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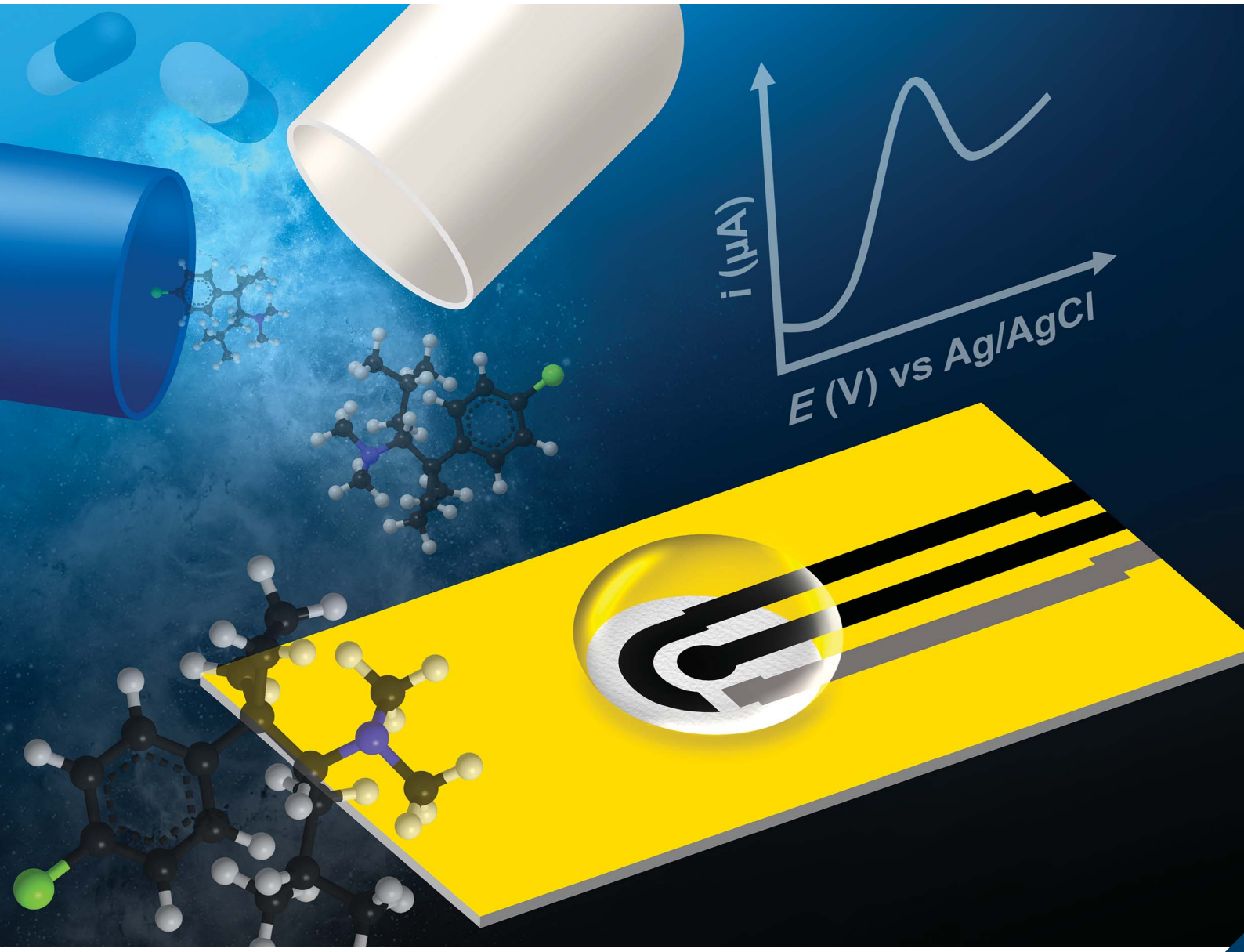

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PAPER
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A simple cost-effective paper-based electrochemical device for detection of adulterated sibutramine in slimming products†

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This work presents the first paper-based electrochemical device, or ePAD, for direct detection of adulterated sibutramine in slimming products. The ePAD was fabricated using a screen-printing technique for defining the hydrophilic area for sample loading and for the working, reference and counter electrodes. The ePAD gave reproducible responses comparable to both conventional rod electrodes and commercial screen-printed electrodes (SPEs). Use of paper to fabricate the ePAD device provides advantages over the conventional SPE platforms (*e.g.* glass, ceramics and polymers) in terms of biocompatibility, strong capillary action and environmental friendliness. To detect sibutramine, square wave voltammetry was employed after sample loading on the circular hydrophilic area. The linear range is 2.51 to 83.7 mg L^{-1} sibutramine, with a precision of 6 %RSD ($n = 3$) and an instrumental limit of detection (3SD of intercept/slope) of 2.46 mg L^{-1} sibutramine. Recovery of spiked samples ranged from 83 to 116%. The samples were capsules, slimming coffee powders and nutraceutical beverages. The samples were appropriately diluted to give concentrations within the linear calibration range. Filtration of undissolved solids found with the capsules and coffee powder samples was not required, demonstrating that the method is not susceptible to solid particles. The ePAD is cost-effective (<US$1 per device) and suitable for on-site analysis.

Introduction

Weight control is a desired goal that many people strive to achieve. Slimming products such as dietary herbal mixtures, coffee powder and beverages, are attractive to consumers because weight-loss drugs do not require medical prescription.[1] Such products are not only sold in pharmacies but also in retail stores and on internet sites. However, some slimming products are adulterated with illegal weight-loss substances to enhance their effectiveness, leading to high risks to consumers' health.

A common adulterant in slimming products is sibutramine, an inhibitor of reuptake of the neurotransmitters serotonin and norepinephrine. Sibutramine was originally developed in 1997 as an antidepressant and was approved as an anti-obesity drug by the United States Food and Drug Administration (FDA).[2] Even though sibutramine helps to lower appetite and stimulate metabolism in the human body, an overdose of sibutramine can cause side effects, such as headaches and an increased blood pressure and heart rate, leading to risk of heart attack and stroke.[3] In 2010, sibutramine was withdrawn from the European, American and Korean markets, based on data from the Sibutramine Cardiovascular Outcomes Trial (SCOUT).[3] In 2016, Thailand's Health Ministry withdrew sibutramine and classified the drug as a Category 1 narcotic,[4] due to reports of death of consumers taking sibutramine adulterated in slimming pills and food supplements.[5] Therefore, a simple and inexpensive analytical method is needed to provide a screening method for consumer protection.

