

Chapter 21

Electrochemical Activities of C/GNP Electrode for Ultrasensitive Immunosensors



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Abstract Graphene is well known for its electroanalytical activity due to the presence of enormous π -electrons in the structure. For this reason, it is believed that the graphene used for modification of the conventional electrodes have a great potential to become a good electrode. The drop-casting method is the most widely used approach due to simple observation in dispersion composition of the substrate and it is capable to tailor the detection limit and reproducibility of the samples. There are two main factors to ensure a good drop-casting material; the solvent used to disperse and the temperature during drying the substrate. Water has been used as vector to carry graphene in this study. Drying in an oven at 100 °C displayed a good electroanalytical activity compared to unmodified screen-printed carbon electrode. The sample with 100% graphene nanoplatelet (GnP) has been selected as an excellent candidate for producing a good analytical study and excellent current densities.

Keywords Graphene nanoplatelets · Cyclic voltammetry · Electrochemistry · Screen-printed electrode

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21.1 Introduction

A Graphene sheet is defined as a single layer of graphite with high conductivity, rapid heterogenous electron transfer and intrinsic flexibility [1–4]. One of the most promising applications of graphene is in electrochemical sensing. Since every atom in a graphene sheet is a surface atom, the molecular interaction causes rapid electron transfer and eventually becomes highly sensitive to adsorb molecules [5, 6]. For this reason, it is believed that graphene used for modification of the conventional electrodes has equal great potential for screen-printed electrode in producing good electrodes for sensing purposes. Several methods of SPE modification have been done such as spin coating, aerosol, and drop-casting.

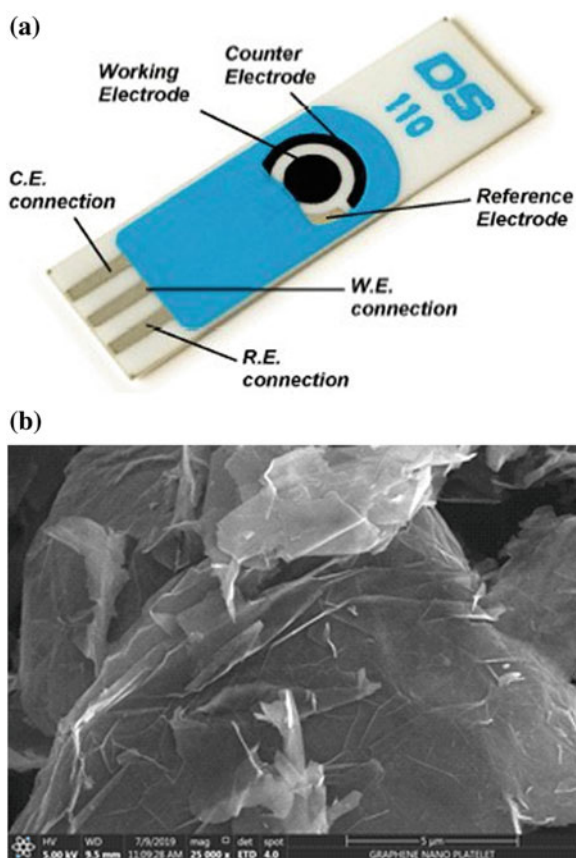
Drop-casting is the most widely used method in modification of SPE where graphene is the underlying substrate. Besides, this method enables to observe the effect of dispersion, composition, and reproducibility of the sample that tailors the limit of detection compared to others. Several organic solvents such as ethanol, acetone, hexane, and dimethylformide have been used to disperse graphene molecules [7, 8] whilst some researchers used di-water as medium to disperse graphene. Both ways work through wonders. However, once dried, the formation of a coffee ring is observed. This effect, which also known as coffee ring effect, occurs during the drying process, where drop edges became pinned to the substrate and capillary flow outward from the centre of the drop brought suspended chemical to the edge as evaporation occurs. Therefore, two main factors that may contributed to the coffee ring effect were substrate temperature and the solvent system [9]. Most of the studies described the coffee ring effect is due to organic solvents [4, 9, 10] and some because of water [9]. Temperature also played an important role in producing the effect of coffee ring where several studies have been conducted on drying properties of the coffee ring effect by inkjet printing [4, 9], but not much studies reported drying by oven.

