CHAPTER 3

Results and Discussion

3.1 Electrochemical properties of the SPCEs modified with GPO and GP

Modification of electrodes by GP possessing large specific surface area could result in a better electrochemical reactivity of the modified electrodes. GP is a promising candidate to improve the electrochemical properties of electrodes. GP has been shown to be a component of good sensors, achieving sensitivity at low concentration for the detection of biochemicals [8]. Figure 3.1 shows the CV (a) and DPV (b) curves of the GPO- and GP-modified SPCEs in comparison with those of the treated-SPCE. The experiment was performed in 10 mM $K_3Fe(CN)_6$. For the CV curves, the typical redox reaction occurred at the electrode surface indicating the presence of anodic and cathodic peaks due to the oxidation and reduction process. It is noted that GP modification showed a higher current value for redox reaction than those of the GPO-modified and those of the treated-SPCEs, respectively. Therefore, GP was employed to increase sensitivity of the electrochemical detection of the SPCE.

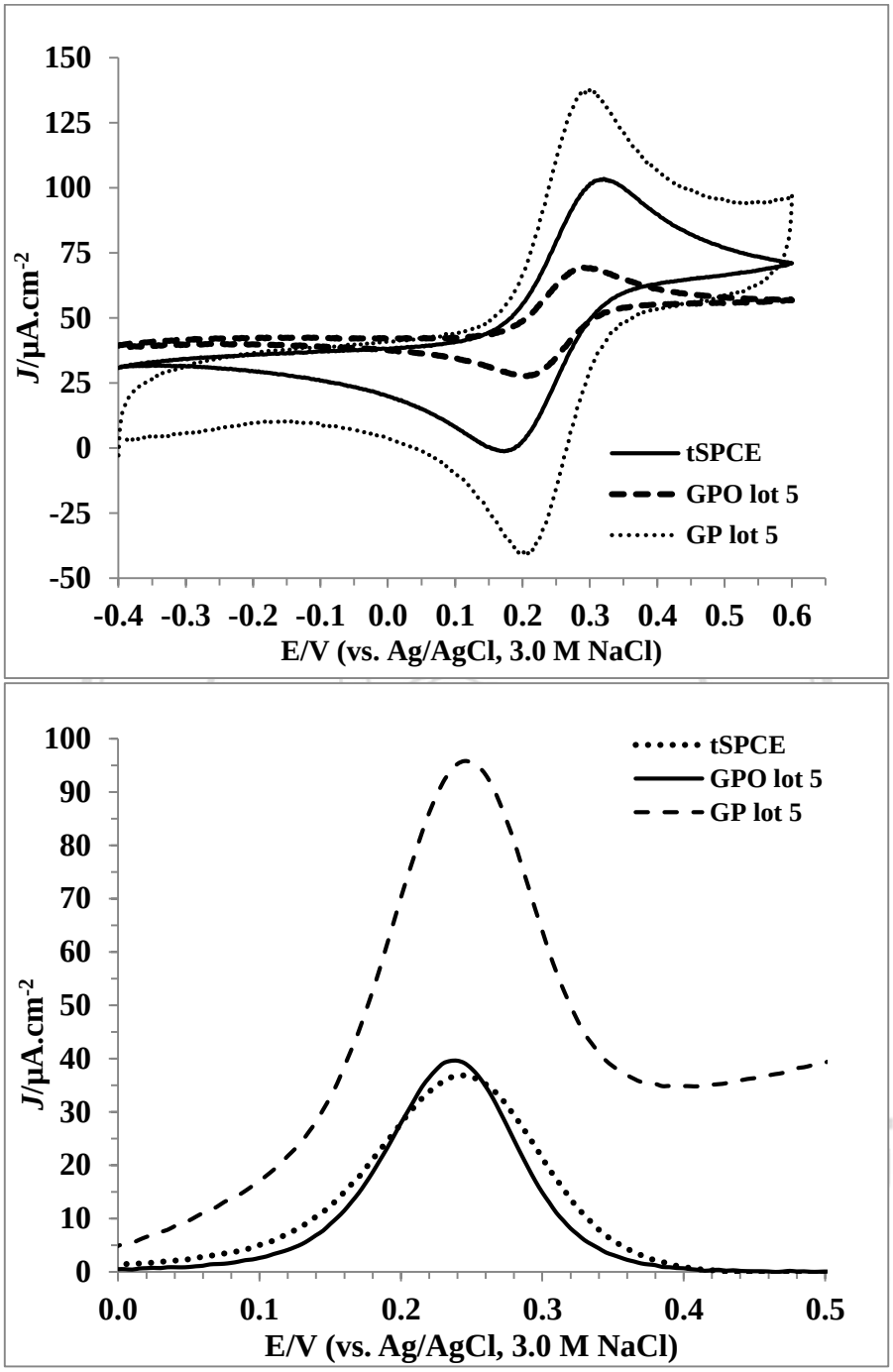


Figure 3.1 (a) CVs and (b) DPVs of bare SPCE, GPO- and GP-modified SPCEs in 10 mM $K_3Fe(CN)_6$. CV condition: scan rate = 50 mVs^{-1}, scan potential range = -0.4-0.6 V. DPV condition: pulse amplitude = 30 mV, pulse width = 30 ms, scan range = -0.4-0.6 V.

The effect of the GP loading on the electrochemical sensitivity of the GP-modified SPCEs was investigated by CVs using the commonly used standard redox probe, 10 mM $K_3Fe(CN)_6$. As shown in Figure 3.2, the first anodic peak current is increased rapidly upon increasing the concentration of GP from 0 to 10 µg, which GP significantly improves the electrical conductivities of GP-modified SPCEs. However, the electrochemical response tends to be decreased at higher GP contents due to a hindered mass transfer process or the agglomeration of GP. Therefore, modification of SPCE with 10 µg GP was chosen for next studies.

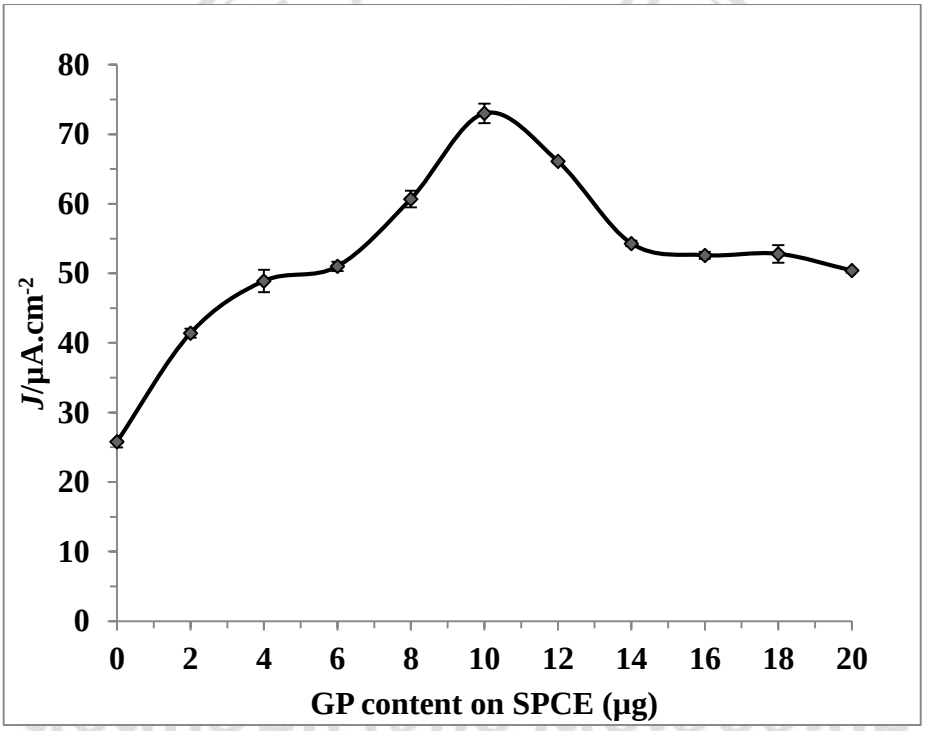


Figure 3.2 anodic current responses of GP-modified SPCEs at different concentrations of GP in 5 mM $K_3Fe(CN)_6$ containing 1 M KNO_3