There are several analytical methods for determination of sibutramine, including spectrometry,[6] infrared spectroscopy,[7,8] liquid chromatography–mass spectrometry (LC–MS/MS),[9] high performance liquid chromatography (HPLC),[10,11] capillary zone electrophoresis (CZE),[12,13] and thin layer chromatography (TLC).[14,15] Visual detection has been reported employing either a paper-based analytical device (PAD) measuring the reaction-band length[16] or smartphone-based colorimetric detection with aggregation of gold nanoparticles.[17] Electroanalytical methods have also been reported for sibutramine detection including potentiometry[18,19] and voltammetry.[20–24]

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With regard to the voltammetric technique, the traditional polarography, with the hanging mercury drop electrode (HDME) as a working electrode, was proposed for determination of sibutramine in beverages and pharmaceutical formulations.[20] Due to the toxicity of mercury, modified glassy carbon was reported as an alternative environmentally-friendly working electrode. Modification of the glassy carbon was carried out using either an electro-reduced graphene oxide (ERGO) film[21] or a porous graphene ink (PGr-ink)[24] to catalyze the electro-oxidation of sibutramine and enhance the sensitivity by increasing the surface area of electrodes, respectively. Another working electrode proposed for analysis of sibutramine was a boron-doped diamond electrode (BDDE).[22] Both modified glassy carbon and BDD electrodes employ liquid samples of 10–20 mL for each measurement, resulting in large sample consumption and waste disposal. To overcome these limitations, a commercial carbon graphite screen-printed electrode (SPE-Gr), using samples on the microliter scale (100 μL), has been employed.[23] Sibutramine is detected by adsorptive stripping voltammetry, using either differential pulse voltammetry (DPV) or square wave voltammetry (SWV).

In this work, a screen-printed electrode on filter paper (the ePAD) was employed coupled with square wave voltammetry for analysis of sibutramine in slimming products, including capsule formulations, slimming coffee powder and beverages. The ePAD is fabricated by a screen-printing technique for forming hydrophobic barriers and three electrode strips. A diluted sample is loaded onto the hydrophilic area (defined by the hydrophobic barrier), and the electrical current is monitored at the working electrode. The measured current is proportional to the concentration of sibutramine in the sample. Solid samples do not require filtration prior to application on the ePAD.

Experimental section

Chemicals and materials

All chemicals were of analytical grade, and solutions were prepared with deionized water (DI) (18.2 MΩ cm) from a Milli-Q® water purification system (Darmstadt, Germany). Sibutramine hydrochloride monohydrate (SHM) was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Potassium hexacyanoferrate ($K_3Fe(CN)_6$), citric acid ($C_6H_8O_7$) and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Sodium hydrogen phosphate (Na_2HPO_4) was purchased from Fluka (Steinheim, Germany). Filter paper was Whatman® Grade 4 (46 × 57 cm) (Buckinghamshire, UK). The screen-printing polymer (resin, hardener and yellow resin color) was purchased from Chaiyaboon Brothers Co., Ltd (Pathum Thani, Thailand). Carbon paste and silver/sliver chloride (Ag/AgCl) paste were purchased from Sun Chemical (Bath, UK).

A 1000 mg L^{-1} stock SHM solution, equivalent to 837 mg L^{-1} sibutramine, was prepared by dissolving 10.00 mg of SHM in deionized water and making up to the mark in a 10.00 mL volumetric flask. A set of working standard sibutramine solutions was obtained by appropriate dilution of the stock solution with McIlvaine buffer (pH 4.0). The McIlvaine buffer solutions (pH 2.2–8.0) were prepared by mixing various volumes of 0.1 M citric acid and 0.2 M sodium hydrogen phosphate[25] and adjusting the pH by addition of a few drops of 0.1 M sodium hydroxide when necessary.

Design and fabrication of the electrochemical paper-based analytical device (ePAD)

The pattern and dimensions of the ePAD were designed with the Adobe Illustrator CS5 software. The pattern of a single ePAD (21 mm × 40 mm) is shown in Fig. 1a. Each ePAD consists of two major areas: a circular hydrophilic area (10 mm diameter) for sample loading (the white area) and a hydrophobic area (the yellow area). The widths of the three electrode strips (RE, WE and CE) are 1.5 mm. The terminal of the working electrode, located in the hydrophilic zone, is circular with a diameter of 2.5 mm (see Fig. 1a). The terminal of the counter electrode, also in the hydrophilic zone, is an arc which is parallel to the circular working electrode.

Screen printing employed a polyester screen mesh (325 mesh) with the photolithographic pattern produced by a commercial firm.[26] As shown in Fig. 1b, there are three steps in the ePAD fabrication:[27] step 1: printing of the hydrophobic barrier onto the Whatman filter paper, except for the circular area of the sample loading zone; step 2: two strips of electrodes (WE and CE) are screen printed with carbon paste; step 3: the RE strip is then screen printed with Ag/AgCl paste. After steps 2 and 3, the filter paper is dried in an oven at 60 °C for 5 min. To

