Supporting Information

Versatile Sarcosine Biosensing Schemes Utilizing Layer-by-Layer Construction of Carbon Nanotube-Chitosan Composite Films

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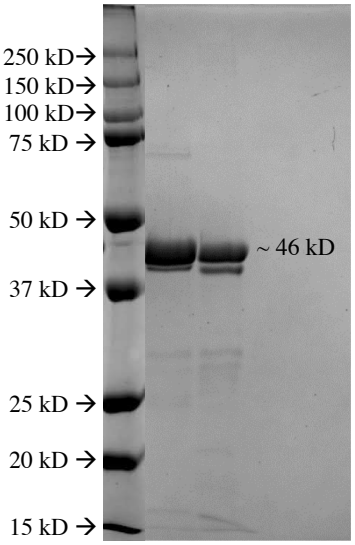

Figure SI-1. SDS-PAGE gel of house-produced SOx (lane 2) and Sigma Aldrich-purchased SOx (lane 3), run with a final SOx concentration of 0.667 mg/mL. A Precision Plus Protein standard (BioRad) was included in the gel for size verification (lane 1), confirming the predicted 46 kD size of the protein from both origins. The gel indicates that house-produced SOx has equivalent yield and purity to store-bought SOx.

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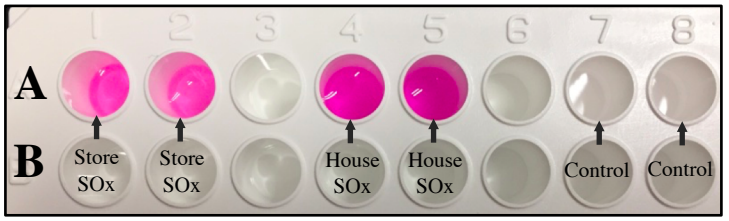

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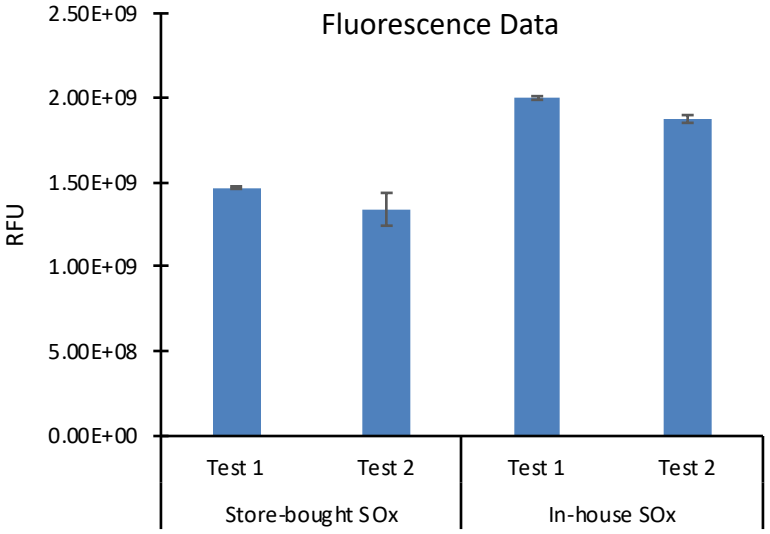


Figure SI-2: **(A)** A fluorimetric hydrogen peroxide assay (Sigma Aldrich MAK165) was performed according to procedure, using a final sarcosine concentration of 0.1 mM. Wells A1-A2 contained Sigma-Aldrich-purchased SOx (100 μg/mL), and wells A4-A5 contained house-made SOx (100 μg/mL). A control mixture with no enzyme added was included in wells A7-A8 in order to provide the background fluorescence, which was subtracted from fluorescence readings; (**B**) A histogram depicting the Relative Fluorescence Units (RFU) of the In-house produced SOx and the purchased SOx from Sigma Aldrich (41 units/mg). The data the produced enzyme is approximately 1.3 times as active as the purchased SOx.

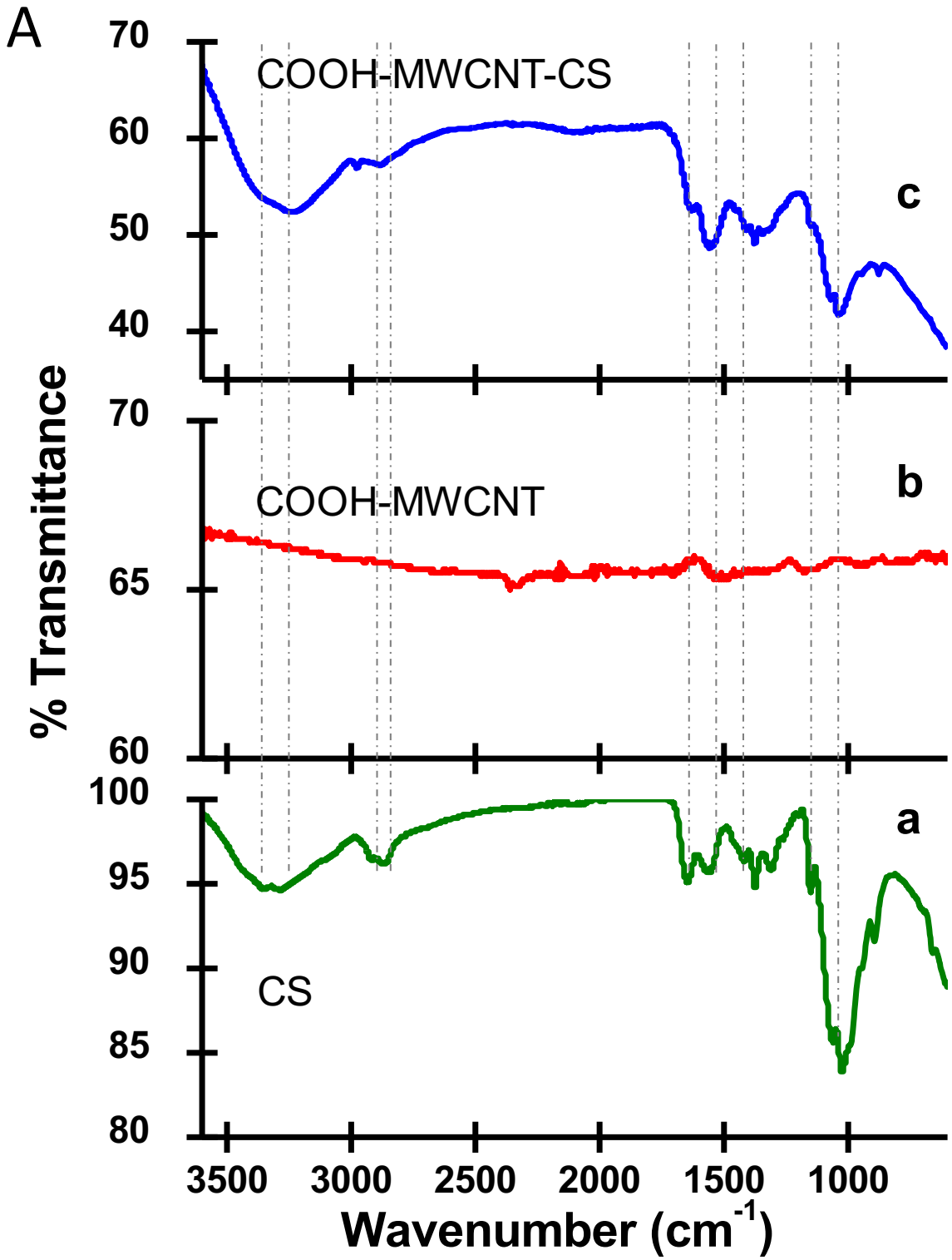


Figure SI-3A. Characterization of of a) CS, b) COOH-MWCNT, c) COOH-MWCNT-CS FTIR spectroscopy

