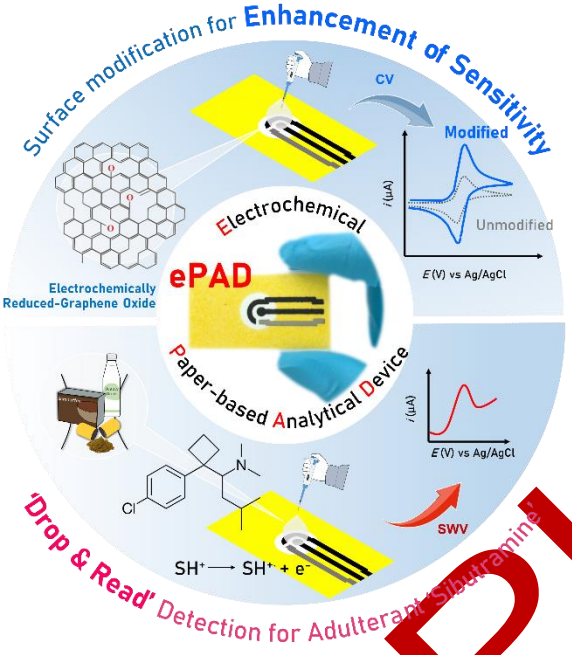
Development of electrochemical paper-based analytical device for sibutramine analysis in slimming product

Candidate:

Thesis advisor:

Duration:

Research output:



1. Rational of thesis research topic

This thesis work aimed to develop a home-made and portable sensing device using the concept of electrochemical paper-based analytical device or **'ePAD'**. The ePAD device are as in finger-held size as straightforward for on-site analysis. Application of the ePAD approached to detection of sibutramine, a banned substance illegally added to some slimming products for weight loss. Implementation of the ePAD as an alternative method for detection of sibutramine will have an impact in the set of regulations and law for the control of slimming products.

2. Novelties of thesis research work

We proposed our home-made ePAD device with the concept of electrochemical detection on paper substrate using a simple screen-printing method. Investigation of enhancement of sensitivity for analysis with surface modification on working electrode using reduced-graphene oxide was carried out. To the best of our knowledge, we proposed ePAD method is the first for employing unmodified electrochemical sensor on a paper platform for detection of sibutramine in slimming products. Our ePAD is easy-to-use and straightforward to develop for on-site analysis.

3. Research objectives

3.1. To propose the fabrication method for ePAD using the screen-printing technique

3.2. To investigate the signal enhancement using reduced-graphene oxide modified carbon working electrode on ePAD

3.3. To develop the ePAD for sibutramine analysis in slimming products

4. Research methodology

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

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

Screen printing employed polyester screen mesh (325 mesh size) with the photolithographic pattern produced by a commercial firm. As shown in Figure 1b, there are three steps in the ePAD fabrication: ***step 1:*** printing of the hydrophobic barrier onto the Whatman filter paper, except for the circular area for sample loading zone, ***step 2:*** two strips of electrodes (WE and CE) are screen printed with carbon paste, ***step3:*** the strip of the RE is then screen printed with Ag/AgCl paste. After step 2 and step 3, the filter paper is dried in an oven at 60 °C for 5 min. To prevent leakage of the sample solution, the underside of the ePAD is sealed with a transparent adhesive sheet. The pattern gives 16 devices per sheet and single units of the ePAD is cut from the sheet. The ePAD device can be fabricated with effective reproducibility.

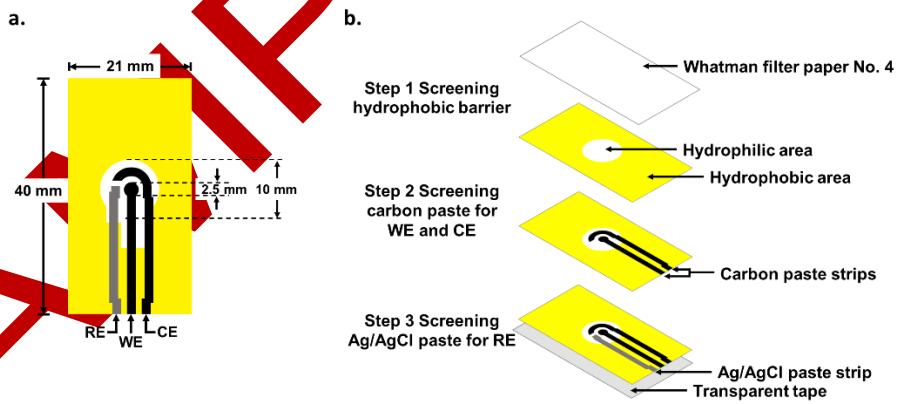


Figure 1. (a) Design and dimensions of the ePAD for sibutramine determination and (b) steps in fabrication of ePAD device using screen printing technique. Note: Sixteen ePAD devices are screen printed per sheet. All dimensions are in millimeter. RE: reference electrode, WE: working electrode, CE: counter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