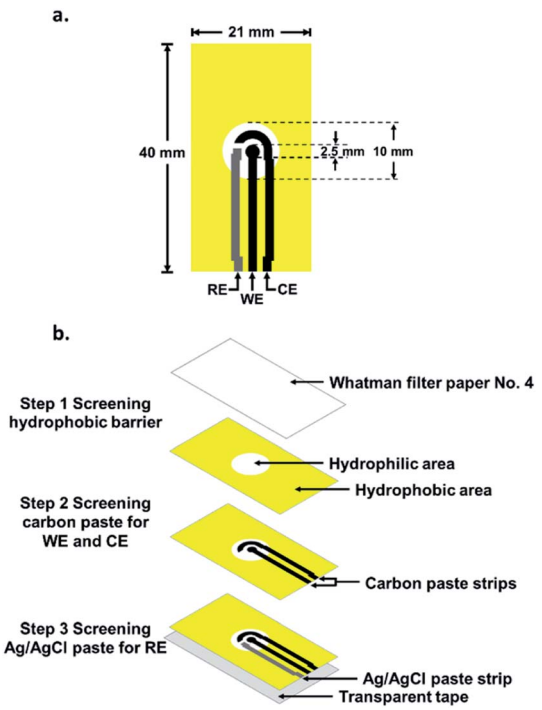

Fig. 1 (a) Design and dimensions of the ePAD for sibutramine determination, and (b) steps in fabrication of the ePAD device using the screen printing technique. Note: sixteen ePAD devices are screen printed per sheet. RE: reference electrode, WE: working electrode, and CE: counter electrode.

prevent leakage of the sample solution, the underside of the ePAD is sealed with a transparent adhesive sheet. The printed pattern gives 16 devices per sheet with single units of the ePAD cut out from the sheet.

Operating procedure

Voltammetric measurements are carried out using a portable PalmSens4 potentiostat, (PalmSens BV, Houten, the Netherlands). An 8-pin IC test clip (Electronic Source, Thailand) was modified for connecting the ePAD to the potentiostat. The potentiostat setting involves voltage scan from −0.5 to +1.2 V (*vs.* Ag/AgCl) at a frequency of 20 Hz and a 10 mV step interval. Measurements are carried out by dispensing 60 μL of sample solution onto the circular hydrophilic area (see Fig. 1a). This volume had been shown to be sufficient to cover all three electrode strips.

Preparation of the samples

Three types of slimming products, *i.e.* capsule formulation, coffee powder and beverages, were purchased from local convenience stores in Bangkok, Thailand.

For the analysis of capsule formulation and coffee powder, an accurate weight of *ca.* 0.5 g of sample was dispersed in 5.0 mL McIlvaine buffer (pH 4) and shaken to obtain a 10% w/v stock sample solution. All sample solutions were stored at 4 °C until analysis. The stocks of the capsule samples were diluted 200-fold in McIlvaine buffer (pH 4) prior to analysis. The coffee samples were directly analysed without further dilution. Beverage samples were diluted 20-fold in McIlvaine buffer (pH 4) prior to analysis.

Recovery study and method validation

Accuracy of the developed method was determined from the recovery of spiked sample solutions. This was carried out by adding 20.0 μL of 837 mg L^{-1} standard sibutramine into the diluted sample and making up to a final volume of 2.00 mL with McIlvaine buffer pH 4.0. Percent recovery was calculated using the following equation:

$$\%\ \text{Recovery} = \left(\frac{C_{\text{spiked sample}} - C_{\text{sample}}}{C_{\text{standard}}}\right) \times 100$$

where $C_{\text{spiked sample}}$ is the concentration of sibutramine found in the spiked samples, C_{sample} the concentration of sibutramine found in the samples and C_{standard} the concentration of the standard.

UV-Vis spectrophotometric absorbance measurements at 223 nm[6] of sample solutions were employed to validate the results of the ePAD method. The samples were filtered through a 0.2 μm cellulose acetate membrane before measurements.

Results and discussion

Reproducibility of fabrication and repeat use of the ePAD

Reproducibility of sensors is a major concern for any in-house fabrication process. Three screen printed sheets, with 16 ePAD devices per sheet, were tested for reproducibility of their performance by testing each ePAD by cyclic voltammetric measurement of 6.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl supporting electrolyte (see Fig. S1 in Appendix A in the ESI†). The redox reaction of $K_3Fe(CN)_6$ is a reversible reaction, and thus the peak current ratio between anodic (i_{pa}) and cathodic (i_{pc}) scans should be close to unity (see Table S1 in Appendix A in the ESI†). Reproducibility of the ePAD was tested for electrodes within a sheet and between sheets. Reproducibility was evaluated from the measured peak current which should be within the control range of mean ± 3SD for 48 ePAD devices (see Fig. S2 in Appendix B†). The results in Fig. S2† show that the in-house screen-printing method produces reproducible ePADs.

The repeat use of an ePAD was evaluated by carrying out repetitive CV scans of the test solution (6.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl supporting electrolyte) for 30 cycles. After each scan, the test solution was wiped off with tissue paper. A new aliquot of the test solution was then dropped on the ePAD device for the next CV scan. The voltammograms in Fig. S3 (Appendix A in the ESI†) clearly show that the ePAD sensor gave reproducible results for 30 scan-cycles. The inset of Fig. S3† shows comparable values of both the i_{pa} and i_{pc} of the 30 scans with %RSDs of 4.6 and 4.3 for i_{pa} and i_{pc}, respectively. Leakage of the testing solution onto the surface underneath the electrode strips was observed when the number of scans was more than 30, leading to a decrease of precision, *viz.* 5.5–6.0 %RSD after 35 cycles, 7.0–7.3 %RSD after 40 cycles: and 8.4–10.3 %RSD after 50 cycles.

Performance of the ePAD for $K_3Fe(CN)_6$ solution

The analytical performance of the proposed ePAD was investigated by comparing with a set of conventional rod electrodes immersed in a vessel containing the test solution and with two different brands of commercial plastic-based screen-printed electrodes (SPEs). Details of the electrochemical systems used in the comparison study are described in Table S2 (Appendix B in the ESI†).