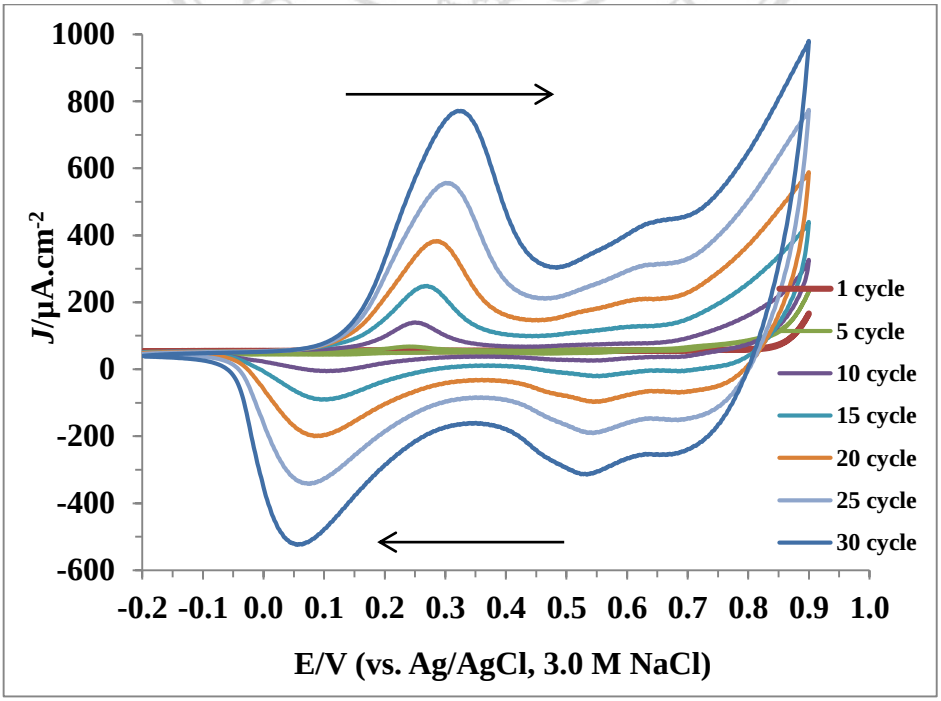
3.2 Electropolymerisation, optimisation and characterisation of MIPANI-GP-modified SPCEs

Molecular imprinting is a powerful technique introducing recognition properties into synthetic polymers using the appropriate templates. The molecular imprinted polymers, denoted as MIPs, possess high selectivity towards the template molecules and can thus be applied as recognition elements able to recognise and rebind the target molecule

specifically. MIPs are generally synthesised and deposited directly on to the transducer surface in a single step. The great advantage of this method is that the thickness of the polymers can be controlled by varying the polymerisation condition. Moreover, a uniform coating can be deposited on complex geometric substrates.

3.2.1 Electropolymerisation of MIPANI film on GP-modified SPCEs

Electropolymerisation of the ANI monomer was demonstrated with the deposition of PANI onto the surface of the GP-modified SPCEs in acidic solution. Figure 3.3 shows the CVs taken during the electropolymerisation of 0.2 M ANI onto the surface area. The formation and growth of the polymer film developed can be easily seen in the Figures 3.3a and 3.3b, corresponding to non-MIPANI and MIPANI, respectively. The peaks according to the oxidation and reduction of the PANI film increased in intensity with increasing number of polymerisation cycle. During the electropolymerisation process, DA template molecules diffused together with ANI towards the surface of the GP-modified SPCEs and were trapped in the polymer metrix in which the DA was embedded inside PANI as a result of this molecule's ability to interact with the ANI units (Figure 3.4).