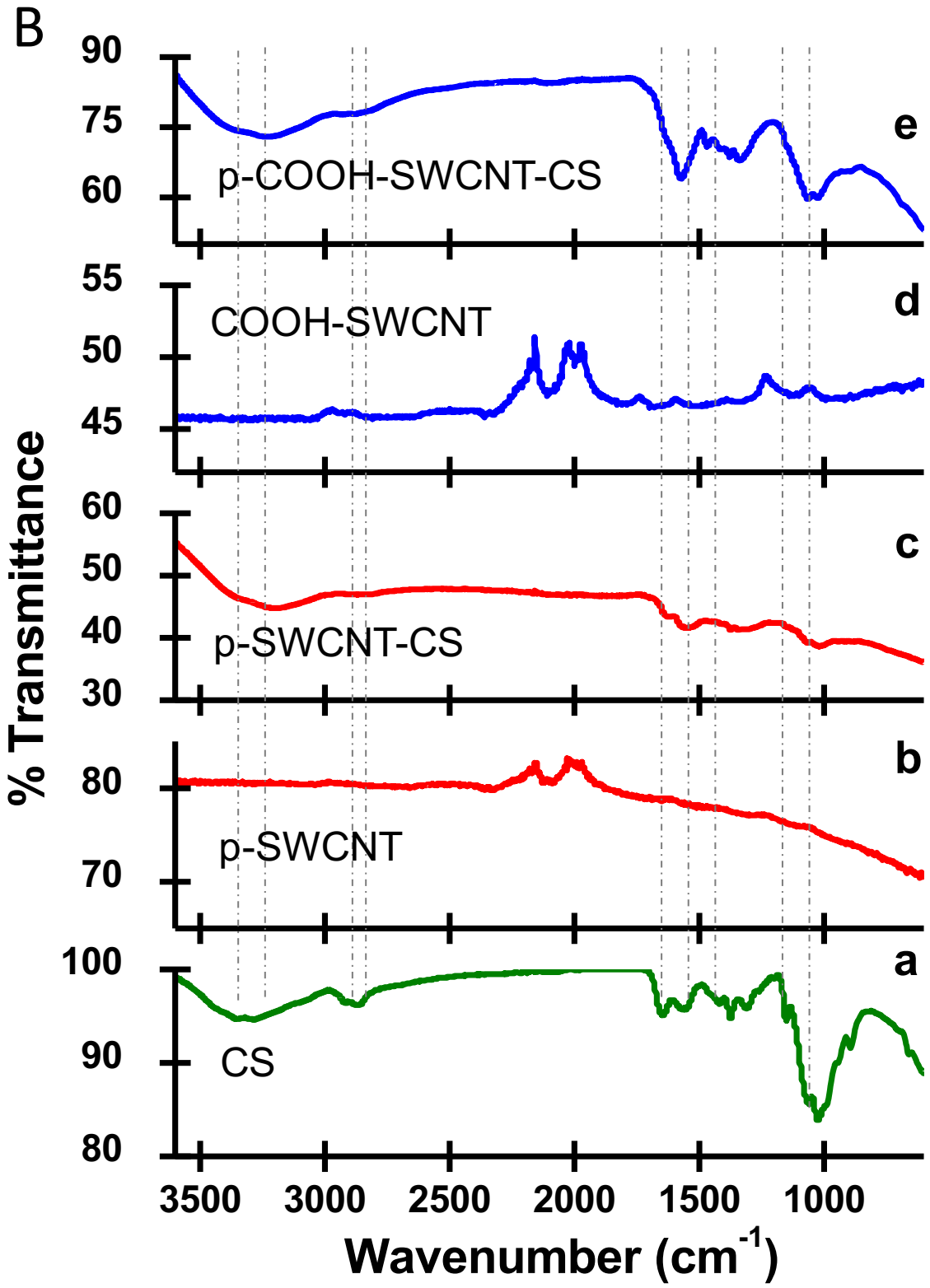


Figure SI-3B. Characterization of of a) CS, b) p-SWCNT, c) p-SWCNT-CS, d) COOH-SWCNT, e) COOH-SWCNT-CS FTIR spectroscopy

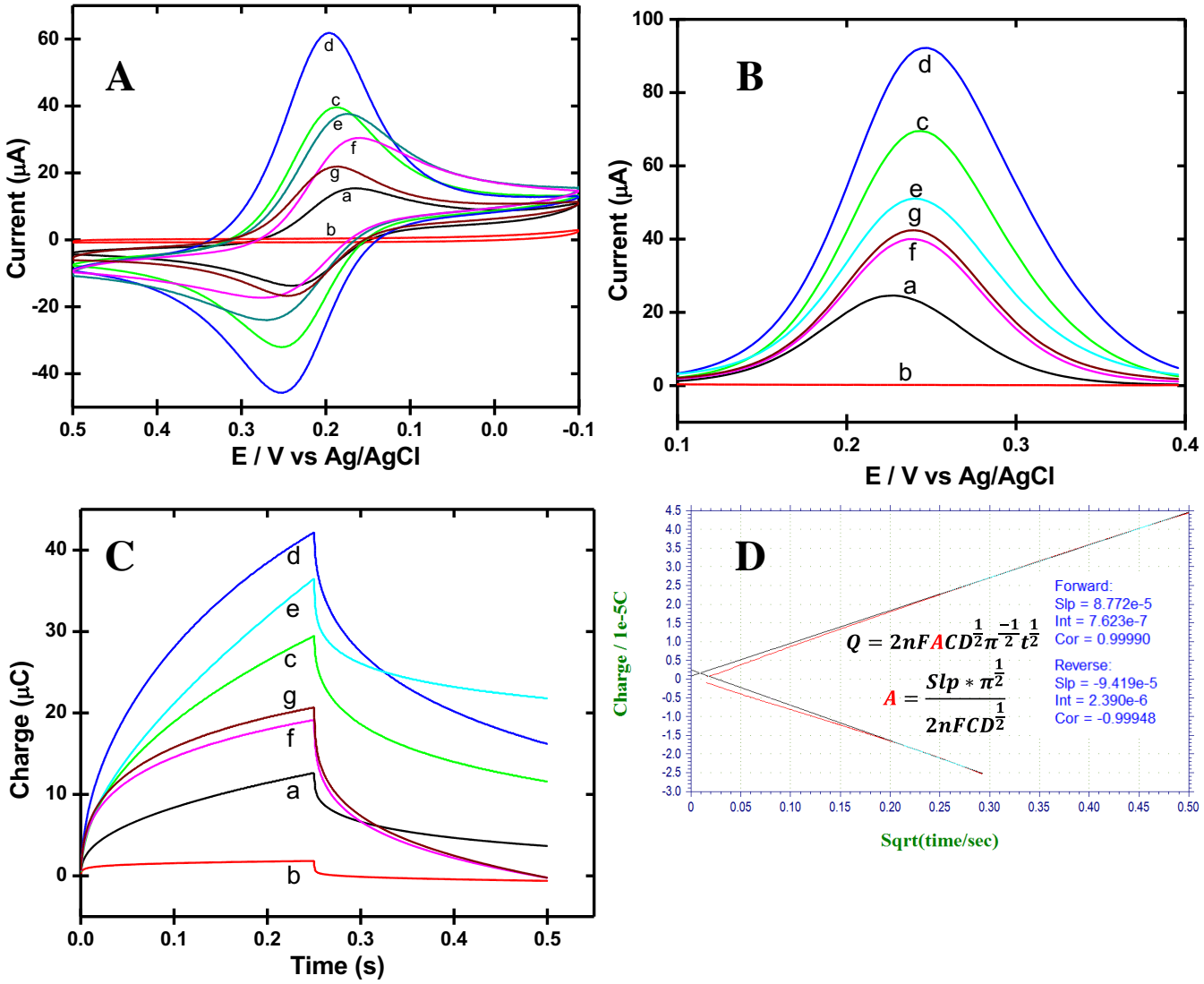


Figure SI–4. **(A)** cyclic voltammetry; **(B)** Differential pulse voltammetry (cathodic sweep) and **(C)** chronocoulometry (CC) of 5 mM potassium ferricyanide (0.5 M KCl) at **(a)** bare Pt, and **(b)** Nafion, **(c)** CS/Nafion, **(d)** CS-COOH-SWCNT/Nafion, **(e)** CS-p-SWCNT/Nafion, **(f)** CS-COOH-MWCNT/Nafion, **(g)** CS-p-MWCNT/Nafion modified Pt; **(D)** Typical Anson plot (charge vs. time$^{1/2}$) to determine the area of the electrode (inset equations). **DPV parameters:** Potential window = 0 +0.4 V; Pulse width = 0.05 s; Amplitude = 0.05 V; Period = 0.5 s; Sensitivity 1E–4 A/V

Table SI-1. Chronocoulometry Summary as a Function of Voltammetric Scans During modification of Pt electrodes. Cyclic Voltametry (CV) and Chroncoulometry (CC) measurements with 5 mM potassium ferricyanide (0.5 M KCl).

