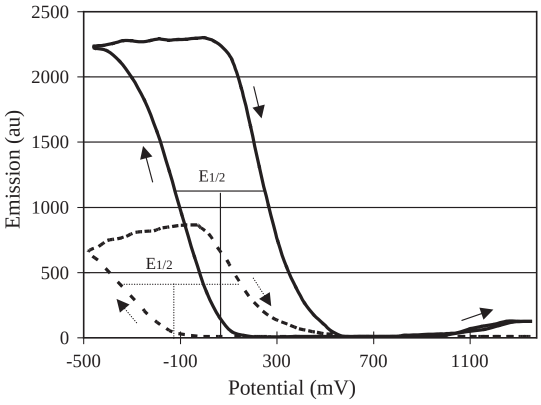

Fig. 2. Emission intensity observed as a function of potential at working electrode for Fe^{III}TMPyP (–) and buffer electrolytes (– – –) in the aqueous solution of pH 2.5. Scan rate 20 mV/s.

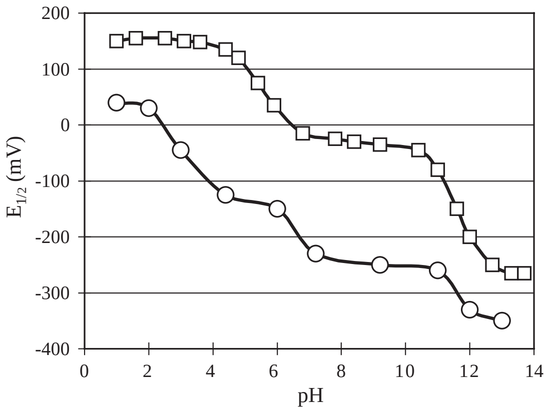

Fig. 3. Effect of pH upon half-wave potentials ($E_{1/2}$) for electrochemical reduction of Fe^{III}TMPyP (□) and buffer electrolytes (○) in aqueous solutions.

Ferryl formation induced by electrochemical reduction of Fe^{III}TMPyP has been confirmed using luminol chemiluminescence in aqueous solutions. Fe^{II}TMPyP, electro-generated by one-electron ($1e^-$) reduction of Fe^{III}TMPyP, was autoxidized in the presence of molecular oxygen to form $O{=}Fe^{IV}$TMPyP [11]. The emission in case of electrochemical reduction of only buffer solutions has been arisen at more negative potential as well as the intensity was differed from that of Fe^{III}TMPyP reduction. As usual, emission was not produced at electrochemically inactive region (100–500 mV) but interestingly no emission was observed at anodic region (>900 mV) while in the case of Fe^{III}TMPyP as expected, it has developed anodic emission. The half-wave potential calculated for both cases revealed that the redox potential for buffer electrolytes shifted towards cathodic side by >100 mV and the trend was continued over the whole pH examined. The comparative values of half-wave potential for the buffer and Fe^{III}TMPyP reduction process as a function of pH have been plotted in Fig. 3. It showed the similar behavior of the curves relative to the buffer and the porphyrin but the potentials for buffer reduction have been shifted towards negative at all pH examined. It may be considered as an indication of at least no electrochemical reduction of Fe^{III}TMPyP took place over there. Moreover, two pKa values were calculated for Fe^{III}TMPyP as 5.7 and 11.7, which are necessarily in agreement with those previous reports [10]. So, the luminol chemiluminescence experienced in this process might be come out as a result of electrochemical reduction of some other species. However, the maximum emission intensity in comparison with that of Fe^{III}TMPyP reduction process was varied by different magnitudes over the whole pH range observed (shown in the Fig. 4).

