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Supplementary Information

Graphene based Multiplexed disposable Electrochemical biosensor for on-farm rapid monitoring of NEFA and βHBA Dairy biomarker

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Table S1: Analysis of standard and spiked blood, (containing known NEFA concentrations) and clinical serum samples (containing unknown NEFA concentrations) using DPV (Differential Pulse Voltammetry) technique.

NEFA Conc. (mM)	Current (µA) in Standard (n=3)	Current (µA) in Spiked Blood Samples (n=3)	Conc. Calculated (mM)	% Recovery	Real Clinical Samples	Current (µA) Measured (n=3)	Estimated Conc. (mM)
0.1	70.14	69.89	0.112	112	Unknown Sample- 1	59.55	0.151
0.25	61.43	58.24	0.299	119.6	Unknown Sample- 2	55.45	0.616
0.5	57.18	55.47	0.614	122.8	Unknown Sample- 3	51.53	1.060
1	51.57	50.69	1.156	115.6	Unknown Sample- 4	50.21	1.21
1.25	48.03	46.21	1.664	133.12			
1.5	46.83	43.14	2.012	134.13			
2	41.66	38.48	2.540	127			
3	34.33	32.14	3.259	108.63			
4	25.95	25.39	4.024	100.5			
5	17.8	18.14	4.846	96.92			
7.5	16.62						
10	7.39						

Table S2: Analysis of standard and spiked blood, (containing known βHBA concentrations) and clinical serum samples (containing unknown βHBA concentrations) using DPV (Differential Pulse Voltammetry) technique.

βHBA Conc. (mM)	**Current (µA) in Standard (n=3)**	**Current (µA) in Blood Samples (n=3)**	**Conc. Calculated (mM)**	**% Recovery**	**Real Clinical Samples**	**Current (µA) Measured (n=3)**	**Estimated Conc. (mM)**
0.7	74.52	75.16	0.609	87	Unknown Sample- 1	72.14	0.7179
0.8	70.55	70.53	0.775	96.87	Unknown Sample- 2	68.54	0.846
1	62.46	65.14	0.968	96.8	Unknown Sample- 3	61.27	1.107
1.2	57.14	57.41	1.245	103.75	Unknown Sample- 4	46.89	1.6223
1.4	52.32	50.58	1.490	106.42			
1.6	47.62	45.86	1.659	103.12			
1.8	42.72	41.49	1.81	100.55			
2	36.96	39.11	1.900	95			
3	33.71	36.21					
4	28.83	30.41					
5	23.18	22.54					
7.5	19.91	18.95					
10	7.22	8.24					

Table S3: Analysis of standard and spiked blood samples with known concentration of NEFA using chronoamperometry

NEFA Conc. in Standard (mM)	Current Measured (µA) (n=3)	Conc. in Spiked Blood (mM)	Current Measured (µA) (n=3)	Calculated conc. of antigen using standard calibration (mM)	% Recovery
Ab	127.94	Ab	128.40		
0.1	121.63	0.1	120.24	0.108	108
0.2	115.35	0.2	116.18	0.204	102
0.4	106.52	0.4	108.47	0.386	96.5
0.6	99.94	0.6	101.14	0.559	93.16
0.8	93.31	0.8	95.53	0.691	86.37
1	83.61	1	90.21	0.817	81.7
1.2	70.58	1.2	75.14	1.173	97.75
1.4	63.71	1.4	62.34	1.475	105.35
1.6	60.89	1.6	60.21	1.525	95.31
1.8	46.29	1.8	47.54	1.824	101.33
2	41.09	2	39.59	2.012	100.6
3	36.42	3	37.12		
4	30.91	4	30.25		
5	25.69	5	26.57		
10	17	10	16.59		

Table S4: Analysis of standard and spiked blood samples with known concentration of βHBA using chronoamperometry.

βHBA Conc. in Standard (mM)	Current Measured (µA) (n=3)	Conc. in Spiked Blood (mM)	Current Measured (µA) (n=3)	Calculated conc. of antigen using standard calibration (mM)	% Recovery
Ab	102.64	Ab	102.75		
0.7	92.95	0.7	94.35	0.591	84.42
0.8	87.20	0.8	90.54	0.686	85.75
0.9	80.92	0.9	82.47	0.887	98.56
1	75.52	1	75.96	1.049	104.9
1.2	68.65	1.2	65.47	1.310	109.16
1.4	61.64	1.4	60.11	1.443	103.07
1.6	51.83	1.6	48.72	1.722	107.62
1.8	46.42	1.8	46.21	1.789	99.38
2	39.80	2	42.18	1.88	94
3	32.28	3	30.11		
4	26.44	4	24.55		
5	17.82	5	19.47		
10	13.42	10	12.76		

Table S5: Analysis of standard and spiked blood samples with known concentrations of NEFA using color based absorbance (at 450 nm) with a commercial colorimetric kit. The clinical samples of unknown concentrations were also measured, and the concentrations were estimated by using the linear plot equation shown in Figure 7(c).

