Supplementary Information

Sample pre-treatment free electrochemical immunosensor with negative electro-pulsion for quantitative detection of acrylamide in coffee, cocoa and prune juice

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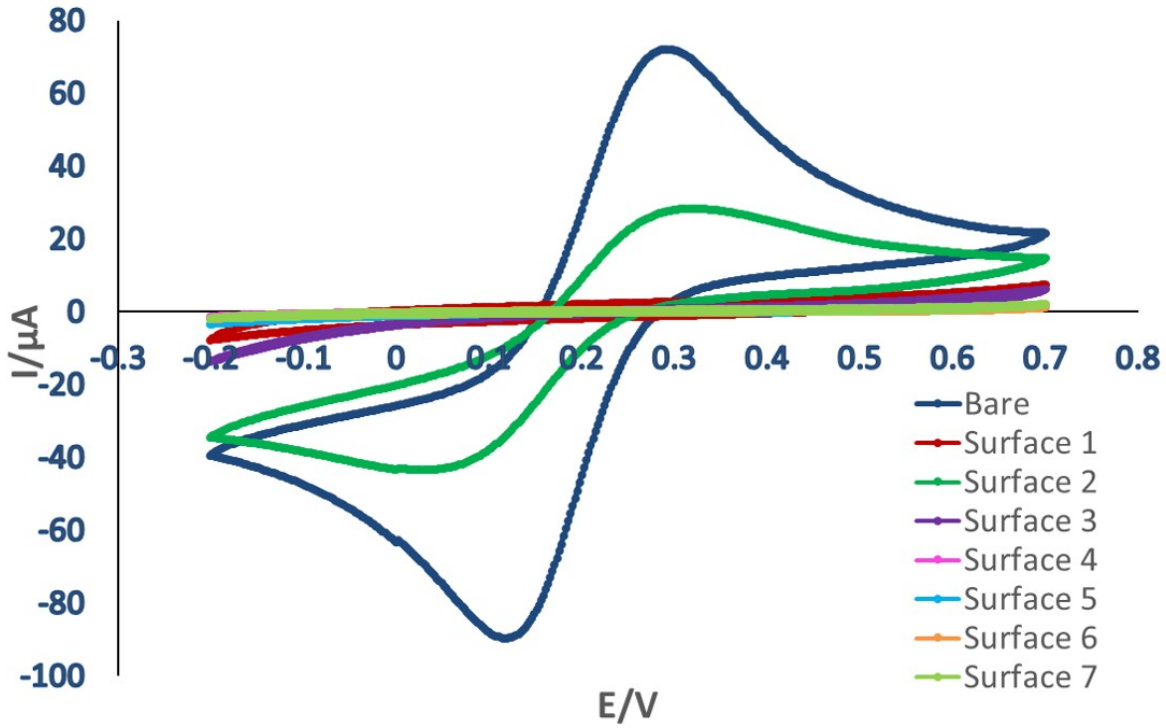

Fig S1. Corresponding zoom of Fig 1(a) − Cyclic voltammograms of glassy carbon plate (GCP) after each modification steps from bare electrode to surface 7 in PB containing 1 mM $[Fe(CN)_6]^{3-/4-}$ and 0.05 M KCl with scan rate of 100 mV s^{-1}.

