Electronic Supplementary Information

Single-step electropolymerization on a printed sensor towards a conductive thin film polymer for the simultaneous determination of drug metabolites: 5-aminosalicylic acid and sulfapyridine

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2. Experimental

2.1. Apparatus, chemicals, reagents, and materials

Voltammetric measurements were conducted using a model CHI660D electrochemical analyzer (CH Instrument, Austin, TX, USA). Electrochemical impedance spectroscopy (EIS) experiments were carried out using PalmSens4 (Houten, The Netherlands). A field emission scanning electron microscope and energy-dispersive X-ray spectroscopy (FESEM-EDS) were performed with an electron microscope at 5 kV (JSM-7610F; JEOL, England, UK). X-ray photoelectron spectroscopy (XPS) measurements were executed using the Kratos Axis Ultra DLD X-ray Photoelectron Spectrometer. All experiments were operated using a three-electrode system, comprising the working, counter, and reference electrodes, which were all screened on the same PVC substrate.

Sulfapyridine (SPD) was acquired from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 5-Aminosalicylic acid (5-ASA) was purchased from Glentham Life Sciences (Corsham, UK). L-methionine, uric acid, ascorbic acid, citric acid, bovine serum albumin, creatinine, urea, ammonium persulfate, and potassium ferricyanide ($K_3[Fe(CN)_6]$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glucose, sodium chloride, potassium chloride, magnesium chloride, sodium hydroxide, methanol, sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$), and di-sodium hydrogen orthophosphate ($Na_2HPO_4 \cdot 2H_2O$) were purchased from Ajax Finechem Pty., Ltd. (New South Wales, Australia). Calcium chloride and zinc sulphate were supplied by Carlo Erba Reagents (Val de Reuil, France). Acetone was acquired from CaHC Co., Ltd. (Bangkok, Thailand). Graphene ink was received from Serve Science Co., Ltd. (Product code: SSG-1760A, Bangkok, Thailand). Silver/silver chloride (Ag/AgCl) ink was obtained from Sun Chemical Ltd. (Product code: C2130809D5, Slough, UK).

The stock standard solution of SPD was prepared in methanol, and 5-ASA was arranged in Milli-Q water, then stored in the dark at 2°C–8°C. The SPD and 5-ASA working solutions were prepared in a mixture of the supporting electrolyte containing methanol (MeOH) and 0.1 M phosphate buffer solution (PBS). PBS was prepared by mixing two stock solutions of $NaH_2PO_4\cdot$and Na_2HPO_4 following the standard recipes. All aqueous solutions were arranged in Milli-Q water from a Millipore water purification system ($R \geq 18.2$ MΩ cm at 25°C).

2.4. Real sample preparation

The poly(L-Met)/SPGE sample application was investigated using various real human urine samples. All human urine samples were well received by the volunteers in our laboratory. Informed consent was obtained from all human subjects, and they already knew what would happen to the human subjects in a trial. To verify the capability of the proposed sensor and developed method, poly(L-Met)/SPGE was utilized to simultaneously determine 5-ASA and SPD in real urine samples using a standard addition method. For the sample preparation, first, real urine samples were diluted with a mixture of the supporting electrolyte containing MeOH and 0.1 M PBS in a proportion of 5%:95% v/v. Next, the standard solutions of 5-ASA and SPD (10 and 30 μM) were spiked into the samples. Then, the simultaneous determination of 5-ASA and SPD was performed by SWV under the optimized conditions as followed by Section 2.3. Finally, the evaluations of accuracy and precision were subsequently approved by determining the collected and prepared samples on the same day (intra-day study) and on three different days (inter-day study).