NEFA Conc. in Standard (mM)	Absorbance Measured (O. D.) (n=3)	NEFA Conc. in Spiked Blood (mM)	Absorbance Measured (O. D.) (n=3)	Calculated Conc. of NEFA using standard calibration plot (mM)	% Recovery	Real Serum Sample	Absorbance Measured (n=3)	Conc. Calculation (mM)
0.1	0.115	0.1	0.115	0.152	100	Sample-1	0.156	0.168
0.2	0.225	0.2	0.277	0.217	108.5	Sample-2	1.222	0.597
0.3	0.467	0.3	0.402	0.267	89	Sample-3	2.356	1.054
0.4	0.69	0.4	0.625	0.357	89.25	Sample-4	2.356	1.218
0.5	0.956	0.5	0.997	0.507	101.4			
0.6	1.124	0.6	1.254	0.610	101.67			
0.7	1.456	0.7	1.541	0.726	103.71			
0.8	1.668	0.8	1.745	0.808	101			
0.9	1.958	0.9	1.897	0.869	96.56			
1	2.379	1	2.347	1.050	105			

Table S6: Analysis of standard and spiked blood samples with known concentrations of βHBA using color based absorbance (at 450 nm) with a commercial colorimetric kit. The clinical samples of unknown concentrations were also measured, and the concentrations were estimated by using the linear plot equation shown in Figure 7d.

βHBA Conc. in Standard (mM)	Absorbance Measured (O. D.) (n=3)	BHBA Conc. in Spiked Blood (mM)	Absorbance Measured (O. D.) (n=3)	Calculated Conc. of BHBA using standard calibration plot (mM)	% Recovery	Real Serum Sample (Diluted 10 times)	Absorbance Measured (n=3)	Conc. Calculation
0.025	0.128	0.025	0.132	0.0255	102	Sample-1	0.455	0.700
0.05	0.312	0.05	0.335	0.0531	106.2	Sample-2	0.527	0.802
0.1	0.667	0.1	0.684	0.105	105	Sample-3	0.669	1.005
0.2	0.975	0.2	0.974	0.199	99.5	Sample-4	0.882	1.690
0.3	1.378	0.3	1.45	0.321	107			
0.4	1.710	0.4	1.85	0.431	107.75			
0.5	2.152	0.5	2.41	0.576	115.2			
1	3.844	1	3.98	1.04	104			

Table S7: The comparison of results of NEFA obtained by using chemical analyzer, a colorimetric kit, and by our proposed electrochemical method.

Sr. No.	Clinical Serum Samples of Unknown conc. of NEFA	Detection by Chemical Analyzer	Detection by Commercial Colorimetric Kit	Our Proposed Electrochemical Method
1	Sample-1	0.165 mM	0.168 mM	0.151 mM
2	Sample-2	0.621 mM	0.597 mM	0.616 mM
3	Sample-3	1.058 mM	1.054 mM	1.060 mM
4	Sample-4	1.226 mM	1.218 mM	1.21 mM

Table S8: The comparison of results of βHBA obtained by using chemical analyzer, a colorimetric kit, and by our proposed electrochemical method.

Sr. No.	Clinical Serum Samples of Unknown conc. of βHBA	Detection by Chemical Analyzer	Detection by Commercial Colorimetric Kit	Our Proposed Electrochemical Method
1	Sample-1	0.784 mM	0.700 mM	0.712 mM
2	Sample-2	0.824 mM	0.802 mM	0.846 mM
3	Sample-3	0.958 mM	1.005 mM	1.107 mM
4	Sample-4	1.721 mM	1.690 mM	1.622 mM