The performance of ePAD was investigated by measuring cyclic voltammogram of 10 mM $K_3Fe(CN)_6$ in 0.1 M KCl as supporting electrolyte. The results in Figure 2 show both peak potentials and measured currents obtained from ePAD (red line) provided a great potential in measurement comparing to other kinds of electrochemical cell, including three conventional three electrode setup (solid black line) and commercial screen-printed electrodes (dash black line).

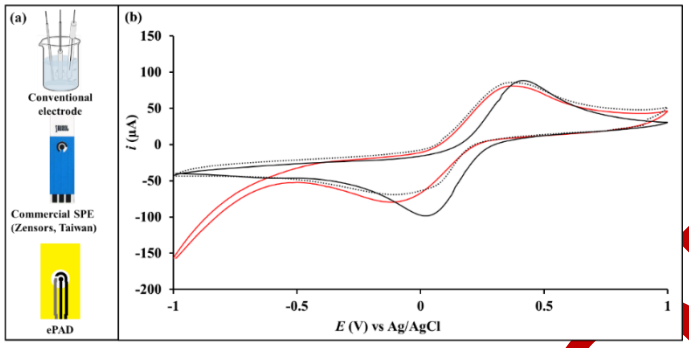


Figure 2. (a) Schematic representatives of three types of electrochemical cells: conventional electrode, commercial plastic-based SPE, and our ePAD. (b) Cyclic voltammogram of 10 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte solution obtained using conventional three-electrode setup (solid black line), commercial plastic-based SPE (dash black line), and ePAD (red line). Voltammetric conditions: scan range of -1.0 V to 1.0 V and scan rate of 100 mV s^{-1}.

4.2. Signal enhancement using an electrochemically reduced graphene oxide (ERGO) modified carbon electrode for electrochemical paper-based analytical device (ePAD)

The enhancement of voltammetric signal using an electrochemically reduced graphene oxide (ERGO) modified carbon electrode for ePAD was studied. The GO was synthesized from graphite powder via Hummers' method and modified onto the WE surface of ePAD. After that, the electrochemical reduction of GO was carried out by applying a constant potential at -1.2 V (vs. Ag/AgCl electrode strip) in 0.1 M KCl electrolyte solution for 800 s. The surface morphology of electrode was observed and characterized using the FE-SEM instruments as shown in Figures 3. This result demonstrated an excellent dispersion of graphene oxide attaching on the WE surface.

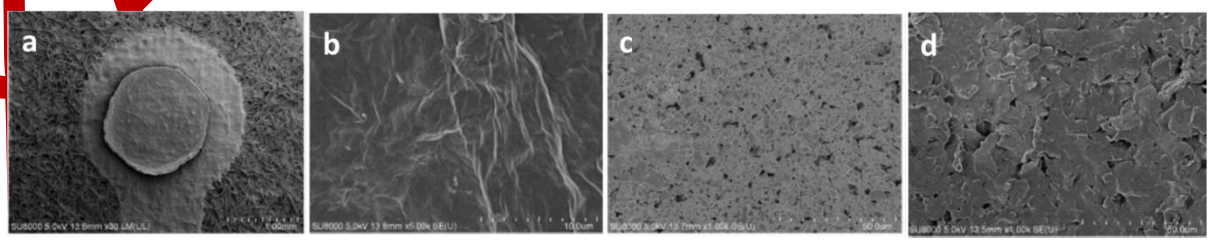


Figure 3. FE-SEM images of (a) GO coated on carbon working electrode, (b) GO-modified working electrode on ePAD, (c) Ag/AgCl reference electrode, and (d) carbon electrodes for working and counter electrodes.

The enhancement study of ERGO revealed that both anodic and cathodic peak current of 6 mM $K_3Fe(CN)_6$ in 0.1 M KCl increases approximately 2 folds. The increase in the peak currents is due to the existence of freely delocalized electrons in the ERGO film results in better conductivity and higher electron transfer rate. Based on these experimental results, the ERGO has potential to enhance the voltammetric signal in chemical analysis.

