Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary material for

Optimization of a Lipase/Reduced Graphene Oxide/Metal-Organic Framework Electrode using Central Composite Design-Response Surface Methodology Approach

Nur Aina Izzati Mohd Mokhtar[1], Siti Efliza Ashari[2,3], Ruzniza Mohd Zawawi[1,*]

[1]*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

[2]*Integrated Chemical BioPhysics Research, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

[3]*Centre of Foundation Studies for Agricultural Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

*****Corresponding author: ruzniza@upm.edu.my*

Table S1 The experimental runs with the actual and predicted current response obtained from RSM model

Run	Parameters			Current response (μA)	
	A: rGO weight (mg)	B: Ultrasonication time (min)	C: Lipase concentration (mg/mL)	Actual	Predicted
1	1.5 (0)	45 (0)	20 (0)	98.79	100.55
2	1 (-1)	45 (0)	20 (0)	105.6	104.6
3	1.5 (0)	45 (0)	20 (0)	100.31	100.55
4	1 (-1)	30 (-1)	10 (-1)	101.59	102.07
5	1.5 (0)	45 (0)	30 (+1)	112.36	111.43
6	2 (+1)	30 (-1)	30 (+1)	113.38	113.83
7	2 (+1)	60 (+1)	10 (-1)	103.78	103.56
8	2 (+1)	30 (-1)	10 (-1)	101.73	101.59
9	1 (-1)	60 (+1)	30 (+1)	112.03	112.37
10	1.5 (0)	45 (0)	20 (0)	99.71	100.55
11	1 (-1)	30 (-1)	30 (+1)	116.93	117.35
12	1 (-1)	60 (+1)	10 (-1)	104.35	104.10
13	1.5 (0)	45 (0)	20 (0)	100.26	100.55
14	2 (+1)	45 (0)	20 (0)	102.36	102.56
15	1.5 (0)	30 (-1)	20 (0)	101.13	99.92
16	1.5 (0)	45 (0)	20 (0)	100.52	100.55
17	1.5 (0)	45 (0)	10 (-1)	101.04	101.17
18	1.5 (0)	60 (+1)	20 (0)	98.01	98.42
19	2 (+1)	60 (+1)	30 (+1)	109.07	108.79

20	1.5 (0)	45 (0)	20 (0)	102.08	100.55

Table S2 The regression coefficient estimate results

Variable	Coefficient estimate
Standard deviation	1.04
Mean	104.25
Coeffiecient of variation (C.V.) %	0.9955
R^2	0.9812
Adjusted R^2	0.9642
Predicted R^2	0.9235
Adequate precision	25.8022