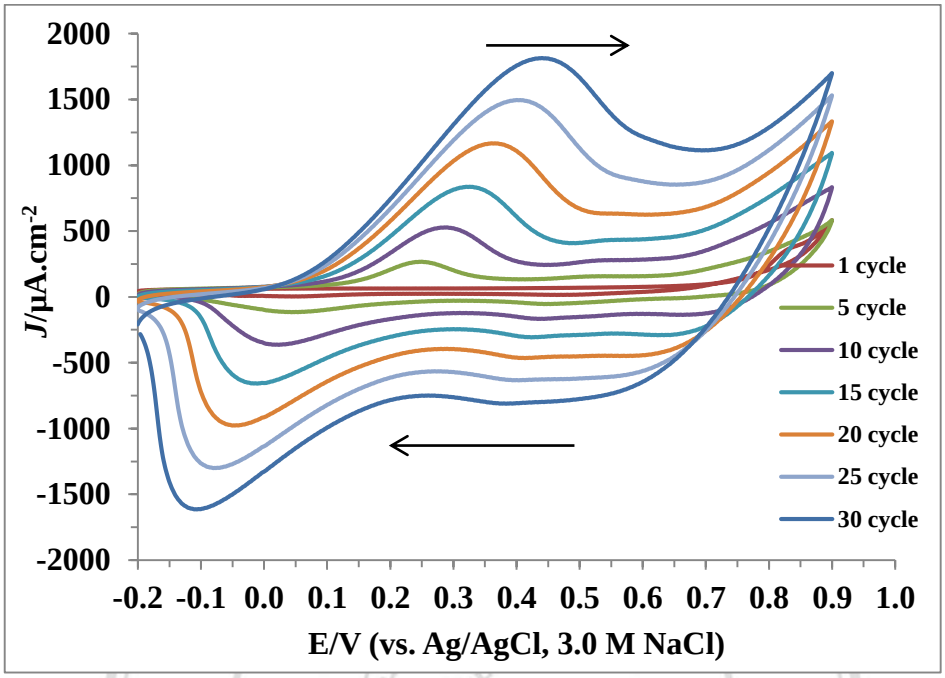


Figure 3.3 Electropolymerisation of ANI (a) without and (b) with the presence of DA on the GP-modified SPCE at a scan rate of 100 $mV \cdot s^{-1}$ and 30 cycles in 1.0 M HCl solution.

From a theoretical stand point, intermolecular hydrogen bond formations between the polymer chian and the template are possible as follwing: (1) between the hydrogen atom in the N-H group of the ANI unit and the nitrogen atom in the N-H group of the DA molecule, and (2) the hydrogen in the hydroxyl group of the DA molecule and the nitrogen atom of the N-H group of ANI units. A schematic representation of the imprinting and removal of DA from DA imprinted PANI modified GP electrode is shown in Figure 3.4. In this study, the DA template was removed by over oxidation with CV in the potential range of -0.9 to +0.9 V in PBS (pH 7.4). Non-MIPANI-GP-modified SPCEs were also prepared under the similar condition without template for a comparison with the properties of the MIPANI-GP-modified SPCEs.