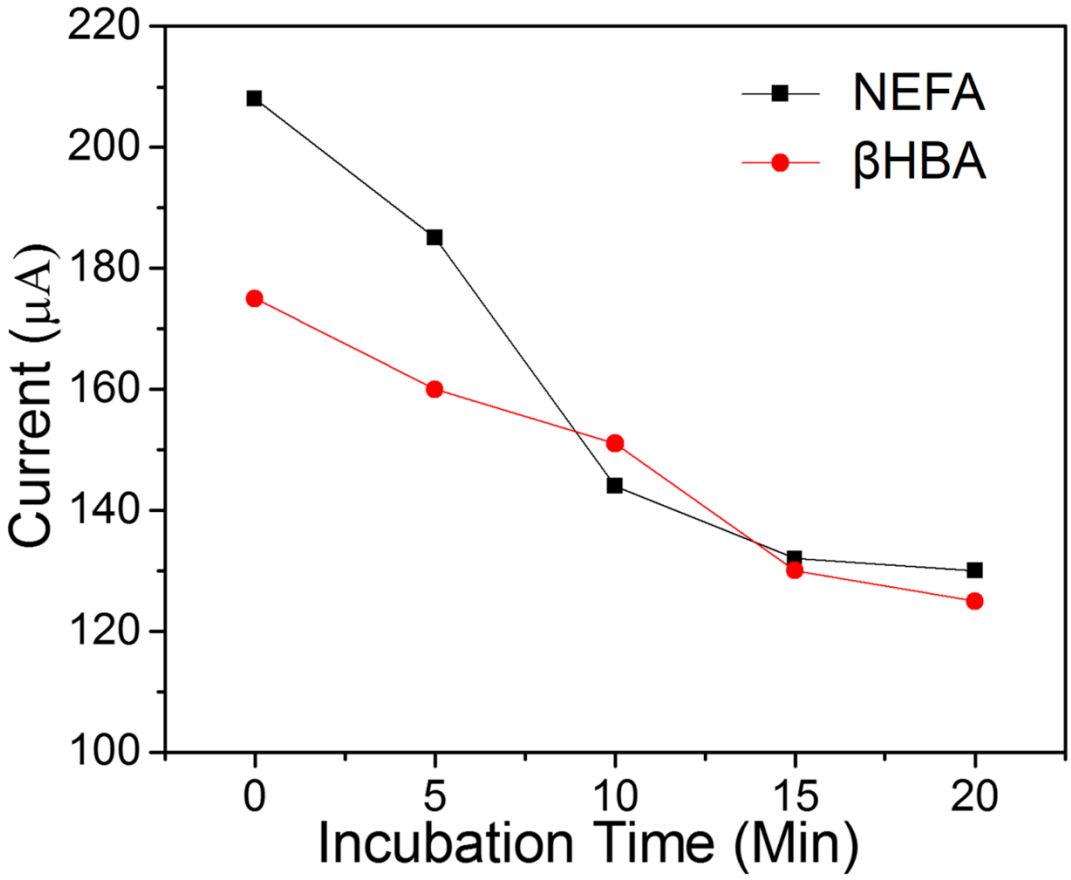

Figure S9: The incubation (interaction time) study of NEFA and βHBA over the Ab@E-rGO/SPE

