Open Access : : ISSN 1847-9286
https://pub.iapchem.org/ojs/index.php/JESE

Supplementary material to

Screen-printed electrode for electrochemical detection of sunitinib malate for therapeutic drug monitoring

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J. Electrochem. Sci. Eng. **14(6)** (2024) 803-822; http://dx.doi.org/10.5599/jese.2478

Sensor fabrication

AUTOCAD designs used for different layers of screen printed electrodes (SPE) is shown in Figure S1. A single SPE has a dimension of 5 cm length and 2 cm width. The electrode has a geometric area of 3.14 mm^2 (r = 1 mm).

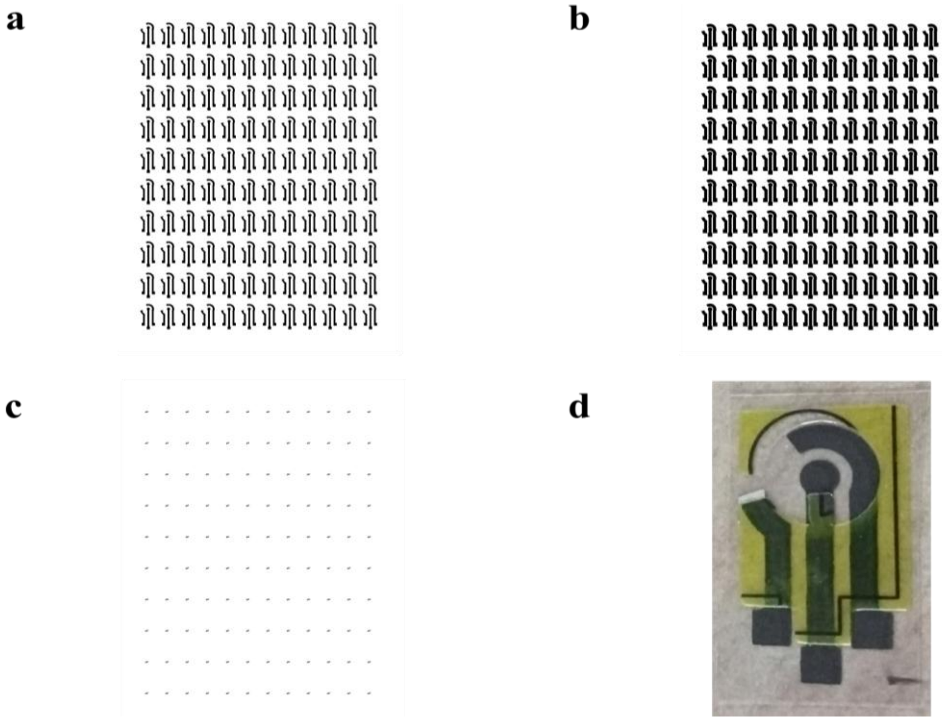

Figure S1. *AUTOCAD design for the fabrication of SPE:first layer of silver (a), second layer of carbon (b), third layer of Ag/AgCl (c) and fully developed SP (d)*

EIS measurement

The EIS Nyquist spectra usually include two parts, semi-circular and linear. A semi-circular part which is located at higher frequencies reflects the charge transfer process (faradaic process) whereas linear part positioned at lower frequencies is characteristic to the diffusion process [1]. The Nyquist plot obtained for our indigenously developed electrode in presence of $[Fe(CN)_6]^{3-/4-}$ redox couple is shown in Figure S2.

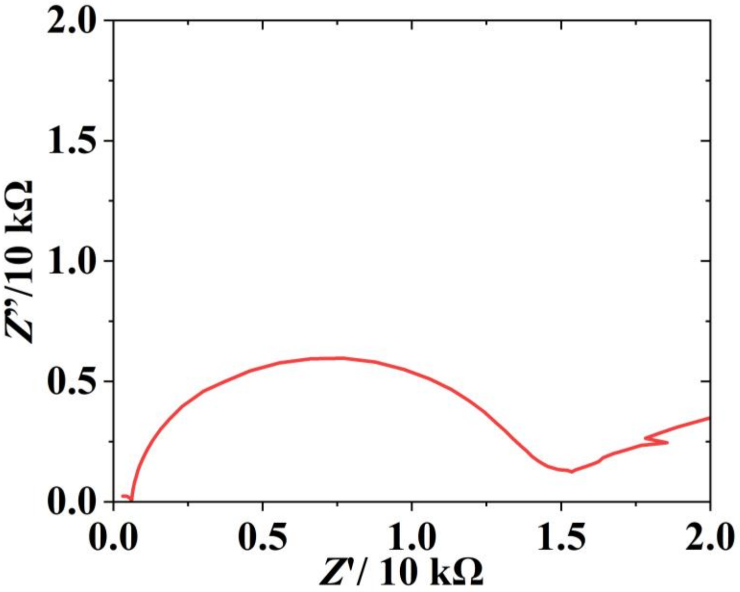

***Figure S2**. Nyquist plot of impedance spectra (100 kHz to 0.01 Hz) of SPE in 0.1 mol L^{-1} KCl containing 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$*

The semi-circular portion usually indicates a charge transfer process. The diameter of the semicircle can give insights into the charge transfer resistance (R_{ct}). As the semicircle is centered around the real axis, this suggests a single time constant process, which is common in electrochemical systems [2]. The linear sloping portion after the semicircle (often referred to as Warburg impedance) typically indicates diffusion-limited processes. This suggests that at lower frequencies, the system behavior is dominated by diffusion rather than charge transfer. Generally, low charge transfer resistance value is favorable for good electrode conductivity and fast reaction kinetics [2].

