Electronic Supplementary Information

Ultrasensitive detection of NSE employing novel electrochemical immunosensor based on conjugated copolymer

Muhammet Aydın[a*], Elif Burcu Aydın[a], Mustafa Kemal Sezgintürk[b]

[a]Tekirdağ Namık Kemal University, Scientific and Technological Research Center, Tekirdağ-TURKEY

***Corresponding Author:**

e-mail: muhammetaydin@nku.edu.tr

mhmmtaydin@outlook.com

Tel:+90 282 250 11 32

Preparation of the Monomer and Copolymer

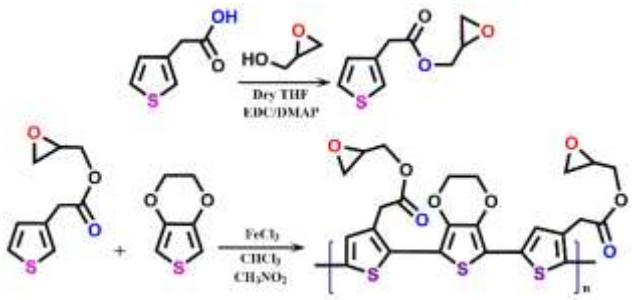

Figure SI-1. Synthesis pathways of thiophene monomer (ThEp).

Synthesis of monomer (ThEp)

The thiophene monomer (ThEp) carrying epoxy functional groups was synthesized by using glycidol and 3-thiopheneacetic acid at room temperature according to Steglich esterification method [1, 2]. FTIR (cm^{-1}): 3109; 2956; 2922; 2853; 1738(C=O); 1512; 1459; 1375; 1260; 1143; 1013; 909; 833; 761; 685; 610 (Figure SI-2B). ^{1}H-NMR ($CDCl_3$): 7.32ppm (Ha), 7.20ppm (Hb), 7.07ppm (Hc), 4.48ppm and 3.98ppm (Hd), 3.74ppm (He), 3.24ppm (Hf), 2.86 and 2.65ppm (Hg). (Figure SI-3).

Synthesis of copolymer

A chemical oxidative polymerization reaction was performed between the synthesized thiophene monomer (ThEp) and the 3,4-Ethylenedioxythiophene (EDOT) monomer in the presence of anhydrous ferric chloride. ThEp monomer (1.98 g, 0.1 mol) and EDOT (1.48 g, 0.1 mol) were added to a 100-mL three-neck round bottom flask and solved in anhydrous chloroform ($CHCl_3$; 70 mL). This mixture was stirred with a magnetic stirrer and incubated at −10/-15 °C in the presence of an ice-salt mixture. Afterwards, anhydrous $FeCl_3$ (12.9 g, 0.8 mol) was solved in nitromethane (CH_3NO_2; 45 mL), and it was added to the mixture in a 20-min period, and the mixture was stirred at room temperature for 90 min. The resulting black polymer solution was concentrated and precipitated in large excess cold methanol. The copolymer was purified by using fresh methanol, and it was dried in a vacuum at 30 °C overnight.

(Yield: %38.1, 0.75g). FTIR (cm^{-1}): 3007; 2945; 2883; 1717(C=O); 1551; 1385; 1311; 1176; 1036; 965; 926; 835; 757; 674; 620; 503(Fig. SI-2C).

Characterization of the monomer and copolymer

The synthesis steps of the conjugated thiophene copolymer are shown in Fig. SI-1. The FT-IR spectra of 3-thiopheneacetic acid, thiophene monomer (ThEp), and copolymer *P(ThEp-co-EDOT)* are given in Fig. SI-2.

