Supporting Information

In Situ Detection of Melatonin and Pyridoxine in Plants Using a CuO-Poly(L-lysine)/Graphene-Based Electrochemical Sensor

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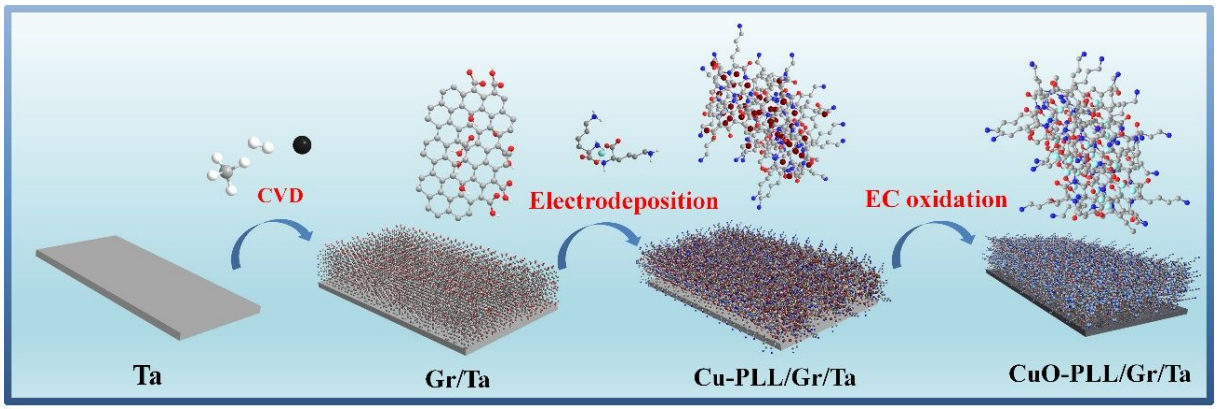
■ S1. EXPERIMENTAL SECTION

Materials and chemicals. Melatonin (MT), pyridoxine hydrochloride (PN, 99%, $C_8H_{11}NO_3$•HCl), dopamine (DA), and $Ni(NO_3)_2$•$6H_2O$ were purchased from Alfa Aesar (USA). Na_2HPO_4, NaH_2PO_4, L-lysine, L-cysteine, ascorbic acid, L-tyrosine, L-tryptophan, serine, KCl, and $ZnCl_2$ were purchased from the Tianjin Guangfu Fine Chemical Research Institute (China). Abscisic acid and indole-3-acetic acid were purchased from Macklin (China). Glucose was purchased from the Shanghai Yuanye Biotechnology Co., Ltd. (China). $CaCl_2$, $CuSO_4$•$5H_2O$, H_3PO_4 and salicylic acid were purchased from the Tianjin Jiangtian Unified Technology Co., Ltd. (China). K_2SO_4, $Mg(NO_3)_2$•$6H_2O$, Na_2S•$9H_2O$, hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6]$•$3H_2O$), $K_3[Fe(CN)_6]$, and $NaNO_2$ were purchased from the Tianjin Kewei Co., Ltd. (China). NaCl, H_3BO_3, NaOH, and NH_4F were purchased from the Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (China). Phosphate-buffered saline (PBS) (pH 5–8) was prepared from 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4. Ultrapure water was used throughout.

■ S2. EXPERIMENTAL RESULTS



Scheme S1. Schematic diagram of the CuO-PLL/Gr-electrode fabrication process.

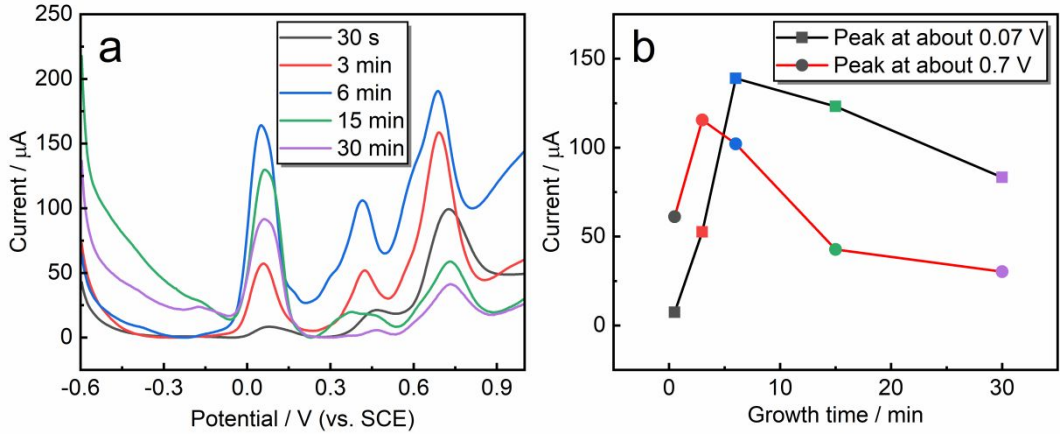


Figure S1. (a) DPV curves of the Gr/Ta sheet with different growth times in PBS (pH 6.0) containing 100 μM MT and 1000 μM PN. (b) Plot of the growth time as a function of the current.

Figure S2. Showing the parameter interface of the signal generator for Cu-PLL electrodeposition.