It has been shown that the emission intensity induced by buffer reduction dropped as the pH increase (pH 3–5) but the emission further intensified very unpredictably in basic aqueous solutions (>pH 7) with a maximum observed at pH 12.0. These results were obtained under aerated conditions and were found largely dependent on the concentration of dissolved molecular oxygen. Luminol chemiluminescence under cathodic condition bears great significance as the intensity increased noticeably at pH<4. This phenomenon has not been reported before but it has been suggested that this higher intensity of chemiluminescence may be generated by cumulative effect of some other species along with oxo-ferryl porphyrin [8]. One possibility is the formation of $Fe^{III/II}$TMPyP redox couples via reduction of Fe^{III}TMPyP by electro-generated hydrogen peroxide. It has also been found that intermediate like compound I, oxo-ferryl porphyrin radical cations are formed via proton-assisted di-oxygen bond cleavage of $Fe^{III/II}$TMPyP couples. This is then transformed into compound II, oxo-ferryl porphyrin [8]. It was further suggested that compound I-like species were formed favorably in acidic conditions and their activities for intensifying luminol emission was much stronger. Therefore, the strong emission experienced in this work at lower pH can be explained essentially by any of the above two observations.

3.2. Effect of O_2 on chemiluminescence

In order to examine the effect of dissolved molecular oxygen on luminol chemiluminescence, buffer electrolytes solutions were deoxygenated by bubbling N_2 gas vigorously. All other conditions were kept the same. Fig. 5 shows the maximum emission intensity for the buffer electrolytes reduction under aerated and deaerated conditions as a function of pH. It is observed that the emission intensity under deaerated conditions decreased by more than 70% through the whole pH range. Although the emission was still observed after N_2 gas bubbling, a trace of the unresolved molecular oxygen somehow would be responsible for the emission. Moreover, the redox potentials obtained for the buffer reduction in this study were closely consistent with those reported for $2e^-$ electrochemical reduction of molecular oxygen to form hydrogen peroxide [18,19]. Therefore, the electrochemical reduction of O_2 or its active species can be taken into consideration for this process. If the emission is considered to be generated by the electrochemical formation of H_2O_2, the big emission at alkaline pH can be explained as it was reported [15,19] that the efficiency of H_2O_2 production by electrochemical reduction of O_2 is higher at alkaline condition. But the big emission observed also at acidic pH is interesting and new to us.

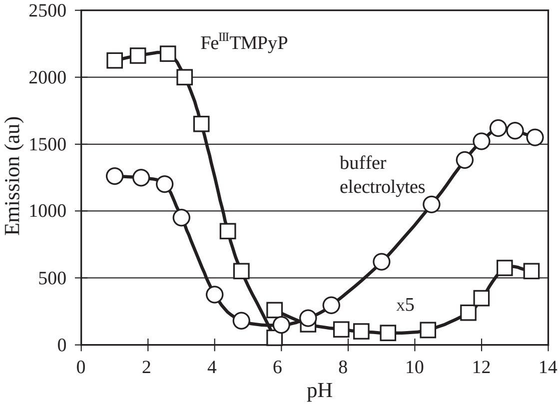

Fig. 4. Emission intensity observed as a function of pH for reduction of Fe^{III}TMPyP (□) and buffer electrolytes (○) under aerated condition.

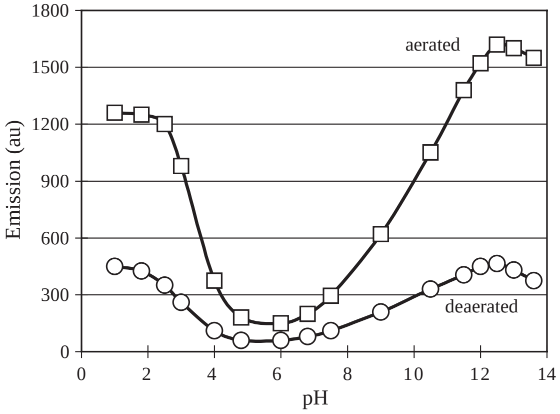

Fig. 5. Maximum emission intensity observed for buffer reduction under aerated (○) and deaerated (□) conditions in different pH buffer solutions.