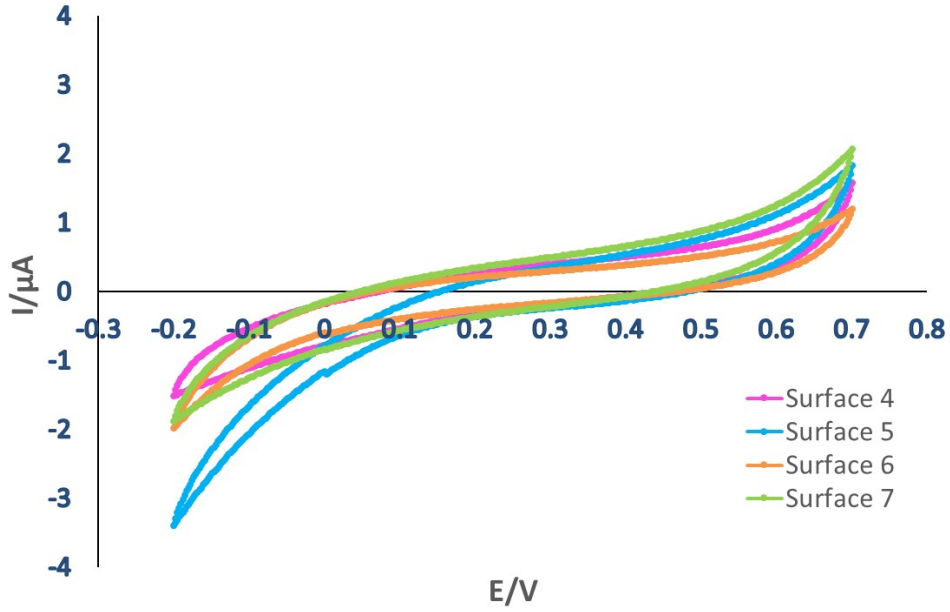

Fig S2. Corresponding zoom of Fig 1(a) − Cyclic voltammograms of glassy carbon plate (GCP) after each modification steps from surface 4 to surface 7 in PB containing 1 mM $[Fe(CN)_6]^{3-/4-}$ and 0.05 M KCl with scan rate of 100 mV s^{-1}.

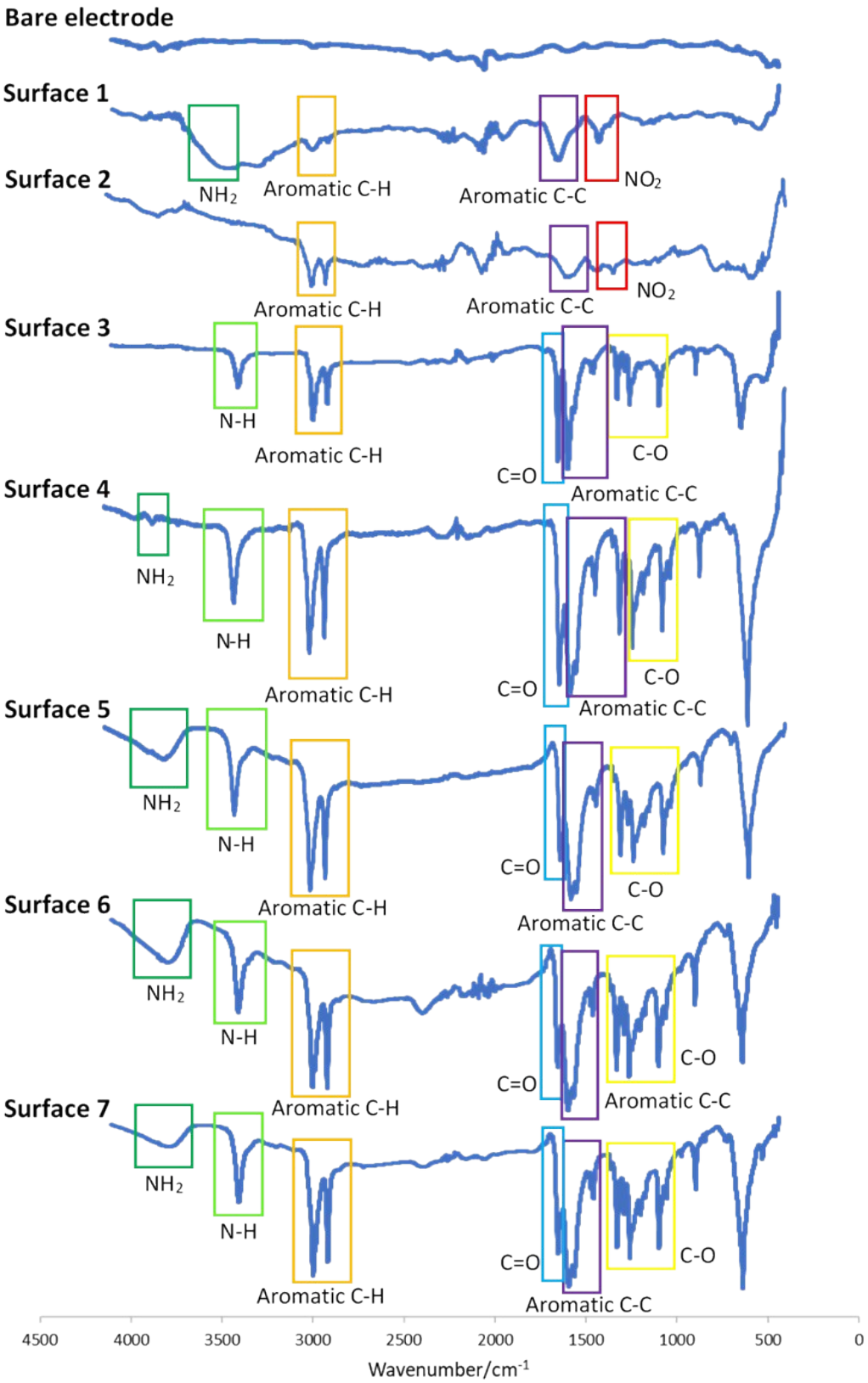

Fig S3. FT-IR spectra of immunosensor surface, from bare GCP to surface 7 after each modification step [NH_2 (green), amide N-H (light green), aromatic C-H (orange), C=O (light blue), aromatic C-C (purple), ether C-O (yellow) and NO_2 (red)].