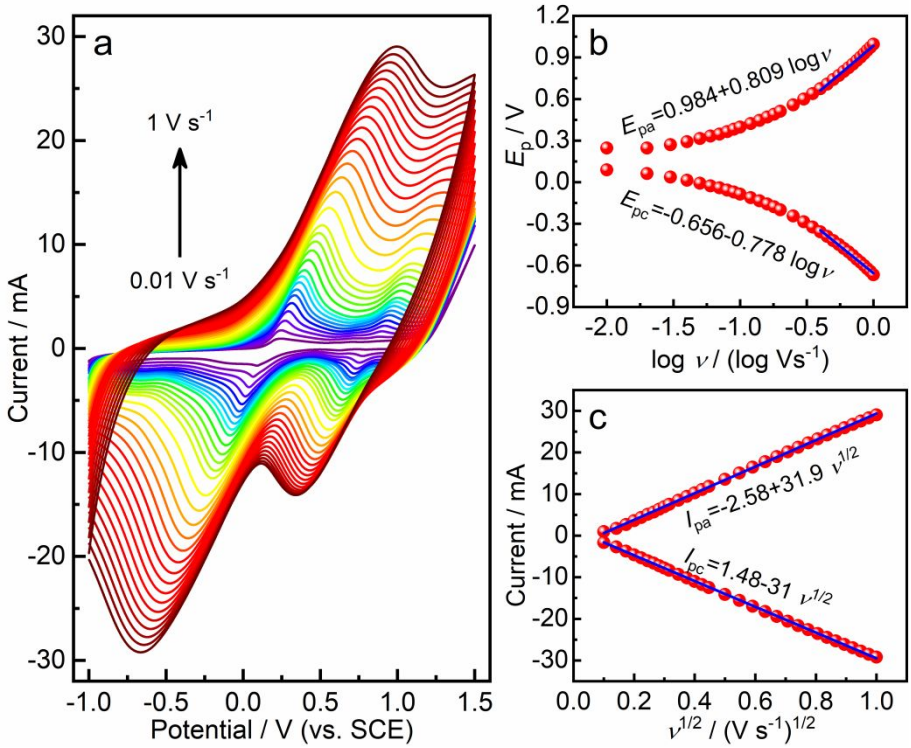


Figure S3. (a) CV curves of the CuO-PLL/Gr electrode in a mixed solution of 0.1 M KCl, 5 mM $K_4[Fe(CN)_6]$, and 5 mM $K_3[Fe(CN)_6$. (b) Peak potentials (E_p) as functions of the scan rate (log v). (c) Peak currents (I_p) as functions of the square root of the scan rate ($v^{1/2}$).

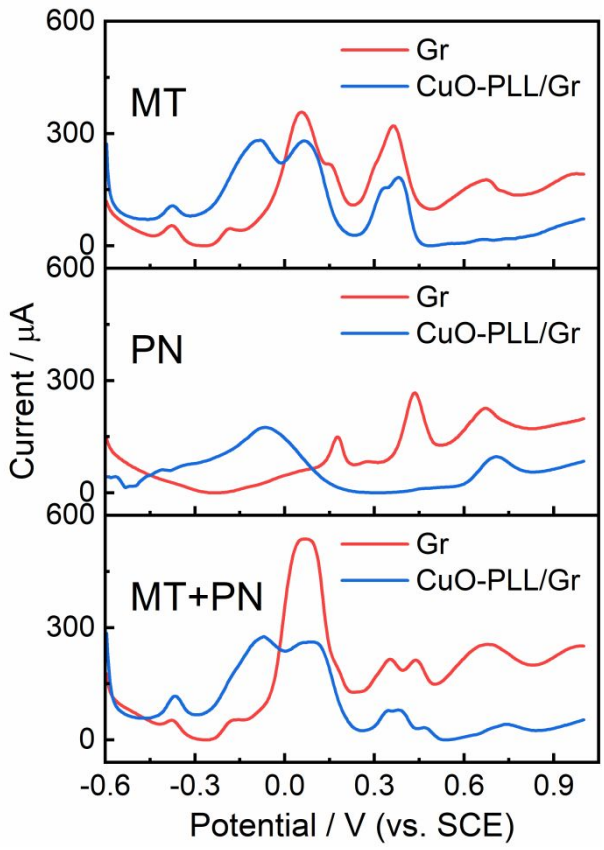


Figure S4. DPV traces recorded using Gr and CuO-PLL/Gr electrodes in 300 μM MT, 800 μM PN, and a mixed solution of MT and PN.

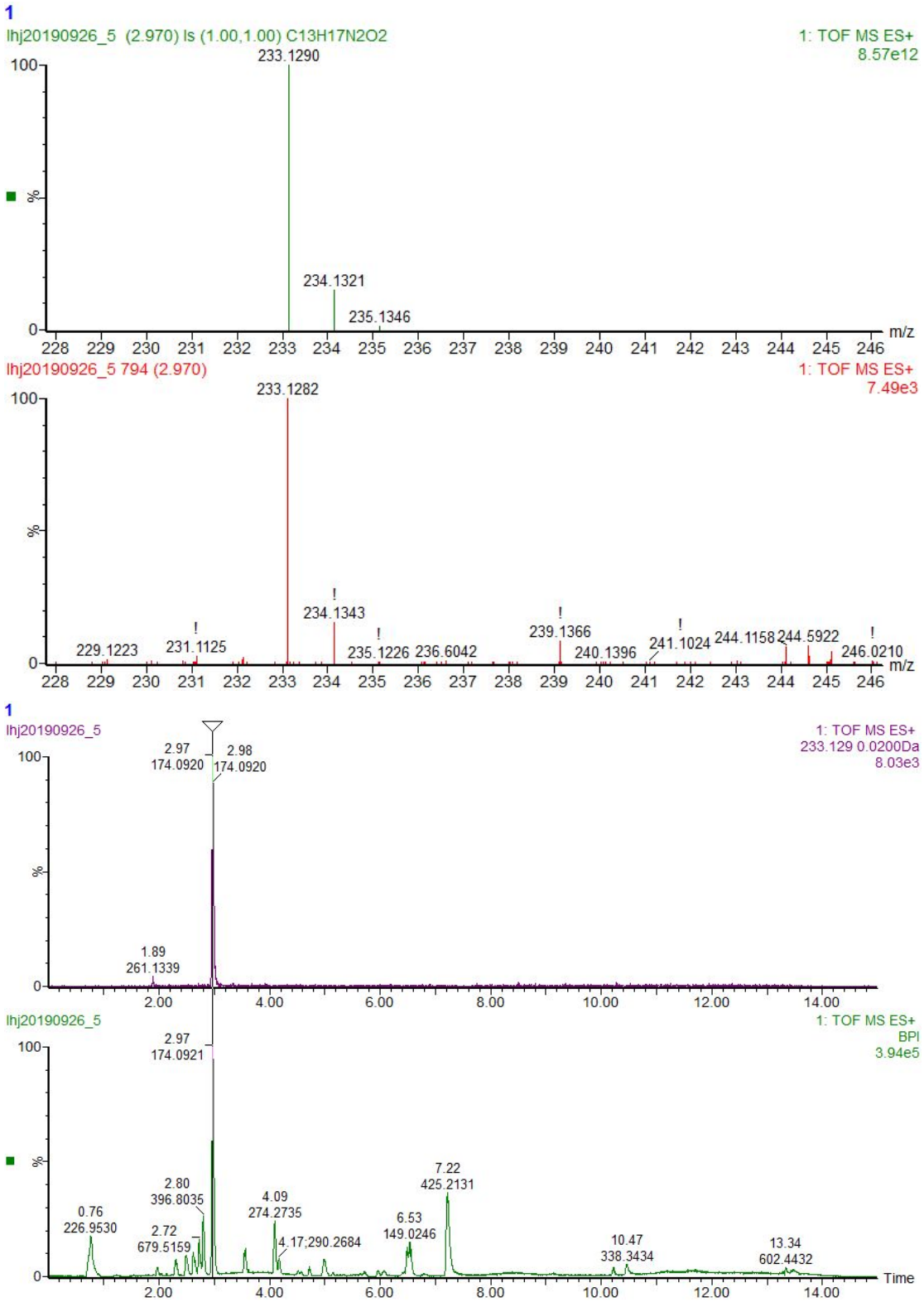


Figure S5. LC-MS spectra of MT solutions before test (MT: m/z = 233, ES^+).