First, a test solution of $K_3Fe(CN)_6$ was used. Fig. 2a–c show the cyclic voltammograms of $K_3Fe(CN)_6$ (solid lines) together with background signals (dotted lines), obtained from the commercial SPEs and the ePAD. The voltammograms obtained for the ePAD (Fig. 2c) are the same as those obtained from the conventional triple rod electrodes (Fig. 2a) and the commercial SPEs (Fig. 2b). The background responses (dotted lines) are similar for all electrochemical cells (Fig. 2a–c). The ratio between the oxidation peak current and reduction peak current (i_{pa}/i_{pc}) of the in-house ePAD was found to be 1.05–1.10. The ratio of i_{pa}/i_{pc} given by the conventional rod electrodes was also close to 1 (1.09–1.12) and comparable to the values from the SPEs (Dropsen™: 0.96–0.99 and Zensor™: 1.05–1.09). Fig. 2d displays the CVs of the ePAD for a series of $K_3Fe(CN)_6$ solutions (2–10 mmol L^{-1}). As shown in the inset of Fig. 2d, the calibration plots for the anodic and cathodic current are linear with a coefficient of determination (r^2) of 0.9999. The slopes of calibration from anodic and cathodic peak currents are equal, indicating that the ePAD sensor is suitable for quantitative analysis.

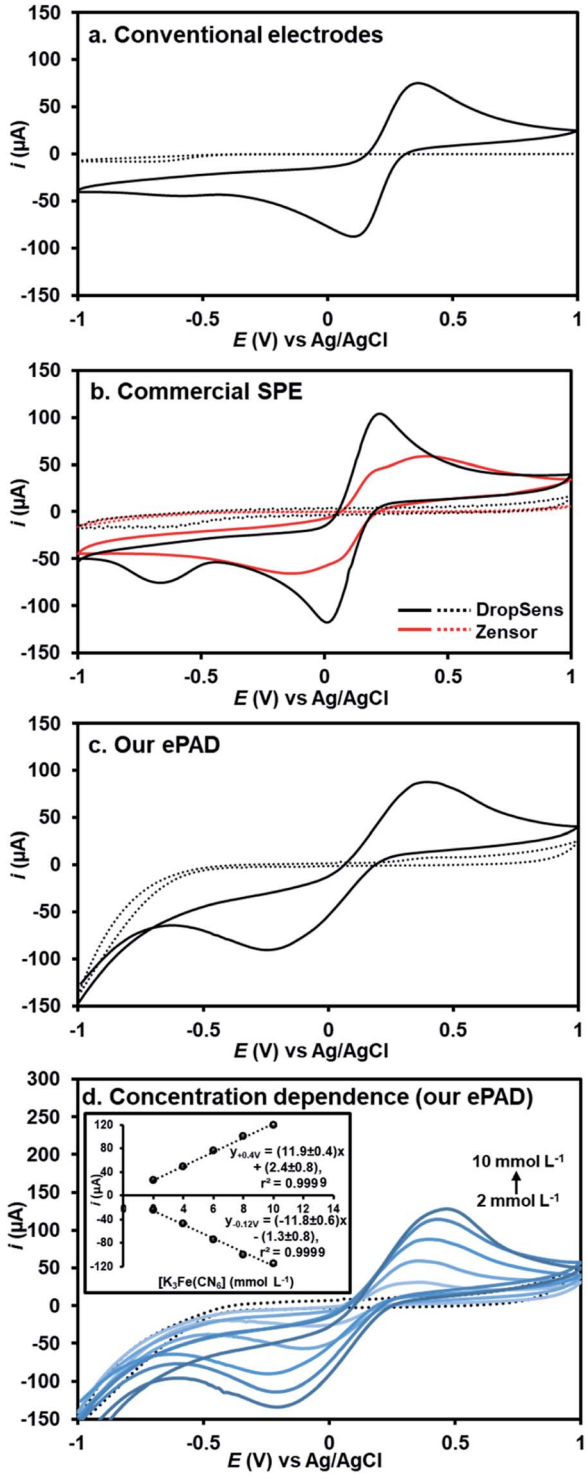

Fig. 2 Cyclic voltammograms (solid lines) of 6 mmol L^{-1} $K_3Fe(CN)_6$ in 0.1 mol L^{-1} KCl obtained from the (a) set of conventional electrodes consisting of a glassy carbon working electrode (diameter 3 mm), platinum rod counter electrode and silver–silver chloride reference electrode; (b) commercial screen-printed electrodes (SPEs) and (c) our ePAD. (d) Concentration dependence of voltammograms of the ePAD for 2–10 mmol L^{-1} $K^3Fe(CN)^6$. Insets are the linear calibration curves for the anodic and cathodic current. Dotted lines are the background signals for 0.1 mol L^{-1} KCl.

Performance of ePAD for sibutramine detection

A solution of 83.7 mg L^{-1} sibutramine in McIlvaine buffer pH 4.0 was used to compare the performance of the ePAD with the commercial electrodes. Fig. 3a–d show the cyclic