3. Results and discussion

3.2. Electrochemical characterization of the poly(L-Met)/SPGE

- **Calculation of the electroactive surface area values (*A*)**

The electroactive surface area values of the SPGE and poly(L-Met)/SPGE were calculated using the Randles–Sevcik equation as shown below:

$$I_p = (2.69 \times 10^5) n^{3/2} A D_0^{1/2} v^{1/2} C_0^*$$

where I_p denotes the peak current; n denotes the number of electrons; A denotes the electroactive surface area; D_0 denotes the diffusion coefficient of redox probe species; v denotes the scan rate; and C_0^* denotes the concentration of redox probe species (5.0×10^{-6} mol cm^{-3}). Based on known parameters for the $[Fe(CN)_6]^{3-/4-}$ (n = 1 and $D_0 = 7.6 \times 10^{-6}$ cm^2 s^{-1}) [1], the electroactive surface area values of the sensors were calculated to be 0.064 and 0.068 cm^2 for the SPGE and poly(L-Met)/SPGE.

- **Calculation of the surface coverage (*Γ*)**

The surface coverage value of the modified sensor was calculated using the following equation [2]:

$$\Gamma = \frac{Q}{nFA}$$

where *Γ* denotes the surface coverage of the electrode (mol cm^{-2}); Q denotes the electric charge obtained via the integration of the oxidation peak (C); n denotes the number of electrons involved; F denotes Faraday's constant (96,485 C mol^{-1}); and A denotes the surface area of modified electrode (cm^2). By substituting the respective data in the equation, the Γ value of poly(L-Met)/SPGE was calculated to be 14.921 nmol cm^{-2}.

3.5. Optimization of electropolymerization for L-Met

Investigation of the polymeric electropolymerization optimization parameters is significant to the formation of the film thickness on the electrode surface, resulting in the electrochemical response of an analyte. In this work, the concentration of L-Met solution, the number of scans for electropolymerization, and the scan rate for electropolymerization were studied. From the results in Fig. S7, the optimal parameters for electropolymerization were the concentration of L-Met solution of 6 mM, the number of scans at three cycles, and the scan rate of 100 mV s^{-1}.

Fig. S1.

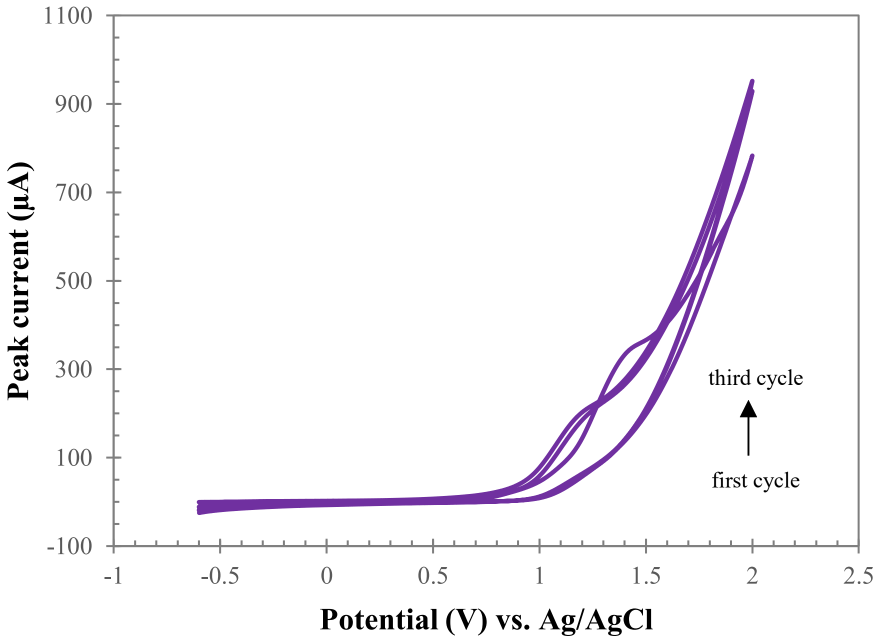

Fig. S1. Cyclic voltammograms for the 6 mM L-Met electropolymerization on the SPGE at a scan rate of 100 mV s^{-1} for three cycles.

