

# Electrochemical sensors to investigate the redox behavior of plasma treated liquids

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**Abstract:** An electrochemical sensor is described to control the performance and quality of plasma treated liquids during and after cancer therapies. This sensor consists of an immobilized reversible redox couple, which is potentiostatically preconditioned before the measurement to transform to the completely reduced form, and then the electrochemical reaction of the reduced form with plasma treated liquid is followed. High stability, portability and possibility of miniaturisation for *in situ* measurements are special features of this sensor.

**Keywords:** Cold atmospheric plasma, plasma treated liquids, electrochemical sensor.

## 1. Introduction

Cold atmospheric plasma (CAP) produced by specific devices (jets or dielectric barrier discharges) has recently shown promising anti-cancer activities and seems to be a powerful tool for cancer treatment [1]. Various studies revealed that CAPs can be applied in direct contact with a living tissue to affect the cell cycle and cause cell apoptosis or necrosis in tumor cells dependent on the CAP device and doses [2]. Depending on the type, composition and energy of CAPs, different types of radicals ( $\text{OH}\cdot$ ,  $\text{NO}_2\cdot$ ,  $\text{NO}$  radicals) and long-lived chemical products ( $\text{O}_3$ ,  $\text{H}_2\text{O}_2$ ,  $\text{O}_2\cdot^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ) can be formed at the CAP-liquid interface which will then penetrate into the liquid and start a chemical process [3]. Some of these chemical processes can initiate or continue in plasma-treated liquid after the CAP is switched off and in the absence of any external energy source. These *post-discharge reactions* may contribute significantly to the biological effects induced by CAPs [4]. Prolonged antibacterial activity of plasma treated aqueous solutions is reported for electrical discharges generated in gas-liquid environments even several days after the solution's exposure to the plasma [5]. Thus it is very important to follow the production of reactive species in the liquid environment during plasma treatment and also monitor the plasma activated liquids after treatment.

The aim of this work is to design an electrochemical sensor to investigate the redox behavior of plasma treated liquids in order to control their performance and quality during and after treatment with plasma. Electrochemical sensors have this superiority to be used *in situ* which is very important for detecting short-lived radicals before their disproportionation reactions. High sensitivity, low-cost and possibility of miniaturisation are other factors that make electrochemical sensors an appropriate choice for monitoring of the plasma treated liquids.

Recently, an electrochemical sensor is designed in our research group based on gold supported 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) lipid bilayers to study the effect of plasma treatment on the structure of the lipid bilayer. Fig. 1 shows the cyclic voltammograms (CVs) of  $\text{K}_4[\text{Fe}(\text{CN})_6]$  as a redox probe on unmodified gold, gold supported lipid bilayer and plasma treated lipid bilayer electrodes. The recorded CVs show that after 30 min of plasma treatment, the reactive species produced by plasma will damage the lipid bilayer on the surface of the electrode which leads an increase in the signal intensity of the redox probe. Moreover, mass spectrometry demonstrated the effect of CAP on POPC alkyl chain by formation of PoxnoPC, which might be attributed to the presence of  $^1\text{O}_2$ .

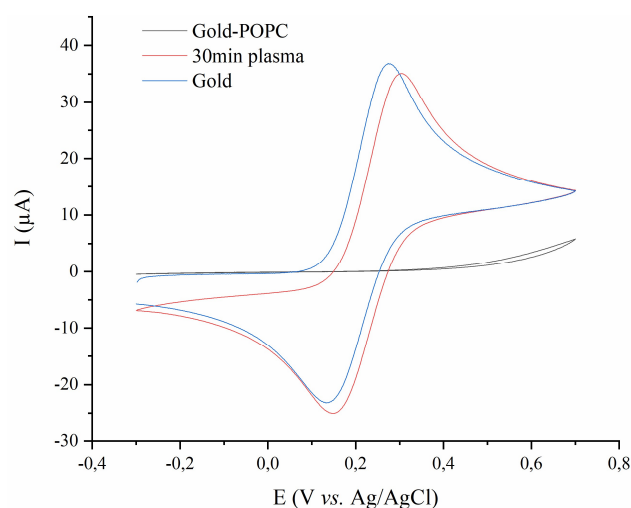


Fig. 1. CVs of 10 mM  $\text{K}_4[\text{Fe}(\text{CN})_6]$  in pH 7.4 phosphate buffer solution on gold electrode and gold supported POPC lipid bilayer before and after 30 min plasma treatment.