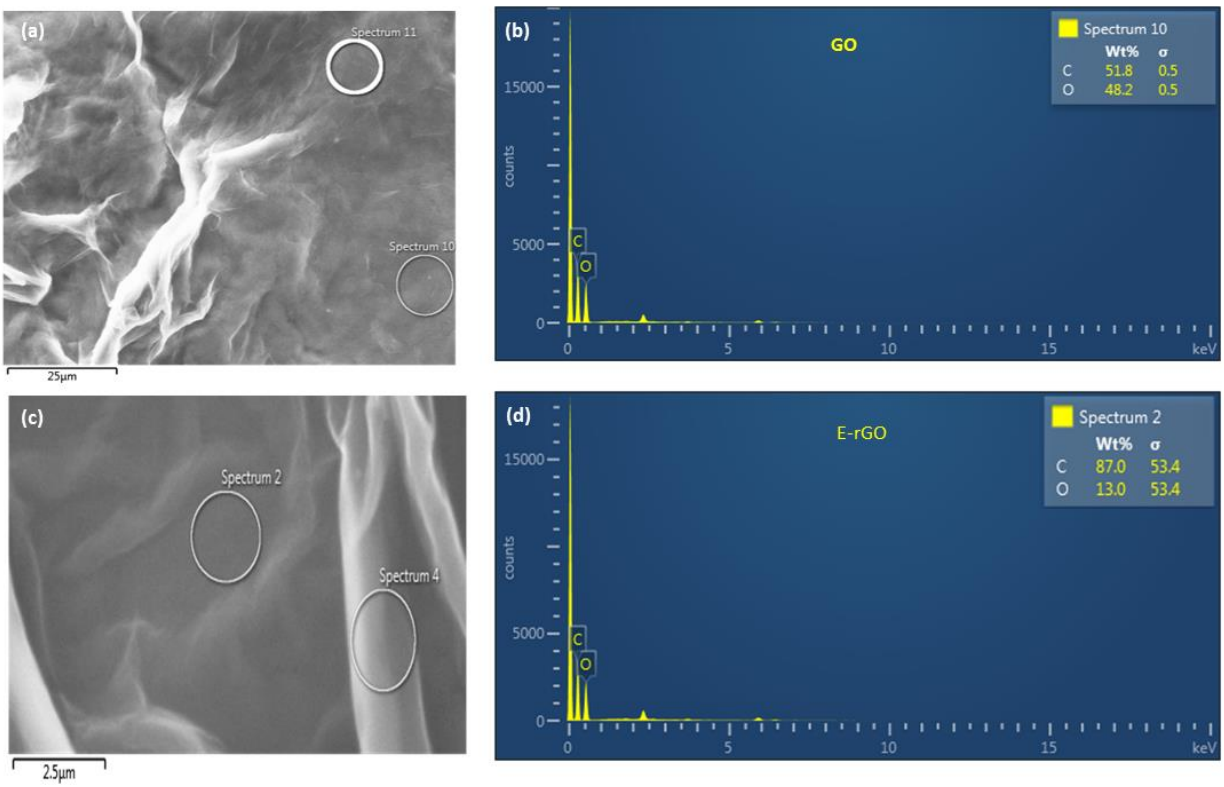

Figure S10: EDX analysis of exfoliated GO and electrodeposited E-rGO on the surface of SPEs.

