PAPER

Study of oxygen effects on electrochemiluminescence using dye-doped oxygen-resisting nanobeads†

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Tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II) (Rudpp) doped oxygen-resisting nanobeads were synthesized and applied in order to study the effects of oxygen on electrochemiluminescence.

Due to the important and irreplaceable effects of oxygen on all the living organisms on earth, oxygen sensing has encouraged many scientists to devote themselves to uncovering its mystical function. Year after year, their efforts have been rewarded with fruitful results and have led to various applications in all possible areas.[1–3] However, most of the research has been based on pure electrochemistry or fluorescence (FL).[4–10] As is well-known, electrochemiluminescence (ECL) is triggered by an applied potential on a fluorophore and its relaxation process is the same as that of FL after excitation.[11,12] Generally, oxygen is a strong quencher in FL, indicating that oxygen affects ECL intensity. Although some studies have been concerned with oxygen sensing based on ECL,[13–16] few have focused on how oxygen affects ECL in an aqueous solution during the whole process, apart from Dong's group.[17] They reported the oxygen effects on tris(2,2-bipyridine)ruthenium(II) during a cathodic ECL process in an aqueous solution. The reason for this situation is possibly that it is hard to identify the oxygen effects between the radical quenching and excited state quenching, because the ECL process includes both radical reaction and excited state relaxation. In this study, we developed a method to study the oxygen effects during the ECL process by the synthesis of an oxygen-resisting polymer nanobead doped with Ru(Ph$_2$phen)$_3$(ClO$_4$)$_2$ [Ph$_2$phen: 4,7-diphenyl-1,10-phenanthroline (Rudpp)]. Using the synthesized bead, the oxygen effects exerted on the fluorophore during the ECL process were successfully revealed.

Since fluorophores with a longer life time would be quenched by oxygen more easily, instead of Ru(bpy)$_3^{2+}$, Rudpp with a longer life time of 5.3 μs[18] was selected as the ECL reagent. During the study, isolating Rudpp from the oxygen became a problem. Coprecipitation of fluorescent probes and polyacrylonitrile (PAN) derivatives from a solution in *N,N*-dimethylformamide can result in an oxygen-resisting polymer bead doped with dyes.[19,20] Based on this method, Rudpp was successfully doped inside poly(vinylidene chloride-*co*-acrylonitrile) (PVDC-*co*-PAN) beads (RPN) with a typical diameter around 200 nm (Fig. S1 and S2†).

Preliminary ECL experimental results showed that under the same conditions, the ECL intensity emitted from an RPN-modified glassy carbon electrode (RPN-GCE) (Fig. 1a) was stronger than that emitted from an Rudpp directly modified GCE (TR-GCE) with a Nafion membrane (Fig. 1b), when the effective Rudpp concentration was kept the same (Fig. 1a′ and b′). Because only the same effective amount of Rudpp, TPA and oxygen participated in the ECL process, any ECL difference between the RPN-GCE and the TR-GCE must have been caused by the oxygen quenching efficiency. Hence, the ECL intensities from the two differently modified electrodes should become the same if the aqueous electrolyte is deoxygenated. This deduction was supported by the experimental results. As shown in Fig. 2a and b, when the solution was deoxygenated, the same ECL intensities could be obtained using the two modified electrodes.
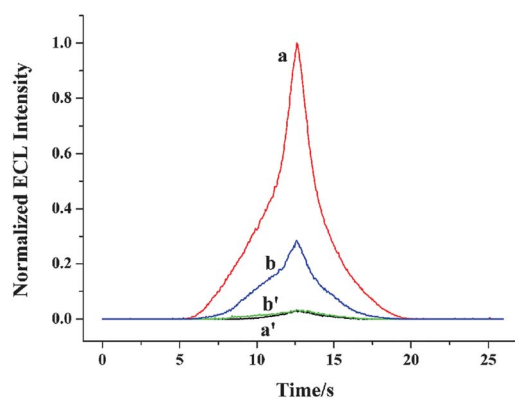
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† Electronic supplementary information (ESI) available: SEM images of RPN and experiment details. See DOI: 10.1039/c2an00026a
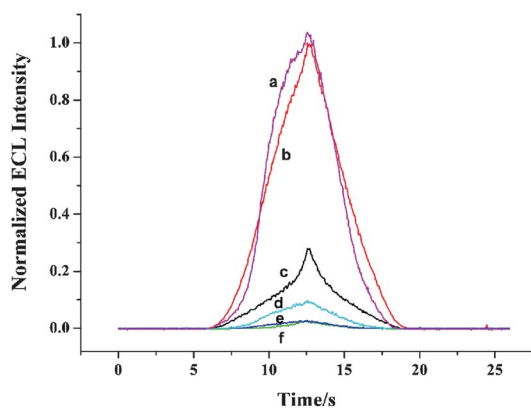
Fig. 1 Normalized ECL curve of RPN-GCE (a,a′) and TR-GCE (b,b′) in different electrolytes: (a′) RPN-GCE in 0.1 M blank PBS; (b′) TR-GCE in 0.1 M blank PBS; (a) RPN-GCE in 0.1 M PBS containing 1 mM TPA, and (b) TR-GCE in 0.1 M PBS containing 1 mM TPA. Scanning potential range: 0–1.3 V.