Here in this study, di-water was used as medium to disperse graphene nanoplatelets (GNP). Two methods of drying were conducted using both oven and hot plate. Hence, two objectives were carried out in these studies; (1) to observe the morphology of SPE and GNP in water and (2) to determine the electrochemical activities via cyclic voltammetry (CV) on different drying methods.

21.2 Methodology

Three-electrode system screen-printed the carbon electrode (SPCE), which acts as medium for the electrochemical sensor (Metrohm DropSens). A carbon working electrode is surrounded by another carbon as counter electrode and silver/silver chloride as reference electrode (Fig. 1a). GNP was obtained from GO Advanced Sdn Bhd. The GNP produced is stable in aqueous solution and ready to be used as monolayer

Fig. 21.1 **a** Screen-printed carbon electrode before surface modification. *Reprinted from [11].* **b** Scanning electron microscopy image of GNP before drop-casting



or multilayer graphene sheets. Figure 1b shows the scanning electron microscope (SEM) image of GNP at 5 μm , respectively.

All chemicals were purchased from Sigma -Aldrich (Gillingham, UK) of analytical grade and were used as received without any further purification. All solutions were prepared with deionized water of resistivity not less than 18.2 $\Omega\text{ cm}$.

Voltammetric measurements were carried out using PC portable BiPotentiostat/Galvanostat (Spain) with a connector for screen-printed electrode purchased DropSens. Experiments were performed using 20 μL (amount after drop-casted) of GNP onto 1 mm diameter carbon working SPE (ref 110).

21.3 Results and Discussion

To understand the principal of this work, 1 mg/mL of GNP was dispersed in water and drop-casted onto SPCE. Later, drying took place by means of hot plate or oven at

Table 21.1 The parameter of C/GNP at different drying method and time

Temperature (°C)	Time	Drying method	Peak separation (mV)	Current density (mA/cm ²)
RT ^a	ON ^b	Hot plate	86 ± 0.00	0.012 ± 0.000
60	1 h	Hot plate	84 ± 0.00	0.006 ± 0.000
100	1 h	Oven	70 ± 0.00	0.005 ± 0.000
100	ON ^b	Oven	74 ± 0.02	0.010 ± 0.001
120	1 h	Hot plate	92 ± 0.03	0.006 ± 0.000

^aRT room temperature (27 °C)

^bON overnight (16–18 h)

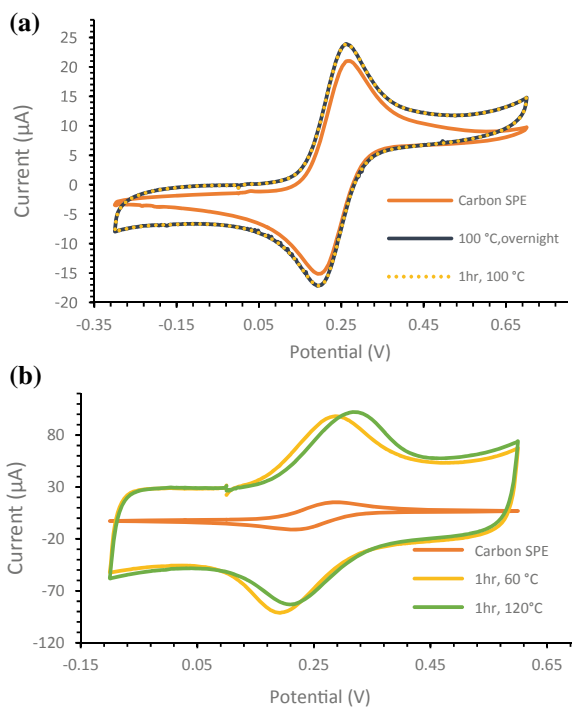
four different temperatures (Table 21.1). All electrochemical activities of GNP were conducted through CV using 1 mM Ferrocene carboxylic acid (FCA) in phosphate buffers solution (pH 7.4). FCA was used as redox solution due to excellent electrochemical properties and stability in both oxidation and reduction state [5, 9]. Raman was conducted for determination of different GNP percentage fingerprints.