The spectrum of 3-thiophene acetic acid showed a broad peak around 3300 cm^{-1}, originating from the -OH end groups, which disappeared completely after the reaction with glycidol. The successful synthesis of monomer ThEp was approved by the presence of a carbonyl stretching peak (Fig. SI-2B). The peak at 1738 cm^{-1} originated from the C=O stretching vibration of ester groups in the thiophene monomer 3-ThEp[3, 4]. The aromatic C-H stretching was seen at 2922 cm^{-1} [5]. Moreover, the characteristic signals of the epoxy group in the monomer were seen at 909 and 833 cm^{-1}.The two bands observed around 1143 cm^{-1} and 685 cm^{-1} illustrated C-S-C asymmetric and symmetric stretching vibrations in thiophene ring [6]. After the copolymerization reaction, the peaks of copolymer were broader than those of monomers, as shown in Figure SI-2C. The aliphatic C-H peaks of the copolymer were seen between 2945-2883 cm^{-1}. The strong signal observed around 1717 cm^{-1} was attributed to the C=O stretching vibration of carbonyl groups (Fig. SI-2C). Furthermore, the characteristic signals of the epoxy group in the copolymer were seen at 926 and 835 cm^{-1} [7].

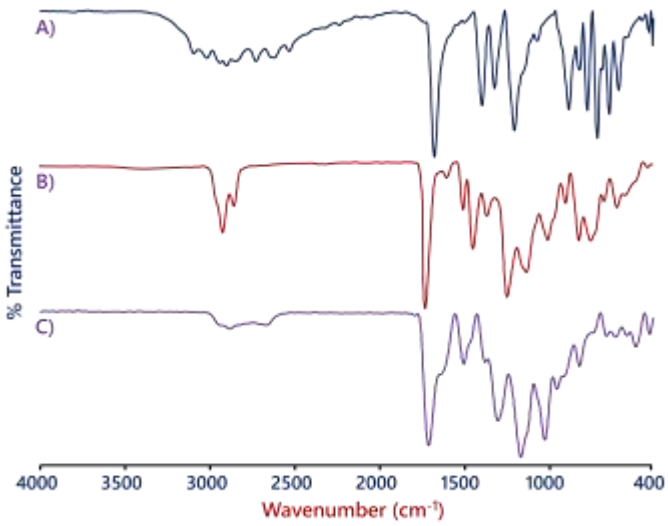

Figure SI-2: FT-IR spectra of 3-thiopheneacetic acid(A), thiophene monomer (ThEp)(B) and copolymer Poly(ThEp-co-EDOT) (C).

The chemical structure of the thiophene monomer (ThEp) was determined by ^{1}H NMR spectroscopy, and before the analysis, the ThEp monomer was dissolved in deuterated chloroform ($CDCl_3$) (Figure SI-3). The three peaks in thiophene rings appeared in the aromatic region at 7.32 ppm (Ha), 7.20 ppm (Hb), and 7.07 ppm (Hc), which were with the integration ratios 1:1:1. The peaks at 3.74 ppm (He), 4.48 ppm, and 3.98 ppm ($Hd_{1,2}$; 2H) were attributable to the methylene group on the monomer side group. The protons of the oxirane ring were seen at 3.24 ppm (Hf), 2.86 ppm, and 2.65 ppm ($Hg_{1,2}$), respectively. The integration ratios of Ha:Hb:Hc:Hd:He:Hf:Hg were calculated as 1:1:1:2:2:1:2.

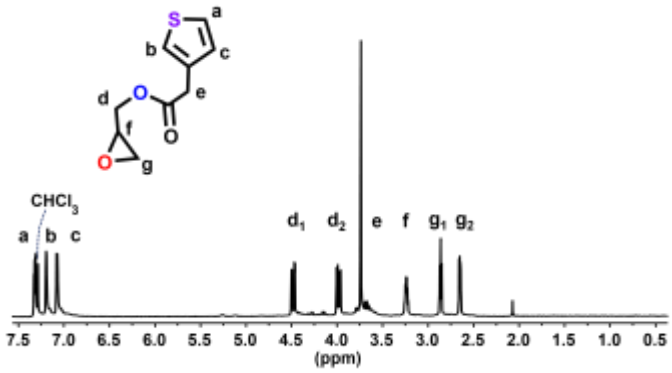

Figure SI-3. ^{1}H NMR spectra of epoxy-substituted thiophene monomer (ThEp).