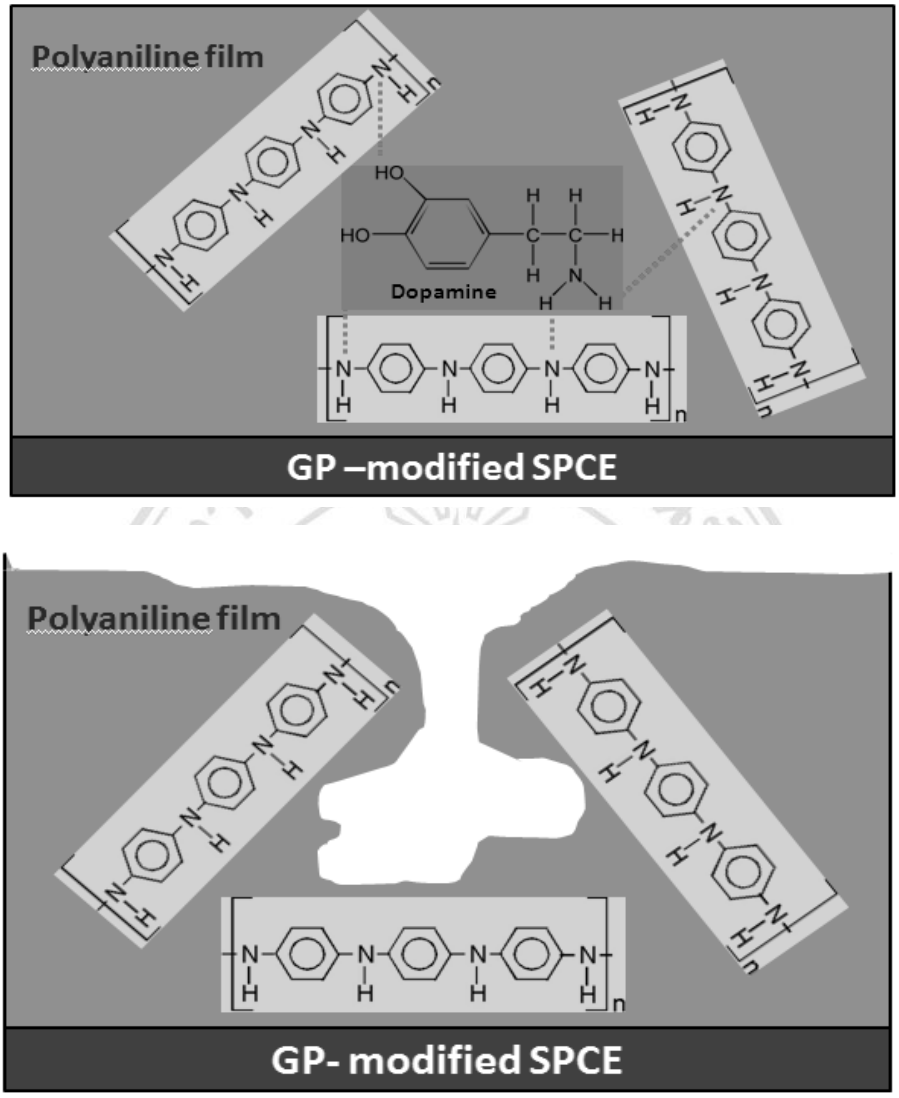
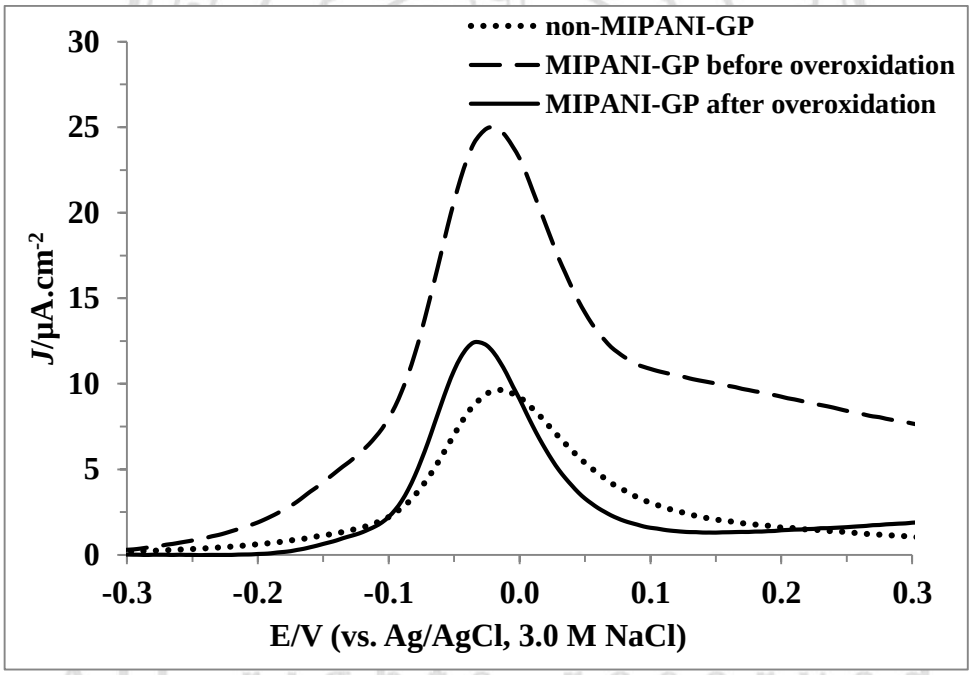


Figure 3.4 Schematic representation of (a) imprinting and (b) removal of DA from the molecularly imprinted PANI-GP-modified SPCE.