4.3. Development of ePAD for detection of sibutramine in slimming products

Sibutramine is a neurotransmitter (serotonin and norepinephrine) reuptake inhibitor. This compound is a banned substance illegally added to some slimming products for weight loss. According to the performance of ERGO-modified WE, we then applied for sibutramine detection. The result was found that using ERGO-modified WE was also enhanced the anodic peak current for sibutramine detection approximately 2 folds. However, we selected to use unmodified carbon WE on ePAD for sibutramine detection in order to reduce time-consumption in the step of electrochemical reduction for activation of GO.

Assessment of the electrochemical response of sibutramine with the developed ePAD was carried out, including (i) type of supporting electrolyte, (ii) pH of supporting electrolyte and (iii) scan rate. The analytical parameters for sibutramine detection with square-wave voltammetry (SWV) technique including (i) amplitude, (ii) potential step and (iii) frequency were then optimized.

Using the optimized parameters of SWV, Figure 4 shows the square-wave voltammograms of sibutramine from 2.51 to 83.7 mg sibutramine L^{-1} in Mcllvaine buffer pH 4.0. A linear relationship between peak current and concentration of sibutramine was observed (inset of Figure 4) with a corresponding linear regression equation I_{pa} (µA) = (0.182 ± 0.003) [sibutramine] (mg L^{-1}) + (0.899 ± 0.125), r^2 = 0.999. Instrument limit of detection (LOD) of 2.46 mg sibutramine L^{-1} was calculated from 3xSD intercept/slope. Precisions of the ePAD were 6.4%and 5.4% RSD for 8.37 and 41.9 mg sibutramine L^{-1}, respectively. The ePAD method offers rapid analysis with sample throughput of 240 samples h^{-1}.

The results of the analysis of the three types of slimming products using the ePAD are shown in Figure 5. The results obtained from UV-vis spectrophotometry are also shown in Table 1. Sibutramine was found in the capsule formulations, but not in slimming coffee powder or the beverages. The table also gives data that demonstrated that the ePAD requires no filtration of sample prior to analysis, which is required for UV-vis spectrophotometric measurement. The contents of sibutramine in the capsule formulation are not statistically different for filtered sample and non-filtered sample (capsule 1: paired *t*-test, t_{stat} = 1.42, t_{crit} = 12.7, P = 0.05; capsule 2: paired

t-test, t_{stat} = 5.00, t_{crit} = 12.7, P = 0.05). The percent recovery of standard sibutramine spiked in diluted samples to the concentration of 8.37 mg sibutramine L^{-1} was found to be 83-116 %.

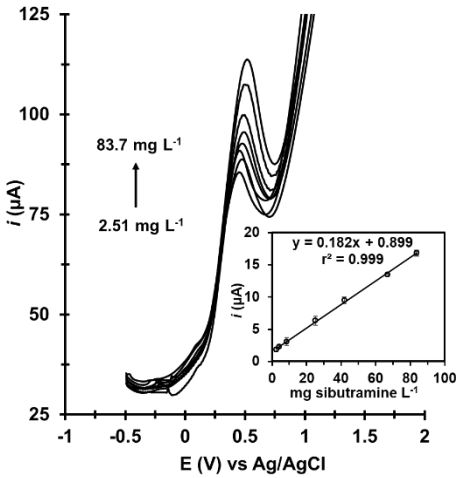


Figure 4. Square-wave voltammogram of increasing concentrations of sibutramine from 2.51 to 83.7 mg L^{-1} in Mcllvain buffer pH 4.0. Inset is a corresponding linear regression. Voltammetry conditions: potential scan from -0.5 to 1.2 V; 200 mV amplitude; 10 mV step potential; 20 s^{-1} frequency.

Table 1. Amount (mg sibutramine g^{-1}/mL^{-1} sample) and recovery of sibutramine found in slimming products using the ePAD and UV-vis spectrophotometry (n=3).

Slimming product	ePAD		Spectrophotometry	Percent recovery
	Filtration	No filtration		
Capsule 1	79.1 ± 3.6	80.0 ± 2.6	81.0 ± 1.7	106
Capsule 2	31.1 ± 2.2	30.7 ± 1.2	31.4 ± 1.9	83
Coffee #1-2	n.d.		n.d.	84 - 92
Beverage #1-4	n.d.		n.d.	91 -116

5. Research conclusions

5.1. The ePAD was successfully fabricated using the screen-printing technique and showed good electrochemical performance comparable to conventional setup and commercial plastic-based screen-printed electrodes.

5.2. ERGO modified WE on ePAD provided 2-fold enhancement of sensitivity in analysis.

5.3. A simple and inexpensive analytical method for detecting sibutramine in slimming products using the ePAD was carried out with non-modification of electrode surface. The ePAD has the capability of measuring solutions of sibutramine containing solid particles without the need for sample filtration. Our ePAD is cost-effective and suitable for on-site analysis.