The chemical structure of the thiophene monomer (*ThEp*) was also investigated by ^{13}C NMR spectroscopy. The three different carbon peaks in thiophene rings appeared in the aromatic region at 125.9 ppm (a), 123.1 ppm (b), 128.5 ppm (c) and 133.2 ppm(d). The peak at 35.6 ppm (e) was attributable to carbon of the methylene group. The carbon peak of the carbonyl group was seen at 170.9 ppm (f). The peak at 65.3 ppm (g) was attributable to the carbon of the methylene in the epoxy group. The carbon peaks of the oxirane ring were seen at 49.3 ppm (h) and 44.7 ppm (j), respectively.

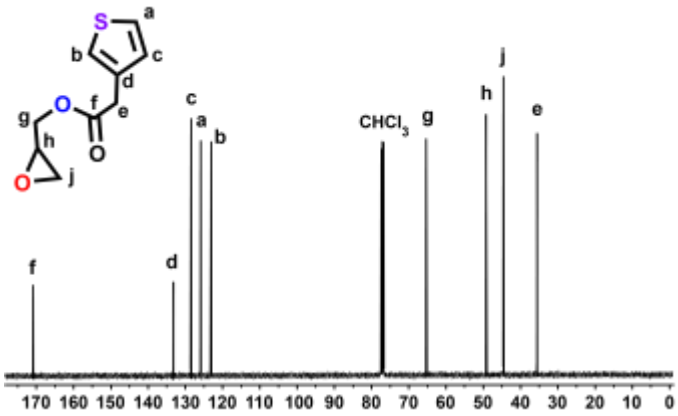

Figure SI-4. ^{13}C NMR spectra of epoxy-substituted thiophene monomer (ThEp).

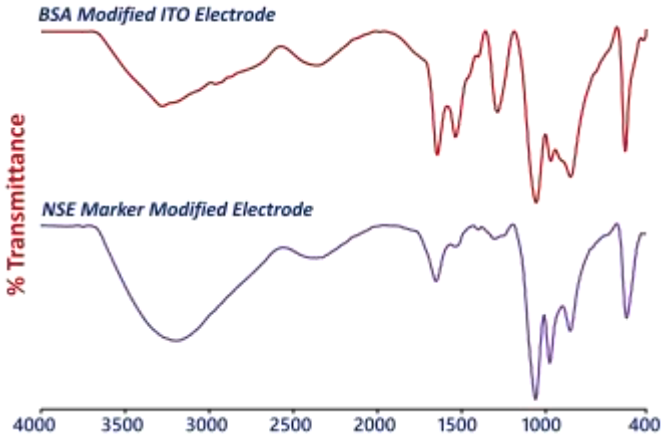

Figure SI-5. FTIR spectra of BSA modified electrode(A) and NSE antigen modified (B) ITO electrode surface.

As seen in Fig. SI-5, the peaks of proteins are visible in both spectra of BSA modified and NSE antigen modified electrodes.

