Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPLEMENTARY INFORMATION

Synthesis and Grafting of Diazonium Tosylates for Thermoplastic Electrode Immunosensors

Cynthia P. McCord†, Tugba Ozer§, and Charles S. Henry*,†,‡

† Department of Chemistry, ‡ School of Biomedical Engineering, Colorado State University, Fort Collins, Colorado 80523, United States

§ Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, Istanbul 34220, Turkey

Index

1. Impact of pre-ground p-nitroaniline modified electrode voltammetry

Diazonium pastes used to modify the 1 mm diameter array electrodes were synthesized with un-ground p-nitroaniline crystals and with p-nitroaniline pre-ground to a fine powder with a mortar and pestle. Cyclic voltammograms were taken before and after modification.

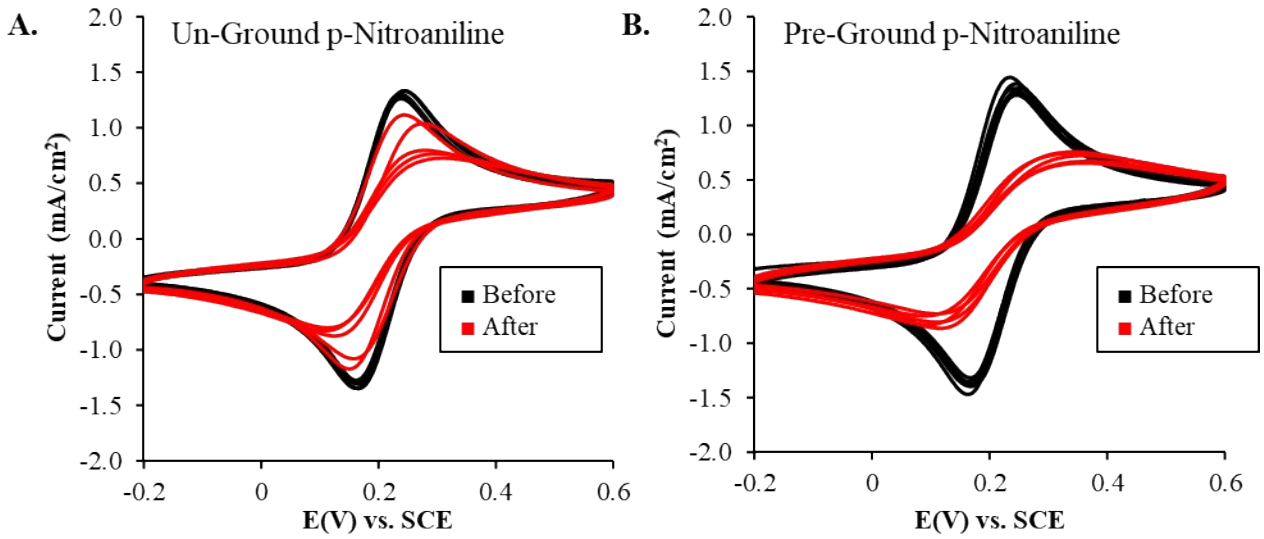

Figure S.1. Cyclic voltammetry for 5 mM ferri/ferrocyanide in PBS on 1 mm diameter electrode arrays v=100 mV/s before and after modification with p-aminophenyl diazonium paste.

2. Cyclic voltammetry of the different electrode pre-treatments

Cathodic pre-treatments were performed at -1.5 V vs. SCE for 30 seconds in 0.1 M sulfuric acid to reduce electrode surfaces. Anodic pre-treatments were performed at +1.5 V vs. SCE for 30 seconds in 0.1 M sodium hydroxide to oxidize electrode surfaces. Sanded electrodes were modified as is following 600 grit sandpaper. Cyclic voltammetry of the modified electrodes is shown in Fig. S.3.