Fig. S2.

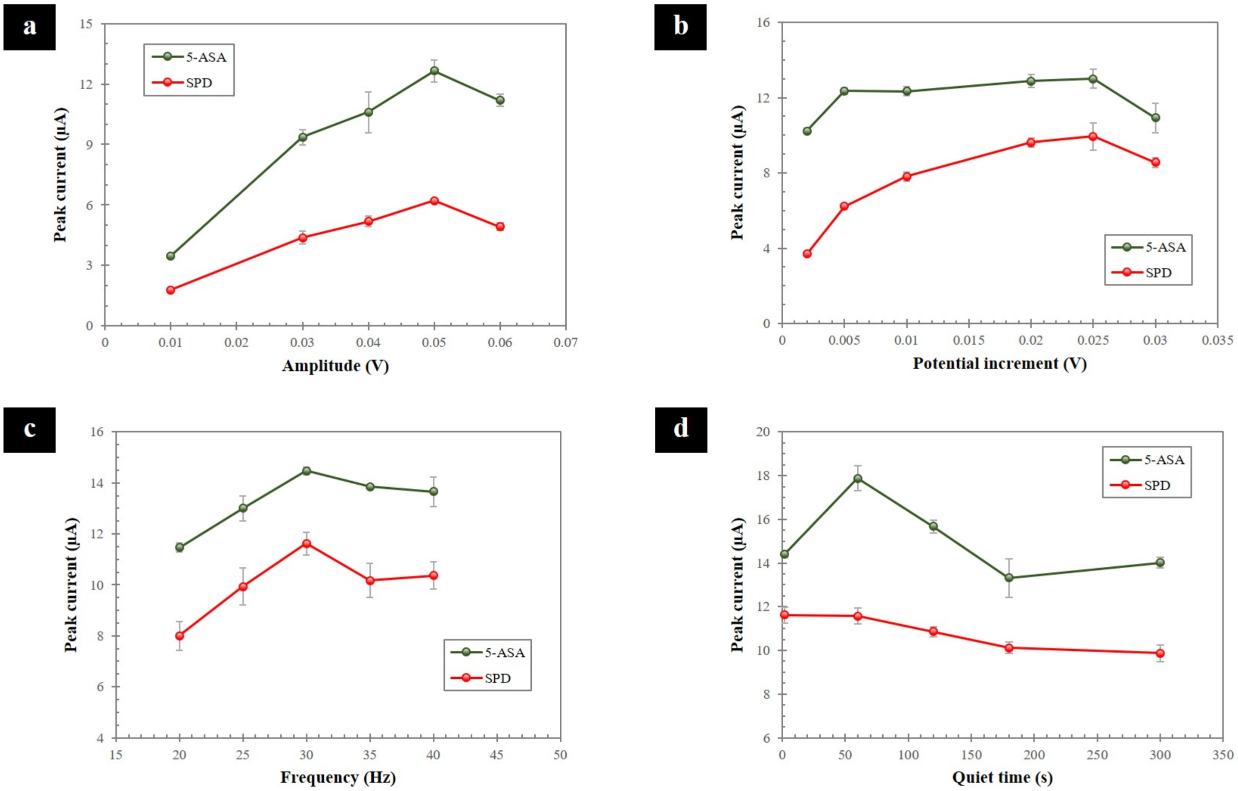

Fig. S2. Effect of an amplitude (a), a potential increment (b), a frequency (c), and a quiet time (d) for the simultaneous detection of 5-ASA and SPD at the poly(L-Met)/SPGE.

Fig. S3.