The DPV results in Figure 3.5a show the current responses of the DA-MIPANI-GP-modified SPCE before and after overoxidation, and nonMIPANI-GP-modified SPCE, respectively, in 0.1 M PBS (pH 7.4). The DPV of the MIPANI-GP-modified SPCE with presence of DA in the polymer film showed the highest response resulted from the oxidations of DA and PANI. After the DA template was removed by overoxidation of the composite film in 0.1 M PBS (pH 7.4) using CV, the DPV response in PBS solution was decreased due to the lack of oxidation of DA in the composite film, remaining the response of the polymer as same as that of nonMIPANI-

GP-modified SPCE. This indicates that MIPANI film was successfully prepared on the surface of the GP-modified electrode. The DPV peak of the nonMIPANI electrode is located at higher potential of -0.02 V, whilst those of DA-MIPANI-GP-modified SPCE before and after overoxidation are observed at the potentials of -0.04 and -0.025 V, respectively. Oxidation of DA at MIPANI-GP-modified SPCEs in PBS solution was also investigated with the DPV technique. Figure 3.5b shows the DPV of the MIPANI electrode after overoxidation in the PBS with and without the presence of 0.10 mM DA. It was found that the DPV current of MIPANI electrode was increased with the presence of 0.10 mM DA, suggesting the existence of the oxidation process of rebinding DA.



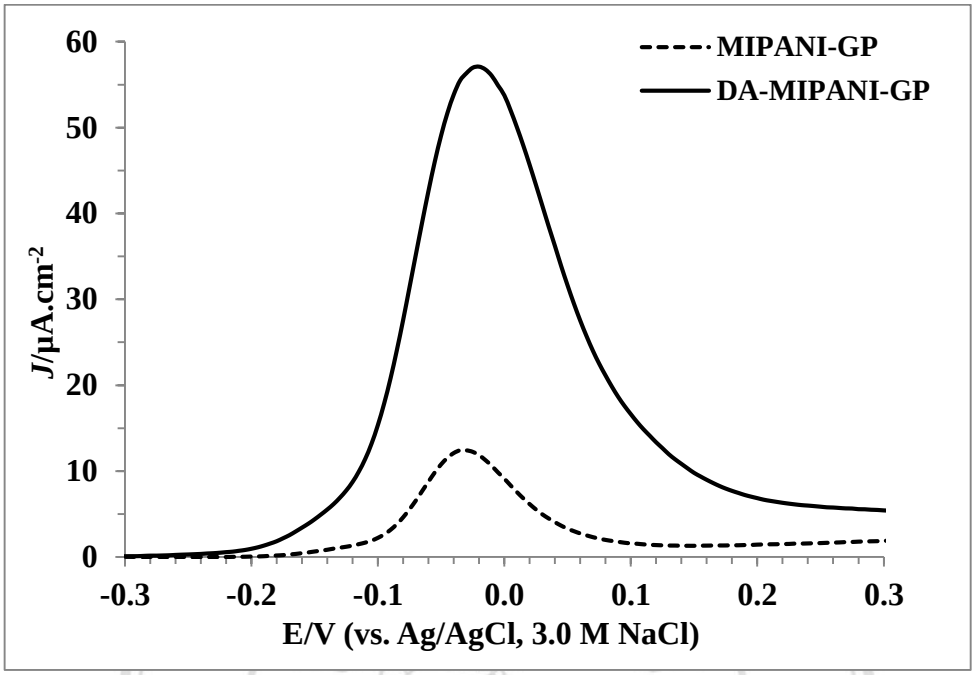


Figure 3.5 (a) DPVs of DA-MIPANI-GP-modified SPCE before and after overoxidation and nonMIPANI-GP-modified SPCE, respectively, in 0.10 M PBS (pH 7.4) and (b) DPVs of the resultant MIPANI-GP-modified SPCE in 0.10 M PBS (pH 7.4) with and without the presence of 0.10 mM DA. Pulse amplitude = 30 mV, pulse width = 30 ms, scan range = -0.6-0.8 V.