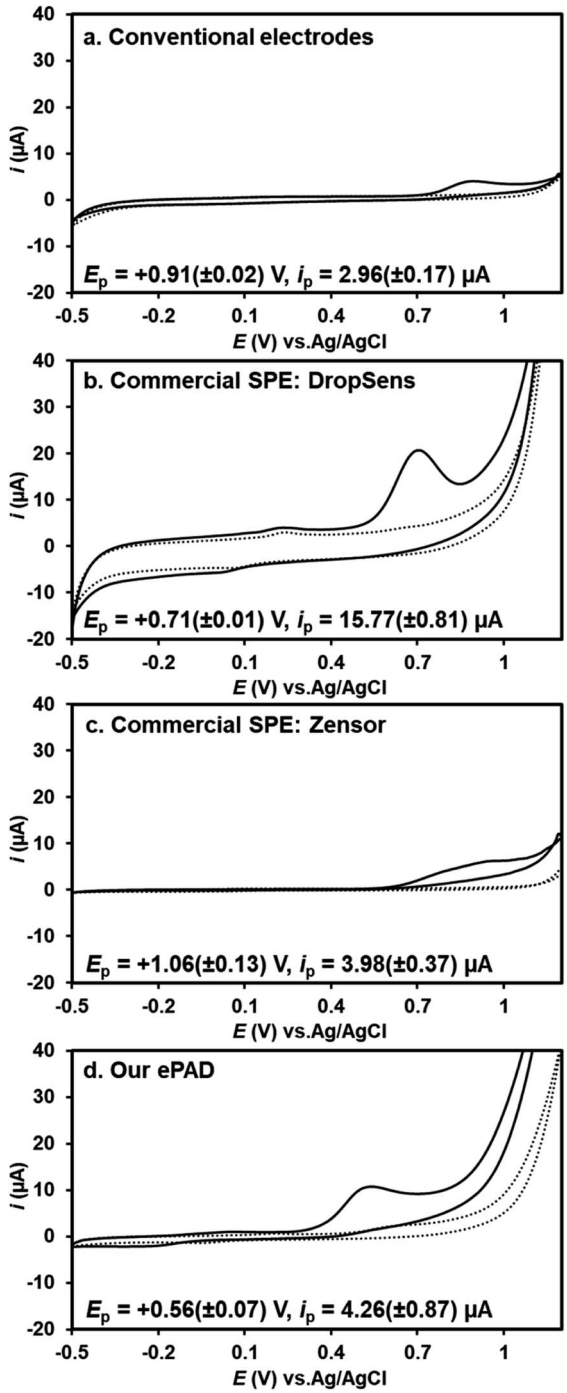

Fig. 3 Cyclic voltammogram (solid lines) of 83.7 mg L^{-1} sibutramine in McIlvaine buffer pH 4.0 obtained for a (a) set of conventional electrodes consisting of a glassy carbon working electrode (diameter 3 mm), platinum rod counter electrode and silver–silver chloride reference electrode; (b) commercial DropSens SPE, (c) commercial Zenzor SPE and (d) the ePAD. Dotted lines are the background signals for McIlvaine buffer pH 4.0.

Table 1 Voltammetric properties of sibutramine obtained from four types of electrochemical cells[a]

Cell (WE area, mm^2)	Voltammetric properties of 83.7 mg L^{-1} sibutramine ($n = 3$)		
	E_p (V) (±SD)	i_p (μA) (±SD)	Current density (μA mm^{-2}) (±SD)
Conventional electrode (4.91)	+0.91 (±0.02)	2.96 (±0.17)	0.60 (±0.03)
Commercial SPE, DropSens (12.6)	+0.71 (±0.01)	15.8 (±0.8)	1.25 (±0.06)
Commercial SPE, Zensor (7.07)	+1.06 (±0.13)	4.0 (±0.4)	0.57 (±0.06)
ePAD (4.91)	+0.56 (±0.07)	4.3 (±0.9)	0.88 (±0.18)

[a] WE: working electrode.

voltammograms of the test solution obtained from the conventional electrode rods, commercial SPEs and the ePAD. All cells exhibited only one oxidation peak and no peak in the reverse scan, showing that the redox reaction of sibutramine is an irreversible process. Data for oxidation peak potentials (E_p) and peak currents (i_p) are shown in Table 1. Different electrochemical cells gave different E_p and i_p values (see Table 1). The ePAD (Fig. 3d) gives an E_p of +0.56 V (*vs.* Ag/AgCl), while the other cells give positive shifts of the oxidation peaks (varying from +0.71 to +1.06 V *vs.* Ag/AgCl). This is due to the differences in the electron transfer capabilities depending on the working electrode[28] and reference electrode. The type of carbon ink used in the ePAD and SPEs (*i.e.* Zensor™ and DropSens™) and the porosity and diameter of the carbon working electrode of each SPE can affect the electron transfer process.[29,30] The experimental results confirm that the ePAD is capable of detecting sibutramine as shown by the number of oxidation peaks which are comparable to that of the conventional three electrodes and the two commercial plastic-based SPEs. The current density from the ePAD is also comparable to those of the conventional rods and SPEs (see Table 1). As compared to conventional SPE platforms (*e.g.* glass, ceramics and plastics), paper has been considered a versatile material for analytical platforms because of its biocompatibility and strong capillary action. The paper material is also abundant, cost-effective, disposable and environmentally friendly (*i.e.* by incineration).[31]

Study of electrochemical oxidation of sibutramine at the carbon working electrode of the ePAD

Assessment of the electrochemical response of sibutramine with the developed ePAD was first carried out using various supporting electrolytes. Two supporting electrolytes, *i.e.* Britton–Robinson buffer and McIlvaine buffer, were selected. As shown in Fig. 4a, both supporting electrolytes display only one well-defined oxidation peak. McIlvaine buffer (black line) provided larger oxidation current than the Britton–Robinson buffer. Therefore, McIlvaine buffer solution was chosen as the supporting electrolyte in further experiments.

The electrochemical behaviour of 83.7 mg L^{-1} sibutramine in McIlvaine buffer solution at different pH values was investigated and is shown in Fig. 4b. The oxidation potential shows a negative shift with increasing pH from 2.2 to 8.0, indicating that the oxidation reaction of sibutramine involves a proton