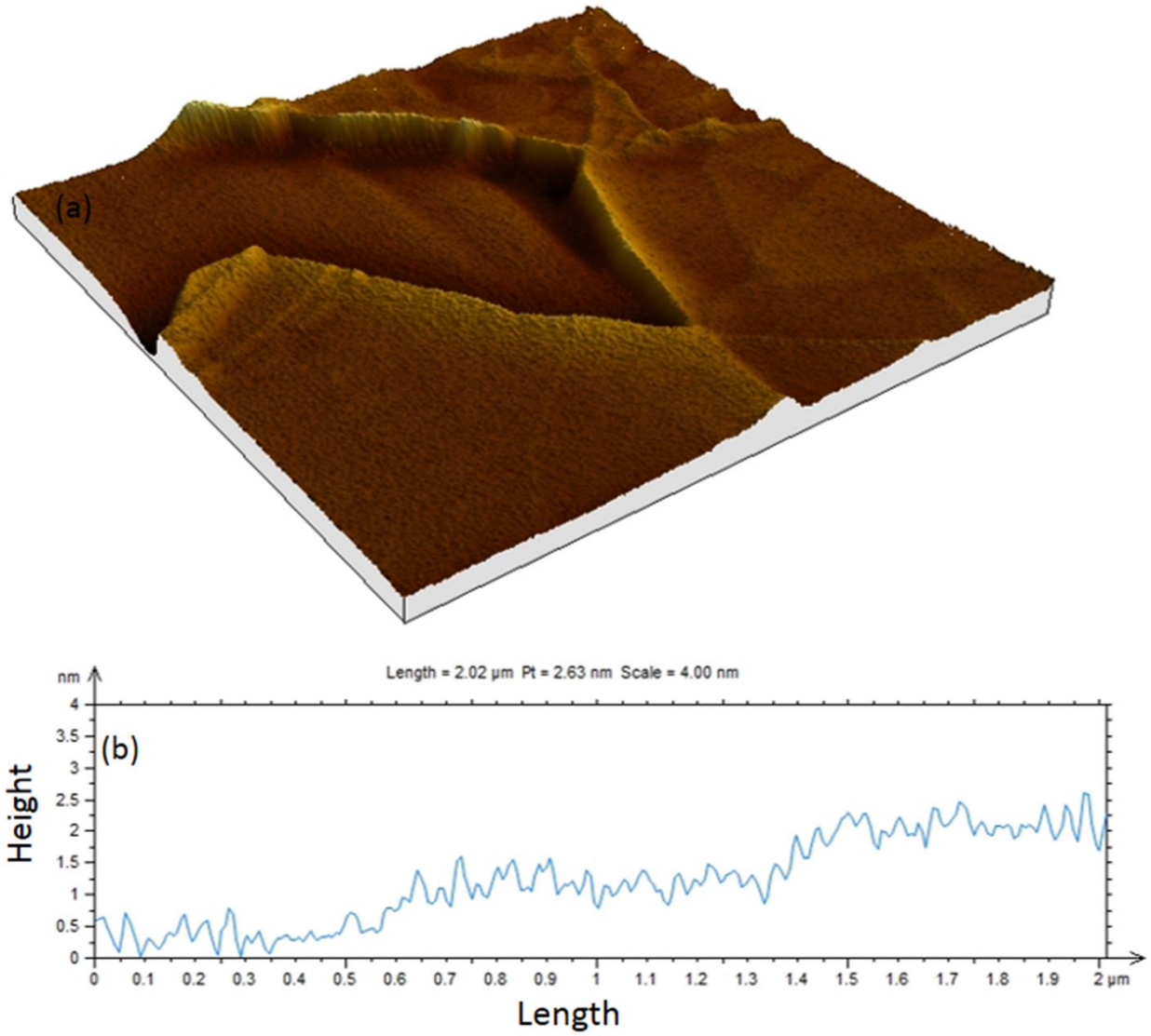

Figure S11: (a) 3D view of AFM analysis of E-rGO nanosheets; (b) Corresponding Line mapping.