In spite of the ability of this sensor for reacting with reactive species output of CAP, low stability of the modified electrode and long modification process made us to design a simple, stable and portable sensor in order to follow the reactivity of the plasma treated liquids. For this purpose, a toluidine blue (TB) modified electrode is proposed. TB is one of the phenathiazine mediators which has a reversible redox reaction and high activity toward many electrocatalytic redox reactions [6-12]. TB is attached covalently to the surface of the electrode by reduction of in situ generated diazonium salt. The immobilized reversible redox system of TB is potentiostatically preconditioned to transform to the completely reduced form of TB. The preconditioned sensor is then introduced to the plasma treated solution containing reactants, which are able to oxidize the reduced form of TB. This leads a change in the potential of the immobilized system. The changes in the potential are measured and used as indicator of the redox behavior of plasma treated solutions [13].

## 2. Experimental

### 2.1. Apparatus

All electrochemical measurements were carried out on an Autolab PGSTAT302N potentiostat by using gold screen-printed electrodes (SPEs). Counter and reference electrodes were made of Platinum and Silver, respectively.

Raman microscopy studies were done using RENISHAW inVia Reflex Raman microscope with 532 nm laser excitation and 0.5% of laser power for 10 seconds.

The kINPen plasma jet consists of a powered pin electrode (1 MHz, 2-6 kVpp, pulsed @ 2.5 kHz) [14] shielded by a dielectric tube from the outer electrode. The dissipated power in pure Ar was around 500 mW. 3 slm Ar was used as the feed gas, either without any admixture or with O<sub>2</sub> (1%, Ar/O<sub>2</sub>) or N<sub>2</sub> (1%, Ar/N<sub>2</sub>). For the experiments, the sources were mounted vertically above the treated liquid sample at a distance of 9.0 mm in open atmosphere.

### 2.2. Electrode preparation

As TB is an aromatic amine, diazonium chemistry was used for covalent attachment of TB to the electrode surface. For in situ generation of TB diazonium salt, TB was dissolved in 0.5 M HCl. Then, ice-cold sodium nitrite aqueous solution was added drop by drop into this mixture. Final concentrations of TB and sodium nitrite were 1 mM and 2 mM, respectively [15]. After stirring the mixture for 1 h at 0 °C, 200 µl of this solution was added to the electrochemical cell and then the gold electrode was derivatized by cyclic voltammetry scanning from 0 to -0.35 V at 100 mV s<sup>-1</sup> scan rate for 5 scans. Finally the modified electrode was rinsed with water several times to remove the loosely adsorbed TB molecules.

## 3. Results and discussion

### 3.1. Electrochemical reduction of TB diazonium salt onto the gold electrode

The grafting possibility of TB molecules onto the gold electrode surface was investigated by the electrochemical reduction of the in situ generated diazonium salt. Fig. 2 shows the CVs recorded under in situ conditions. The first scan has a broad irreversible peak at  $E_p = -0.2$  V, indicating that an irreversible reaction is associated with the reduction of the diazonium function and this is the loss of dinitrogen and the formation of radicals. In the following scans, the peak disappears nearly completely due to the covalent bonding of highly reactive radicals to the gold surface and formation of an organic layer blocking further reduction of diazonium salt [16]. After washing the modified electrode with water for several times, the resulting CV in phosphate buffer saline (PBS) pH 7.4 shows a reversible well-defined peak (Fig. 3) attributed to the redox reaction of bonded TB molecules.

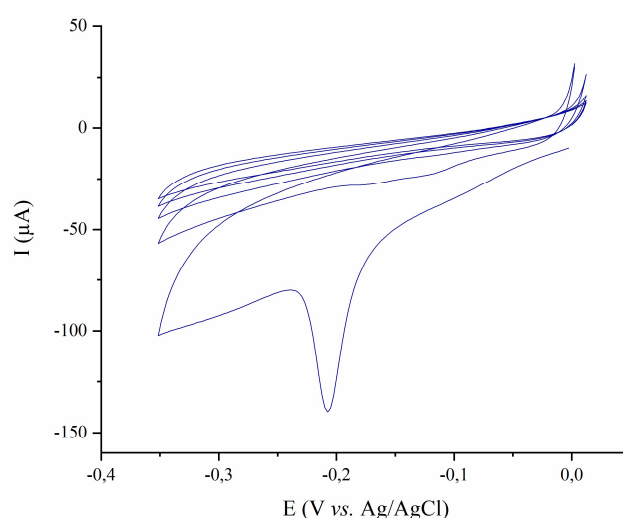


Fig. 2. CVs of the reduction of in situ generated TB diazonium salt on gold SPE at scan rate of 100 mV s<sup>-1</sup>.