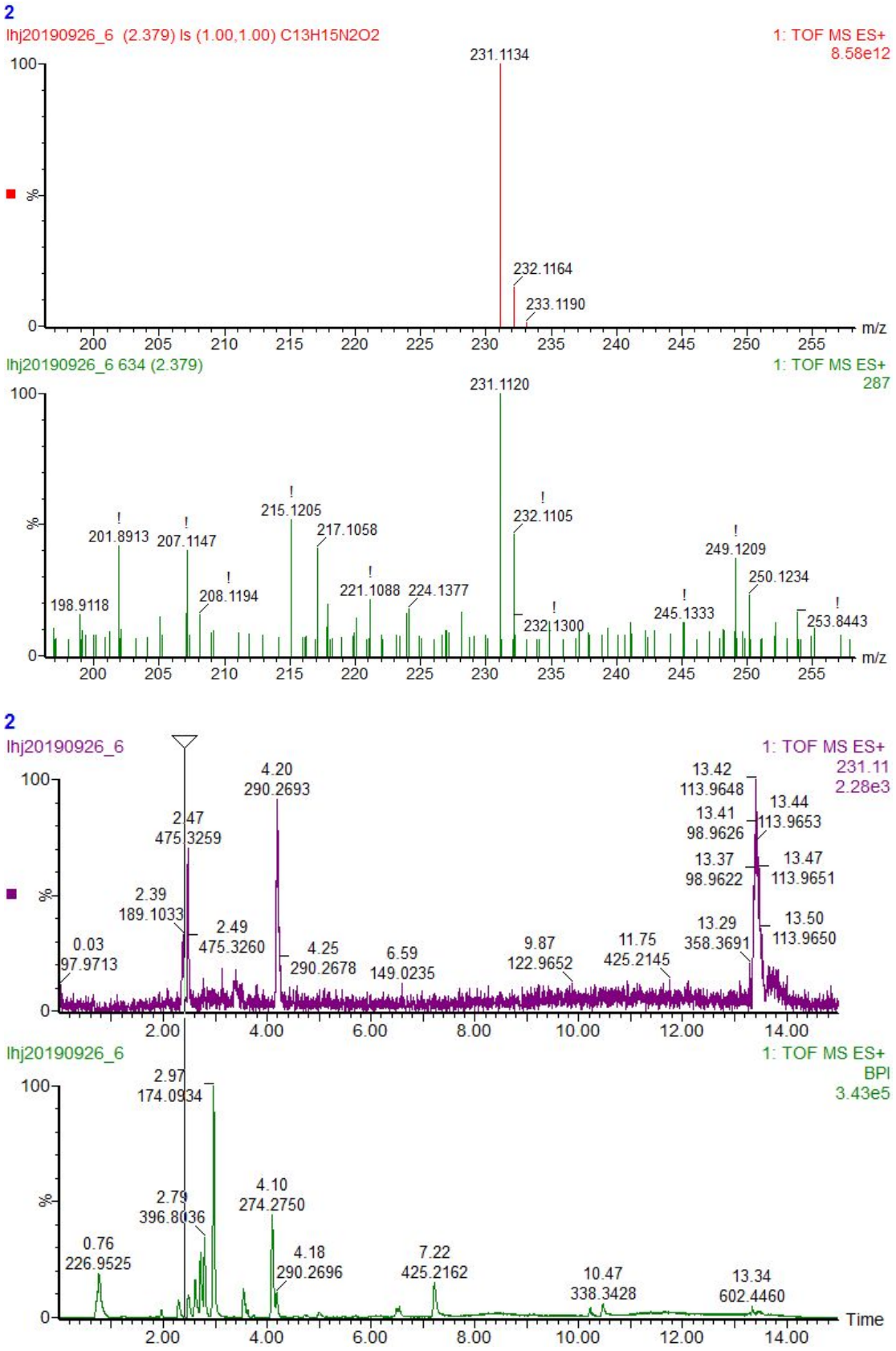


Figure S6. LC-MS spectra of MT solutions after the test (oxidized MT : m/z = 231, ES^+).