Effect of ascorbic acid

Experiments were performed with a constant concentration of ascorbic acid (AA) of 80 µM and varying STB concentration (12, 24 and 36 µM). Experiments were also performed with 0.537 µM STB (plasma concentration) and varying AA concentration (40, 80 and 100 µM). The results shown in Figure S3a show that AA does not affect the concentration of STB if its concentration is kept constant (RSD = 1.67 %). If, however, AA concentration was increased, its oxidation current is abruptly increased, while keeping the peak height of STB constant, which suggests that there are not detrimental effects on the drug oxidation Figure S3b clearly shows that even low concentrations of STB can be detected with minimal variations in anodic currents at high concentrations of AA.

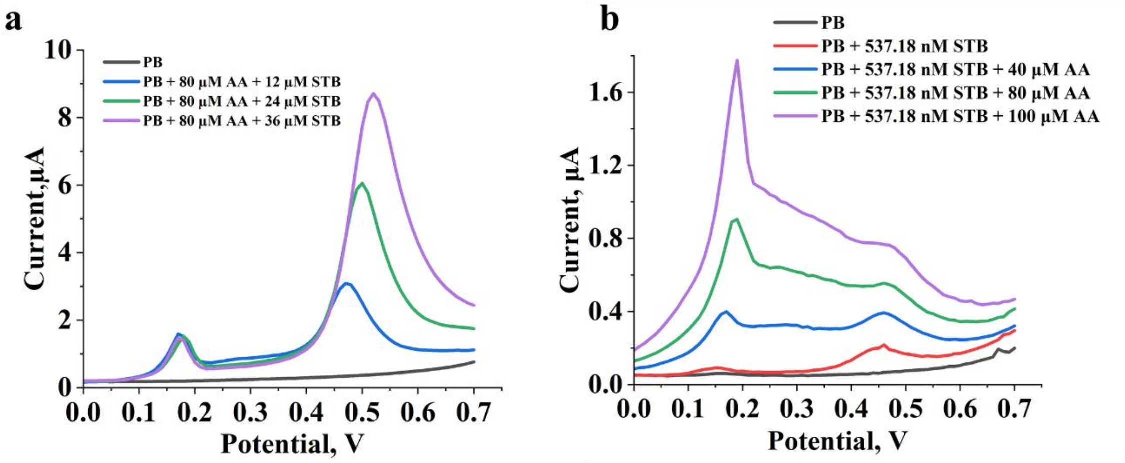

Figure S3. *Differential pulse voltammograms of SPE in 0.1 M PB, pH 7.4, with 80 µM of AA and varying STB concentrations (b) and with 537 nM of STB and varying AA concentrations (a)*

Matrix effects

The sample matrix will change the sensitivity of the sensor. Molecules in the matrix can also interact with the sensor surface. which can restrict the access of redox molecules to the sensors and lower sensitivity. Especially serum can vastly differ in refractive index, salinity, protein concentration, and optical properties from individual to individual which will induce baseline shifts for sensors and thus increase the noise of sensors as these baseline shifts are difficult to compensate for [3]. These baseline shifts were seen when we added serum and urine to the supporting electrolyte (Figure S4).

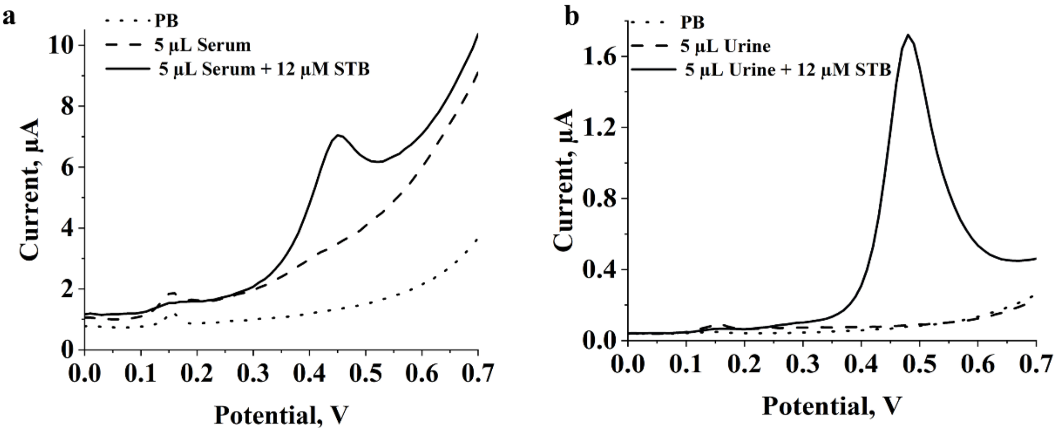

Figure S4. *Differential pulse voltammograms of SPE with serum (a) and urine (b), spiked with 12 µM STB in 0.1 M PB*