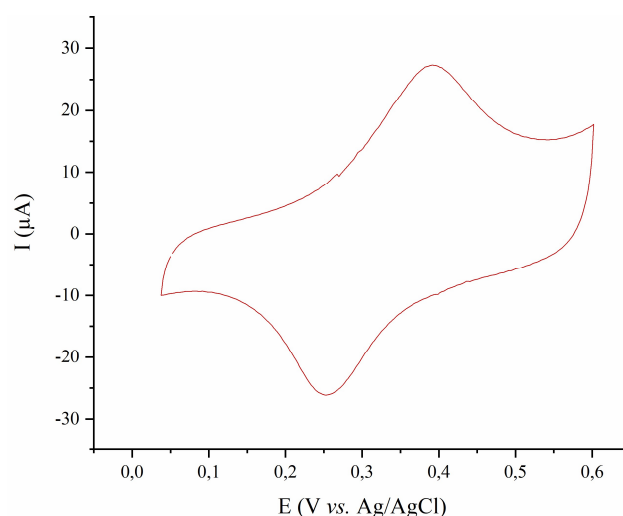


Fig. 3. CV of TB modified gold SPE in PBS solution (pH 7.4) at scan rate of 100 mV s<sup>-1</sup>.

### 3.2. Raman microscopy analysis

To prove the existence of TB molecules on the surface of the modified electrode, Raman spectra of gold electrodes were recorded before and after modification with TB (Fig. 4). The presence of known Raman bands [17, 18] in the spectra of TB modified electrode, indicates that TB is successfully attached to the surface of the electrode.

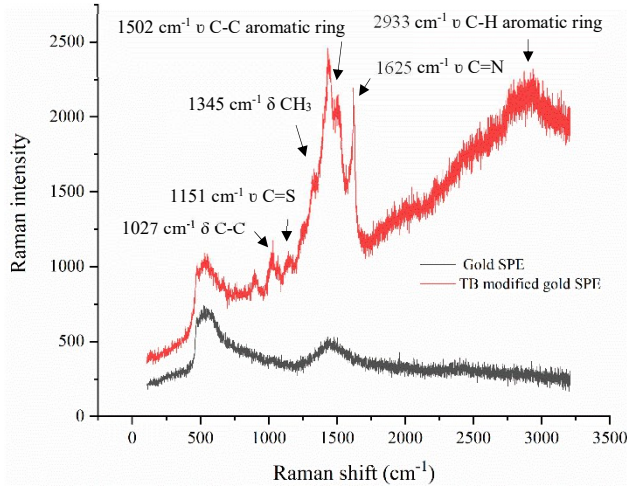


Fig. 4. Raman spectra of unmodified and TB modified gold SPE obtained with 532 nm laser excitation.  $\delta$  denotes a bending vibration and  $\nu$  denotes a stretching vibration.

### 3.3. Monitoring the redox behavior of plasma treated liquids

The reversible redox system  $TB_{red}/TB_{ox}$  which is bonded to the surface of the electrode can be easily tuned to various starting conditions to obtain a fixed ratio of  $a_{red}/a_{ox}$ , simply by a potentiostatic pretreatment. According to Fig. 3, at  $E = 0.1$  V, the redox system on the surface is nearly completely reduced to the form of  $TB_{red}$ . In the presence of oxidizing agents like plasma treated solutions, the chemical reaction with the immobilized  $TB_{red}$  changes the concentration ratio of oxidized to reduced forms, which in turn, leads to a shift in the electrode potential towards positive values (reaction 1) [13]:

$$E = E^0 + \frac{2.3 RT}{nF} \log \frac{TB_{ox}}{TB_{red}} \quad (1)$$

So the shift in the potential of the electrode is proportional to the redox behavior of the plasma treated solution. After each measurement, the surface of the electrode can easily be renewed again by potentiostatic preconditioning.

Fig. 5 shows the changes of the potential of the unmodified and TB modified gold SPE in the presence of untreated and 30 s and 2 min treated PBS solutions by the kINPen plasma jet with Ar feed gas. As is shown in Fig. 5, by increasing the treatment time, the potential of the TB modified electrode is shifted to more positive values, indicating the elevated production of reactive species in plasma treated solutions by increasing the treatment time.

Further studies were performed by Ar feed gas with  $O_2$  (1%, Ar/ $O_2$ ) and  $N_2$  (1%, Ar/ $N_2$ ) admixtures. The redox behavior of the 30 s treated PBS solutions with Ar feed gas without and with admixtures are compared in Fig. 6.

As a control experiment, the behavior of the TB modified electrode was studied in the presence of different concentrations of  $H_2O_2$ . Fig. 7 indicates that the TB modified electrode shows the same trend with treated PBS solutions in the presence of  $H_2O_2$  solutions.