In order to investigate the development of luminol chemiluminescence, spectroscopic measurements were carried out in various conditions. It was primarily thought that electrochemically generated hydrogen peroxide reacts with Fe^{III}TMPyP to form $O{=}Fe^{IV}$TMPyP which ultimately induced luminol emission. With this view in mind, absorption spectra of 5 μM Fe^{III}TMPyP were measured under aerated and deaerated conditions to assess the role of electro-reduction of O_2 on Fe^{III}TMPyP spectra. The shift of absorption spectra as a function of potential, on aqueous buffer electrolytes solution of pH 12, has been represented in the Fig. 6. It is observed that at aerated condition absorption spectra shifted from 422 to 428 nm at cathodic potentials, indicating the existence of some other active species along with Fe^{III}TMPyP, while the spectra at deaerated condition shifted to an almost insignificant extent. The result is similar to that of emission pattern observed under respective conditions as discussed earlier. So, the electrochemical reduction of molecular oxygen and the reduced active species may be the intermediates in conducting eventual luminol chemiluminescence. The shape of observed spectra under aerated conditions looked to be a composite of Fe^{III}TMPyP ($\lambda_{max}=423$ nm) and $O{=}Fe^{IV}$TMPyP spectra ($\lambda_{max}=438$ nm), similar to that obtained by the direct reduction of Fe^{III}TMPyP (11) or by

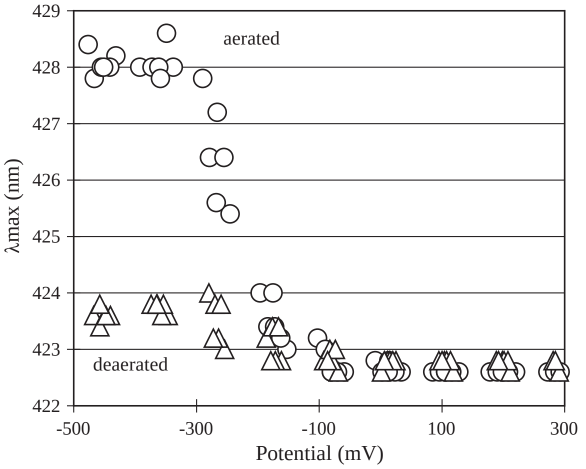

Fig. 6. Shift of wavelength of 5 μM Fe^{III}TMPyP as a function of potential for buffer electrolytes under aerated and deaerated conditions in the pH 12. Scan rate 1 mV/s. Measuring interval 60 s.

the reaction of H_2O_2 with Fe^{III}TMPyP [8]. Actually, the obtained spectra ($\lambda_{max}=428$ nm) were perfectly reproduced by a combination of Fe^{III}TMPyP (72%) and $O{=}Fe^{IV}$TMPyP (28%) spectra at pH 12.0 [8]. Absorption spectrum of each component is shown in Fig. 7.

3.3. Chemiluminescence as a function of delay time

Further chemiluminescence study was carried out as a function of delay tubes at aerated conditions in order to examine the diminishing rate of the electrochemically generated active species. A delay tube of 0∼70 cm long was placed in between the electrode and the inlet of Fe^{III}TMPyP (ch2 in Fig. 1), causing a delay time of 0–90 s before the electrochemically reduced active species mix with supplied iron solutions. Experimental results with three different lengths of delay tube in various pH buffer solutions have been presented in the Fig. 8. The insertion of the delay tubes lowered the emission intensity significantly but not completely. More than 60% and 40% of the original intensity was still observed with the delay time 45 s and 90 s, respectively. This implies that the electrochemically reduced active species should have quite a long lifetime. But except H_2O_2, no probable active species here can exist for so long a period of time because O_2^- or other oxo-anions are supposed to be diminished within 1 s in aqueous solutions [20].

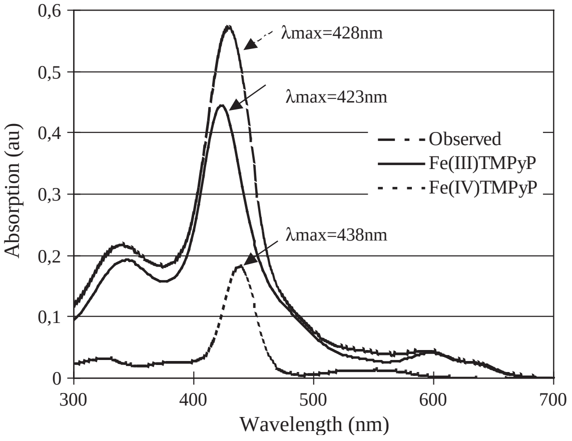

Fig. 7. Deconvolution of absorption spectrum of Fe^{III}TMPyP observed as a result of electrochemical reduction of buffer electrolytes at pH 12.0.