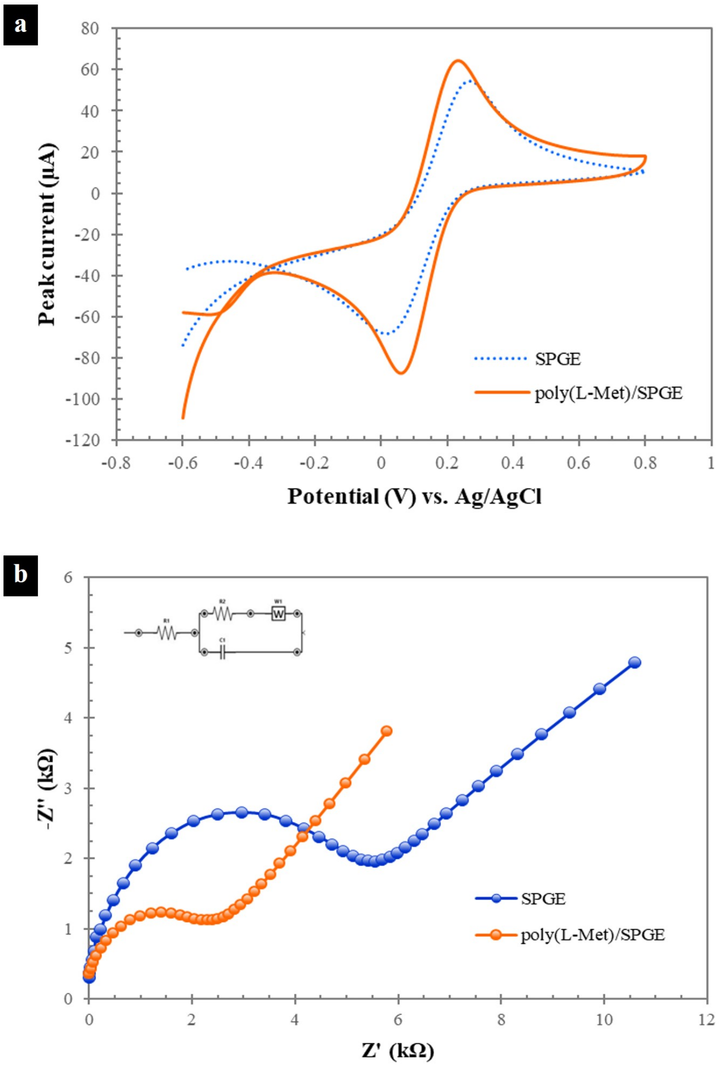

Fig. S3. Cyclic voltammograms (a) and EIS Nyquist plots (b) of 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl at the SPGE and poly(L-Met)/SPGE.

Fig. S4.