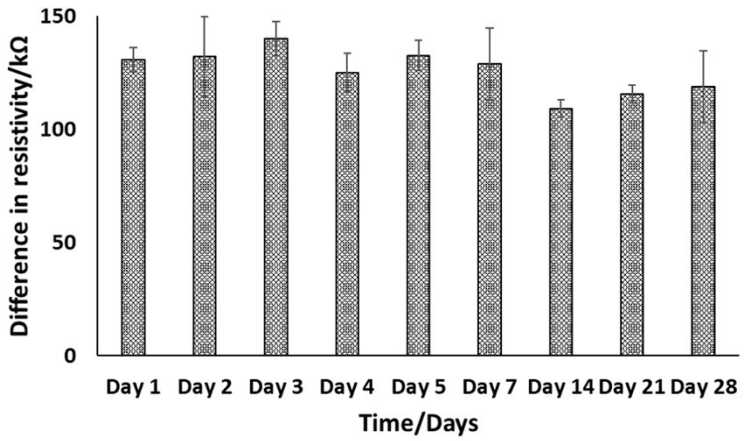

Fig S4. Stability study of the developed immunosensor based on the decrease in resistivity from day 1 to day 28, tested in 10 μg mL^{-1} of free acrylamide in PBS, pH 7.4 after storage of immunosensor at 4°C kept in PBS, pH 7.4.

Table S1. Values of equivalent circuit parameters of fitting curves for the fabrication of electrochemical impedance immunosensor interface by z-view.

Electrode	**R_s/Ω**	**R_{ct}/Ω**	**Q/Mh_o**	**n**	**Cdl/F**
Bare GCP	16.496	9.252×10^{2}	2.576×10^{-6}	0.779	8.903×10^{-5}
Surface 1	13.219	1.056×10^{5}	4.778×10^{-6}	0.774	3.914×10^{-6}
Surface 2	36.095	2.785×10^{3}	5.747×10^{-6}	0.740	1.343×10^{-6}
Surface 3	15.911	1.235×10^{5}	1.975×10^{-6}	0.826	1.468×10^{-6}
Surface 4	43.067	2.280×10^{5}	5.818×10^{-6}	0.843	6.133×10^{-6}
Surface 5	28.296	4.207×10^{5}	1.550×10^{-6}	0.845	1.433×10^{-6}
Surface 6	24.467	7.604×10^{5}	1.251×10^{-6}	0.886	1.244×10^{-6}
Surface 7	18.947	5.129×10^{5}	1.417×10^{-6}	0.855	1.343×10^{-6}

Table S2. Box-Behnken experimental design for three factors used in displacement condition and response.

Run	Factor 1	Factor 2	Factor 3	Response 1
	A:Pulsing Duration min	B:Pulsing Potential mV	C:Concentration of Acrylamide μg mL^{-1}	Difference in resistivity kΩ
1	10	-700	30	161.65
2	5	-800	30	58.67
3	10	-900	30	113.5
4	10	-800	15	163.06
5	5	-800	0	15.2276
6	10	-800	15	187.35
7	10	-900	0	12.1243
8	10	-700	0	12.008
9	15	-900	15	95.176
10	5	-900	15	69.55
11	15	-700	15	64.044
12	10	-800	15	166.96
13	5	-700	15	45.804
14	10	-800	15	187.117
15	15	-800	0	15.4522
16	10	-800	15	164.14
17	15	-800	30	225.812

Table S3. Analysis of variance (ANOVA) for quadratic model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	80250.07	9	8916.67	16.22	0.0007	significant
A- Pulsing Duration	5577.40	1	5577.40	10.15	0.0154	
B- Pulsing Potential	5.86	1	5.86	0.0107	0.9207	
C-Concentration of Acrylamide	31855.39	1	31855.39	57.95	0.0001	
AB	13.64	1	13.64	0.0248	0.8793	
AC	6965.35	1	6965.35	12.67	0.0092	
BC	582.41	1	582.41	1.06	0.3376	
A^2	10761.73	1	10761.73	19.58	0.0031	
B^2	12518.20	1	12518.20	22.77	0.0020	
C^2	8292.59	1	8292.59	15.09	0.0060	
Residual	3847.74	7	549.68			
Lack of Fit	3231.37	3	1077.12	6.99	0.0455	significant
Pure Error	616.37	4	154.09			
Cor Total	84097.81	16				