Fig. 2 ECL curve of RPN-GCE (a,c,e) and TR-GCE (b,d,f) in different oxygen concentrations: (a,b) 0% O_2 with 1 mM TPA; (c,d) 20% O_2 with 1 mM TPA; and (e,f) 0% O_2 without TPA. Scanning potential range: 0–1.3 V.

This indicated that the excited state of Rudpp was well protected by PVDC-*co*-PAN. This result was confirmed by further experiments. There were no significant changes of the FL intensity when the RPN were exposed to different oxygen concentrations (Fig. S3†).

In addition, it was found that the RPN-GCE emitted weaker ECL before deoxygenation (Fig. 2c). Because the Rudpp was protected by PVDC-*co*-PAN, oxygen did not affect the excited state of Rudpp and Rudpp radical species, which hinted that the TPA radical could also be greatly quenched by oxygen during the process.

In order to focus our study on oxygen and Rudpp, TPA was removed from the electrolyte, and the whole electrochemical system was exposed to different oxygen concentrations to monitor the effect of oxygen on the Rudpp ECL. For comparison, RPN-GCE and TR-GCE were used. As shown in Fig. 3, the ECL intensity from TR-GCE directly decreased with increasing oxygen concentration. This indicated that oxygen played a role as a quencher during the ECL process. This result sounded reasonable, because Rudpp in TR-GCE was directly exposed to oxygen without any protection. Under general considerations, the ECL curve of the RPN-GCE should be a relatively horizontal line, since Rudpp in the RPN-GCE was well protected by PVDC-
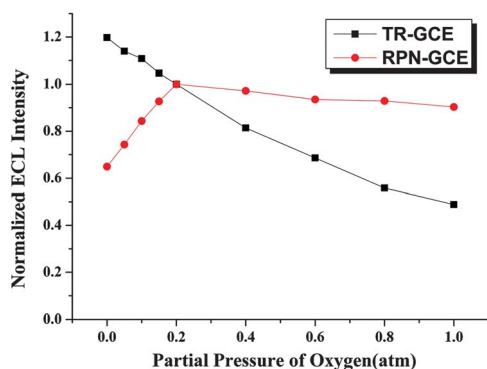
co-PAN. However, when we came to the ECL curve analysis, a strange phenomenon was found. The ECL intensity increased with increasing oxygen concentration, when the concentration of oxygen was below 20%, and then the ECL intensity reached a relative balance with just a small decrease. The decreasing was the same as the FL experiments (Fig. S3†). It might be caused by the penetration of oxygen through the membrane under high oxygen pressure. As a result, the excited state of Rudpp was quenched. For the increasing part before the oxygen concentration reached 20%, because the effective amount of Rudpp was fixed, an increase of the ECL intensity could only result from the increasing concentration of co-reactant according to the ECL mechanism. In this experiment, the only possible co-reactant was the hydroxy ion.[21,22] The hydroxy ion can be generated by the reaction between oxygen and water when the potential is above 0.4 V under a standard state.[23]

$$O_2 \text{ (g)} + 2H_2O + 4e^- \rightleftharpoons 4OH^- \text{ (aq)}$$
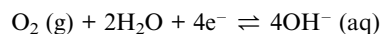
Hence, the concentration of the hydroxy ion might increase with increasing oxygen concentration during the ECL process. However, this raised the problem of whether the hydroxy ion could penetrate the PVDC-*co*-PAN polymer to affect the ECL process. As shown in Fig. 4, the ECL intensity changed with changing pH, when an RPN-GCE was applied as the working electrode and blank PBS of different pH values was used as the electrolyte. The results showed that the RPN-GCE was sensitive to pH, since no other co-reactant, except the hydroxy ion, existed in the electrolyte. The only way for it to be affected by pH was the penetration of hydroxy ions through the PVDC-*co*-PAN polymer. The result indicated that, in addition to being a quencher, oxygen also acted as a co-reactant as TPA to enhance ECL, during the ECL process.

In conclusion, RPN were successfully synthesized and applied in the study of oxygen effects on the Rudpp ECL process in aqueous solution. The result from the comparison experiment based on RPN-GCE and TR-GCE showed that oxygen was a quencher to two important species in the ECL process, which were the excited state of Rudpp and TPA radicals. However, it was found that oxygen was transformed into hydroxy ions and functioned as a co-reactant to enhance the ECL, during the ECL process based on RPN-GCE when the oxygen concentration was
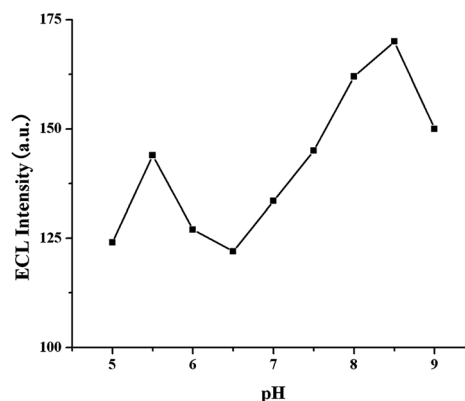

Fig. 3 Normalized ECL intensity of RPN-GCE and TR-GCE under different oxygen partial pressures. Electrolyte: 0.1 M PBS, pH 7.5.

Fig. 4 ECL Intensity of RPN-GCE in blank PBS solution at different pH values.

below 20%. This indicated that RPN might be applied in the determination of oxygen at low concentrations.

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