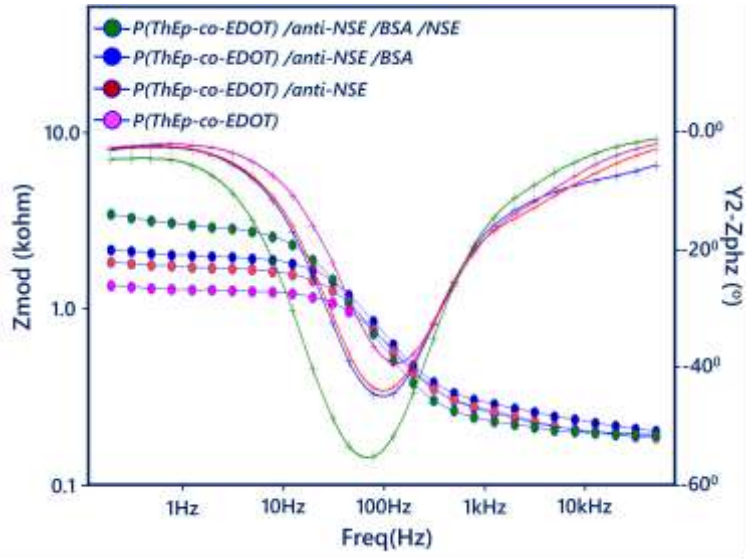

Figure SI-6. Bode plots of P(ThEp-co-EDOT), anti-NSE, BSA and NSE modified electrodes.

Fig. SI-6 represents the Bode plots of the different modified electrodes, and this provides monitoring of the direct relationship between impedance, phase degree, and frequency. As seen in this figure (the impedance-frequency curve), copolymer-coated electrode had a lower Rct than other fabrication steps. These results supported the obtained EIS results and the successful fabrication of the proposed biosensor.

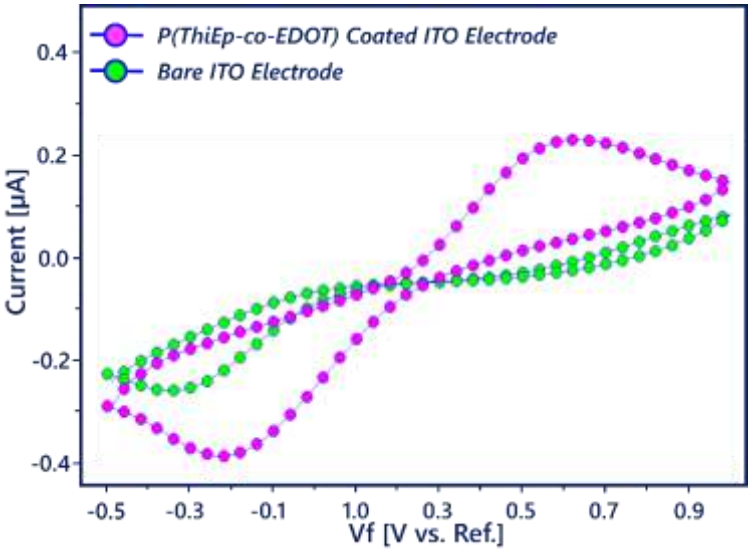

Figure SI-7. CVs of bare and copolymer coated ITO electrode.

Cross-sectional images of the electrodes coated with different concentrations of the copolymer solutions are recorded and given in Figs. SI-8. Fig. SI-8A, 8B, and 8C illustrate the cross-sectional images of 0.5 mg/mL, 1 mg/mL, and 2.5 mg/mL copolymer-coated electrodes, respectively. The approximate thicknesses of copolymer films obtained from cross-sectional images were found to be 250 nm (0.5 mg/mL), 840 nm (1.0 mg/mL), and 1.84 µm (2.5 mg/mL).