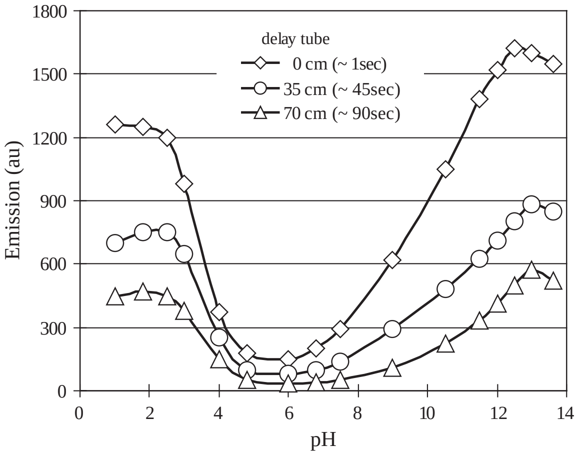

Fig. 8. Maximum emission intensity observed as a function of various delay times under aerated conditions in buffer solutions of different pH.

However, the decrease of emission intensity by about 30% demonstrated that some compounds other than only H_2O_2 might have generated there. In this context, the formation of HO_2^- or the reduction of oxygen to water or that of protons to hydrogen can be taken in consideration. Moreover, the crude surface of the laboratory constructed carbon electrode might be involved in generating some unknown active species i.e. metal ions. Any of them, or in combination with other species, may contribute to the maximum emission in some extent. However, it was assumed that the electrochemical reduction of molecular oxygen into stable peroxy anions or hydrogen peroxide could be the predominant intermediates for the formation of ferryl species. Therefore, further study is required for detecting the active intermediates precisely.

3.4. Effect of catalases on chemiluminescence intensity

In order to examine the generation of H_2O_2 by electrochemical reduction of buffer solutions, an experiment was carried out with hydroperoxy catalase as a catalyst for H_2O_2 decomposition. Catalase at 2000 units/ml solution was added by making another channel in between working electrode and the inlet of iron solutions (ch2) of flow-injection system (shown in Fig. 1). Fe^{III}TMPyP solution (10 μM) and other conditions were kept constant. The emission intensity of iron porphyrin as a function of potential with and without catalase in pH 7.5 aqueous buffer solutions has been shown in the Fig. 9. It is clear from the figure (Fig. 9) that almost no emission was

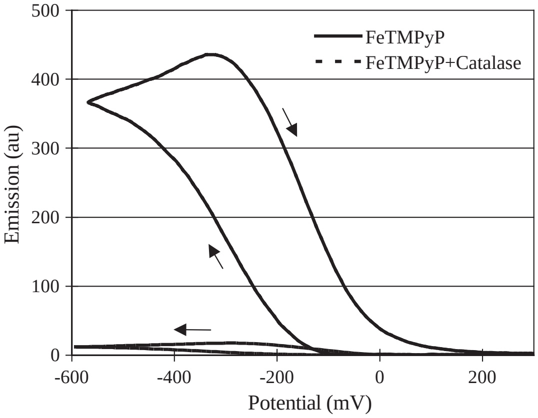

Fig. 9. Emission intensity observed as a function of potential with and without hydroperoxy catalase (2000 units/ml) in the pH 7.5 buffer solutions. Scan rate 20 mV/s.

observed when hydroperoxy catalase was added. Similar results were found in all other pH solutions examined as shown in the Table 1. It shows the emission, as expected, quenched completely in neutral pH (pH 7–9) while in strong acid (pH 2–4) and in alkaline (pH 11) conditions the intensity decreased by about 70–80%, which may be due to the lower activity of catalase in that particular conditions. With the views in mind, experiments were carried out in pH 7.5 to detect the catalytic decomposition rate of electro-generated active species by using various other catalysts (results shown in Fig. 10).