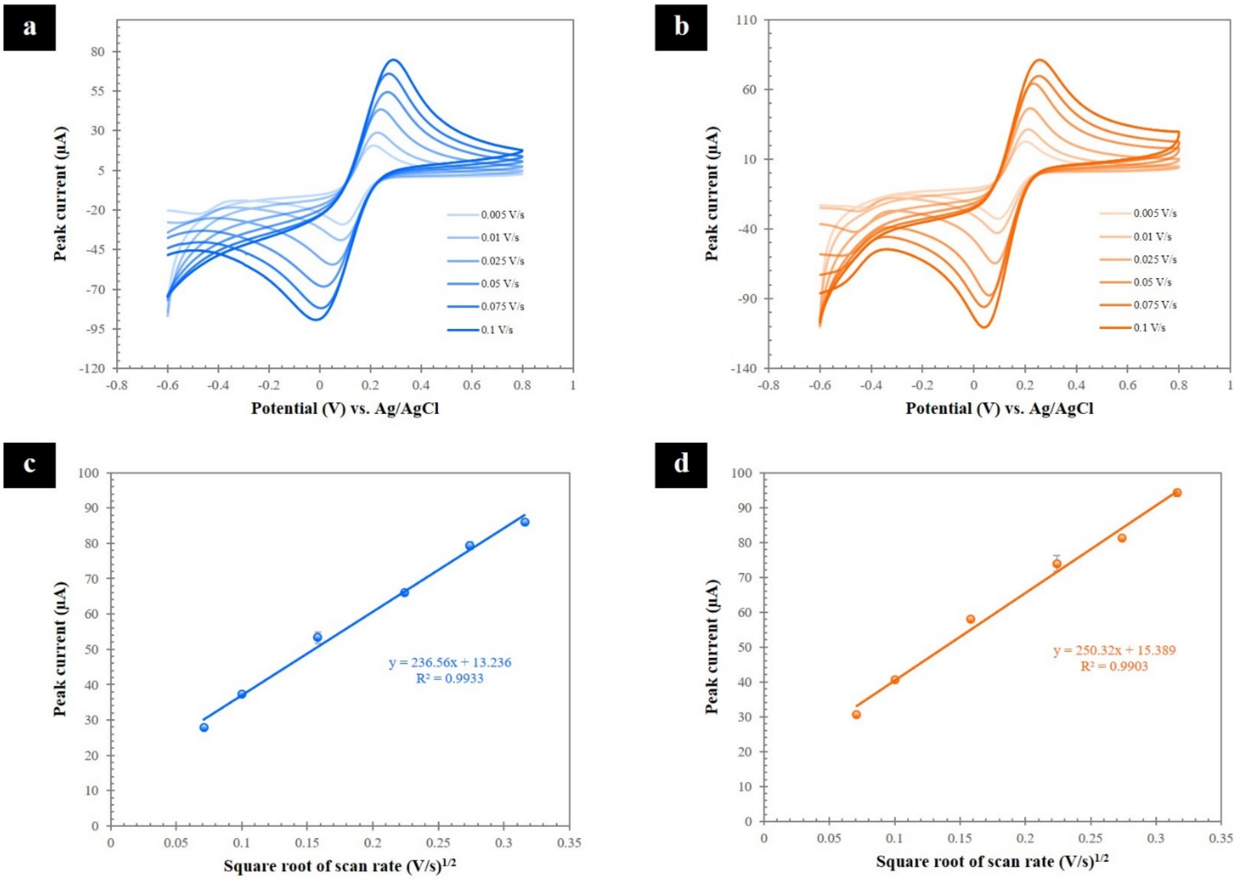

Fig. S4. Cyclic voltammograms of 5 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl using the SPGE (a) and poly(L-Met)/SPGE (b) at different scan rates in the range of 5–100 mV s^{-1} and the plots between the anodic peak current and square root of scan rate on the SPGE (c) and poly(L-Met)/SPGE (d).

Fig. S5.