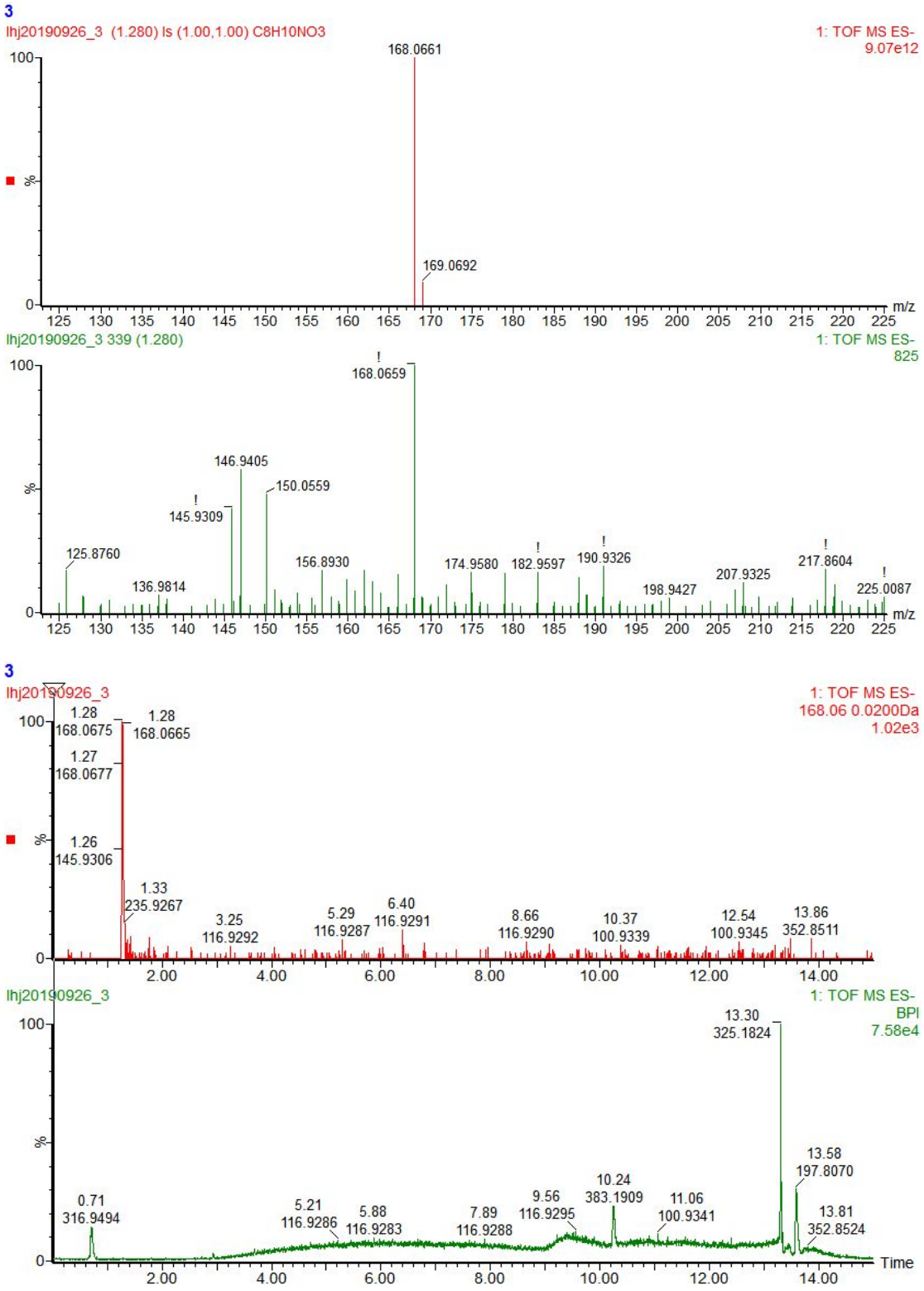


Figure S7. LC-MS spectra of PN solutions before the test (PN: m/z = 168, ES^-).

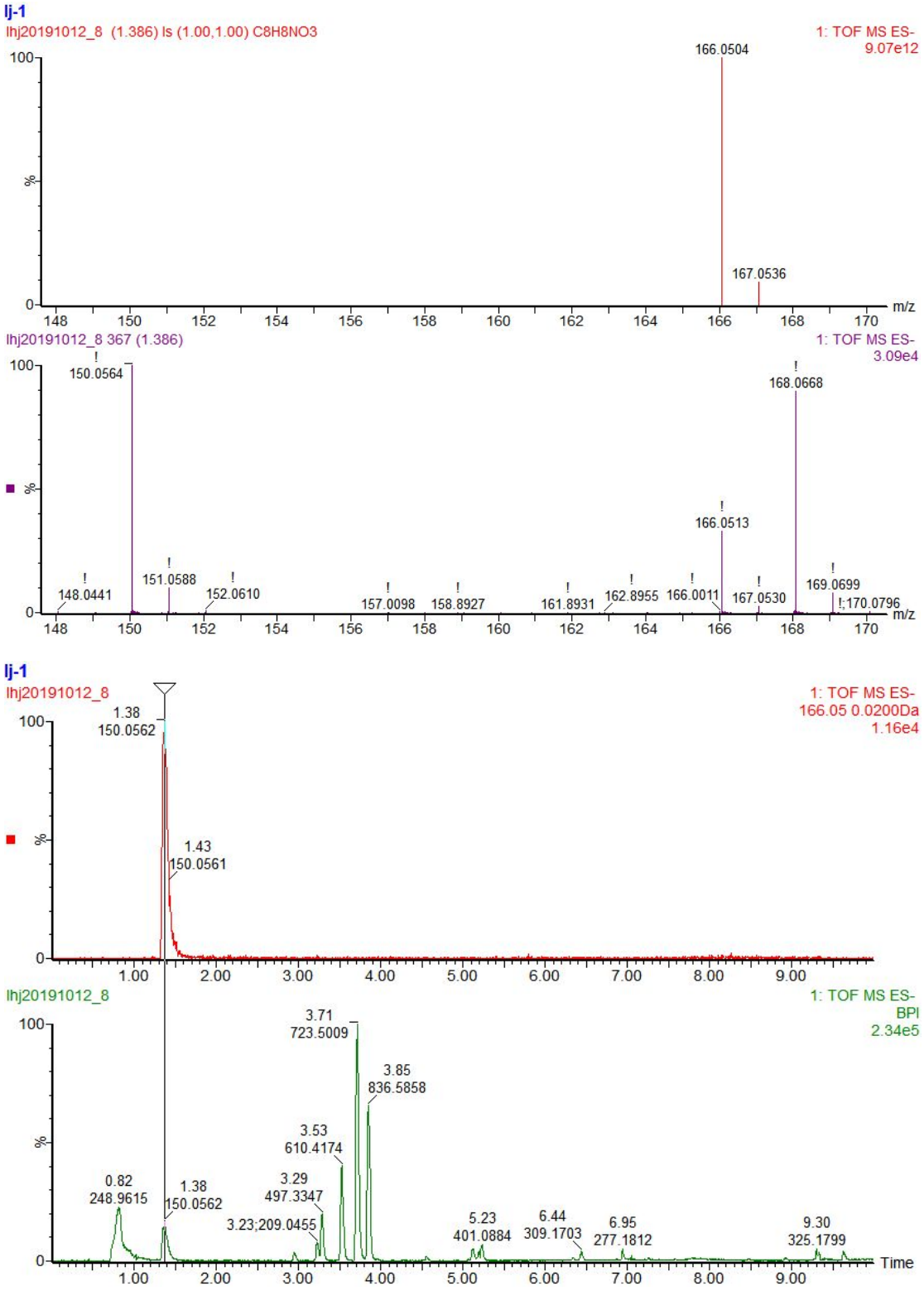


Figure S8. LC-MS spectra of PN solutions after the test (pyridoxal: m/z = 166, ES^-).

To explore the reaction mechanism, we analyzed the reaction products of MT and PN by LC-MS. In the LC profile, MT and PN showed retention time at 2.97 and 1.28 min, respectively (Figures R15, and R17). In the MT solution after the DPV test, a new peak at 2.379 min appeared on the LC spectrum, and a predominant peak at m/z = 231.2461(ES^+) was observed from the HR-MS spectrum of the solution (Figure R17). We detected an oxidation product (deprotonated MT) with a molecular weight of 230 in the MT solution after the test. Similarly, a reaction product with a molecular weight of 167 was detected in the tested PN solution (Figure R18). The PN was oxidized to pyridoxal with a molecular weight of 167.18 (m/z = 166.05, ES^-). The measured values are in agreement with the standard values. MS measurement confirmed the molecular weights of the reaction products. Based on the LC-MS results, the redox reactions could be proposed as shown in **Scheme 1c**.