It is observed that emission quenched considerably with the addition of hydroperoxy catalase, which confirmed the formation of H_2O_2. Again, SOD also resulted quenching of emission slightly, indicating there might have been an existence of O_2^- at working electrode but clearly the main product formed in solution was H_2O_2. However, no effect was observed with mannitol on emission, which ensured that there was no hydroxyl radical generated in the process. Thus it can be suggested that the chemiluminescence observed in this process was a result of electrochemical formation of H_2O_2, which then oxidizes iron porphyrin to form oxo-ferryl porphyrin to stimulate luminol.

In this context, it can be mentioned here that the formation of H_2O_2 by electrochemical reduction of O_2 at carbon electrode is a common ancient practice. Various reactions have been reported for electro-reduction of molecular oxygen at different potentials [21,22] depending upon the conditions, but the experimental results in this study suggested that the electrochemical reduction proceeds through 2-electron transfer process because the redox potentials obtained for buffer electrolytes (shown in Fig. 3) were identical with the reported values [18,23] for the 2e$^-$ reduction of molecular oxygen at corresponding pH. Taking all of the above discussions in consideration, the possible reaction mechanisms can be proposed as follows:

$$O_2 + e^- \rightarrow O_2^- \text{(electrochemical reduction of } O_2) \quad (1)$$

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \ \text{(hydroperoxide)} \quad (2)$$

$$\text{Fe}^{\text{III}}\text{TMPyP} + H_2O_2 \rightarrow O{=}\text{Fe}^{\text{IV}}\text{TMPyP} \ \text{(absorption)} \quad (3)$$

$$O{=}\text{Fe}^{\text{IV}}\text{TMPyP} + \text{Lu} + H_2O_2 \rightarrow \ \rightarrow h\nu \ \text{(emission)} \quad (4)$$

$$O_2^- + \text{SOD} \rightarrow 1/2H_2O_2 + 1/2O_2 \ \text{(quenching)} \quad (5)$$

$$H_2O_2 + \text{catalase} \rightarrow H_2O + 1/2O_2 \ \text{(quenching)}. \quad (6)$$

The higher chemiluminescence intensity at alkaline pH may be attributed to the conventional synthesis and stability of H_2O_2 as well as its higher reactivity to form ferryl porphyrin in basic conditions. The intensity of the chemiluminescence response as a function of solution pH has been reported previously [24]. The sensitivity continuously increased with increasing pH and a sensitivity maximum was observed at pH 12. The increasing sensitivity of the luminescence response up to pH 12 presumably resulted from the increasing concentration of the more reactive HO_2^- (pKa for H_2O_2 is 11.6). However, their conclusions are essentially in agreement with those observations recorded in this work.

Table 1
Emission (a.u.) observed with and without catalase in different pH

	pH 2	pH 4	pH 7	pH 9	pH 11
Fe$^{\text{III}}$TMPy	1200	1025	425	750	1550
Fe$^{\text{III}}$TMPyP+catalase	450	250	0	0	320

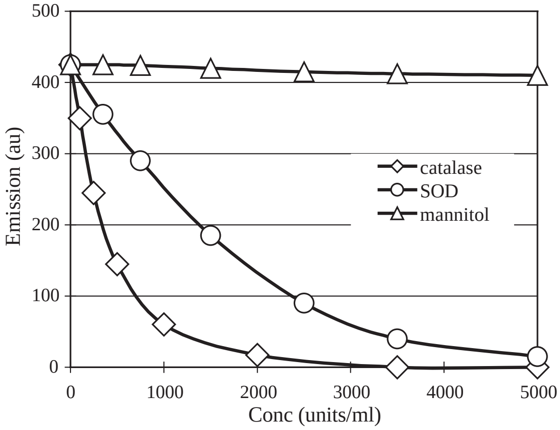

Fig. 10. Maximum emission intensity observed for 10 μM Fe$^{\text{III}}$TMPyP with different concentrations of hydroperoxy catalase, SOD and mannitol in the buffer solutions of pH 7.5.

