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Sensing element based on poly(3,4-(1-azidomethylethylene)dioxythiophene) as electroactive layer of electrochemical DNA sensors

VERA SHAVOKSHINA*, EGOR ANDREEV

Department of Analytical Chemistry, Faculty of Chemistry, Lomonosov Moscow State University, Leninskiye Gory, 119991 Moscow, Russia ✉ veraleksandrovn@mail.ru

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Abstract

In this work, we demonstrate poly(3,4-(1-azidomethylethylene)dioxythiophene) modified electrodes as an advanced sensing element for further DNA sensors elaboration. Conducting poly(3,4-(1-azidomethylethylene)dioxythiophene) combines the advantages of the poly(3,4-ethylenedioxythiophene) and easy surface modification due to azide groups. We have carried out the electrochemical synthesis of poly(3,4-(1-azidomethylethylene)dioxythiophene) on planar screen-printed electrodes in acidic aqueous solution. Electrochemical activity of poly(3,4-(1-azidomethylethylene)dioxythiophene) in $K_3[Fe(CN)_6]$ solution investigated by cyclic voltammetry was compared with poly(3,4-ethylenedioxythiophene). Standard heterogeneous rate constant, limit of detection and sensitivity was determined. According to these studies, poly(3,4-(1-azidomethylethylene)dioxythiophene) exhibits better electroactivity than poly(3,4-ethylene-dioxythiophene).

1. Introduction

In the past decades, DNA sensors has been drawing particular attention due to great significance of their applications including detection of microorganisms [1], DNA damage [2]. At the same time, one of the most promising areas of research is development of electrochemical sensors based on conducting polymers [3]. The main advantages of these devices are their low cost, sensitivity and selectivity. Conducting polymer-based DNA sensors provide express analysis, since they convert the hybridization event into a direct electrical signal. Poly(3,4-ethylenedioxythiophene) (PEDOT) is one of the most widely used conducting polymer. PEDOT is electroactive in aqueous solutions and exhibits good electrochemical stability and relatively high conductivity [4]. To create an electrochemical DNA-sensor it is necessary to immobilize DNA onto modified electrodes. Unsubstituted PEDOT offers no possibility for covalent bonding of other

molecules, its derivative conducting poly(3,4-(1-azidomethylethylene)dioxythiophene) (azido-PEDOT) combines the advantages of PEDOT with facile functionalization. Azido-PEDOT electrodes can be functionalized with acetylene-terminated DNA probe by Cu(I) catalyzed azide-alkyne cycloaddition ("click chemistry") [5]. Thus, azido-PEDOT can be used as electroactive layer of electrochemical DNA sensors.

Polymers can be synthesized chemically and electrochemically. Electropolymerization is preferable, since deposition can be targeted directly to the electrode surface. Also, the properties of the polymer film can be modulated by varying electrochemical polymerization conditions.

DNA sensors commonly work in aqueous solution, thus it would be better to provide the electropolymerization in the same medium. Azido-PEDOT is synthesized by electrochemical oxidation of the monomer only in an organic medium [5]. PEDOT films are generally synthesized in organic solutions [6], but the electropolymerization of 3,4-ethylenedioxythiophene (EDOT) may also be carried out in aqueous solution even in absence of any surfactants [7].

The properties of the PEDOT films are deeply affected by electropolymerization conditions, such as applied electropolymerization methods, polymerization potential, solvent, supporting electrolyte [6]. Therefore, the purpose of the work was to synthesize and study of the properties of azido-PEDOT for further elaboration of DNA sensors.

2. Experimental

2.1 Reagents and chemicals

Experiments were carried out with Millipore Milli-Q water. The monomer, 3,4-ethylenedioxythiophene (>97%), and potassium ferricyanide were purchased from Sigma-Aldrich (USA). The monomer 3,4-(1-azidomethylethylene)dioxythiophene (azido-EDOT) was purchased from Skolkovo Institute of Science and Technology (Russia). Inorganic salts (chloride, hydrophosphate, and dihydrophosphate of potassium) and perchloric acid were obtained of the highest purity from Reachim (Moscow, Russia).

2.2 Instrumentation

The electrochemical measurements and electropolymerization were carried out by means of a universal potentiostat-galvanostat AutolabPGSTAT 128N (The Netherlands). A Renishaw InVia Raman microscope (Renishaw, UK) was used for recording Raman spectra. The latter were acquired with 514 nm excitation laser source, 20× objective in the range from 900 to 2200 cm^{-1} . Scanning electron microscopy (SEM) was performed on Supra 50 VP LEO (Carl Zeiss, Germany).

2.3 Methods

For electrochemical measurements including electropolymerization of EDOT and azido-EDOT we used the method of cyclic voltammetry. Cyclic voltammetry was conducted in a three-compartment electrochemical cell that contained a glassy carbon counter electrode, Ag | AgCl | 1 M KCl reference electrode, planar screen-printed structures (SPE) (Rusens Ltd., Moscow) with carbon working electrode (diameter 0.9 mm).