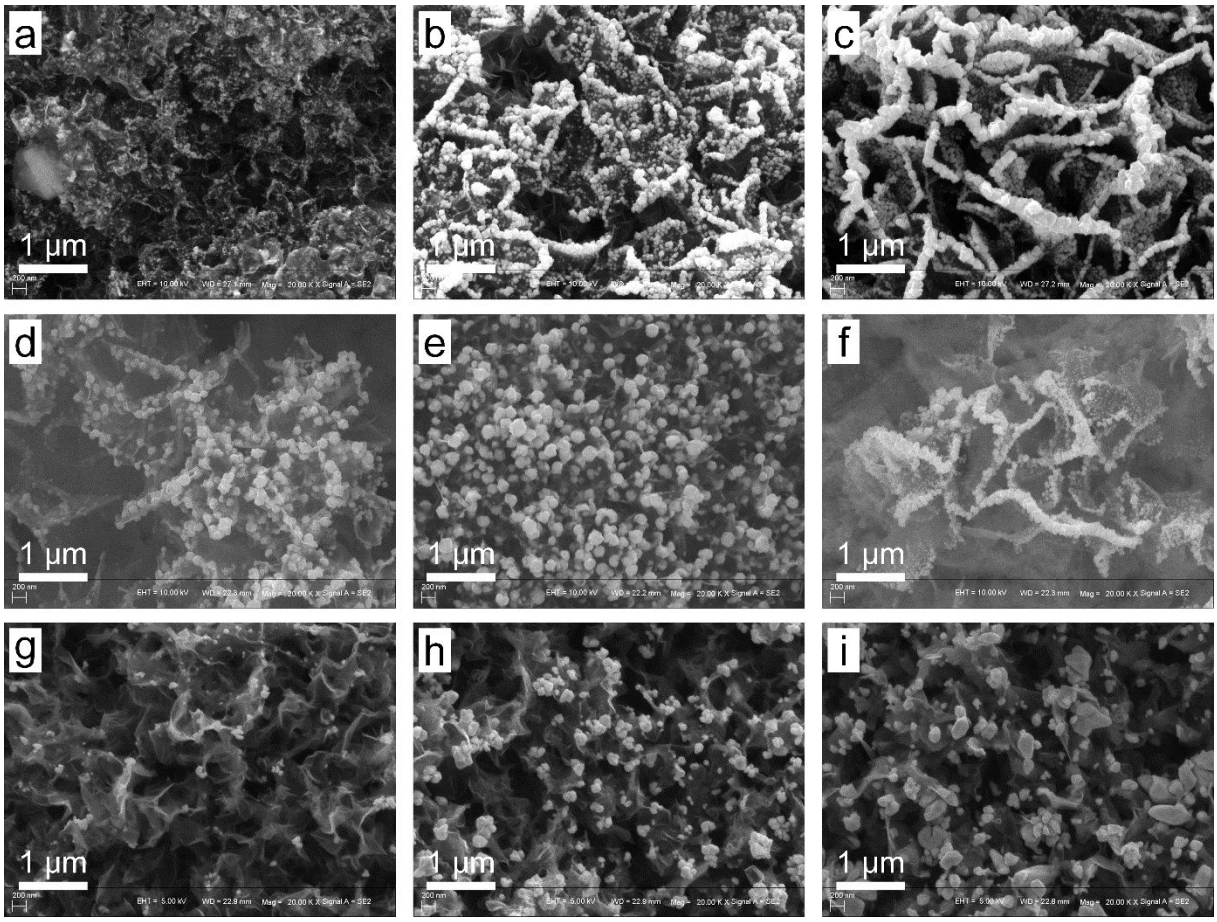


Figure S9. SEM images of various CuO-PLL/Gr electrodes: (a-c) With Cu^{2+} at concentrations of 0.4, 0.8, and 4 mM, respectively; (d–f) With voltage ranges for deposition of Cu-PLL of −1.2–0, −1.2–1, and −3–0 V, respectively; and (g–i) With various deposition times: 2, 6, and 30 min, respectively. Note: (a–f) pH 6, (d–i) 0.16 mM Cu^{2+}, and (g–i) pH 4. Other parameters are those of the optimized conditions.

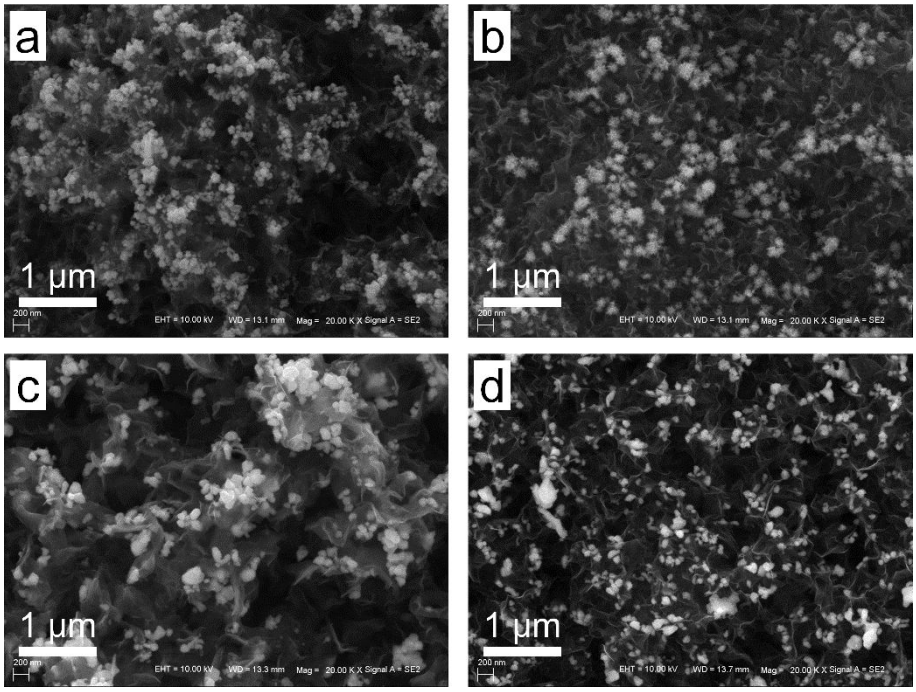


Figure S10. SEM images of CuO-PLL/Gr electrodes with CuO-PLL layers deposited on them at different potential ranges (magnified 20, 000 times). (a) −1.2–0, (b) −1.2–1, (c) −1.2–2, and (d) −1.2–3 V. The electrolyte is a mixed solution of 1 mM $_{L}$-lysine, 0.16 mM $CuSO_4$, 0.8 mM NaCl, 0.072 mM H_3BO_3, and 0.1 M PBS (pH 4.0).