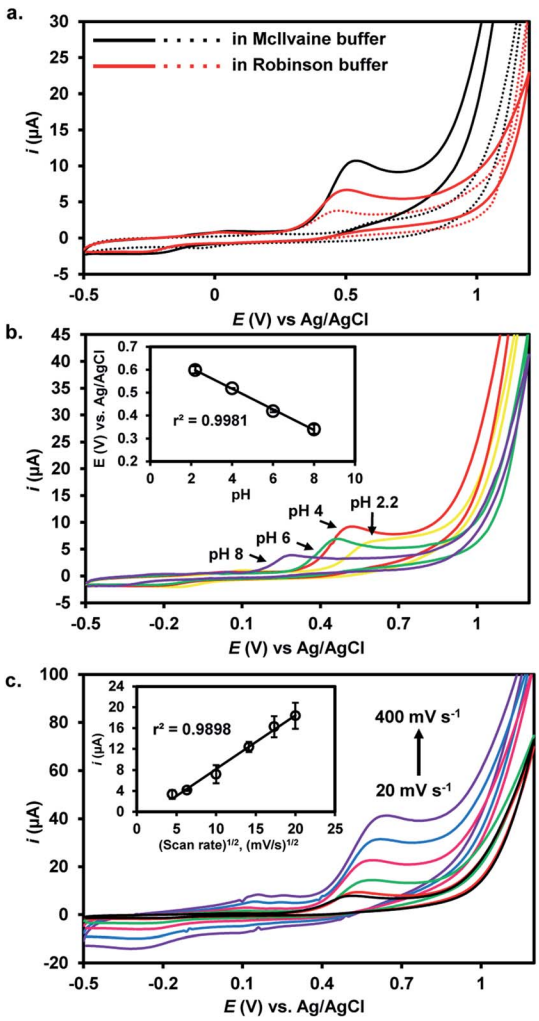

Fig. 4 Cyclic voltammograms of 83.7 mg L^{-1} sibutramine obtained from (a) two supporting electrolyte solutions (solid lines) and from the background electrolyte solutions (dashed lines); (b) for various pH of McIlvaine buffer solution (pH 2.2–8.0) and (c) with different scan rates (20–500 mV s^{-1}) in McIlvaine buffer pH 4.0. Voltammetric conditions are as given in Fig. 3. The inset of Fig. 4b is the linear plot of oxidation potential *E vs.* pH of electrolyte, and that of Fig. 4c is the linear plot of anodic current *i vs.* square root of the scan rate ν. The error bars in the insets correspond to the standard deviation from triplicate measurements.

transfer process. The peak potential is linear with pH, with the equation E_{pa} (V) = (−0.058 ± 0.002)·pH + (0.746 ± 0.012); r^2 = 0.9981 (see the inset of Fig. 4b). The observed slope of (−0.058 ± 0.002) V/pH is equal to the Nernstian slope (−0.0591 V/pH) for one electron redox reaction. Thus, one amine group of sibutramine is oxidized with loss of one electron and one proton, as previously reported for a hanging mercury drop working electrode,[20] electro-reduced graphene oxide film modified glassy carbon electrode,[21] BDDE,[22] carbon graphite screen-printed electrode[23] and porous graphene ink-modified electrode.[24] In Fig. 4b a well-defined peak with the maximum oxidation current is observed for McIlvaine buffer pH 4.0. Hence, McIlvaine buffer solution pH 4.0 was chosen in further studies.

Study of the anodic peak current (I_{pa}) with the scan rate (ν) was investigated for the kinetics of sibutramine oxidation.

The cyclic voltammograms of 83.7 mg L^{-1} sibutramine in McIlvaine buffer pH 4.0 show anodic peak currents increasing with the scan rate from 20 to 400 mV s^{-1} (Fig. 4c); the linearity of the plot between the peak current and square root of scan rate shows that the kinetics of the electrochemical process of sibutramine at the carbon working electrode is a diffusion-controlled process according to the Randles–Sevcik equation[32] (see the inset of Fig. 4c). The linear equation is I_{pa} (μA) = (1.02 ± 0.05)$\nu^{1/2}$ ((mV s^{-1})$^{1/2}$) − (2.1 ± 0.7), r^2 = 0.9898.

Optimization of SWV for sibutramine detection

The variables that affect the sensitivity of the measurement of sibutramine using the SWV mode are the potential amplitude, step potential and frequency. These were optimized for a standard solution of 83.7 mg L^{-1} sibutramine in McIlvaine buffer pH 4.0 as the supporting electrolyte. The target of the optimization procedure is to maximize the peak current. The results are shown in Fig. 5. The optimized conditions for SWV detection of sibutramine are 200 mV amplitude, 10 mV step potential and a frequency of 20 Hz.

Analytical performance of the ePAD for sibutramine detection

Using the optimized settings of SWV, Fig. 6 shows the square-wave voltammograms of sibutramine from 2.51 to 83.7 mg L^{-1} sibutramine in McIlvaine buffer pH 4.0. A linear relationship between the peak current and concentration of sibutramine was observed (inset of Fig. 6) with a corresponding linear regression equation, I_{pa} (μA) = (0.182 ± 0.003) × sibutramine (mg L^{-1}) + (0.90 ± 0.12), r^2 = 0.9991.

The instrumental limit of detection (LOD) of 2.46 mg sibutramine L^{-1} was calculated from the 3SD intercept/slope. Precision of the ePAD was 6.4% and 5.4 %RSD (n = 10) for 8.37 and 41.9 mg L^{-1} sibutramine, respectively. The ePAD method offers rapid analysis with a sample throughput of 240 samples h^{-1}.

Interference study

To evaluate the possible interferences to the developed ePAD sensor for detection of sibutramine, the measurement of 8.37 mg L^{-1} sibutramine was investigated under the optimal experimental conditions. The possible interfering substances

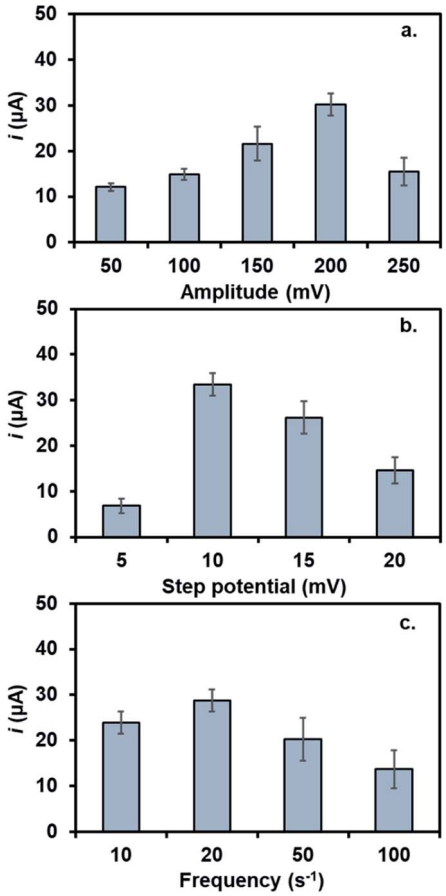

Fig. 5 Effect of square-wave voltammetric parameters on current (i) of 83.7 mg L^{-1} sibutramine using the ePAD. Error bars are from triplicate measurements.