The recovery percentage of all sensors often decreases dramatically as a result of such non-specific adsorption. The biggest difficulty in analyzing urine samples is the variety of pH ranges that might affect the location of peak potential and the current intensity value. So, we performed the recovery studies of the analyte in serum, and urine samples in assessing the performance of our proposed sensor. It was studied in an extensive way and the good recovery values obtained for STB in real samples indicate that even changing the matrix does not induce a mass change in the diffusion flux of STB towards the sensor.

Reproducibility and stability

Reproducibility was examined by detecting a triplicate set of STB. The reaction of STB in 0.1 M PB, pH 7.4 on six distinct electrodes using DPV (Figure S5a). Stability of the developed sensor was estimated every five days for one month. The responses for every 5 days are shown in Figure S5b.

Investigation showed that the domestically produced SPE offers great reproducibility and stability when stored in a sealed condition.

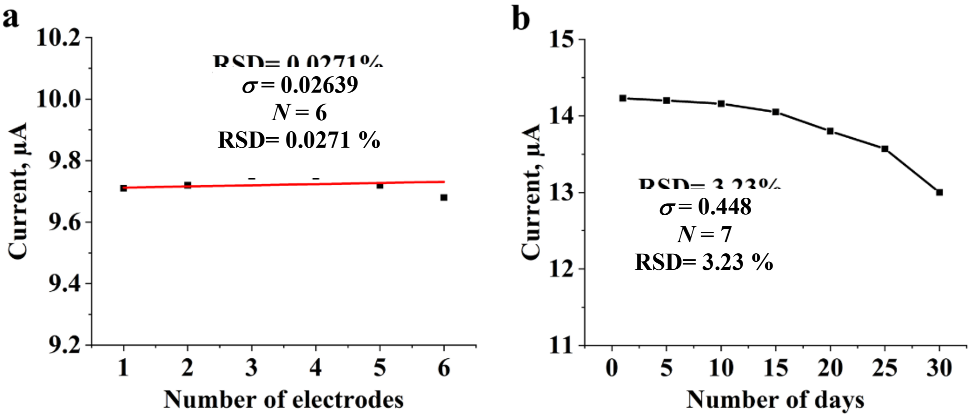

Figure S5. *Peak current obtained on different SPE electrodes (a) and different intervals of days in M PB, pH 7.4 containing 40 µM STB (b)*

Inter and intraday analysis of real samples

To evaluate inter- and intra-day precision, three concentrations of the sunitinib drug were tested, [five replicates for each concentration] were validated using the indigenously developed electrodes on the same day and across different days, respectively, to assess the repeatability and reproducibility of the voltammetric method employing screen-printed electrodes (SPEs). The average standard deviation (SD) and relative standard deviation (RSD) values for the intra- and inter-day measurements in real samples using electrodes are presented in Table S1. The low RSD and SD values further confirm the repeatability, reproducibility, and precision of the SPEs in the determination of STB.

Table S1*: Inter and intraday analysis of STB in real samples like serum, urine and tablets using SPEs*

Sample	Intra-day analysis				Inter-day analysis			
	C_{measured} / µM ± SD	Recovery, %	RSD, %	*t*-test	C_{measured} / µM ± SD	Recovery, %	RSD, %	*t*-test
Serum	12 ± 0.12	100.37	1.35	0.228	12 ± 0.12	100.20	1.57	0.22
	24 ± 0.10	101.53	2.2	1.54	24 ± 0.10	97.7	4.26	-1.24
	36 ± 0.12	97.53	5.34	-1.05	36 ± 0.12	102.87	3.44	1.81
Urine	8 ± 0.09	100.23	2.19	0.234	8 ± 0.09	100.86	4.3	0.45
	11 ± 0.09	100.73	2.03	0.8	11 ± 0.09	101.25	3.6	0.02
	25 ± 0.10	100.33	2.38	0.215	25 ± 0.10	100.15	3.13	0.10
Tablets	12.15 ± 0.12	101.42	2.79	1.14	12.15 ± 0.12	100.26	2.48	0.23
	24.3 ± 0.11	102.76	2.89	2.07	24.3 ± 0.11	101.16	4.53	0.55
	36.45 ± 0.12	103.09	3.03	2.2	36.45 ± 0.12	100.16	3.82	0.09

At *n* = 5; 95 %, *t*-test = 2.571

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