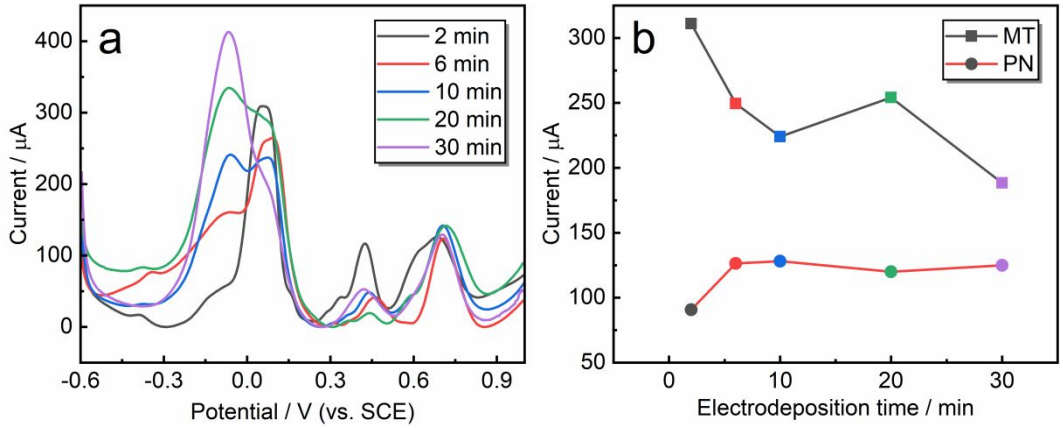


Figure S11. (a) DPV curves of the CuO-PLL/Gr electrodes with various deposition times for CuO-PLL. (b) Relationship between current and deposition time. The electrolyte solution was a mixture of 100 μM MT and 1000 μM PN.

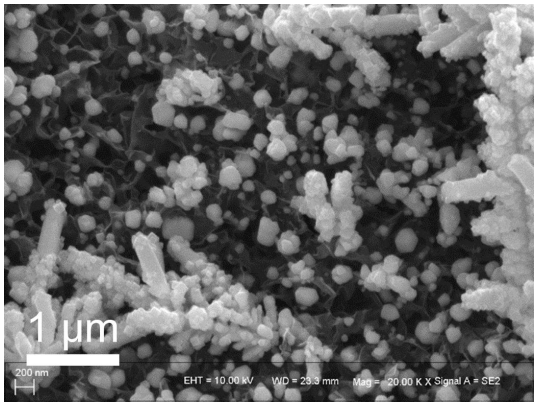


Figure S12. SEM image of the Cu/Gr electrode.

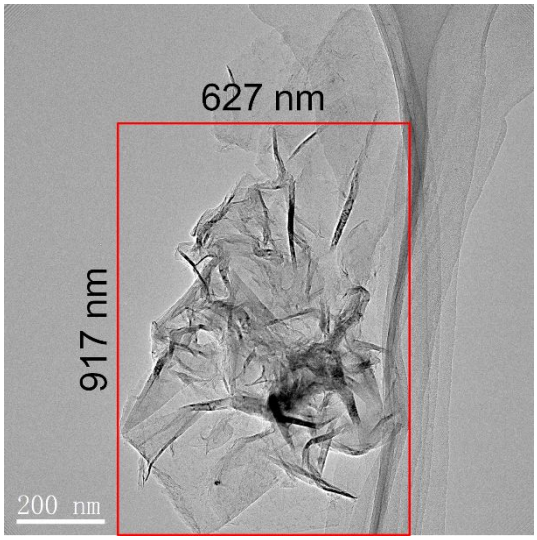


Figure S13. TEM image of a curled Gr sheet.

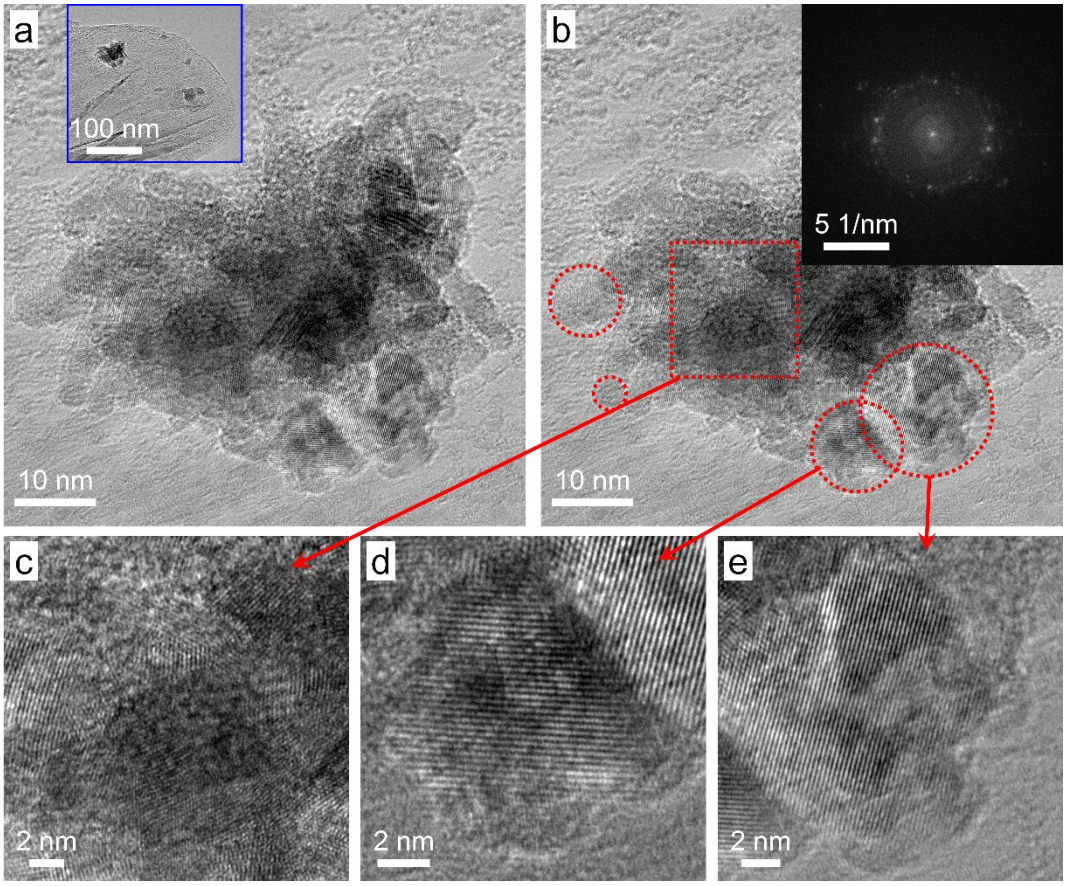


Figure S14. TEM and HRTEM images of the CuO-PLL/Gr nanosheets.