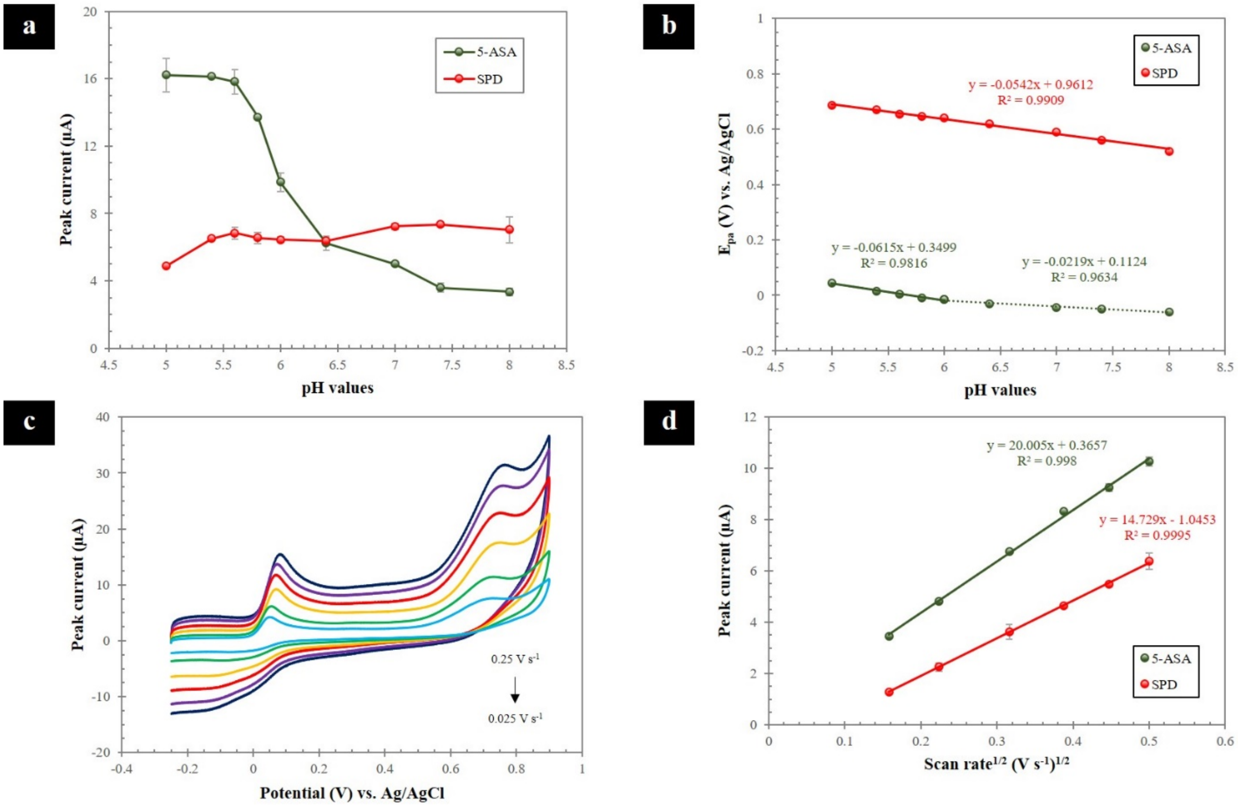

Fig. S5. The plots of I_{pa} vs. pH values (a) and E_{pa} vs. pH values (b) for 5-ASA and SPD. Cyclic voltammograms of the simultaneous detection of 100 μM 5-ASA and SPD in MeOH:PBS (20%:80% v/v) at various scan rates (c) and the plot of I_{pa} vs. the square root of the scan rate (d).

Fig. S6.

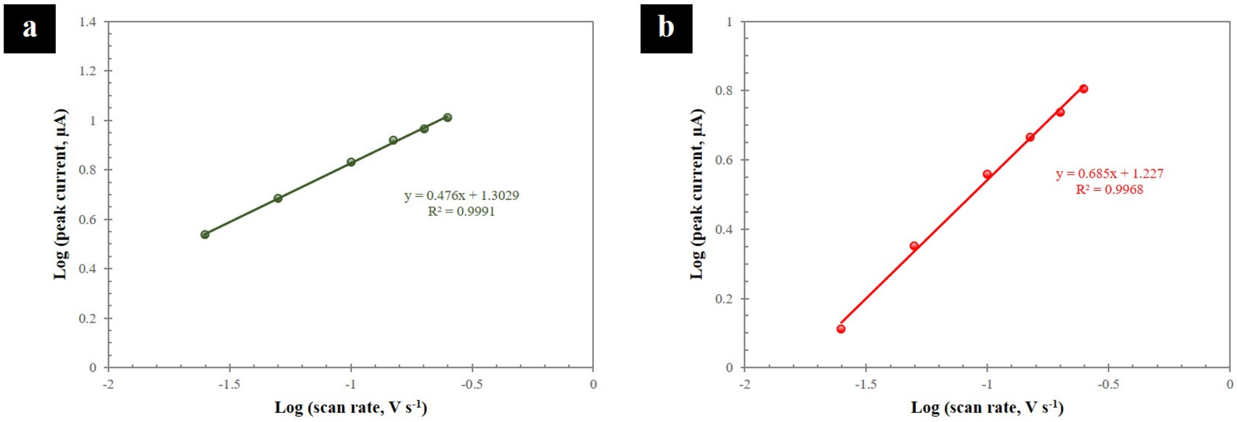

Fig. S6. The plots of log I_{pa} (current) vs. log v (scan rate) with the scan rate studied in the range of 25–250 mV s^{-1} for 5-ASA (a) and SPD (b).