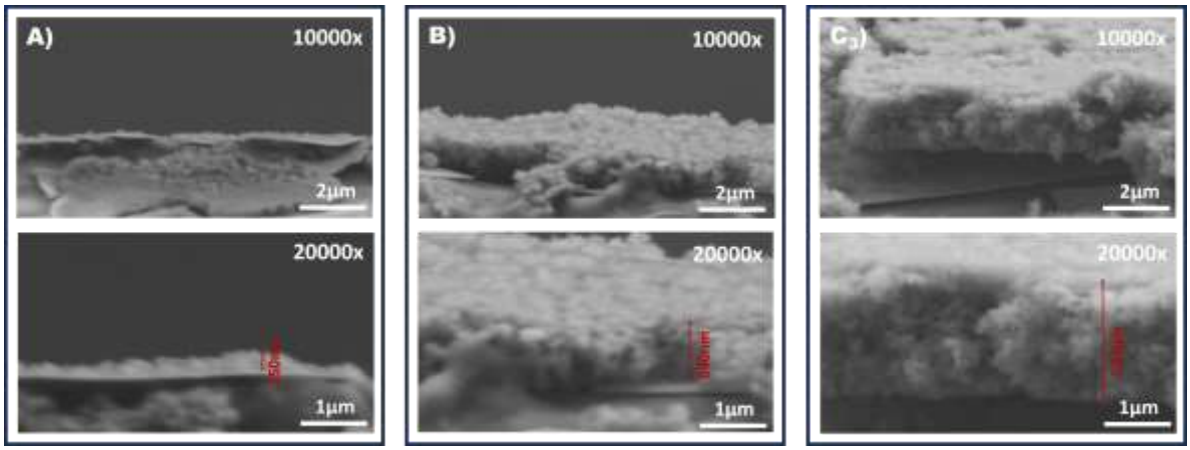

Figure SI-8. Cross- sectional SEM images of polymer films obtained with solutions of different polymer concentrations.

Cross-sectional SEM images of bare ITO, copolymer-coated ITO and anti-NSE antibody-modified ITO electrodes are given in Figs. SI-9A-C, respectively. The approximate thickness values of the ITO (indium/tin oxide) layer on the PET electrode, the copolymer film on the electrode, and the anti-NSE immobilized electrode, as well as the variations of the cross-sectional morphologies, are shown in Figure SI-9.

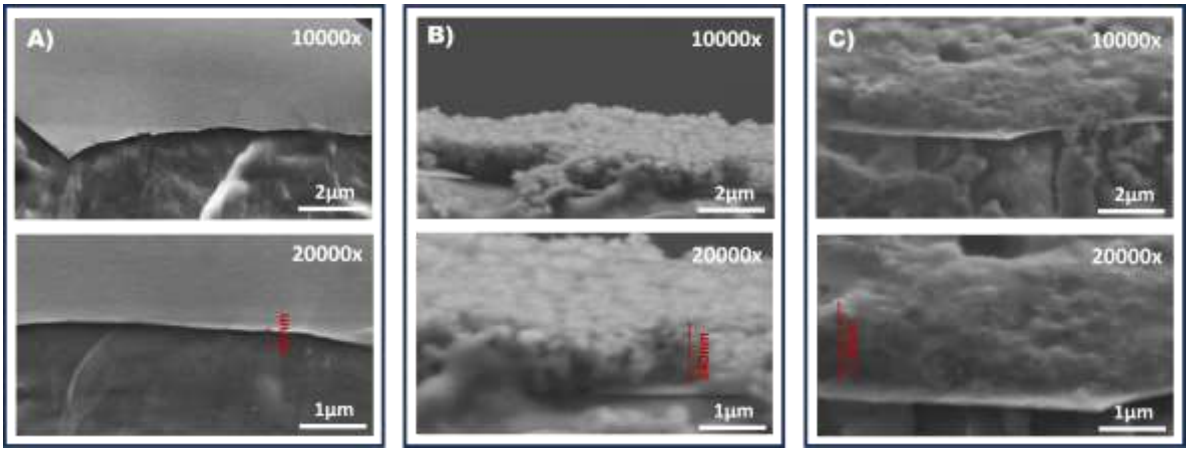

Figure SI-9. Cross- sectional SEM images of bare electrode, polymer coated electrode and anti-NSE antibody modified electrode.

Fig. SI-10 represents the Bode plots of electrodes modified with different amounts of NSE antigens. When the impedance-frequency curve was investigated, the electrode on which a low amount of NSE was immobilized had a lower Rct than other electrodes. The results supported the obtained EIS and CV results.