Electrode Type	Average Area (cm^2) from CC	CV Average I_p (µA) [a]	CV Average I_p (µA) [b]	CV Average I_p (µA) [c]	DPV Average I_p (µA) [d]
Bare Pt	$0.016 \pm 0.00_{08}$	15.9 ± 0.1	12.5 ± 0.2	14.7 ± 0.1	24.2 ± 0.1
Pt/Nafion	$0.001 \pm 0.00_{01}$	-	-	-	-
Pt/CS/Nafion	$0.039 \pm 0.00_{09}$	40.2 ± 1.0	37.8 ± 1.2	38.6 ± 0.7	68.3 ± 2.2
Pt/CS-COOH-SWCNT/Nafion	$0.058 \pm 0.00_{46}$	58.5 ± 7.8	57.7 ± 8.1	58.4 ± 8.0	94.8 ± 6.3
Pt/CS-p-SWCNT/Nafion	$0.054 \pm 0.00_{02}$	30.1 ± 7.6	27.8 ± 7.7	28.8 ± 8.3	46.3 ± 3.0
Pt/CS-COOH-MWCNT/Nafion	$0.023 \pm 0.00_{05}$	25.8 ± 6.4	22.5 ± 5.8	23.5 ± 5.9	37.7 ± 3.1
Pt/CS-p-MWCNT/Nafion	$0.019 \pm 0.00_{04}$	21.0 ± 0.4	19.2 ± 0.9	19.8 ± 0.8	38.7 ± 3.0

Notes: Similar trends were found for both anodic and cathodic wave analysis. In all cases n = 3

[a] Faradaic and non-Faradaic (charging) peak current.

[b] Isolated Faradaic current from individual peak analysis.

[c] Isolated Faradaic current from background subtraction (0.5 M KCl).

[d] **DPV parameters**: Potential window = 0↔ +0.4 V; Pulse width = 0.05 s; Amplitude = 0.05 V; Period = 0.5 s; Sensitivity 1E-4 A/V

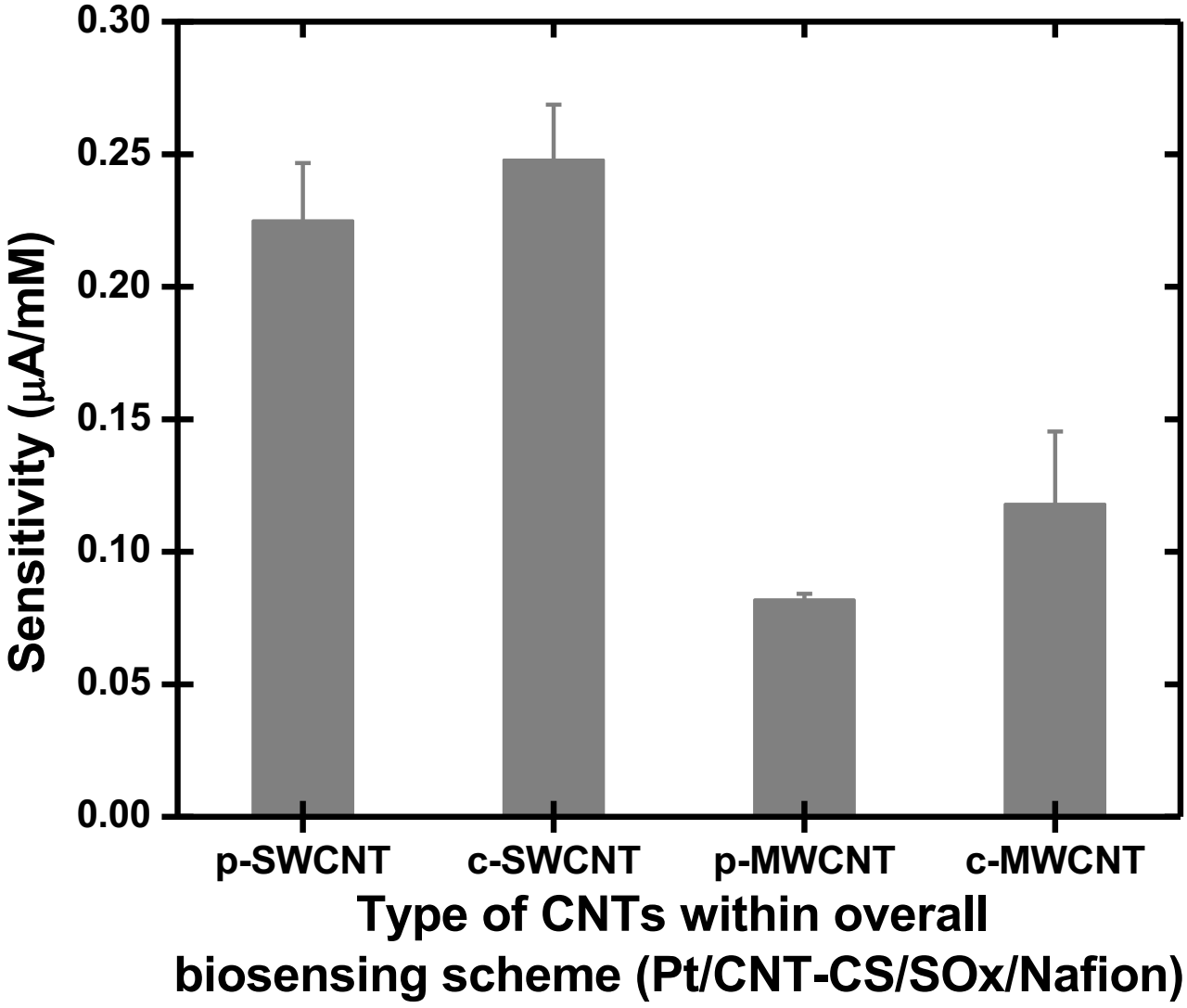
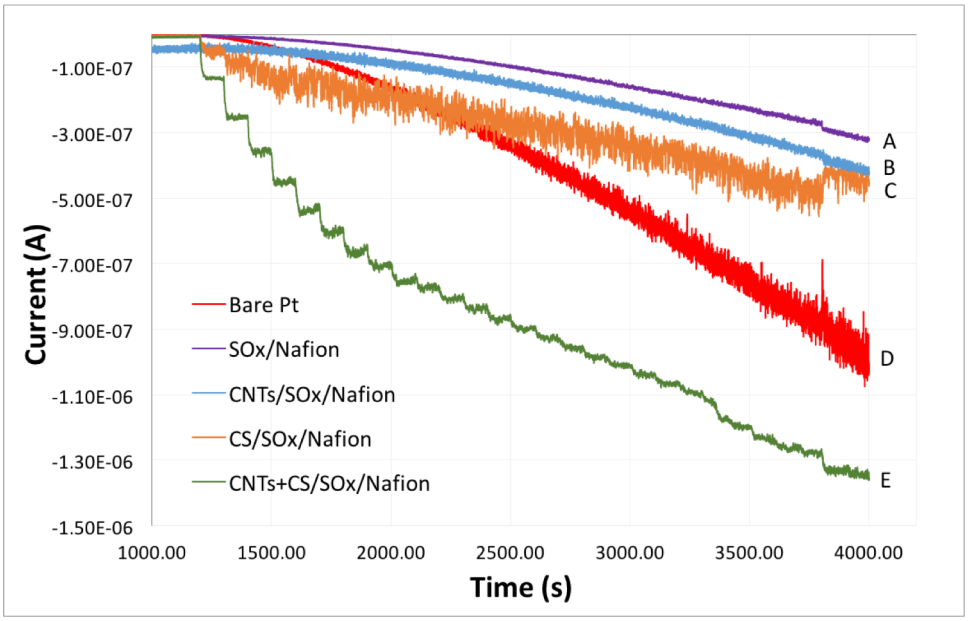


Figure SI-5. Sensitivity of Pt/CS-p-SWCNT/SOx/Nafion, Pt/CS-COOH-SWCNT/SOx/Nafion, Pt/CS-p-MWCNT/SOx/Nafion and Pt/CS-p-MWCNT/SOx/Nafion electrochemical biosensor toward the oxidation of hydrogen peroxide. Nafion concentration was 5% wt. Note: In some cases, standard error bars are smaller than markers for average value (n = 4).