Table S3 The constraints for the independent parameters and the output

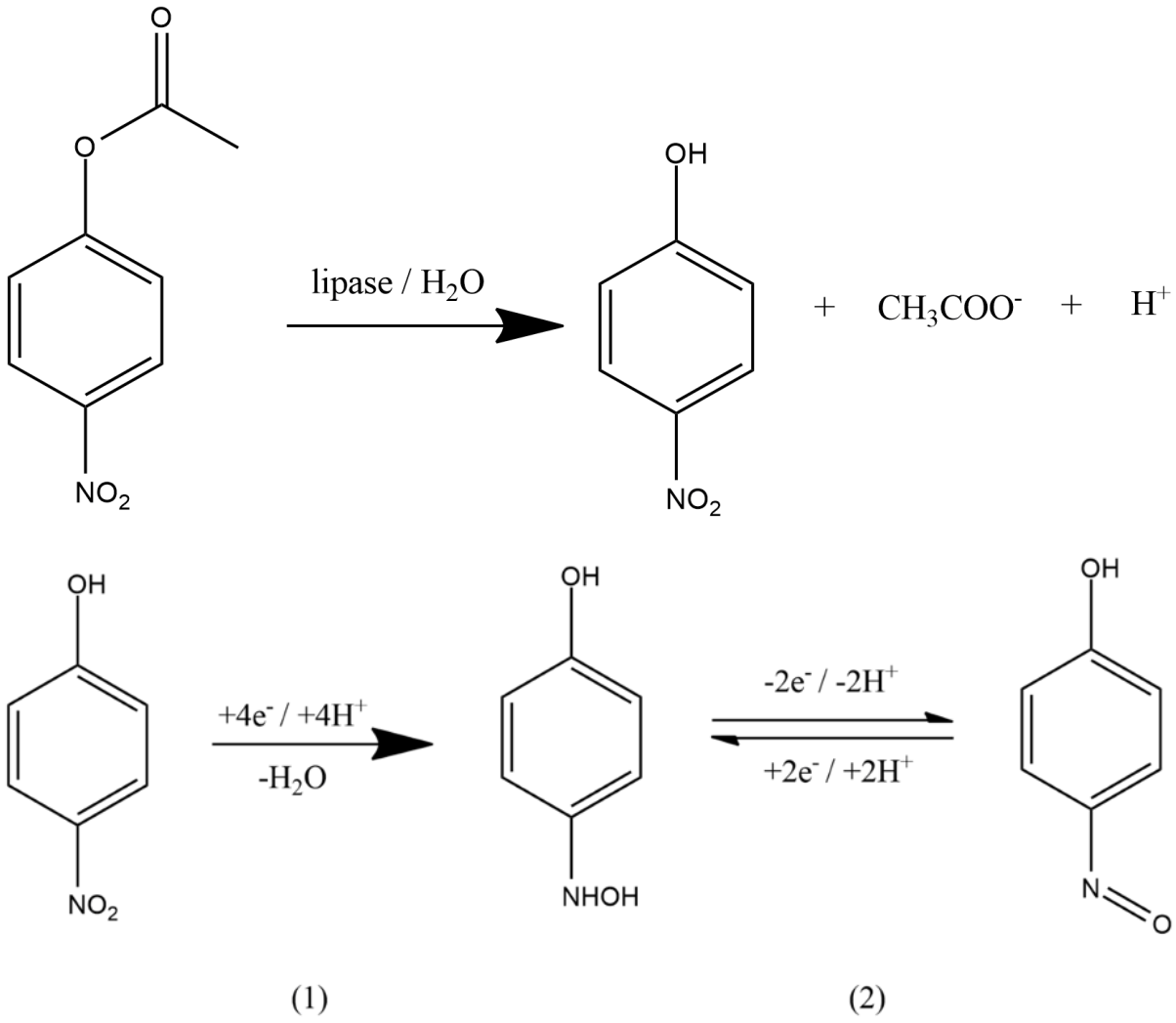
Parameters (unit)	Goal	Lower Limit	Upper Limit
rGO weight (mg)	In range	1	2
Ultrasonication time (min)	In range	30	60
Lipase concentration (mg/mL)	In range	10	30
Current (μA)	Maximize	98.01	116.93

Table S4 The validation set of the lipase/rGO/Cu-MOF/SPCE

Sample	Independent Variables			Current Response (μA)		Residual Standard Error % (RSE %)
	rGO weight (mg)	Ultrasonication time (min)	Lipase concentration (mg/mL)	Predicted	Actual	
1	1	30	20	103.959	103.883 ± 1.29	0.073
2	1.5	30	10	98.795	100.230 ± 3.25	1.453
3	1.5	60	10	100.796	101.395 ± 2.47	0.594
4	1.5	30	30	112.556	112.540 ± 1.08	0.014
5	1.5	60	30	107.547	109.390 ± 0.72	1.714

Table S5 Percentage area of the secondary structure of free lipase and lipase/rGO/Cu-MOF

Sample	Area (%)				
	α-helices	β-sheets	β-turns	Turns and bands	Unordered coils
Free lipase	14.32	37.57	21.80	11.78	14.53
Lipase/rGO/ Cu-MOF	35.60	27.79	23.94	5.12	7.55



Scheme S1 The mechanisms of p-NPA detection using lipase

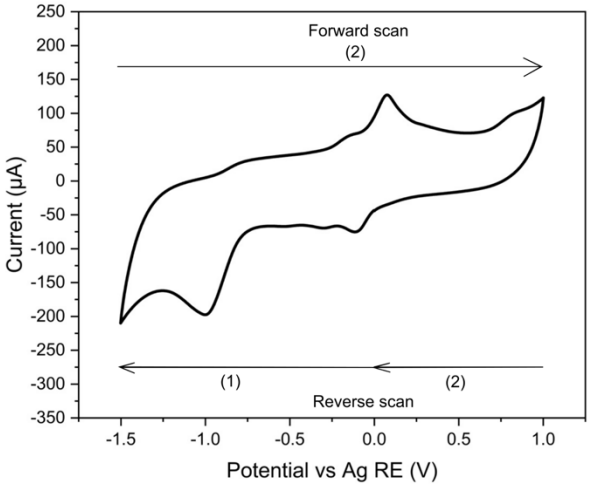


Fig. S1 Cyclic voltammogram of 750 µM p-NPA in 0.1 M PBS (pH 7) using lipase/rGO/Cu-MOF/SPCE at a scan rate of 100 mV/s