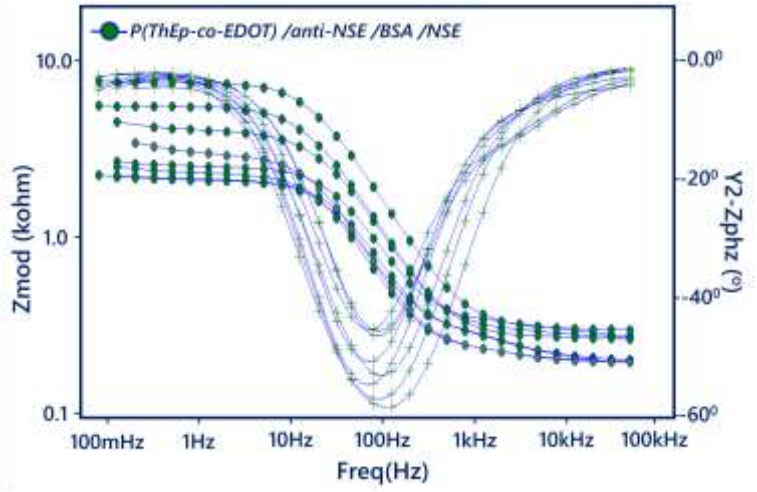

Figure SI-10. The Bode plots of electrodes modified with different amounts of NSE antigens.

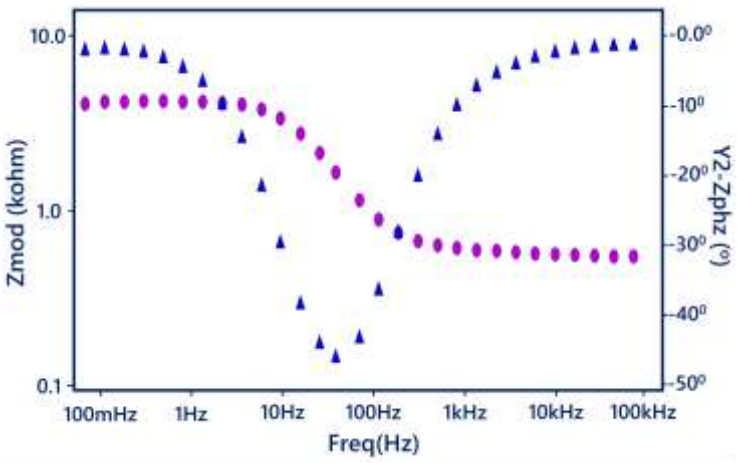

Figure SI-11. Bode plot.

Surface coverage was calculated by using the equilibrium Q=nFAΓ, where Q is the total charge (C), n is the number of electrons transferred, F is the Faraday constant (96,485 C mol^{-1}), A is the electroactive surface area (cm2), and Γ is the surface coverage (mol cm^{-2})[8] (Determining Surface Coverage of Self-Assembled Monolayers on Gold Electrodes). The surface coverage of the bare electrode was found to be 1.051 × 10−6 mol cm−2. After coating the synthesized copolymer on the electrode surface, Γ was found to be 6.797 × 10−5 mol cm−2, and this result indicated that the surface was increased by approximately 65 fold.

References

1. E. B. Aydın and M. K. Sezgintürk, *Anal. Chim. Acta*, 2019, **1077**, 129-139.
2. B. Neises and W. Steglich, *Angewandte Chemie International Edition in English*, 1978, **17**, 522-524.
3. E. B. Aydın, M. Aydın and M. K. Sezgintürk, *Microchim. Acta*, 2023, **190**, 235.
4. M. Wang, P. Baek, L. Voorhaar, E. W. C. Chan, A. Nelson, D. Barker and J. Travas-Sejdic, *Eur. Poly. J.*, 2018, **109**, 237-247.
5. K.-L. Foo, S.-T. Ha, G. Yeap and S. Lee, *Phase Transitions*, 2018, **91**, 509-520.
6. E. Sahin, P. Camurlu, L. Toppare, V. M. Mercore, I. Cianga and Y. Yagˇcı, *Journal of Electroanal. Chem.*, 2005, **579**, 189-197.
7. S. Edmondson and W. T. Huck, *J. Mat. Chem.*, 2004, **14**, 730-734.
8. Alice H. Suroviec, *Chem. Educator* 2012, **17**, 83–85.