3.2.2 Optimisation of synthetic conditions for fabrication of MIPANI-GP-modified SPCEs

The electrochemical properties of MIPANI-GP-modified electrodes depend on acid concentration, number of electropolymerisation cycles, and concentrations of monomer and template. Therefore, the optimisation of the fabrication process was studied as follows.

3.2.2.1 Effect of acid concentration

Different concentration of protonic acid (0.1, 0.5, 1.0, 2.0 M of HCl) was used as dopant and supporting electrolyte for aniline polymerisation. The result showed that 1.0 M HCl is a good supporting electrolyte for aniline polymerisation. The 1.0 M HCl

provided higher current acid among the other concentrations used for electropolymerisation of aniline on GP modified SPCE surface. Therefore, the 1.0 M HCl was selected as the optimal electrolyte for the polymerisation of aniline on the surface of GP modified SPCE.

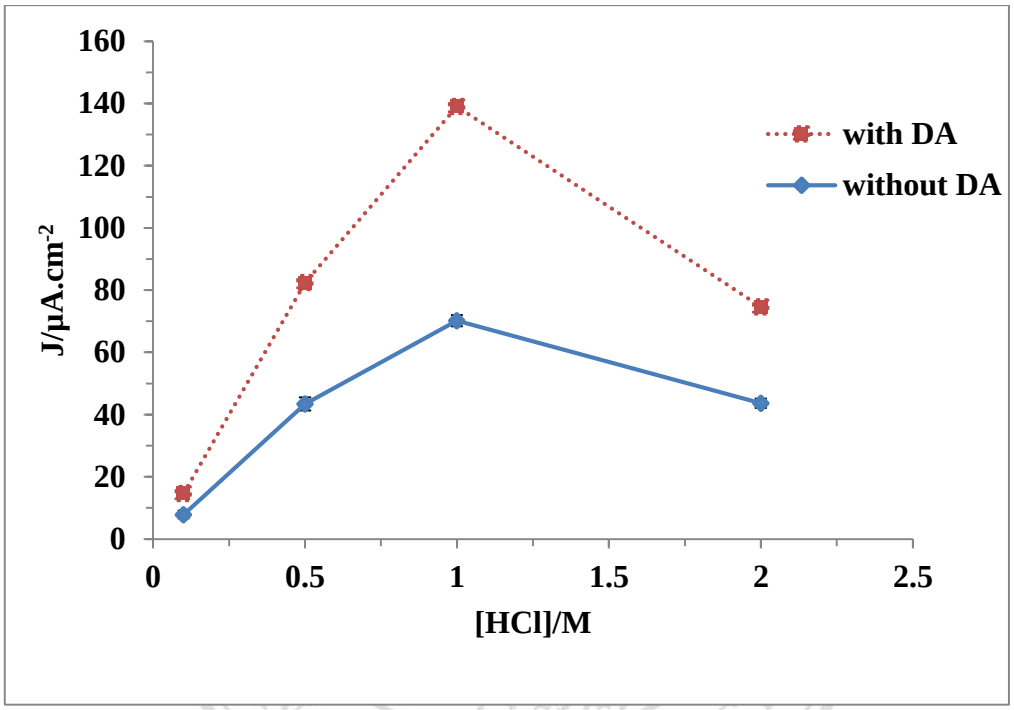


Figure 3.6 Effect of acid concentration on current response of the first wave for oxidation process of PANI on GP-modified SPCEs

3.2.2.2 Effect of the monomer concentration

The monomer concentration in the polymerisation process affects the thickness of the deposit and the amount of the imprinted molecule in the polymer matrix, which in turn further affects the electrochemical behaviour of the sensor. To evaluate the effect of the aniline concentration on the response of the MIPANI-GP-modified SPCE to DA and nonMIPANI-GP-modified SPCE, the MIP were electropolymerised in a solution of constant DA concentration and varying aniline concentration range of the 0.1 to 0.5 M (shown in Figure 3.6 and 3.7). The response of the MIPANI-GP-modified SPCE

to DA was found to increase with an increase of aniline monomer concentration. However, a considerable constant in the current response of the MIPANI-GP modified SPCE and nonMIPANI-GP-modified SPCE above 0.2 M of DA concentration was also observed. Therefore, it could be concluded that optimum monomer concentration was approximately 0.2 M.