A



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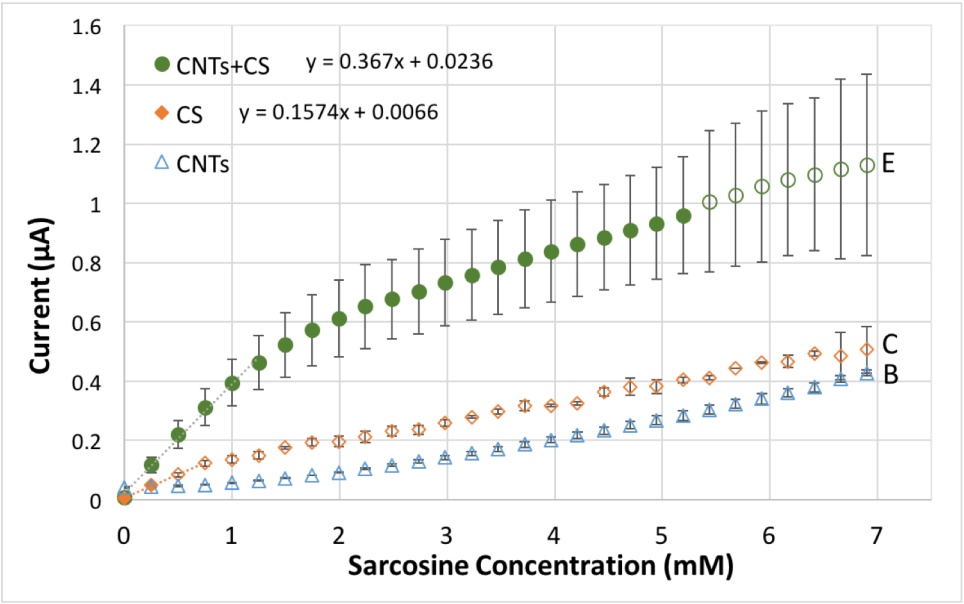

Figure SI-6. I-t curves (A) and corresponding calibration curves (B) of the CS-COOH-SWCNT/SOx/Nafion system, both including and excluding SWCNTs and chitosan. Successive 0.1 mM injections of sarcosine established the I-t curves for platinum (Pt) electrodes modified with (a) Pt/SOx/Nafion, (b) Pt/CNTs/SOx/Nafion, (c) Pt/CS/SOx/Nafion, (d) bare Pt, and (e) Pt/CS-COOH-SWCNT/SOx/Nafion. . Note: In some cases, standard error bars are smaller than markers for average value (n = 4).

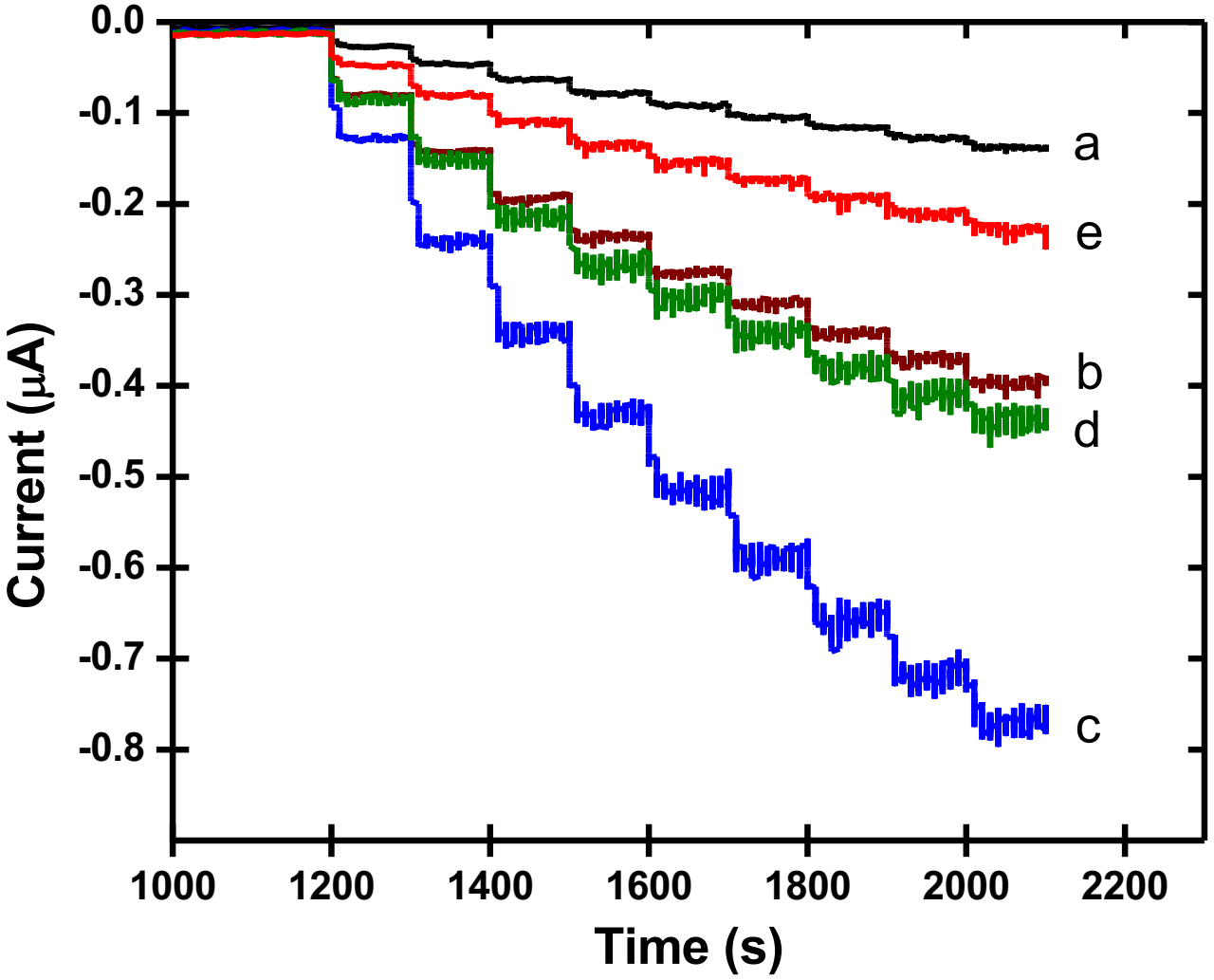

Figure SI-7: Representative amperometric I-t curves during successive 0.1 mM injections of sarcosine at Pt/CS-COOH-SWCNT/SOx/Nafion using various concentrations of SOx: (a) 2 mg/mL, (b) 4 mg/mL, (c) 6 mg/mL, (d) 8 mg/mL and (e) 10 mg/mL. Note: Nafion concentration was 5% wt.