Electropolymerization of azido-EDOT was carried out from its 0.01 M solution in 0.1 M perchloric acid in cyclic voltammetric regime. Potential range was from -0.2 to 0.9 V. Sweep rate was 0.04 V s^{-1} .

Electropolymerization of EDOT was carried out from its 0.01 M solution in 0.1 M perchloric acid in cyclic voltammetric regime. Potential range was from -0.2 to 0.9 V. Sweep rate was 0.04 V s^{-1} .

PEDOT SPE and azido-PEDOT SPE with different number of growing cycles were investigated at different concentrations of the $\text{K}_3[\text{Fe}(\text{CN})_6]$ in 50 mM phosphate buffer (pH = 7.0) containing 0.1 M KCl. The sensitivity was obtained from the slope of the calibration curve (current density vs potassium ferricyanide concentration). Limit of detection was calculated as the ratio of 3 times the standard deviation of the peak currents (10 runs) of the lowest measurable concentration to the sensitivity. To evaluate standard heterogeneous rate constant, cyclic voltammograms were recorded in 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ (in 50 mM phosphate buffer pH = 7.0, containing 0.1 M KCl) at scan rates ranging from 0.01 to 1.0 V s^{-1} . The experiments were performed at room temperature ($24 \pm 2^\circ \text{C}$).

3. Results and discussion

Electropolymerization of azido-EDOT on screen-printed electrodes (SPE) was performed by cyclic voltammetry. Azido-PEDOT had been electrodeposited from aqueous solutions for the first time. Current increase was observed at around 0.9 V corresponding to the formation of radical cations, current intensity increased with increasing number of cycles (Fig. 1). "Nucleation loop" (current crossover) observed in the first scan indicates the beginning of the nucleation process. After electropolymerization, voltammograms of azido-PEDOT SPE with different number of growing cycles were recorded in an electrolyte solution free of modified monomer. The area of voltammograms increased in step with number of growing cycles.

Also, modified electrodes were studied by Raman spectroscopy. The Raman spectra of the PEDOT and the azido-PEDOT films have common bands corresponding to the main polymer chain. There are bands in the Raman spectra of azido-PEDOT corresponding to stretching vibration of the $-\text{N}=\text{N}=\text{N}$ group: 1310 cm^{-1} (sym.), 2120 cm^{-1} (asym.). Polymer morphology was studied using SEM. Synthesized azido-PEDOT has porous morphology. The thickness of the film obtained during 10 electropolymerization cycles is about 120 nm.

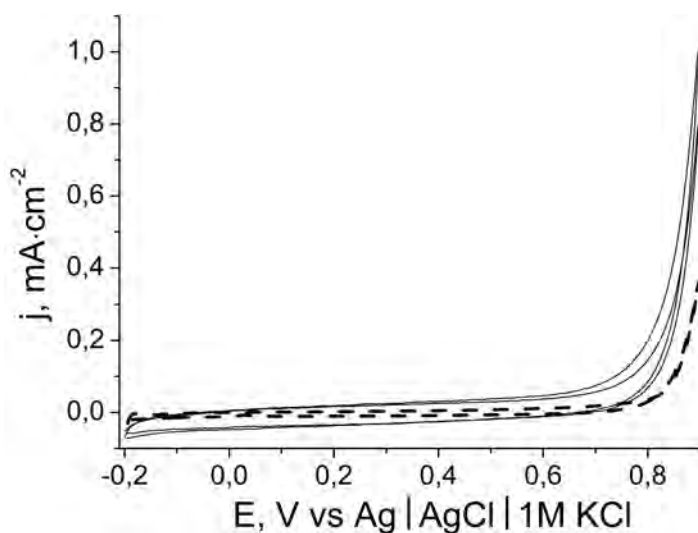


Fig. 1 Potentiodynamic electrodeposition of azido-PEDOT on screen-printed electrode from a solution of 0.01 M EDOT in 0.1 M HClO_4 in the potential range of -0.2 to 0.9 V during 10 cycles (scan rate 0.04 V s^{-1}). Dashed line represents the first electropolymerization cycle.

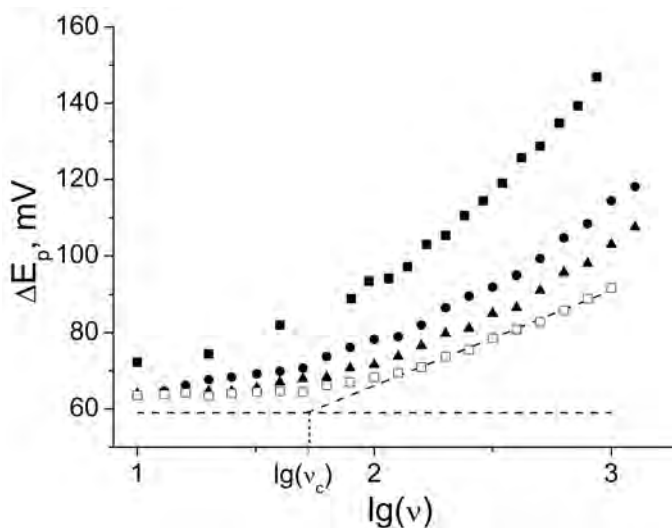


Fig. 2 Plot between the difference of the anodic and cathodic peak potentials (ΔE_p) versus logarithm of scan rate ($\lg v$) for screen-printed electrodes (in $5 \text{ mM K}_3[\text{Fe}(\text{CN})_6]$) modified azido-PEDOT during different numbers of growing cycles: 1 (■), 5 (●), 10 (▲), 20 (□). The dashed line shows the definition of the critical scan rate (v_c) for 20 cycles.