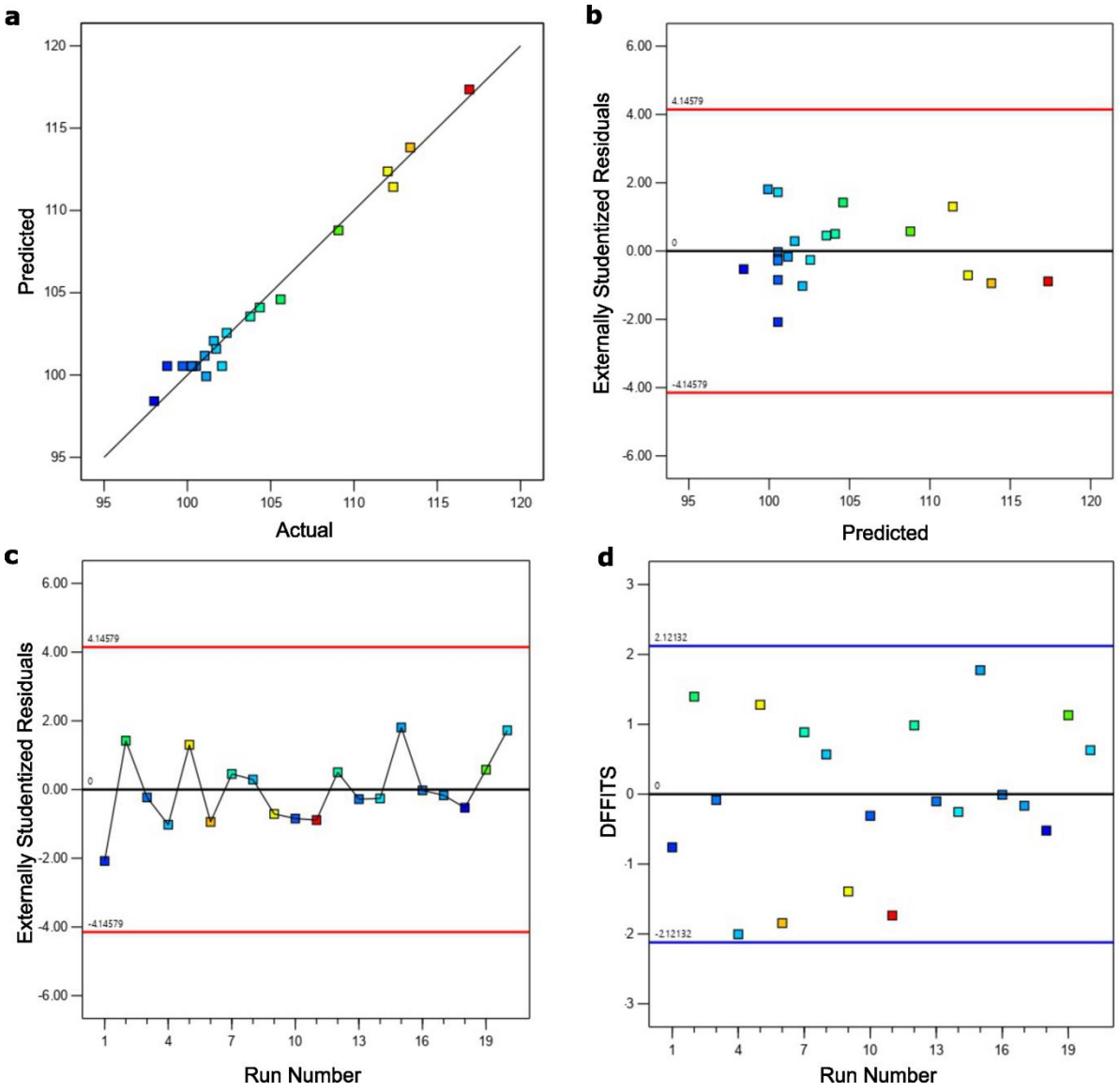


Fig. S2 The diagnostic plots for the electrode optimization. The predicted versus actual response plot (a), the externally studentized residuals response versus the predicted current response plot (b), the studentized residuals response versus run number plot (c), and the DFFITS experimental run versus the run number plot (d)