The substrate temperature plays a role on the behaviour of the droplets which influenced the result of the end products. Table 21.1 shows the experimental conditions of GNP drop-cast. The temperature conditions reported are higher as compared to other studies using the inkjet printing method [9, 10]. Drying using hot plate showed a uniform dome shape after completion. These profiles are suggestive to be a coffee ring effect as reported by Deegan et al. [10]. He described that when higher temperature is employed, the evaporation rates over entire feature increased, and the amount of evaporation time has effects on the substrate [4]. Similar results were seen in Soltman and co-worker using inkjet printing [9]. In contrast, drying by oven which acts as contained environment did not show any coffee ring effect. This suggests that drying in oven method controls the heat as temperature rises, hence reduce the effect of coffee ring.

A rather popular tool to study electrochemical reaction is CV, qualitatively. The experiment was performed at the stationary phase by applying the voltage to the working electrode and scanned linearly within the unstirred solution. Diffusion happens by means of transporting the reactant to the working electrode surface. Hence, estimation of the reduction potentials, formation constants and number of electron transfer per reactant molecule could be made [12, 13]. Figure 21.2 depicts the CV signatures of unmodified SPCE (bare carbon) and GNP-modified SPCE of drying using hot plate and oven. Both displayed oxidation and reduction characteristics at a scan rate of 100 mV s⁻¹. All modified SPCE showed higher peak separation compared to unmodified SPCE in all drying methods. This suggested that graphene nanomaterials provided excellent electrical conductivity and large surface area which facilitated electron transfer activity [8].

The integration area under the anodic and cathodic peaks indicated the optimum electrochemical activity and peak separation was calculated based on the potential differences between cathodic and anodic peaks. The ideal peak separation was selected based on the lowest value of peak separation. Hence, in this study, drying

Fig. 21.2 **a** CV peaks obtained from drying using oven (potential range -0.35 to 0.7 V). **b** CV peaks obtained from drying using hot plate (potential range -0.15 to 0.7 V). Scan rate: 100 mV s^{-1}



by oven at $100 \text{ }^\circ\text{C}$ for 1 h (Table 21.1) with peak separation of $70 \pm 0.00 \text{ mV}$ were ideal drying condition for GNP. A huge peak separation was observed in drying by hot plate compared to oven. This may be due to unstable condition such as moisture from the environment which fluctuated the temperature during incubation time [10].

As described earlier, 1 mg/mL of GNP displayed an excellent electrochemical activity in all drying methods, thus showing a good electroanalytical activity. However, not many studies on lower concentration of GNP were conducted for evaluating electrochemical and electroanalytical activities. CV was conducted on a series of GNP percentages (4, 8, 12, 16, 20, and 100%). Figure 3a exhibits the CV of different GNP percentages drop-casted on single printed working carbon electrode in a potential range between -0.20 and $+0.70 \text{ V}$ at a scan rate of 100 mV s^{-1} . Each percentage displayed excellent electrochemical and electroanalytical activities with typical Nernstian electrochemical reaction. Figure 3b shows the peak separation values between anodic and cathodic peaks based on CV from Fig. 3a. The values were between range of $65\text{--}77 \text{ mV}$. The range of peak separation was significantly higher than 59 mV (theoretical value) and the separation of these two signals increased with faster scan rates. Hence, this reaction is quasi-reversible.

Current densities and diffusion rate occurred during the whole CV process was calculated using the Randles Sevcik equation. In this equation, current peak (I_p) is predicted to be proportional to the square root of the scan rate (Eq. (21.1)).