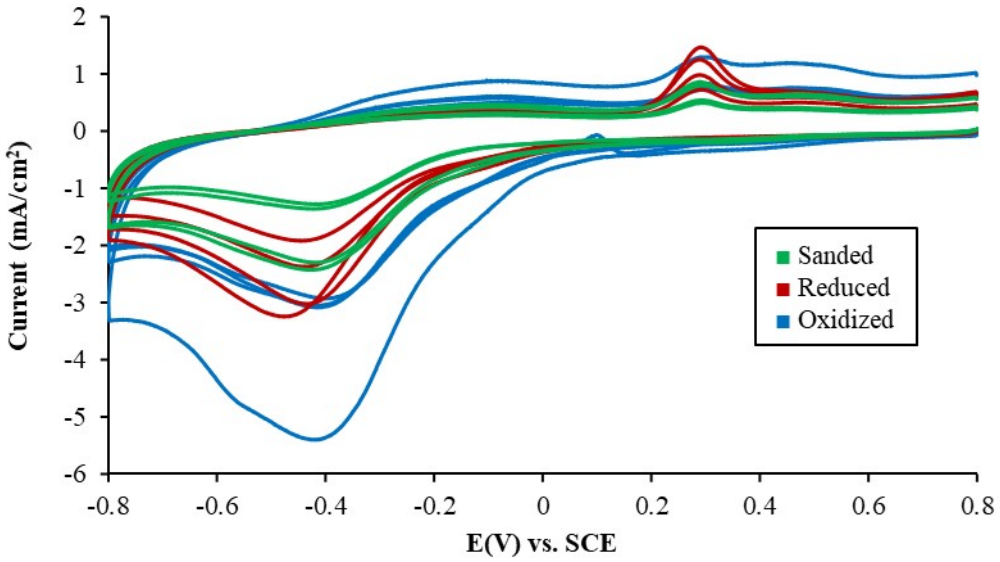

Figure S.2. Cyclic voltammetry for N_2 degassed 0.1 M H_2SO_4 on p-nitrophenyl diazonium salt modified 2.5 mm diameter disk electrodes v=100 mV/s.

3. Coverage and capacitance data for p-nitrophenyl modified electrodes

2.5 mm diameter disk electrodes and 1 mm diameter array electrodes were modified with p-aminophenyl diazonium salt using either single recipe 0.2 mmol p-nitroaniline (PN) paste or triple recipe 0.6 mmol paste with un-ground or pre-ground PN. Before and after modification, capacitance measurements were taken with cyclic voltammetry from 0.15 to 0 V vs. SCE v=100 mV/s in N_2 degassed 0.1 M H_2SO_4. The capacitance was calculated using the following equation:

$$C = \left(\frac{|I_a| + |I_c|}{(2v)A}\right)$$

where C is the geometric area-normalized capacitance, v is the scan rate (V/s), I_a is the anodic current and I_c is the cahodic current at 0.1 V vs. SCE, and A is the geometric surface area of the electrode. Following capacitance measurements, cyclic voltammograms were taken of the modified electrodes from 0.8 to -0.8 V vs. SCE v=100 mV/s in N_2 degassed 0.1 M H_2SO_4 to calculate surface nitrophenyl coverage.