3.5. Effect of alkenes on luminol chemiluminescence

Alkene oxidation catalyzed by oxo-ferryl porphyrin was investigated with different water-soluble carboxylic alkenes [25]. In this experiment we have chosen cyclopent-2-ene-1-acetic acid for its higher reactivity to detect the diminishing rate of electrochemically generated oxo-ferryl porphyrin by measuring luminol chemiluminescence. Alkene solution was added by making another channel between iron (ch2) and luminol (ch3) inlet (Fig. 1). Experimental results with different concentrations of alkene in the pH 12 buffer solutions have been shown in Fig. 11.

It was clearly observed that emission decreased accordingly with the increase of alkene concentrations. Similar results were obtained in all other pH examined. But as reported before [25], the activity was found maximum at higher pH (data not shown). Absorption spectra of Fe^{III}TMPyP, as a function potential, were also taken with and without alkene under aerated conditions. But no shift of wavelength was observed in the presence of alkene (data not shown). All these facts were considered as evidences of the electrochemical formation of oxo-ferryl porphyrin in the system.

3.6. Current efficiency for H_2O_2 formation

It has been demonstrated that hydrogen peroxide can be produced with a current efficiency of 40–70% by the reduction of oxygen at a vitreous carbon electrode in aqueous media of pH 2 [16]. In this study the electrolytic proficiencies of O_2 reduction to form H_2O_2 was measured from observed emission intensities (Fig. 4) in comparison with that of reference experiments. Fig. 12 represented the emission intensities of the reference experiments in different acidic conditions where known amount of H_2O_2 was added from outside.

From Fig. 4, we observed the emission intensity at pH 11 of about 1500, which was attained in the reference experiment when H_2O_2 concentration used was 55 μM (in Fig. 12). The cathodic current was recorded as about 0.8 mA for the generation of mentioned emission. Further the total emission intensity observed here was assumed to be generated by the electrochemical formation of H_2O_2 through Eqs. (1) and (2) as discussed earlier. Comparing the emission intensities with the reference experiments (details in Appendix), we estimated that about 33%, 45% and 62% of the injected electrons were used to reduce O_2 into H_2O_2 at pH 2, 7 and 11, respectively (data shown in Table 2).

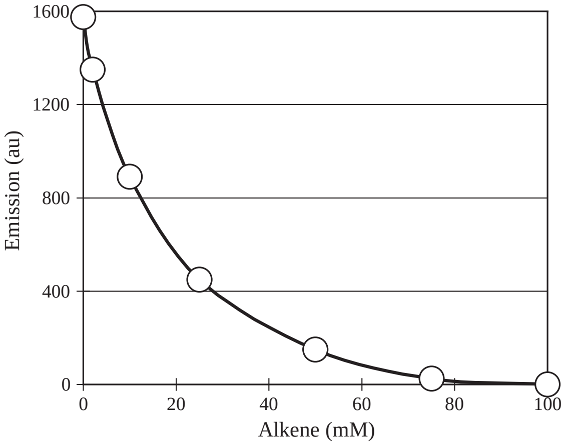

Fig. 11. Emission intensity with different concentrations of alkene at pH 12.0.

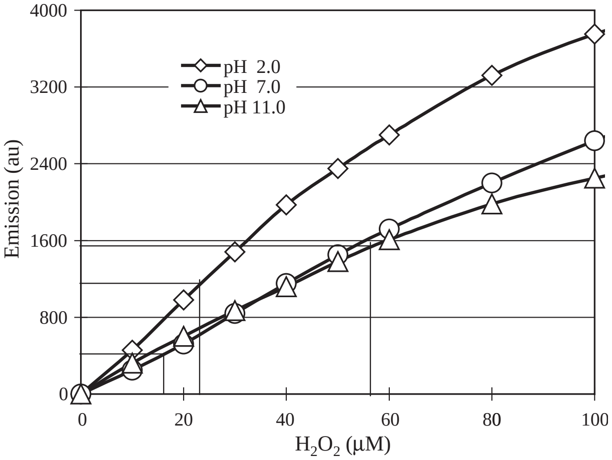

Fig. 12. Emission intensity observed for 10 μM Fe^{III}TMPyP in different pH when known concentration of H_2O_2 solution was added from outside.