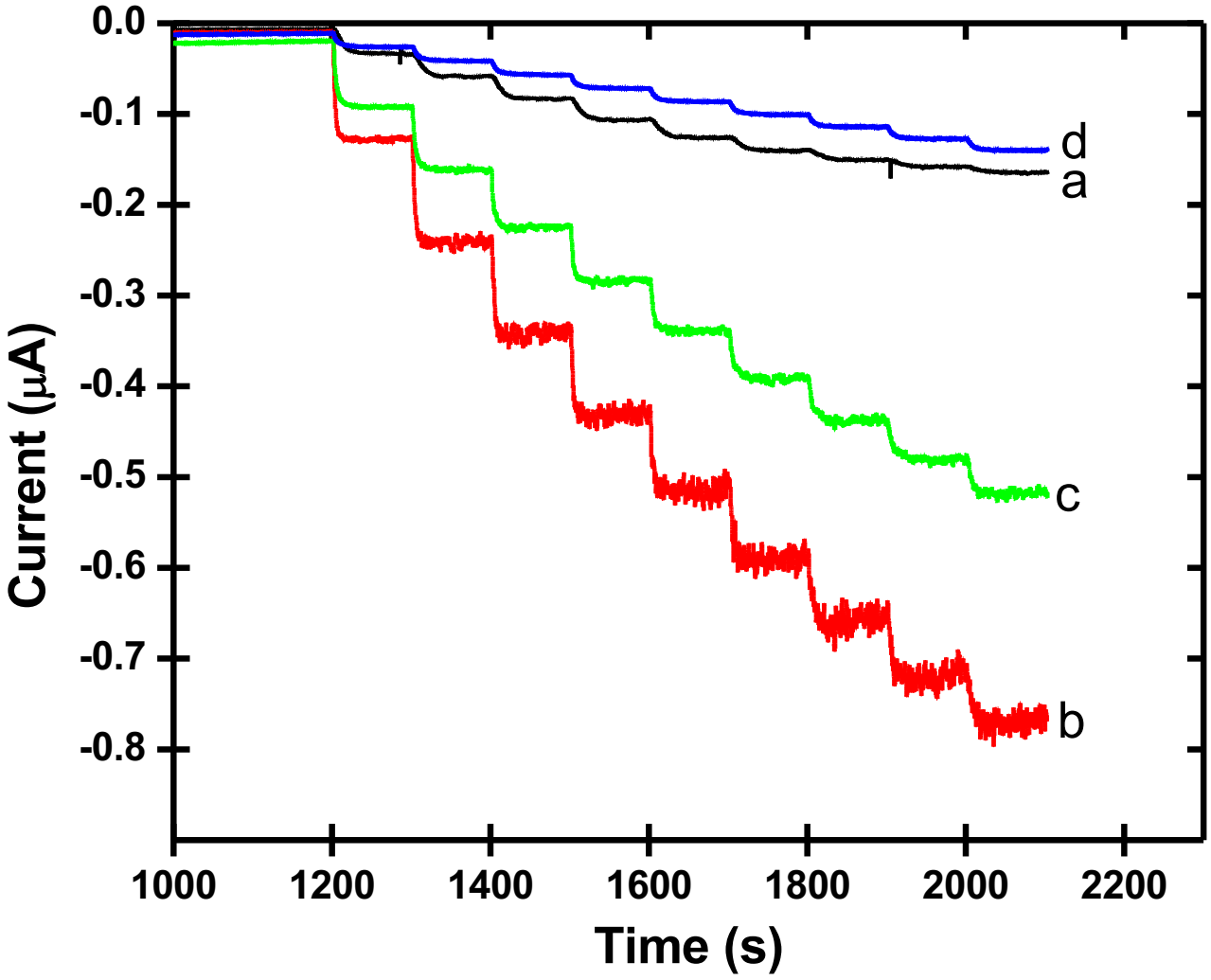

Figure SI-8: Representative amperometric I-t curves during successive 0.1 mM injections of sarcosine at Pt/CS-COOH-SWCNT/SOx/Nafion. Nafion concentration is varied in ethanol as follows: (a) 1 % wt., (b) 3 % wt., (c) 5 % wt. and (d) 10 % wt. Note: [SOx] = 6 mg/mL.

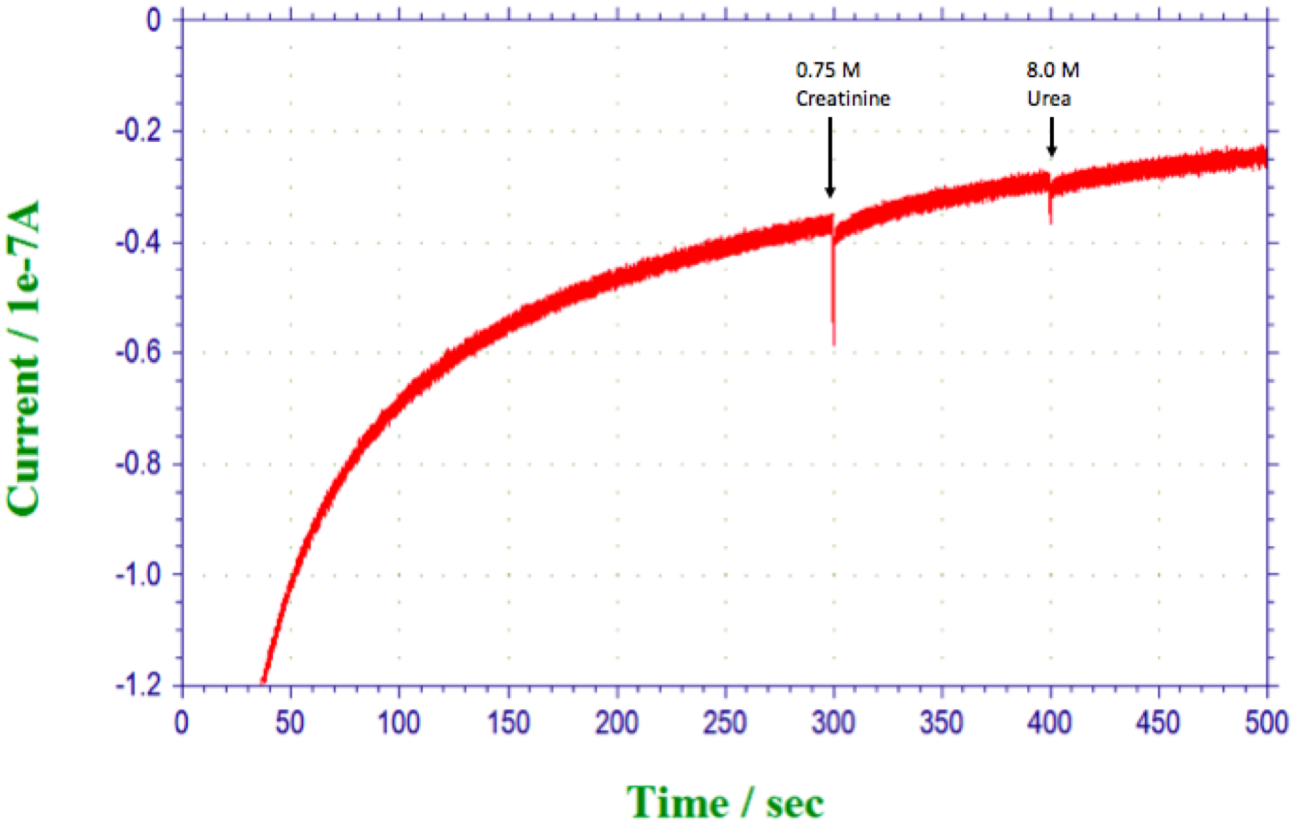

Figure SI-9. Injections of 0.75 M Creatinine (50uL) and 8 M Urea (50uL) at a bare Pt electrode (300 seconds elapsed and 400 seconds elapsed, respectively), creating a similar response to the interferent tests, indicating that the Nafion layer is non-inhibitory for the creatinine to reach the electrode surface.

Table SI-2. Interferent Testing of Pt/COOH-SWCNT-CS/SOx/Nafion System – Avg. Step Current Resp.

Injection (Day 0)	**Concentration** (mM)	**n**	**Average Step Current** (amps)	**Standard Error** (amps)	**Average Selectivity Coefficient**
Urea	400	7	1.04 E-09	2.40 E-10	-4.31
Creatinine	12	7	3.52 E-10	1.09 E-10	-3.28
Sarcosine	0.25	7	2.01 E-08	6.51 E-09	0.167
Sarcosine	0.25	7	2.29 E-08	7.62 E-09	0.21
Sarcosine	0.25	7	1.82 E-08	4.96 E-09	0.14
Sarcosine	0.75	7	4.19 E-08	1.38 E-08	N/A

Table SI–3: Comparison of Sarcosine/Creatine/Creatinine Biosensor Performance Parameters– Literature Comparison

System	Type	Analyte	WE	Sensitivity (μA/mM)	Response Time (s)	Linear Range (mM)	LOD† (μM)	Stability (days)	Ref
Pt/CS/COOH-SWCNT/SOx/Nafion*	1st G	Sarcosine	Pt	0.48 ±.09	8	0.75	6.47 ±0.73	> 12	a
Pt/CS/COOH–SWCNT/SOx/Nafion	1st G	Sarcosine	Pt	0.37 ±0.11	–	1	15.9	–	a
Pt/CS-COOH–SWCNT/SOx/Nafion	1st G	Sarcosine	Pt	0.29 ±0.12	8	1.5	13 $\pm0.00_6$	> 12 (24% loss)	a
Pt/CS-pSWCNT/SOx/Nafion	1st G	Sarcosine	Pt	0.22 ±0.05	–	1.5	13 $\pm0.00_3$	–	a
Pt/CS-COOH–MWCNT/SOx/Nafion	1st G	Sarcosine	Pt	0.12 ±0.05	–	1.5	36.3 ±0.02	–	a
Pt/CS-pMWCNT/SOx/Nafion	1st G	Sarcosine	Pt	$0.08 \pm0.00_{36}$	–	1.5	48.3 $\pm0.00_9$	–	a
Carbon/EDAC+NHS/SOx/Nafion	1st G	Sarcosine	Carbon	3.4×10^4	-	1e–4	.016	60	[27]
Graphene/ PVA–AuNP–pphTEOS+SOx	2nd G	Sarcosine	Graphene	7.87	8	0.5–7	500	–	[28]
Pt/Aniline/SOx	1st G	Sarcosine	Pt	0.05	2	0.01–1	10	–	[4]
Carbon/SOx+CAH+ Glutaraldehyde	2nd G	Sarcosine	Carbon	0.035 ±0.003	>60	4	–	8 (50% loss)	[29]
Pt/CS/COOH-SWCNT/SOx+CA+CI/Nafion/CS	1st G	Creatinine	Pt	0.57 ±0.24	8	0.5	7.8 ±4.2	-	a
Carbon/SOx+CAH+ Glutaraldehyde	2nd G	Creatine	Carbon	0.010 ±0.006	>60	3.5	–	8 (50% loss)	[29]
Pt/Aniline+CS+Fe_3O_4 NPs/Glutaraldehyde /CA+CI+SOx	1st G	Creatinine	Pt	23.3	2	.001–.8	1	200 (10% loss)	[31]
HRP+Fc+AuNPs+MWCNTs+ Teflon/CA+CI+SOx	2nd G	Creatinine	Steel	1.59	19±1	.003–1	0.1	17 (50% loss)	[30]
Pt/Aniline/COOH–MWCNTs/EDC+NHS/CA+CI+SOx	1st G	Creatinine	Pt	0.24	5	.01–.75	0.1	180 (15% loss)	[31]

Notes: Literature reports for spectroscopy-based sarcosine sensors are not included in comparison table; * denotes material doped with enzyme. †LOD = $3\sigma_{blank}/b_1$; EDAC: 1–Ethyl–3–(3–dimethylaminopropyl) carbodiimide; NHS: *N*–Hydroxysuccinimide; PVA: Polyvinyl alcohol; AuNP: gold nanoparticles; pphTEOS: prehydrolyzed Tetraethylorthosilicate; CAH: Creatinase; CI: Creatininase. Platforms presented in the current work designated **(a)**.