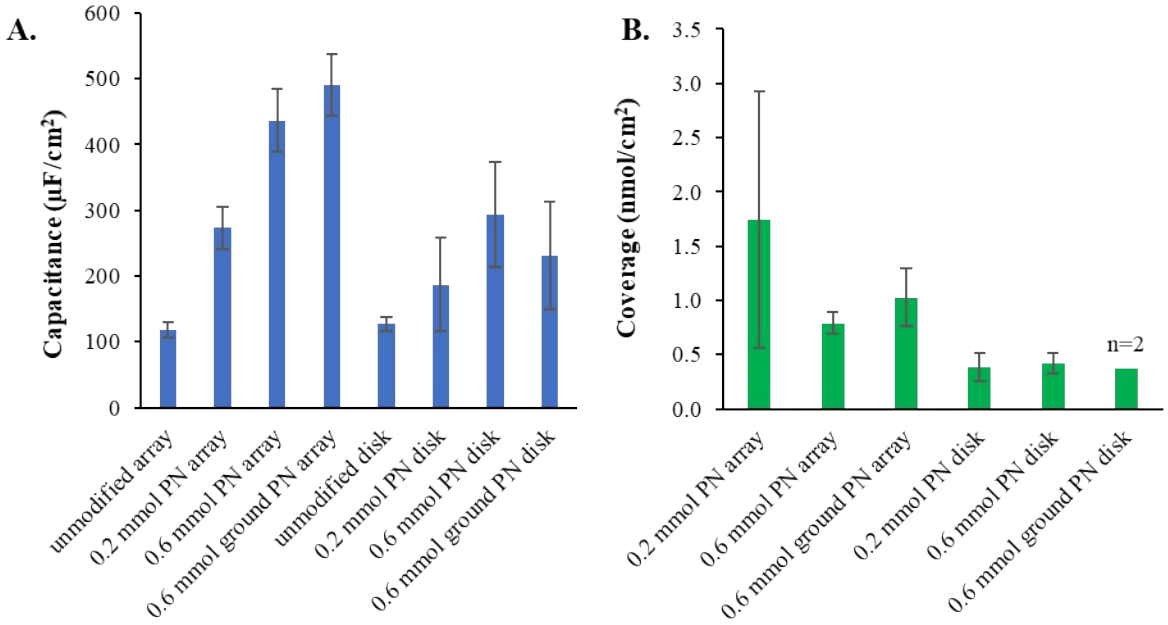

Figure S.3. Data for unmodified and p-nitrophenyl diazonium salt modified electrodes for A) capacitance (n=4), and B) nitrophenyl coverage (n=4).

4. Cyclic voltammetry of p-aminophenyl and p-nitrophenyl diazonium salt modified electrodes

p-Aminophenyl diazonium salt pastes were synthesized with both 1:1 and 3:2 p-phenylenediamine sodium nitrite ratios and used to modify 2.5 mm diameter disk electrodes. The voltammetry of the aminophenyl modified electrodes were compared to that of p-nitrophenyl diazonium salt modified electrodes as shown in Fig. S.5.

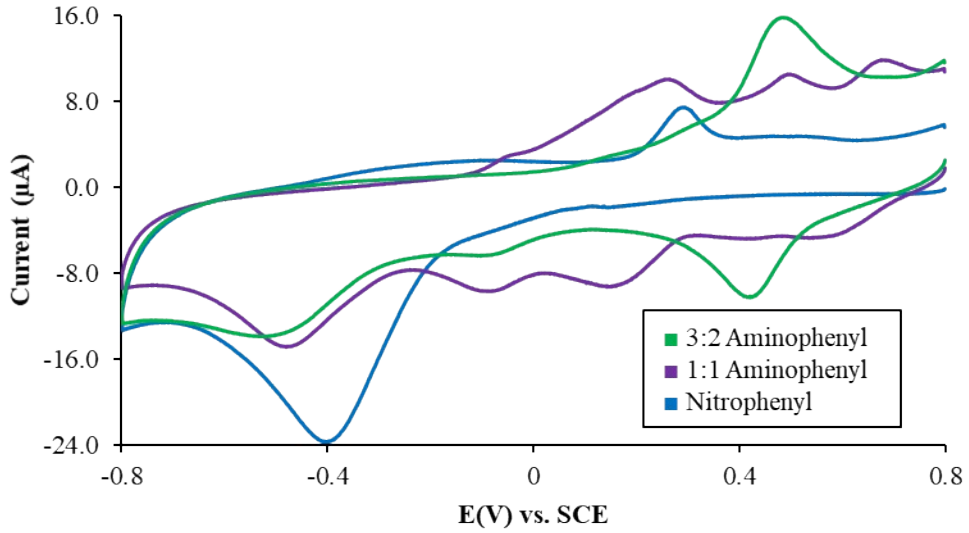

Figure S.4. Cyclic voltammetry for N_2 degassed 0.1 M H_2SO_4 on p-nitrophenyl and p-aminophenyl diazonium salt modified 2.5 mm diameter disk electrodes v=100 mV/s.