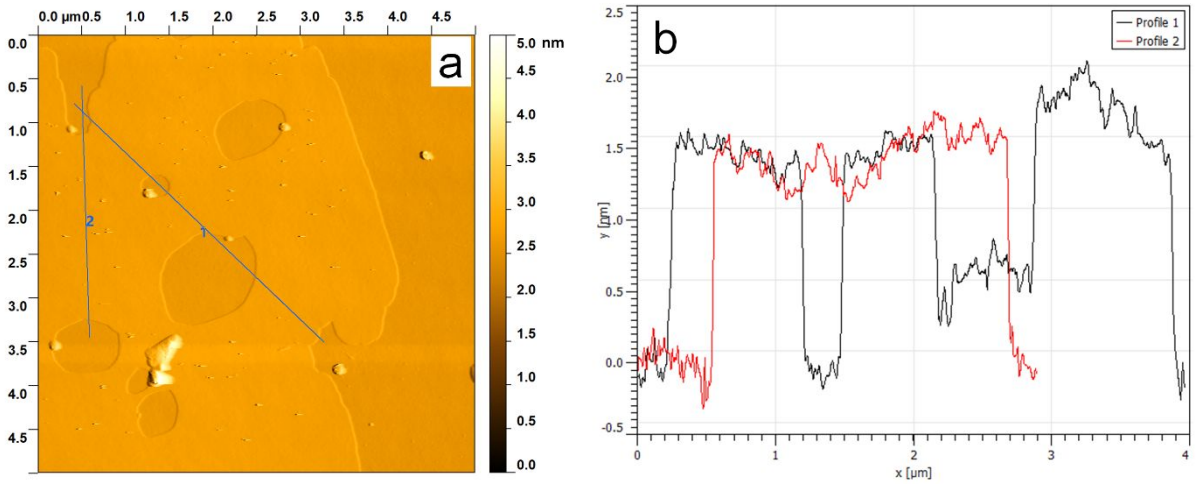


Figure S15. (a) AFM image of graphene sheets and (b) height profile along line.

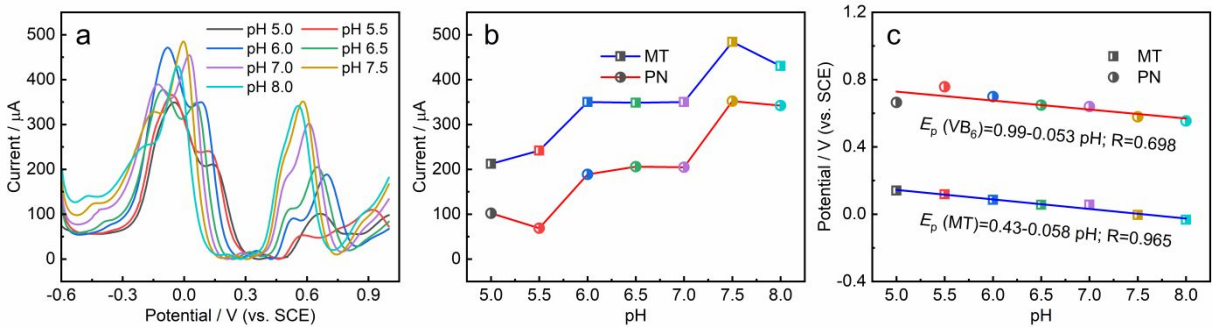


Figure S16. The effect of pH on the signal response of the CuO-PLL/Gr electrode. (a) DPV curves in pH 5–8 PBS containing 500 µM MT and 800 µM PN. (b) Peak currents as functions of pH. (c) Peak potentials as functions of pH.

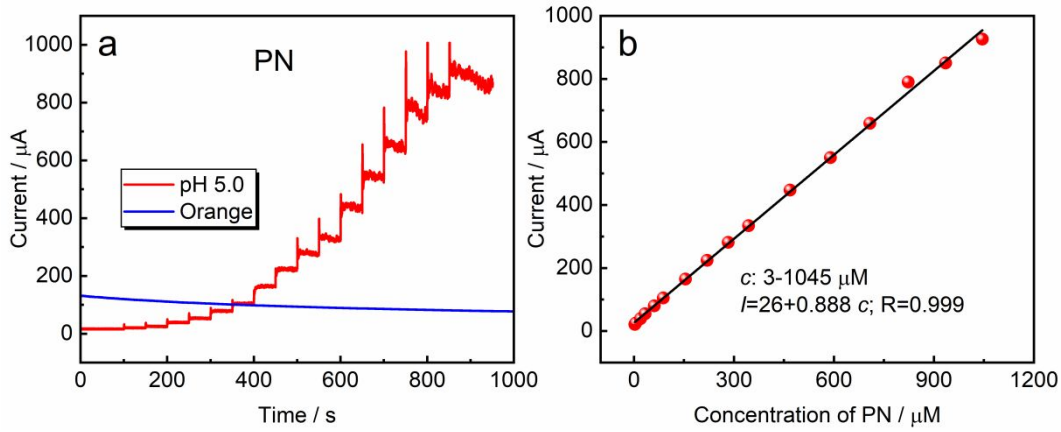


Figure S17. (a) Chronoamperometry traces for PN using the CuO-PLL/Gr electrode at 1 V. (b) The working curve. Electrolyte: pH 5.0 PBS solution.