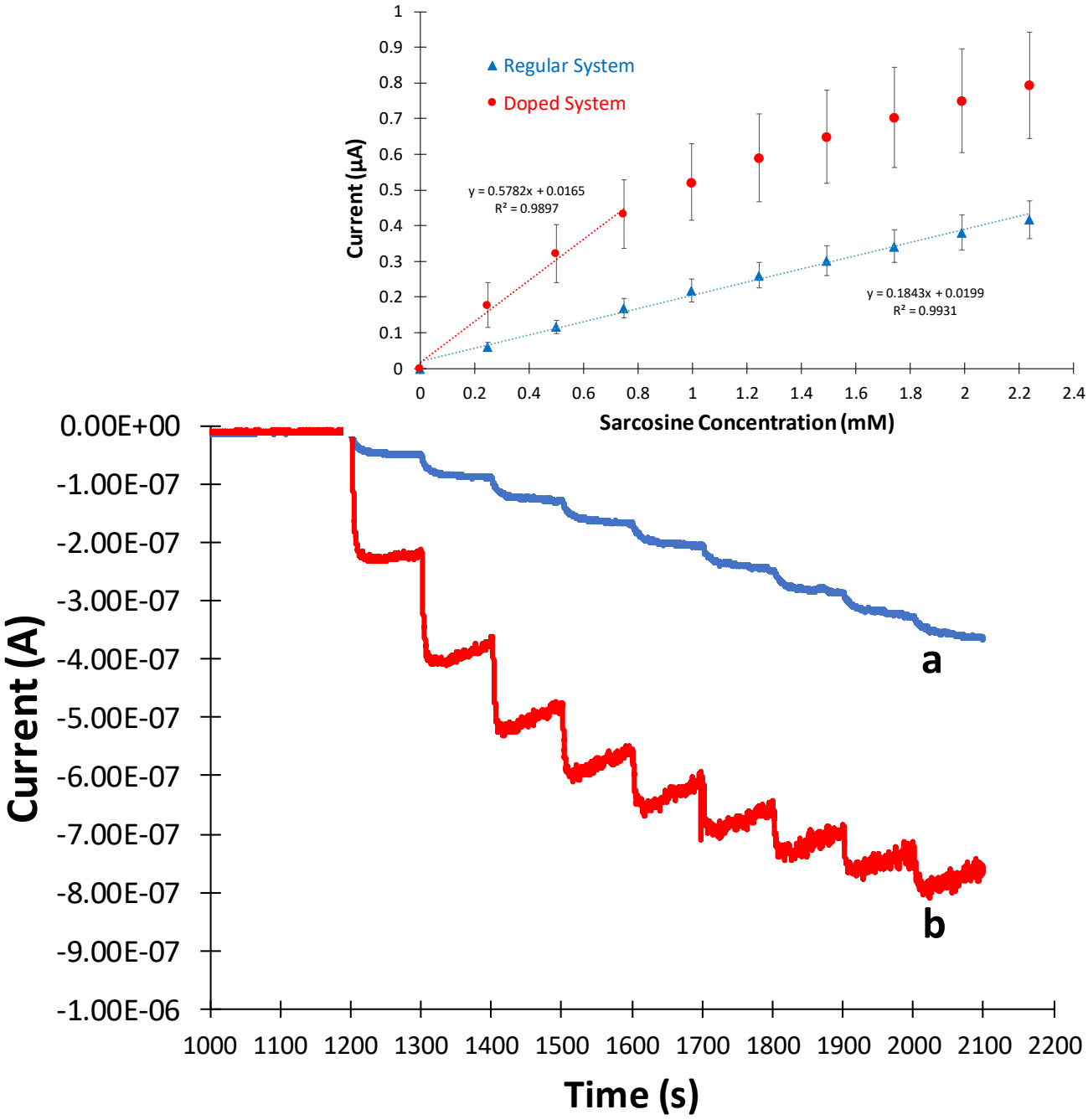

Figure SI-10: Representative amperometric I-t curves and corresponding calibration curves (**inset**) during successive 0.25 mM injections of sarcosine at (a) Pt electrodes modified with (a) Pt/CS/COOH–SWCNT/SOx/Nafion versus (b) Pt/CS/COOH–SWCNT/SOx/Nafion doped with additional SOx. Nafion concentration was 5% in all cases. Note: Solid symbol markers indicate a step-like response and, in some cases, standard error bars are smaller than markers for average value (n = 3).