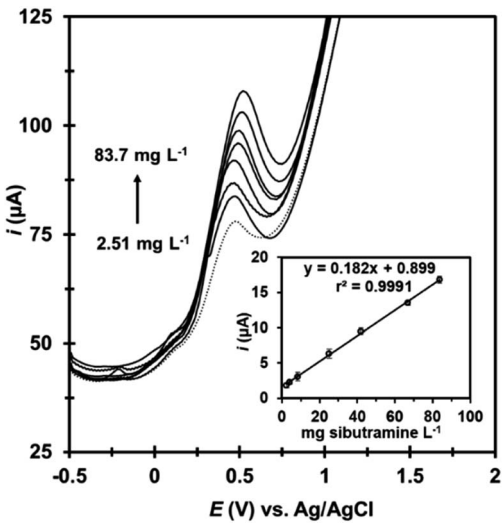

Fig. 6 Square-wave voltammogram of increasing concentrations of sibutramine from 2.51 to 83.7 mg L^{-1} in McIlvain buffer pH 4.0. The dotted line represents the background signal of McIlvain buffer pH 4.0. The inset is the corresponding linear regression between the peak current and sibutramine concentration. Voltammetry conditions: potential scan from −0.5 to 1.2 V; 200 mV amplitude; 10 mV step potential; 20 Hz frequency.

Table 2 Effect of added compounds on the peak current response of 8.37 mg L^{-1} sibutramine using the developed ePAD sensor

Compound	Tolerance limit[a] (mg L^{-1})	Tolerance level [compound]/[sibutramine[b]]	Change in peak response (%)
Ascorbic acid	300	50	−4.70
Glucose	500	50	−4.21
Glutathione	500	100	+4.55
K^+	750	75	−2.76
Na^+	500	50	−3.02
Mg^{2+}	750	75	−4.28
Zn^{2+}	500	75	−4.36
Cl^-	750	75	−2.76
CO_3^{2-}	250	50	−4.12
NO_3^-	500	50	−3.02
NO_2^-	2000	200	+4.91

[a] Maximum concentration for <5% absolute change in the sibutramine signal. [b] Sibutramine concentration at 8.37 mg L^{-1}.

were chosen from organic and inorganic compounds reported to be present in slimming products. The tolerance limit is defined as the maximum concentration of the added compound that resulted in a less than (<) ±5% variation of the peak current of the 8.37 mg L^{-1} sibutramine sample. The results in Table 2 show that organic compounds did not interfere when added at 50 to 100-fold concentration. These compounds include ascorbic acid, glucose and glutathione. For inorganic ions, *i.e.* K^+, Na^+, Mg^{2+}, Zn^{2+}, Cl^-, CO_3^{2-} and NO_3^-, interferences were observed but at concentrations of 50–75 fold. High tolerance was found for NO_2^- reaching a level of 200-fold. The results indicate that the presence of these substances did not interfere with the determination of sibutramine using the optimal conditions of the ePAD sensor.

Application to slimming products for analysis of sibutramine

The results of the analysis of the three types of slimming products using the ePAD are shown in Table 3. The results obtained from UV-vis spectrophotometry[6] are also given in Table 3. Sibutramine was found in the capsule formulations but not in slimming coffee powder or in the beverages. The sibutramine content in capsule 2 agreed with the amount stated on the label (15 mg per capsule). The data in the table also demonstrate that the ePAD requires no filtration of samples prior to analysis, which is required for UV-vis spectrophotometric measurement. The contents of sibutramine in the capsule formulation are not statistically different for the filtered sample and non-filtered sample (capsule 1: paired *t*-test, $t_{stat} = 3.22$, $t_{crit} = 12.7$, and $P = 0.05$; capsule 2: paired *t*-test, $t_{stat} = 2.50$, $t_{crit} = 12.7$, and $P = 0.05$). The percent recovery of standard sibutramine spiked in diluted samples at a concentration of 8.37 mg L^{-1} sibutramine was found to be 83–116% (see Table 3).

As shown in Fig. S4,† the square-wave voltammograms of the capsule formulations (Fig. S4a and b†) display well-defined oxidation peaks at −0.56 V (*vs.* Ag/AgCl) with significant difference of the peak current from the background signal. The same oxidation peaks were observed when standard sibutramine was added. The UV-vis spectra in Fig. S4i and j† show an increase in absorbance at 223 nm on spiking sample solutions with sibutramine. In contrast, coffee and beverage samples display insignificant changes in the oxidation peak current compared to background signals (Fig. S4c–h†). There is also no UV absorption in the samples (Fig. S4k–p†). The results in Fig. S4† show that the ePAD method provides results, on the addition of standard sibutramine, that are consistent with those obtained from UV-vis spectrophotometric measurement.

Comparison with other electroanalytical methods for determination of sibutramine

Our proposed ePAD sensor is the first paper-based platform for analysis of sibutramine, although there are other

Table 3 Amount and recovery of sibutramine found in slimming products using the ePAD and UV-vis spectrophotometry[a]

Slimming product	ePAD ($n = 3$)	Spectrophotometry[6] ($n = 3$)	Percent recovery ($n = 3$)[b]
Solid sample (mg g^{-1} sibutramine; mg sibutramine per capsule)			
Capsule 1	$79 \pm 4^{b,d}$; $19 \pm 1^{b,d}$ $80 \pm 3^{c,e}$; $20 \pm 4^{c,d}$	80 ± 2^{b}; $20 \pm 0.5^{b,d}$	106 ± 6
Capsule 2	$31 \pm 2^{b,d}$; $14 \pm 1^{b,d}$ $31 \pm 1^{c,e}$; $14 \pm 0.5^{c,d}$	31 ± 2^{b}; $14 \pm 1^{b,d}$	83 ± 5
Coffee 1	n.d.	n.d.	92 ± 4
Coffee 2	n.d.	n.d.	84 ± 8
Liquid sample (mg mL^{-1} sibutramine)			
Beverage 1	n.d.	n.d.	116 ± 6
Beverage 2	n.d.	n.d.	112 ± 9
Beverage 3	n.d.	n.d.	91 ± 7
Beverage 4	n.d.	n.d.	108 ± 6

[a] Note: capsule 1: no label for sibutramine content; weight of one capsule is 253 mg. Capsule 2: 15 mg per capsule on the label; weight of one capsule is 469 mg. LODs for the ePAD: 0.0246 mg sibutramine per g solid sample; 0.0491 mg sibutramine per mL liquid sample. LODs for spectrophotometry: 0.003 mg sibutramine per g solid sample; 0.006 mg sibutramine per mL liquid sample. The amount of sample used: 0.5 g solid sample dissolved in 5 mL McIlvaine buffer pH 4; 200-fold dilution for capsules; no dilution for coffee; 20-fold dilution for liquid samples. Recovery study based on sibutramine spiked at 8.37 mg L^{-1}. n.d.: not detected. [b] Filtration of samples with a 0.2 μm cellulose acetate membrane. [c] Direct analysis without filtration. [d] Paired *t*-test for capsule 1: $t_{stat} = 3.22$, $t_{crit} = 12.7$, and $P = 0.05$. [e] Paired *t*-test for capsule 2: $t_{stat} = 2.50$, $t_{crit} = 12.7$, and $P = 0.05$.