Table 1

Standard heterogeneous rate constant calculated from Eq. (1) for polymer-modified screen-printed electrodes with different polymer amount in 5 mM $K_3[Fe(CN)_6]$ solution.

Number of growing cycles	$k_s^0 / 10^{-3} \text{ cm s}^{-1}$	
	Azido-PEDOT	PEDOT
1	3.1	4.3
5	3.3	3.4
10	3.7	3.2
20	3.8	–
average	3.5 ± 0.3	3.6 ± 0.4

Electrochemical DNA sensors based on indirect methods require the use of labels or electroactive indicators, such as ferri-/ferrocyanide redox couple (mediators shuttle the electrons between the double-stranded DNA and the electrode). The study of processes between modified electrodes and a redox couple in the solution is essential for sensors elaboration.

Kinetics of electron transfer between $[Fe(CN)_6]^{3-/4-}$ and azido-PEDOT in aqueous solution was studied by cyclic voltammetry. The standard electrochemical rate constant was evaluated from the difference of the anodic and cathodic peak potentials ($\Delta E_p = E_{pa} - E_{pc}$) measured from the cyclic voltammograms (Fig. 2). This method is based upon the determination of critical scan rate at which the electrode reaction changes from reversible to quasireversible and Matsuda Ayabe parameter Λ is 1 (ref. [8]). Then following equation is used to calculate the value of k_s^0

$$k_s^0 = \Lambda \sqrt{\frac{FDv}{RT}} \quad (1)$$

Diffusion coefficient values, required to evaluate k_s^0 by the methods described above, was $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The value of k_s^0 for PEDOT-modified electrodes was calculated similarly.

According to calculated k_s^0 (Table 1) PEDOT SPE and azido-PEDOT SPE exhibit a similar electrochemical activity in $K_3[Fe(CN)_6]$ solution. However, PEDOT SPE does not have analytical characteristics similar to that of the azido-PEDOT modified electrodes. Azido-PEDOT SPE has shown the higher faradaic/capacitive current ratio (I_f/I_c) (Fig. 3). For example, at scan rate 150 mVs^{-1} I_f/I_c for azido-PEDOT is 6 and for PEDOT –4. The sensitivity of the azido-PEDOT SPE is $3.7 \text{ mA cm}^{-2} \text{ M}^{-1}$, PEDOT SPE $-3.4 \text{ mA cm}^{-2} \text{ M}^{-1}$. For the azido-PEDOT SPE a limit of detection of 0.4 mM was obtained while for the PEDOT SPE a limit of detection of 0.8 mM was achieved.

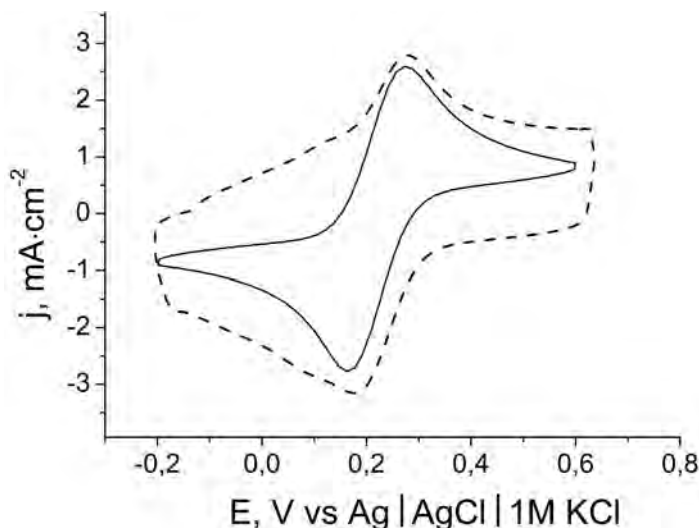


Fig. 3 Azido-PEDOT modified electrode (solid line) and PEDOT modified electrode (dashed line) in 5 mM $K_3[Fe(CN)_6]$. Conditions: 5 electropolymerization cycles, scan rate 1 V s^{-1} .

4. Conclusions

Conducting polymer azido-PEDOT has been electrochemically synthesized from aqueous solution. Azido-PEDOT has porous morphology, that provides anion exchange in polymer film. Nonfaradaic processes at PEDOT modified electrodes contributes significantly to currents. The result is that the analytical characteristics (limit of detection, sensitivity) of azido-PEDOT modified electrodes are better than for PEDOT-modified ones. Thus, azido-PEDOT modified electrode is proper sensing element for further elaboration of DNA sensors.

Acknowledgments

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