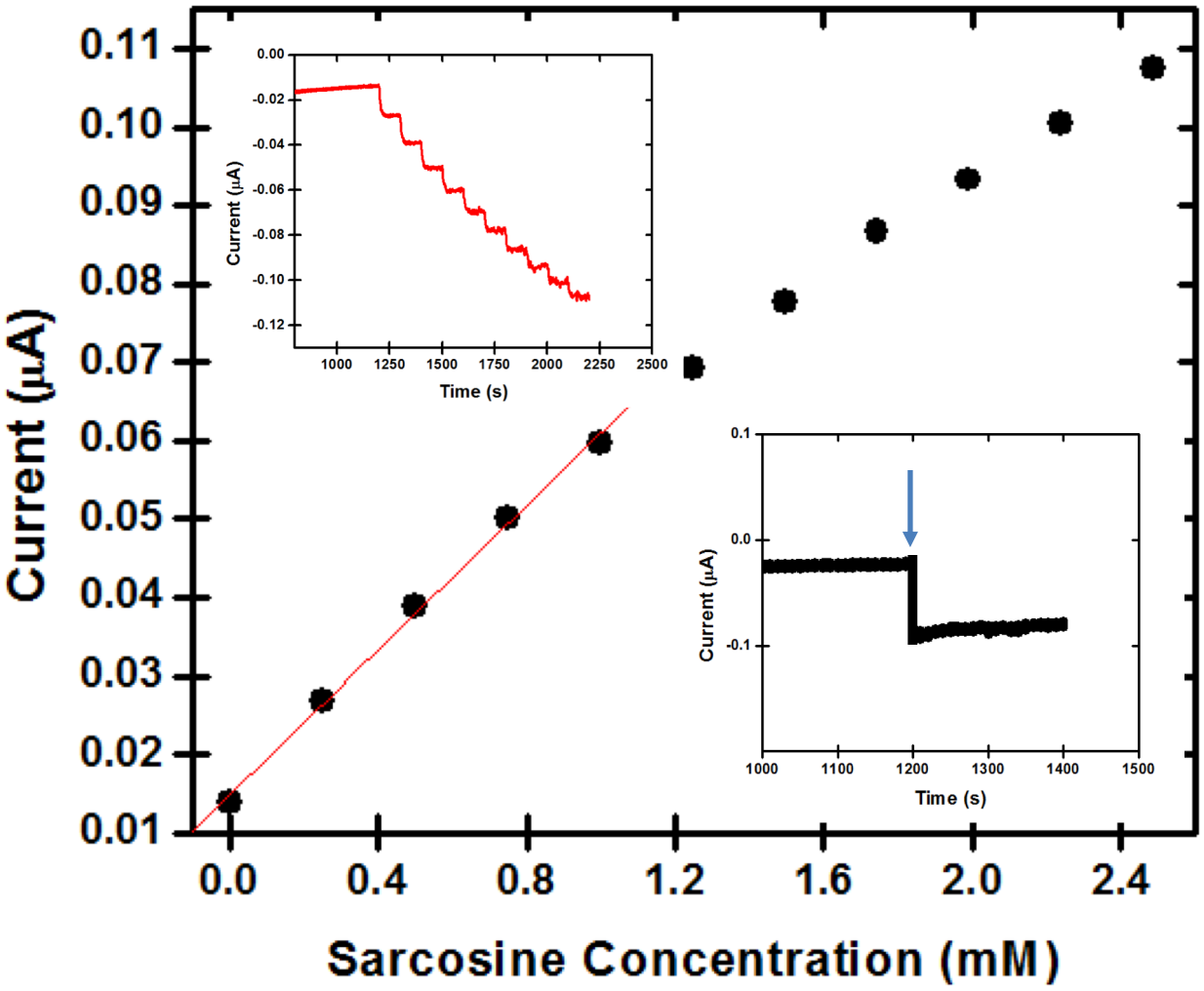

Figure SI-11. Representative amperometric I-t curve (inset, top), background corrected calibration curve of the Pt/CS-COOH-SWCNT/SOx/Nafion system in a synthetic urine matrix. An electrode was charged in room temperature in the urine and an I-t curve was established by performing subsequent 0.1 mM injections of sarcosine. Afterwards, a detection test was performed with a fresh Pt/CS-COOH-SWCNT/SOx/Nafion electrode, which was charged in surine and swapped into a sarcosine spiked urine solution (1.50 mM). The detected concentration was calculated using the calibration curve to be 1.48 mM (RSD = 1.3%).

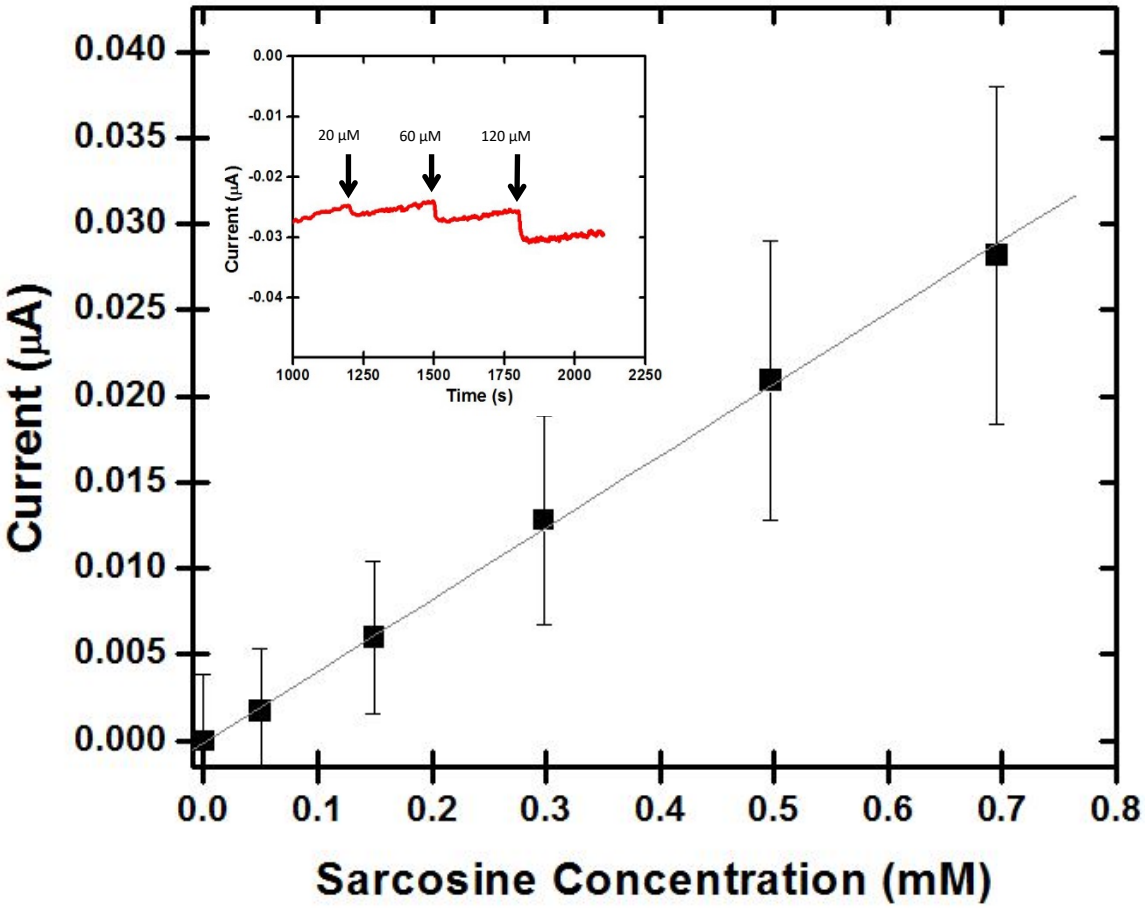

Figure SI-12. Calibration curve in surine with the Pt/CS-COOH-SWCNT/SOx/Nafion (5% wt) system, established by increasing injections of 50 mM sarcosine to obtain concentrations ranging from 50μM to 700μM. The I-t curve (inset) demonstrates the capability of the system to measure low concentrations of sarcosine with relative accuracy when calibrated in surine, detecting 18.9 ± 4.5 μM from a 20.0 μM sarcosine injection (n=5).

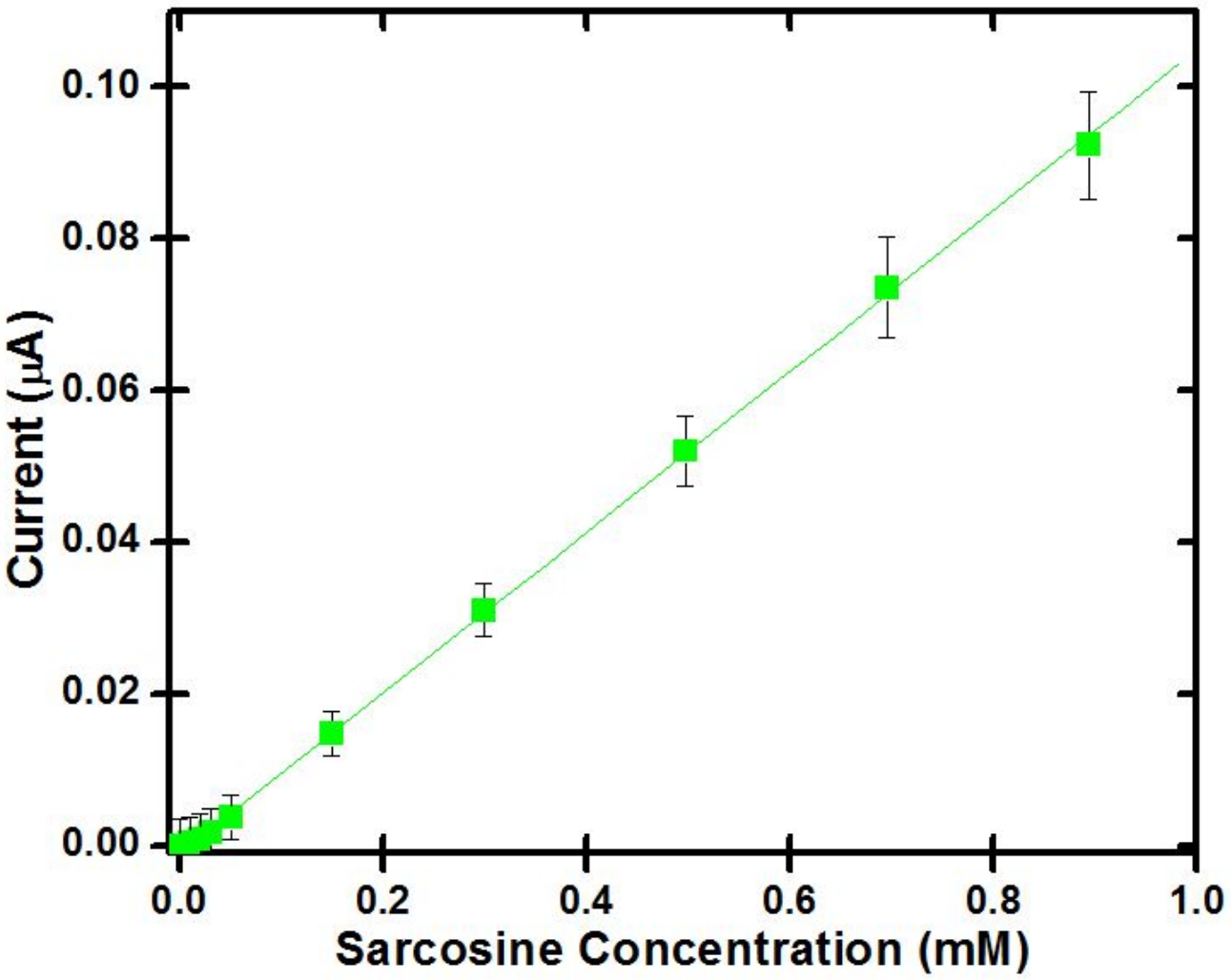

Figure SI-13. Calibration curve in surine with the Pt/CS-COOH-SWCNT/SOx/Nafion* (5% wt) system, established by increasing injections of 50 mM sarcosine to obtain concentrations (n = 5).