Fig. S7.

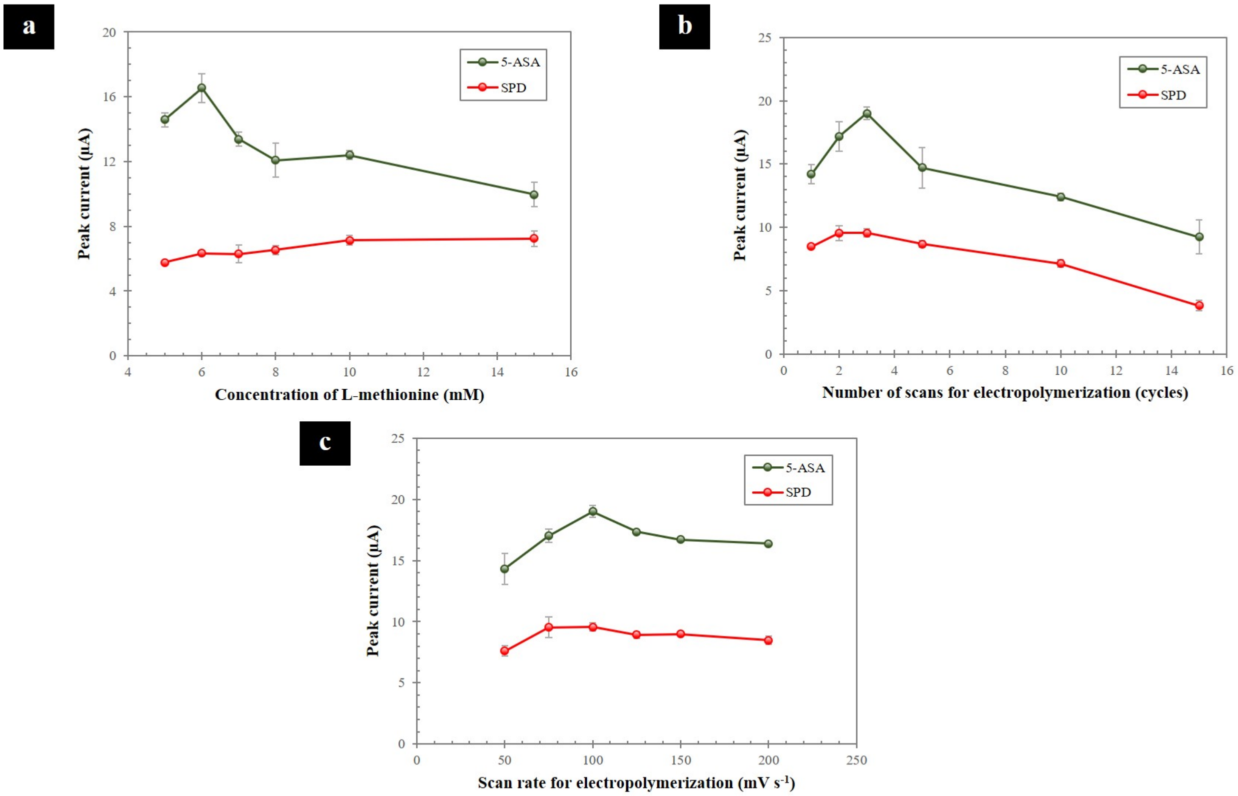

Fig. S7. The L-Met electropolymerization optimization studies consist of the concentration of L-methionine (a), number of scans (b), and scan rate for electropolymerization (c) on the SPGE.

Fig. S8.