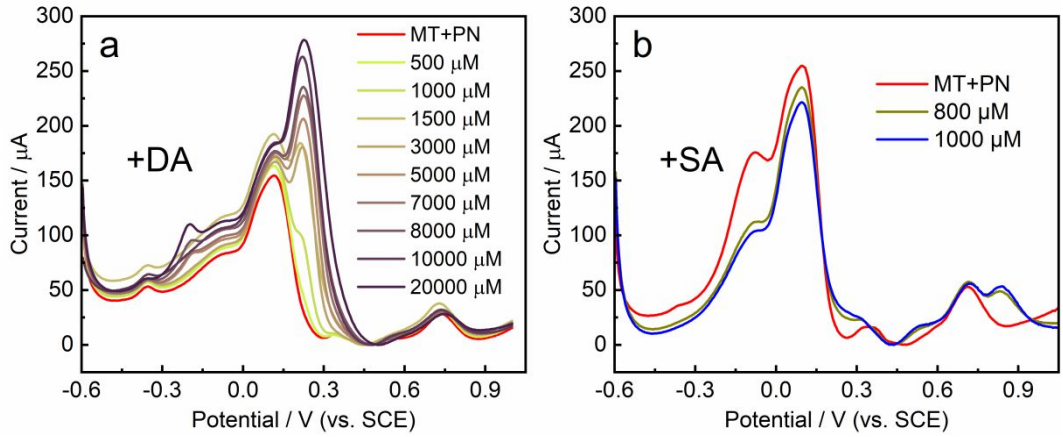


Figure S18. DPV curves for the CuO-PLL/Gr electrode in 0.1 M PBS (pH 6.0) containing 300 μM MT, 800 μM PN, and additional (a) 500–20000 μM dopamine or (b) 800–1000 μM salicylic acid.

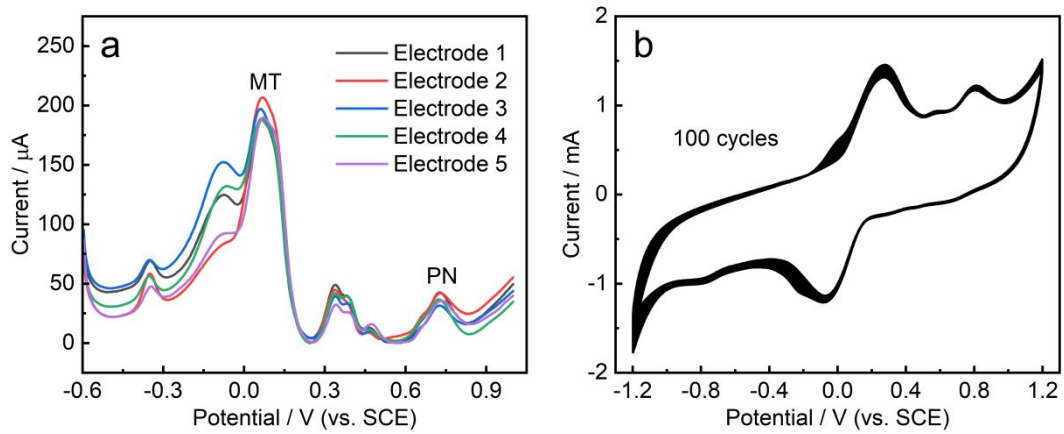


Figure S19. (a) DPV traces separately acquired using five different CuO-PLL/Gr electrodes. (b) CV traces continuously recorded for 100 cycles using a CuO-PLL/Gr electrode. Electrolyte: a solution of 300 μM MT and 800 μM PN.

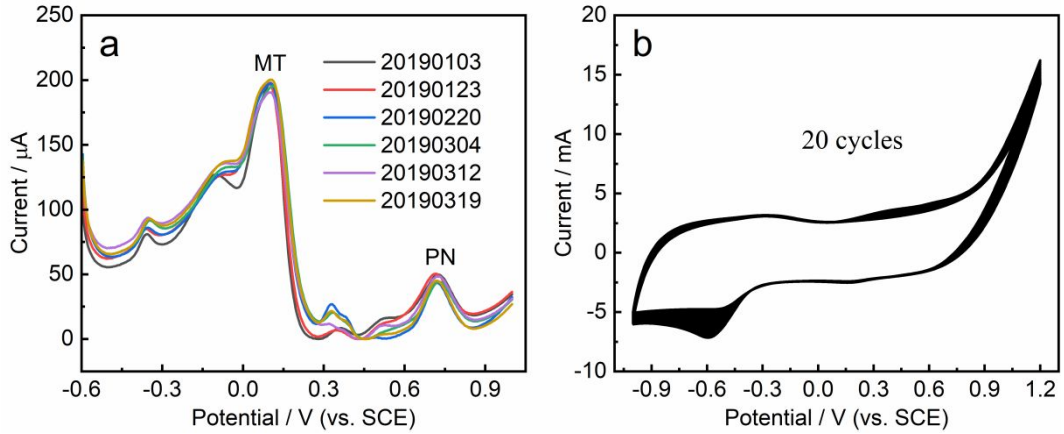


Figure S20. (a) DPV traces for the CuO-PLL/Gr electrode acquired over several months. Electrolyte: a solution of 300 μM MT and 800 μM PN. (b) CV curves acquired during catalyst regeneration. Electrolyte: 0.1 M NaOH.

Table S1. Comparing different methods for the detection of MT and PN.

Method	Real sample	Sample preparation method	Concentration range/μM		LOD/μM		Ref.
			MT	PN	MT	PN	
EC	Orange	Crush or insert directly	0.016-1110	3-2076	0.012	2.3	This work
HPLC-FD	Rice	Pressurized Liquid Extraction	0.75-15; 15-750	-	1.15	-	[1]
EC	Lymph node	Slices	0.05-100	-	0.024	-	[2]
EC	Oxylife capsules; Melatonex tablets	Liquid extraction	4.3-430	49-850	0.6	6.6	[3]
BIA-MPA	Pharmaceutical sample	Oral solution was dissolved in ethanol-buffer solution.	-	10-30	-	0.54	[4]
UV spectra	Laboratory prepared mixtures	-	-	24.3-243.1	-	4.9	[5]
EC	Human serum; tablet	diluted by pH 3 B-R buffer solution	0.02-6	-	4.1	-	[6]

Table S2. Detecting MT in orange puree samples (n=5).

MT in the orange puree/ μM	Added/ μM	Found / μM	RSD/%	Recovery/%
86.9±0.5	48	134.1	4.3	98.3
	91	187.3	3.7	100.1
	130	225.8	4.1	99.7
	170	261.9	2.8	97.4
	200	294.8	3.9	99.3
	231	326.9	4.2	99.9

Table S3. Detecting PN in orange puree samples (n=5).

PN in the orange puree/ μM	Added/ μM	Found / μM	RSD/%	Recovery/%
83.6±0.5	48	132.3	3.1	101.6
	89	173.2	3.7	100.7
	168	253.2	4.8	101.0
	200	283.2	1.9	99.8
	265	352.8	2.7	101.6

■ REFERENCES

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