5. SEM Images of Modified TPE arrays

For SEM sample preparation, the TPE arrays were either unmodified but pre-treated as if they were going to be modified with sanding and reduction in acid followed by 10 min sonication in IPA or modified with the optimized diazonium procedures for nitrophenyl or aminophenyl modification. Array connections were removed with a razor blade and 1 mm diameter electrode samples were laser cut out of the array templates. SEM imaging was performed with a JSM6500F field emission scanning electron microscope (JEOL, Tokyo, Japan) at 15 kV acceleration voltage.

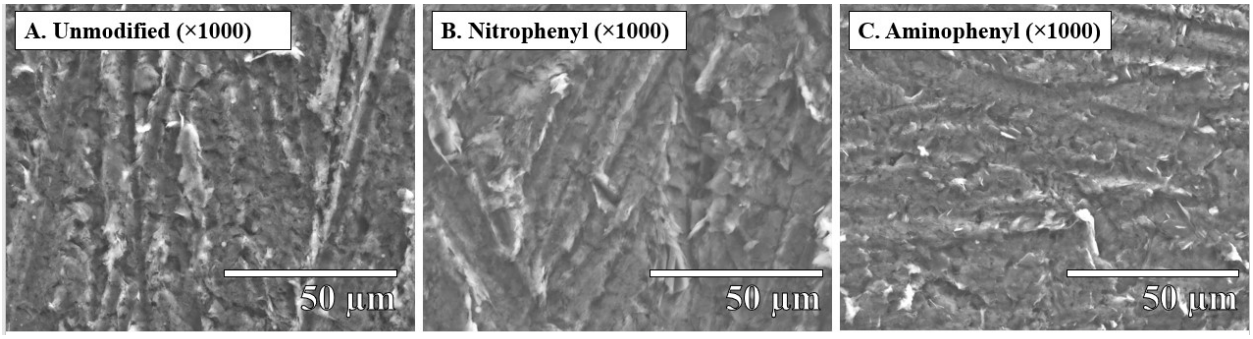

Figure S.5. ×1000 SEM images of A) unmodified B) nitrophenyl modifies, and C) aminophenyl modified TPEs.

6. TPE Immunosensor Design and Setup

TPE immunosensors (Fig. S.5) were made to have 25×57 mm rectangular dimensions. Large 7×3 mm oval shaped CE were added to the top of the arrays to use for electrochemical pre-treatments so that 3× array electrodes could be treated at a time by clipping the wires together. Each of the six individual electrochemical cells contained a 1 mm diameter WE and 1.5 mm diameter CE within an 8×5 mm rastered oval to hold solution droplets in place. To perform SWV measurements, the tip of the homemade Ag/AgCl RE was placed inside the 20 µL droplet.