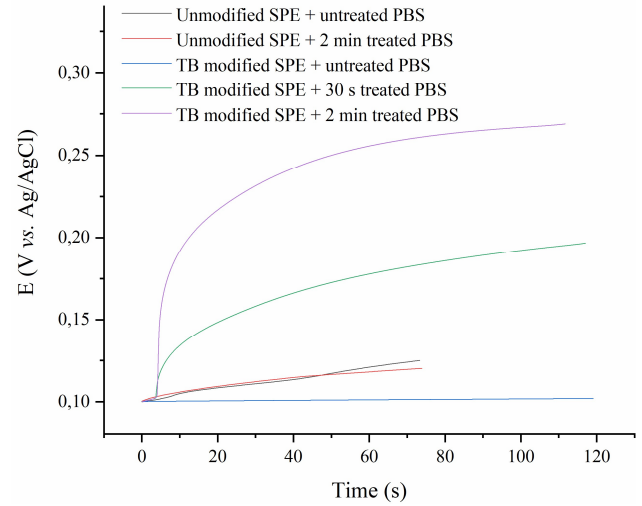


Fig. 5. Changes of the potential of the unmodified and TB modified SPE in the presence of untreated, 30 s and 2 min treated PBS solutions with Ar feed gas plasma jet.

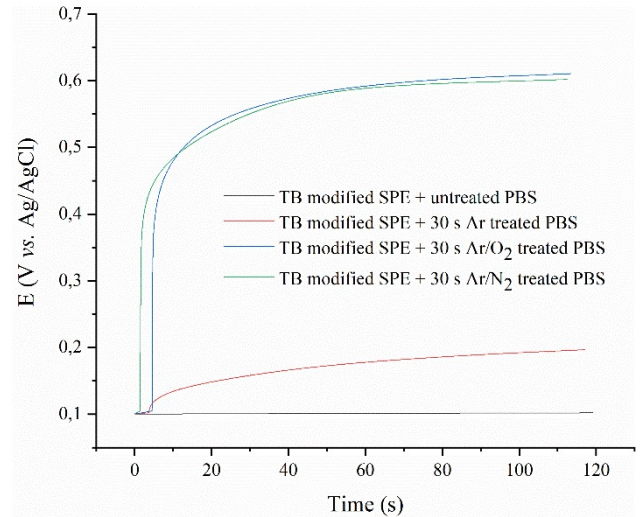


Fig. 6. Changes of the potential of the TB modified SPE in the presence of untreated and 30 s treated PBS solutions with Ar feed gas without any admixture or with  $O_2$  (1%, Ar/ $O_2$ ) and  $N_2$  (1%, Ar/ $N_2$ ).

As the introduced TB modified electrode shows good performance for monitoring the redox behavior of plasma treated solutions after treatment, further studies will be done to use this sensor for *in situ* studies during plasma treatment. The high stability of the immobilized TB layer

on the surface of the electrode and its simple applicability make this sensor an appropriate choice to be used in plasma therapy studies.

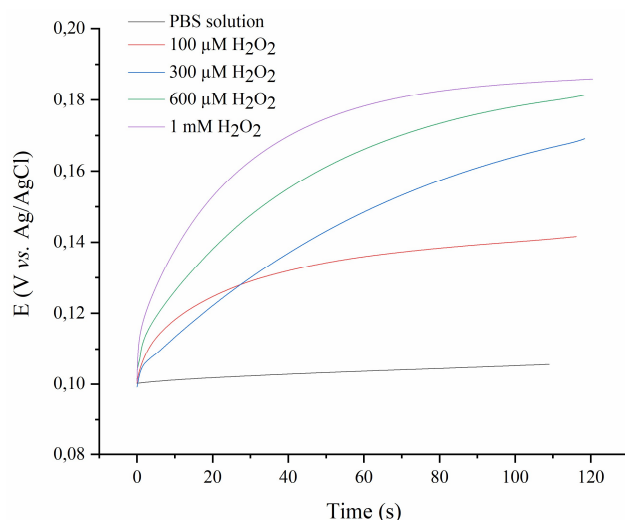


Fig. 7. Changes of the potential of the TB modified SPE in the presence of different concentrations of H<sub>2</sub>O<sub>2</sub>.

#### 4. Conclusions

The evaluation of the formation of redox species through direct treatments with plasma and through post-discharge reactions in plasma-treated liquids is of great importance. For this propose, a simple and quick *in situ* measurement will be of great advantage. Here we describe a modified electrode with an immobilized TB layer as reactive species probe, which opens the way to use such sensors for the assessment of reactive species output of plasma. This sensor has the possibility to be used in cancer therapies for following the redox behavior of plasma-activated liquids.

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