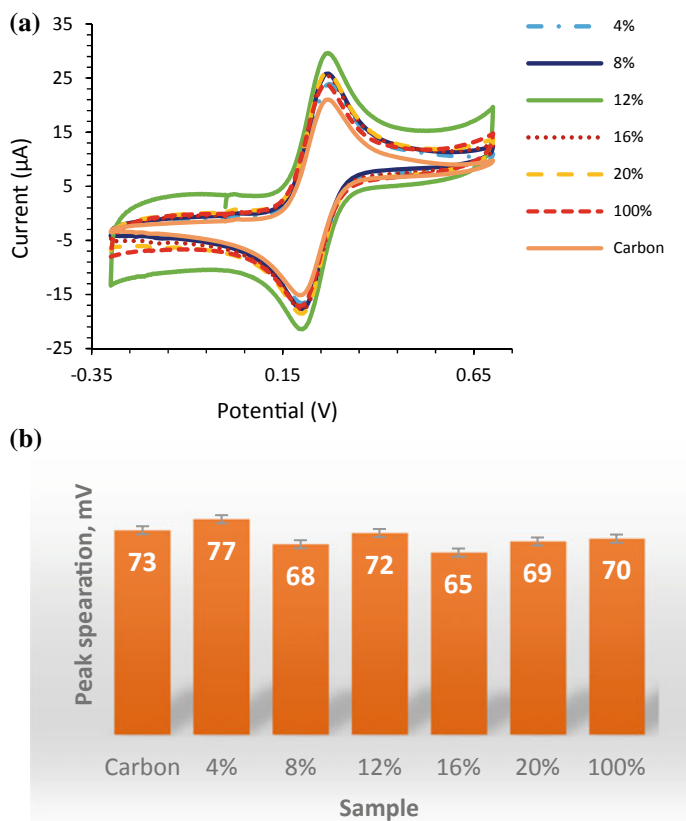


Fig. 21.3 **a** CV peaks presented as voltammograms. **b** Peak separation of GNP-modified SPCE at various percentage and unmodified (carbon) in 1 mM FCA phosphate buffer (pH 7.4) at scan rate: 100 mV s^{-1} presented as peak separation

$$I_p = 2.69 \times 10^5 \times n^{3/2} \times ACD^{1/2}v^{1/2} \quad (21.1)$$

where n is the number of electrons involved in the reaction, A is the electrode surface area (cm^2), C , the bulk concentration (mol cm^{-3}), D , the diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$) and v , the sweep rate (V s^{-1}). Taking Eq. (21.1) is in the resemblance with $y = mx + c$ linear graph equation, the m value or the slope replaced the I_p to produce Eq. (21.2) as follows.

$$\text{Slope} = 2.69 \times 10^5 \times n^{3/2} \times ACD^{1/2} \quad (21.2)$$

For this experiment, the number of electrons involved in this reaction, n , was 1, the electrode area of carbon working SPCE, A , was 0.04 cm^2 with the concentrations of $10^{-6} \text{ mol ferrocyanide}$. The slope and current were given in Table 21.2 for each

Table 21.2 The slope, diffusion coefficient and current densities of unmodified and modified GNP

Sample	Slope	Diffusion coefficient (cm ² s ⁻¹), D	Current densities (mA cm ⁻²)
Unmodified (carbon)	$Y = 67.886x - 2.5876$	1.126×10^{-10}	0.33 ± 0.064
<i>Modified GNP</i>			
4%	$Y = 75.701x - 3.3445$	1.28×10^{-10}	0.33 ± 0.002
8%	$Y = 87.756x - 5.2654$	1.26×10^{-10}	0.53 ± 0.002
12%	$Y = 84.982x - 4.9892$	1.20×10^{-10}	0.54 ± 0.02
16%	$Y = 80.616x - 4.8453$	2.92×10^{-9}	0.54 ± 0.02
20%	$Y = 78.516x - 3.4782$	1.17×10^{-10}	0.55 ± 0.02
100% (1 mg/ml)	$Y = 758.526x - 3.478$	2.52×10^{-9}	0.55 ± 0.00

percentage at 100 mV s⁻¹ scan rates. After the substitution of the parameter and unit, the diffusion coefficient, D values for each percentage were as displayed in Table 21.2. Table 21.2 indicated the slope, diffusion coefficient and current densities of unmodified and modified SPCE at different GNP percentages. Every percentage of GNP displayed higher current densities than unmodified SPCE, suggesting an increase in electroanalytic activity of GNP on carbon working electrode surface. Diffusion coefficient was the highest in 16% GNP compared to others, followed by 100% GNP. In contrast, 100% GNP (1 mg/mL) displayed the highest current densities which proposed that high concentration provided high current densities. Raman spectroscopy in combination with electrochemistry was used in the following analysis to better understand the characteristic of graphene when drop-casted onto SPCE.

21.4 Conclusion

In summary, we have demonstrated proof-of-concept that the method of drying GNP heavily influenced the electrochemical and electroanalytical activities of modified SPCE. Drying in oven at 100 °C for an hour was selected based on better and low peak separation. The highest electrochemical and electroanalytical activities with highest current densities were observed using 1 mg/mL (100%) GNP concentration, thus, reflecting the graphene characteristic of having high electroconductivity further studies on functionalization are yet to be established to produce a sensitive electrochemical biosensor.

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