Clearly the current efficiencies are always significantly below 100% and this is in contrast to electrolysis in alkaline solution where the current efficiencies were generally >90% [26]. The current efficiencies dropped in the present study, which may be due to oxygen reduction occurring at carbon surfaces in competition with other reactions. In this context, Eqs. (7) and (8) can be considered for their involvement in the formation of remaining current.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (7)$$

$$2H^+ + 2e^- \rightarrow H_2. \quad (8)$$

Between them, Eq. (8) is found to be predominantly engaged in cathodic reduction, especially at acidic solutions, which may be the cause of obtaining small current efficiency at lower pH (Table 2). The physico-chemical properties of the carbon electrode surface may also affect the electrolytic efficiency. It was reported that in the presence of metal ions, the efficiency for the production of hydrogen peroxide was negligible over an extended period of time [16]. Therefore, we conclude that the values of current efficiencies obtained in this experiment for the formation of H_2O_2 may not be up to the mark but at least the approximate values can be calculated. Similar conclusions have also been drawn in other reports [20,27].

Table 2
Current efficiency in different pH

pH	$[H_2O_2]$ (μM)	Current (mA)	Efficiency (%)
2	22	0.6	33
7	16	0.3	45
11	55	0.8	62

4. Conclusion

Electro-synthesized hydrogen peroxide-mediated oxo-ferryl porphyrin formation in aqueous media was confirmed by using luminol chemiluminescence method. The process involved a two-step reaction: first, a two-electron ($2e^-$) electrochemical reduction of molecular oxygen to H_2O_2, then a chemical reaction of H_2O_2 and Fe^{III}TMPyP to form $O{=}Fe^{IV}$TMPyP. Luminol chemiluminescence produced only at cathodic potential and the half-wave potential ($E_{1/2}$) shifted towards more negative region. The emission intensity served as the basis of the formation rate and stability of H_2O_2 in different pH. The results of spectrophotometric analysis confirmed the existence of $O{=}Fe^{IV}$TMPyP in solutions. In this study, the rate of efficiency to reduce molecular oxygen into hydrogen peroxide at the various pH used was estimated to be about 30–65%. However, the efficiency can be expected to improve further when oxygen concentration is increased in the solution. Thus, the method can be used for better understanding of the property and application of electrochemically reduced water.

Appendix A.

Since the current production by electrochemical reduction was estimated as 0.8 mA, the number of moles of electron involved in the reduction process per second can be calculated as follows. We know,

$$1\mathrm{A} = 1\mathrm{C/s} = 1/\mathrm{F}\ \mathrm{mol/s} = 1/96500\ \mathrm{mol/s}$$

$$= 1 \times 10^{-5}\ \mathrm{mol/s}$$

where, F = 96,500 C/mol.

So the current, $0.8\ \mathrm{mA} = 1 \times 10^{-5}\ \mathrm{mol/s} \times 8/10{,}000 = 8 \times 10^{-9}\ \mathrm{mol/s}$.

Again the flow rate in this experiment was maintained as 2.7 ml/min = 0.045 ml/s. So the number of moles of H_2O_2 flowing per second can be calculated as:

$$0.045\ \mathrm{ml/s} \times 55\ \mu\mathrm{M} = 2.475 \times 1/1000\ \mu\mathrm{mol/s}$$

$$= 2.475 \times 10^{-9}\ \mathrm{mol/s}.$$

Therefore the current efficiencies for the H_2O_2 formation by $2e^-$ reduction of O_2 can be derived by the following equation:

$$2.475 \times 10^{-9}/8 \times 10^{-9} \times 2 \times 100 = 61.8\%.$$

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