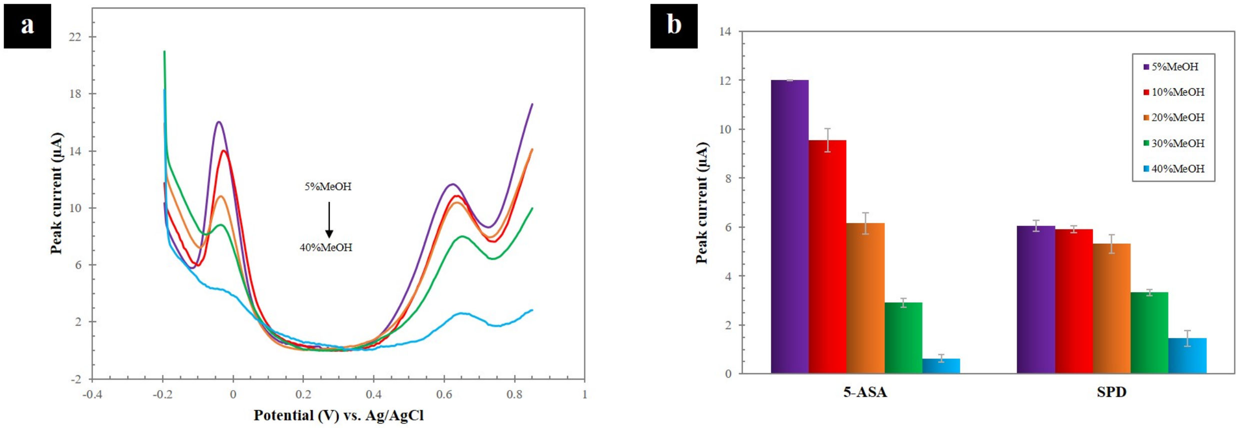

Fig. S8. Square wave voltammograms (a) and a chart of the peak current (b) of the simultaneous detection of 5-ASA and SPD in a mixture of MeOH and PBS at different proportions.

Fig. S9.

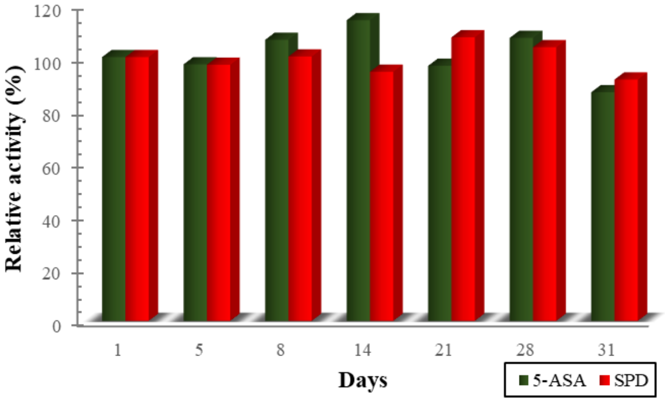

Fig. S9. A chart of the relative activity of 5-ASA and SPD for the stability study of poly(L-Met)/SPGE.

Table S1.

Recovery tests of the simultaneous determination of 30 µM 5-ASA and SPD in real human urine using different dilution factors of a mixture of MeOH and 0.1 M PBS (pH 5.6) (n = 3).

Dilution factor	**5-ASA**		**SPD**	
	Recovery (%)	**RSD (%)**	**Recovery (%)**	**RSD (%)**
100-fold	40.67	5.83	71.99	6.20
300-fold	69.73	21.41	108.84	13.56
500-fold	71.81	6.86	97.86	3.32
600-fold	80.63	12.52	102.72	13.08
800-fold	85.31	20.70	108.60	11.61
1000-fold	78.98	9.25	106.70	17.57

References

1. M. Punjiya, C. H. Moon, Z. Matharu, H. R. Nejad and S. Sonkusale, *Analyst*, 2018, **143**, 1059-1064.
2. P. A. Pushpanjali, J. G. Manjunatha and M. T. Srinivas, *FlatChem*, 2020, **24**, 100207.