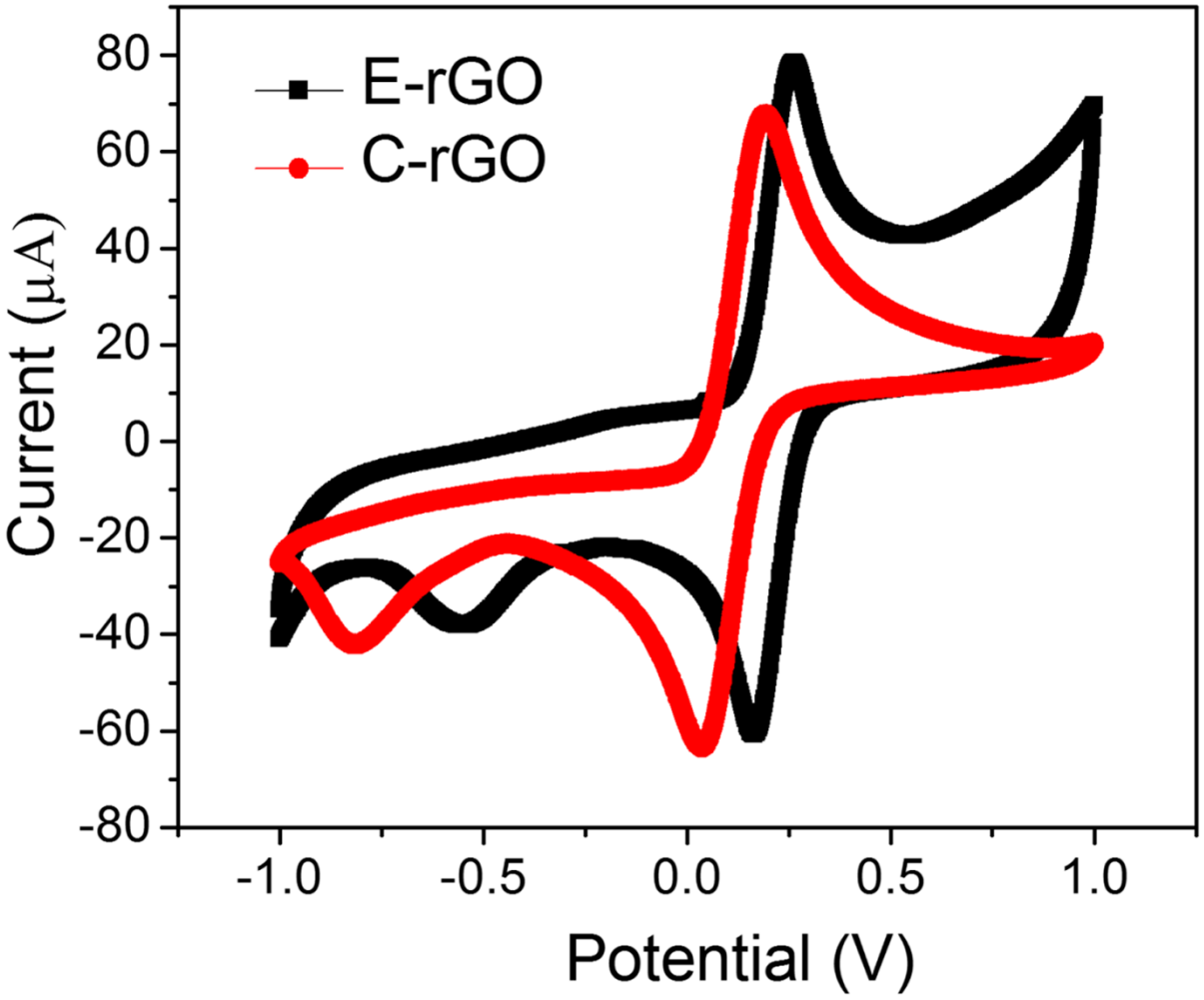

Figure S12: The comparison between the antibodies immobilized over the E-rGO and C-rGO towards electrocatalytic activity for sensor performance.

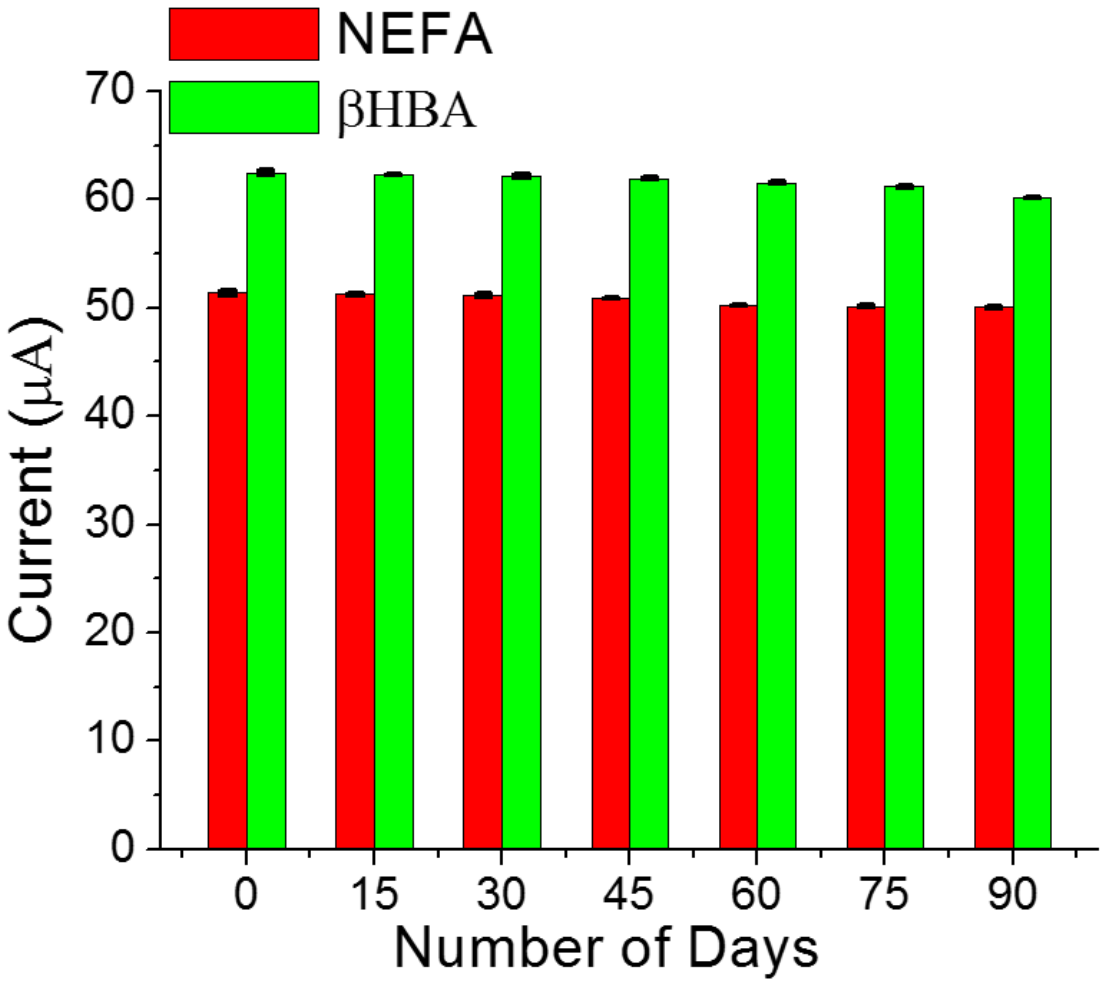

Figure S13: Stability of Ab@ErGO/SPEs during storage and the corresponding variation in response was measured at different time intervals.