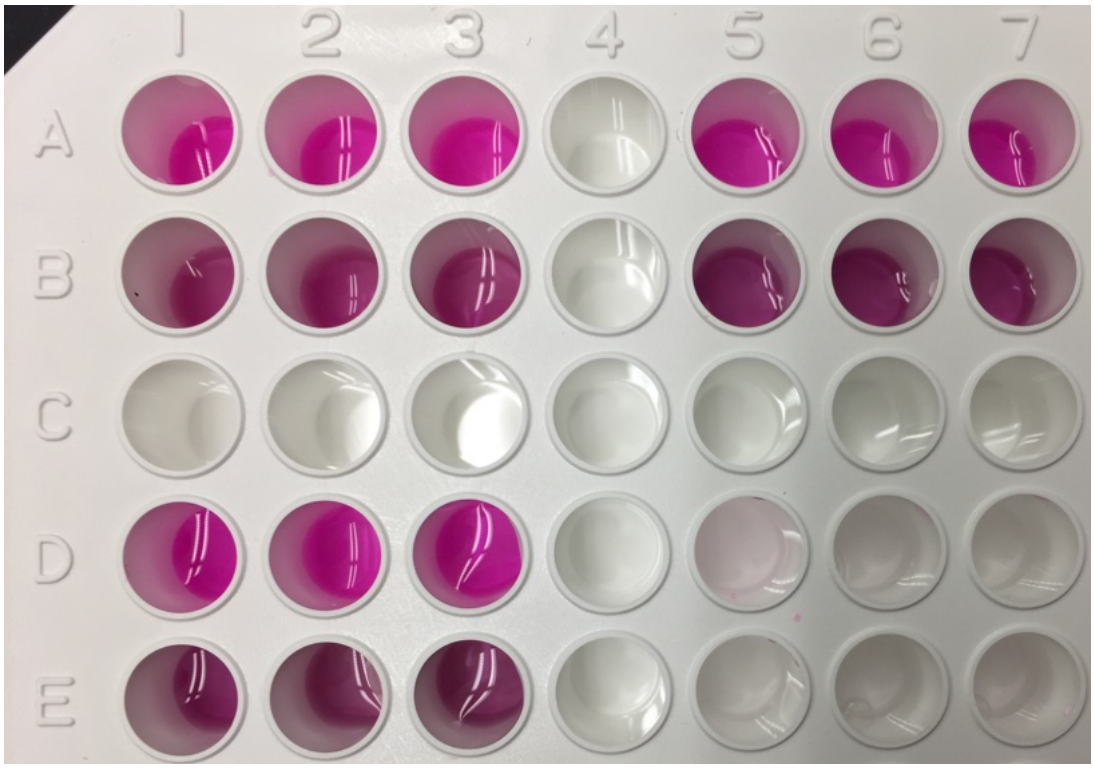

Figure SI-14: A fluorometric hydrogen peroxide assay (Sigma Aldrich MAK165) was performed according to the procedure, using final analyte concentrations of 10 μM (rows A&D) and 100 μM (rows B&E). Wells A1-A3 and B1-B3 contained house-made SOx (100 μg/mL) with sarcosine as the analyte. Wells A5-A7 and B5-B7 contained house-made SOx, Creatinase, and Creatininase (100 μg/mL, 100 μg/mL respectively) with creatinine as the analyte. Wells D1-D3 and E1-E3 contained house-made SOx, Creatinase, and Creatininase (100 μg/mL, 436 μg/mL, and 10 μg/mL respectively) with creatinine as the analyte. Wells D5-D7 and E5-E7 served as controls with no enzyme present, in order to provide the background fluorescence that was subtracted from fluorescence readings.

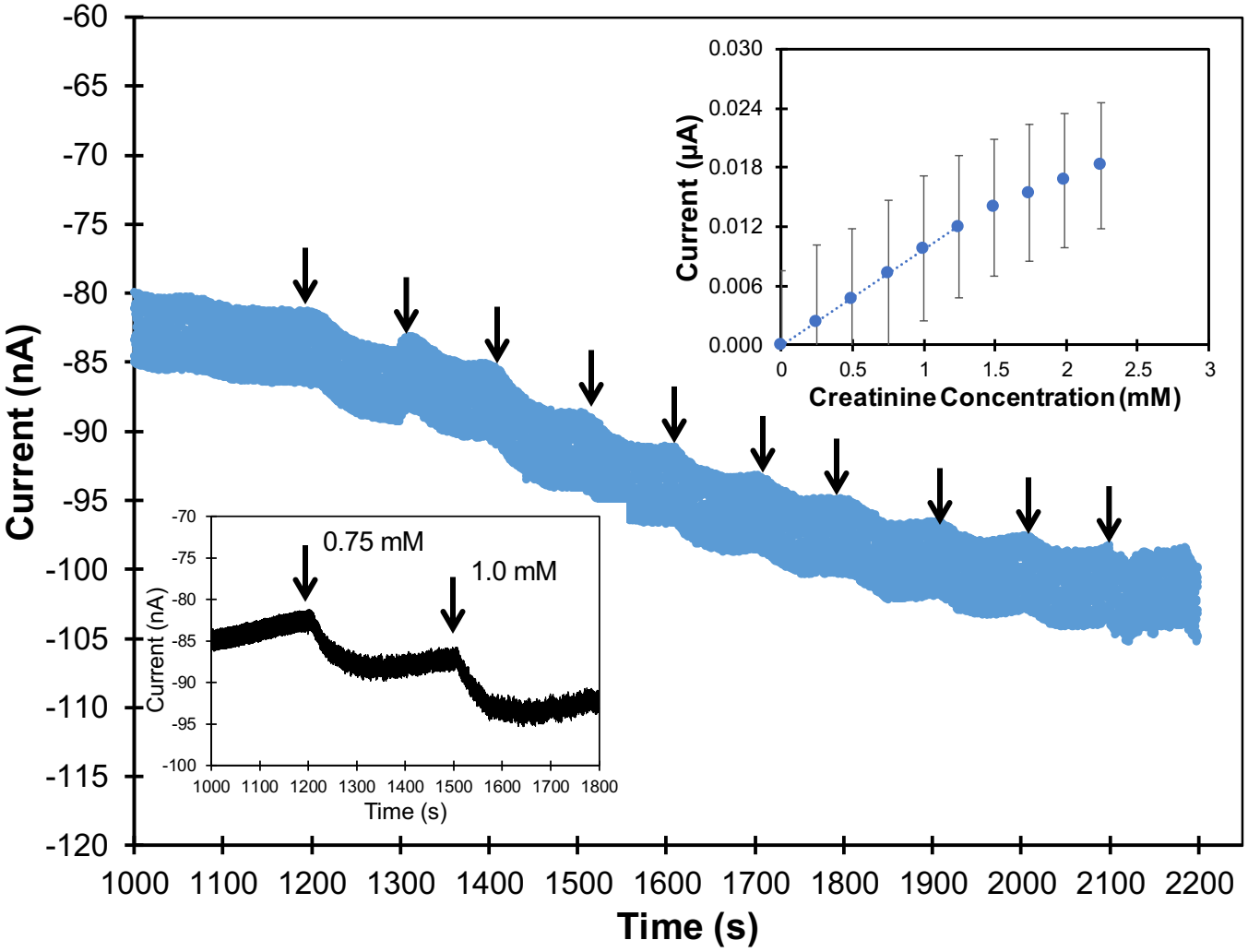

Figure SI-15: Calibration curves obtained from amperometric I-t curves during successive 0.25 mM injections of creatinine at Pt/CS-COOH-SWCNT/CI-CA-SOx/Nafion* in blood serum; **Inset *(top)*** representative calibration curve derived from I-t response and **Inset *(bottom)*** step current response to 0.75 mM and 1.0 mM creatinine spiked blood serum samples used to test for quantitative analysis of creatinine.