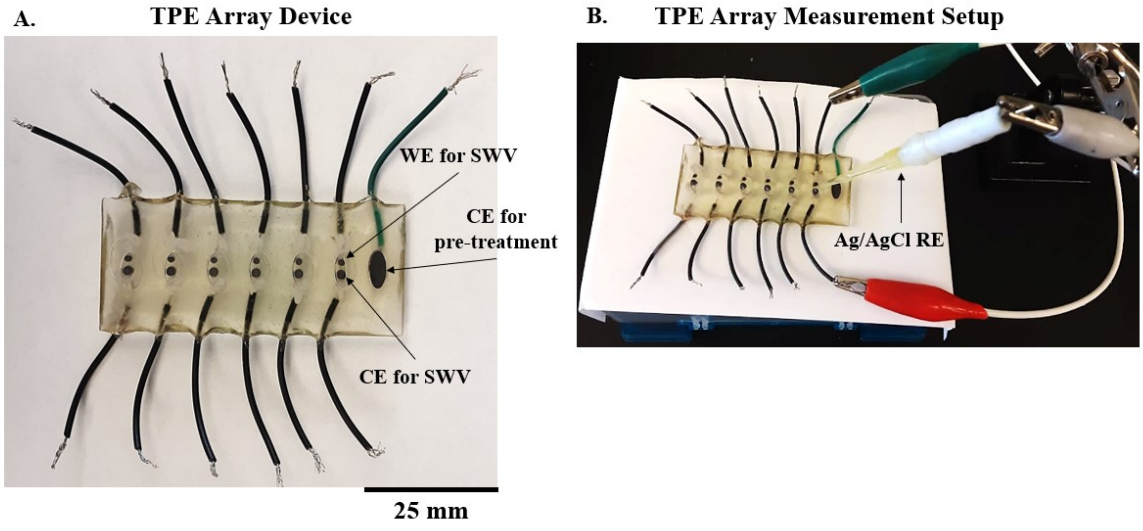

Figure S.6. A) TPE Immunosensor design and B) setup for SWV.

7. SWV Data Processing

To process SWV data, first the background subtraction function in CH Instrument software was used to subtract the tris-HCl voltammogram from the p-AP voltammogram taken after 20 min in p-APP substrate. The resulting peak was integrated using the CH Instrument software to determine the peak area (nVA: nanoVolt×Amps) as shown in Fig. S.5.

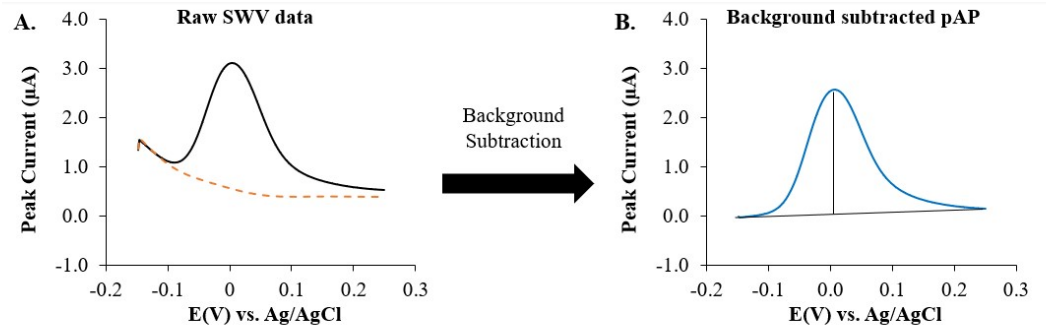

Figure S.7. SWV data processing. A) Raw data of the tris-HCl buffer blank on the modified electrodes overlayed with the data from 20 min in pAPP for CRP ELISA experiments (CRP 10,000 ng/mL), and B) background subtracted data with integration shown.

8. Four-Parameter Logistic (4-PL) Regressions

To fit the CRP ELISA data, 4-PL logistic fits were calculated using MATLAB 2020a using L4P and L4Pinv code sourced from Giuseppe Cardillo:

Cardillo G. (2012) Four parameters logistic regression - There and back again https://it.mathworks.com/matlabcentral/fileexchange/38122

The 4-PL fits for PN, PPD, and PPD in serum are listed below:

General model: cf(x) = D+(A-D)/(1+(x/C)^B)

PN 4-PL: 0.1-10000 ng/mL

Coefficients (with 95% confidence bounds):
A = 16.19 (1.801, 30.57)
B = 0.6628 (0.2096, 1.116)
C = 120.2 (-7.422, 247.8)
D = 131.9 (106.5, 157.3)
R^2=0.9978

PPD 4-PL: 0.1-10000 ng/mL

Coefficients (with 95% confidence bounds):
A = 39.07 (1.408, 76.73)
B = 0.8874 (-0.02673, 1.802)

C = 52.53 (-14.9, 120)
D = 227 (184.1, 269.9)
R^2=0.9943

PPD 4-PL: 0-1000 ng/mL

Coefficients (with 95% confidence bounds):
A = 52.12 (20.73, 83.5)
B = 0.9139 (-0.3075, 2.135)
C = 32.61 (-28.99, 94.2)
D = 181.1 (124, 238.1)
R^2=0.9993