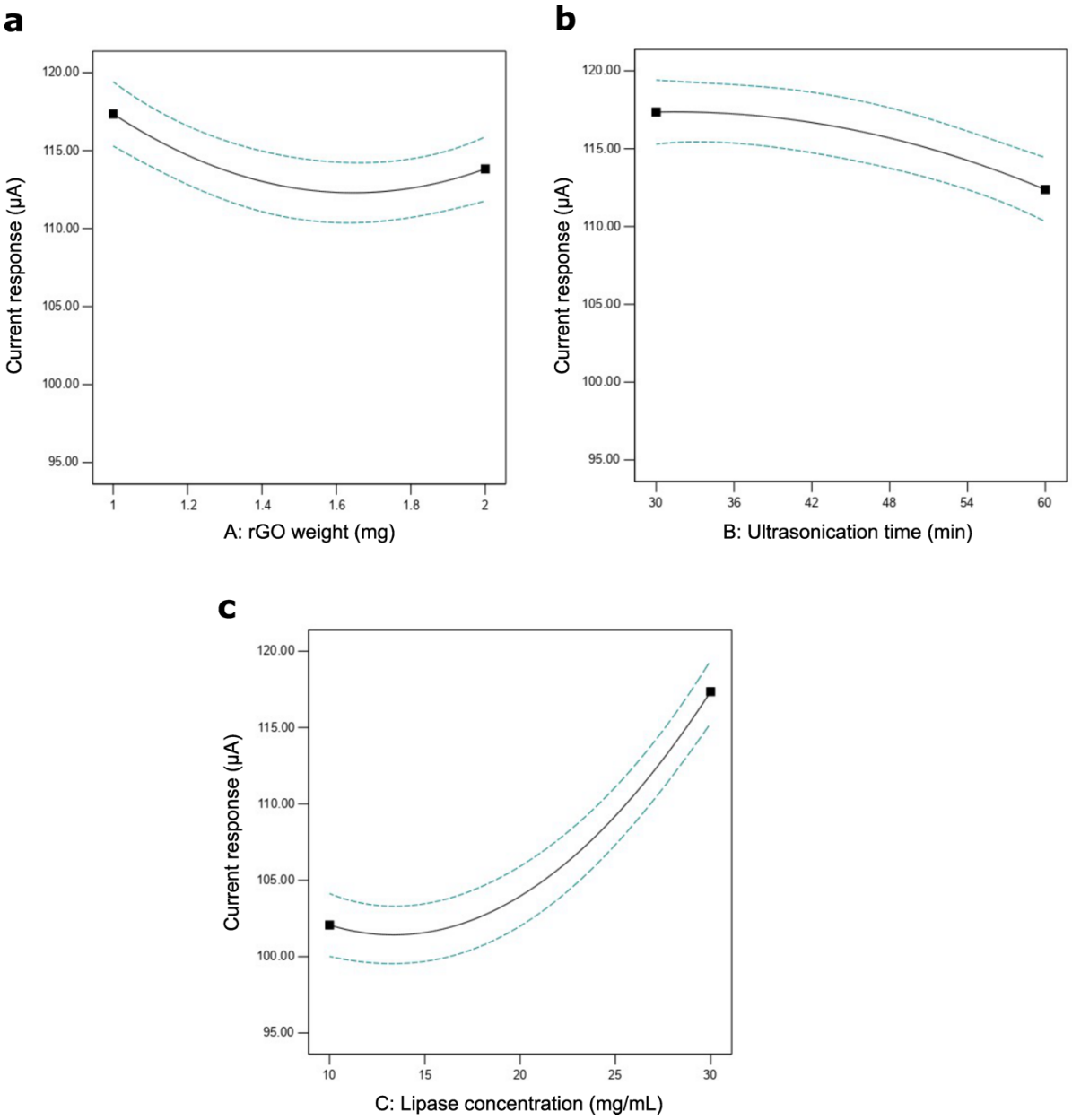


Fig. S3 The effect of rGO weight (a), ultrasonication time (b), and lipase concentration (c) on the current response. The dotted lines represent the 95% confidence limit

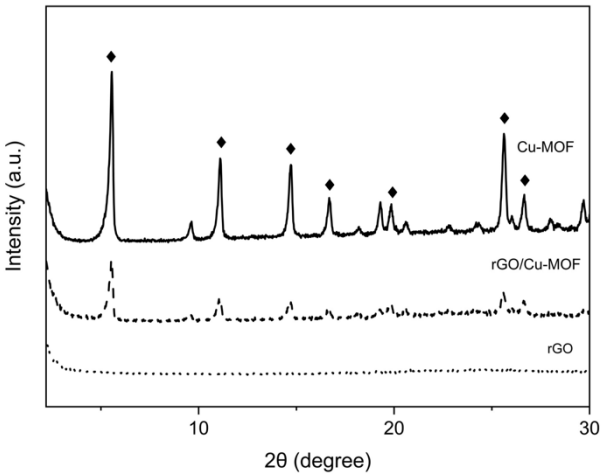


Fig. S4 XRD diffractograms of Cu-MOF, rGO/Cu-MOF, and rGO (the ♦ symbol indicating peak corresponding to Cu-MOF structure)