Table 4 Comparison of our ePAD with other electroanalytical methods for sibutramine determination[a]

Electrode	Technique	Electrode source	Linear range (mg L^{-1} sibutramine)	LOD (mg L^{-1} sibutramine)	Sample volume per analysis	Sample(s)	Sample pretreatment	Cost (USD)
Conventional rod/hanging drop electrode								
(A) [b]ISE[18,19]	(A1) [i]POT (batch)[18]	Lab-made	14–2798	n.a.	50 mL	Pharmaceutical formulations	Dissolution in HCl	n.a.
	(A2) [i]POT ([j]FIA)[19]	Lab-made	2.8–2798	2.2[o]	70 μL			n.a.
(B) [c]HDME[20]	[k]POL	Commercial	1.2–29.1	0.35[q]	750 μL	Pharmaceutical formulations and beverages	Dispersion in DI water (solid sample); not required (beverages)	~2000[s]
(C) [d]ERGO-GCE[21]	[l]AdSDPV	Commercial + modified	0.067–5.36	0.013[q]	n.a.	Fortified human urine and plasma	Precipitation of protein + centrifugation	~400[s]
(D) [e]BDDE[22]	[m]BIA-SWV	Commercial	4.2–41.8	0.067[o]	n.a.	Natural slimming formulations and a multivitamin supplement	Dispersion in DI water + standing to separate insoluble solids	~600[s]
(E) [f]PGr-ink/GCE[24]	[n]SWAdSV	Commercial + modified	0.013–8.37, 8.37–41.85	0.004[p]	n.a.	Illegal slimming capsule	Dispersion in DI + sonication + filtration	~400[s]
Screen-printed electrode								
(F) [g]Gr-SPE[23]	[l]AdSDPV	Commercial (ceramic-based SPE)	0.56–33.5	0.084[p]	100 μL	Slimming tea	Extraction with boiling water	3.57[t]
(G) [h]ePAD (this work)	[n]SWV	Lab-made (paper-based SPE)	2.51–83.7	2.46[r]	60 μL	Capsule formulations, slimming instant coffee and nutraceutical beverages	Dispersion in DI water (solid sample); not required (beverages)	0.08[t]

[a] n.a.: not available. [b] Sibutramine (SBT+) ion-selective electrode. [c] Hanging mercury drop working electrode. [d] Electroreduced graphene oxide modified glassy carbon electrode. [e] Boron-doped diamond electrode. [f] Porous graphene ink modified glassy carbon electrode. [g] Graphite screen-printed electrode. [h] Paper-based electrochemical analytical device. [i] Potentiometry. [j] Flow injection analysis. [k] Polarography. [l] Adsorptive stripping differential pulse voltammetry. [m] Batch injection analysis by square-wave voltammetry. [n] Square wave voltammetry. [o] The definition of LOD is not given. [p] 3SD of the blank/slope. [q] 3SD of the peak current/slope. [r] 3SD of the intercept/slope. [s] Cost of the working electrode rod. [t] Cost of SPEs consisting of working, reference and counter electrodes.

electroanalytical methods for the determination of sibutramine. Table 4 lists the features of these methods and also of our ePAD.

Conventional rod/hanging drop electrodes are commonly used for sibutramine detection. Method A is based on potentiometry employing an in-house ion-selective electrode (ISE) for batchwise and flow-based analyses. Method B employs polarography and the hanging drop mercury electrode (HDME). As for voltammetric measurements, Method C uses commercial glassy carbon electrodes (GCEs) modified with porous graphene ink (PGr-ink) for enhancing the sensitivity. A commercial boron-doped diamond electrode (BDDE) was also used as a working electrode for detection of sibutramine. The screen-printed electrode (SPE) was also proposed to minimize the size of electrochemical cells and consumption of the sample volume. A commercial ceramic-based graphite SPE (Method F) is listed with a sample volume of 100 μL.

Unlike the previous reports (Methods A to F), we developed an in-house paper-based SPE, the ePAD (Method G) to reduce costs of analysis. The ePAD is less expensive than those of previous reports and requires no modification of the working electrode surface to obtain the required voltammetric signal. Most of the samples are slimming products, including pharmaceutical capsules, beverages and tea. Solid samples do not require filtration prior to analysis.

Conclusions

This work presents a simple and inexpensive analytical method for detecting sibutramine in slimming products using a paper-based electrochemical analytical device or ePAD. To the best of our knowledge, this proposed ePAD method is the first employing an unmodified electrochemical sensor on a paper platform for detection of sibutramine. Square wave voltammetry is used for the detection. Although the instrumental detection limit of the ePAD is higher than those of other previous reports, the ePAD has the capability of measuring solutions of sibutramine containing solid particles without the need for sample filtration. The ePAD is cost-effective and suitable for on-site analysis.

Author contributions

Kitima Sirivibulkovit: methodology; investigation; formal analysis; validation; writing – original draft. Prapin Wilairat: supervision; writing – review and editing. Duangjai Nacapricha: conceptualization; supervision. Sineewanlaya Wichit: formal analysis. Phoonthawee Saetear: conceptualization; methodology; writing – original draft; writing – review and editing; supervision.

Conflicts of interest

There are no conflicts to declare.

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