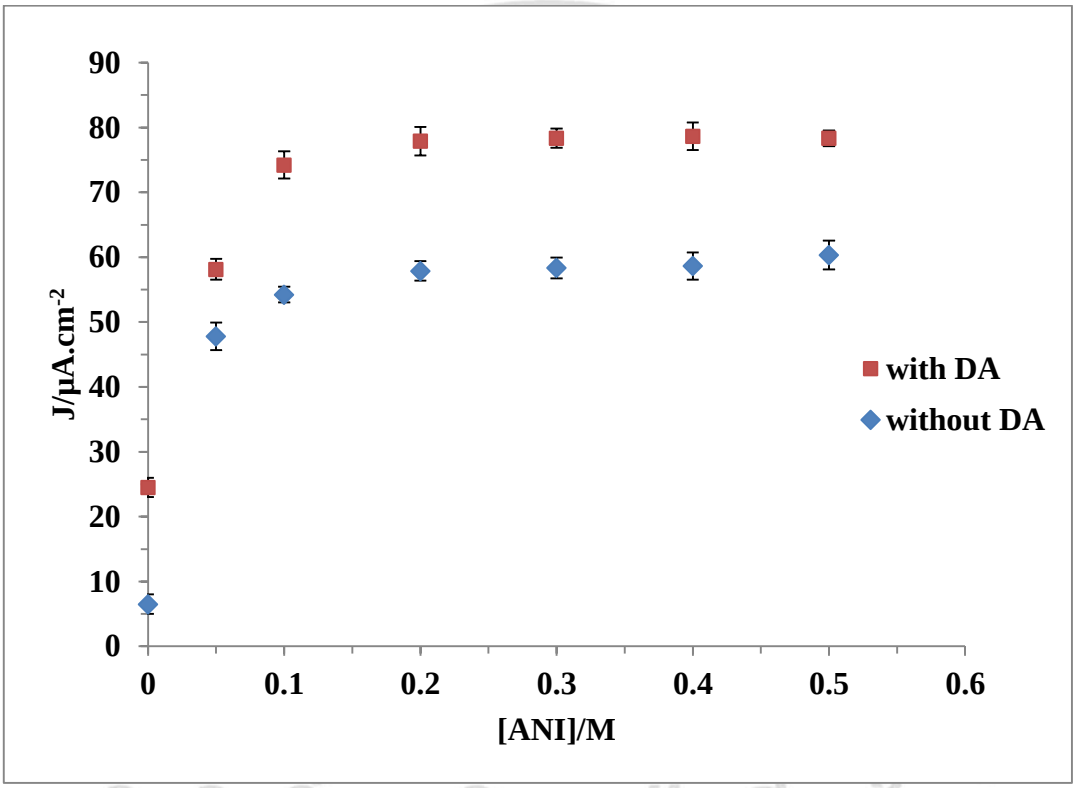


Figure 3.7 Current responses for oxidation process of PANI on GP-modified SPCEs with 0.1 mM DA and without DA in 0.1 M PBS solution (pH 7.4). PANI synthesised with different monomer concentrations in 1.0 M HCl

3.2.2.3 Effect of number of electropolymerisation cycles

The optimum number of cycle applied to the cell, for both MIPANI and non MIPANI -GP-modified electrodes, during the electropolymerisation was found to affect the sensitivity and linearity of the sensor. In order to obtain a sensing layer of the electrode, a series of experiments were performed by electrodes fabricated with

different numbers of layer. With too few cycls, the template would not be adequately embedded for the resulting surface, after removal of DA, to have well-defined recognition structure with too few cycles. On the other hand, higher cycle numbers lead to more extensive electropolymerisation, and therefore to the formation of thicker sensing film with less accessible imprinted site. The response of the MIPANI-GP-modified electrodes and nonMIPANI-GP-modified electrodes to DA was found to increase with increasing the number of cycle (Figure 3.10). The highest current difference between the MIPANI-GP-modified electrode and nonMIPANI-GP-modified electrode for DA was obtained by applying 30 cycles in the electropolymerisation. Therefore, optimal the number of polymerisation cycle was found to be 30.

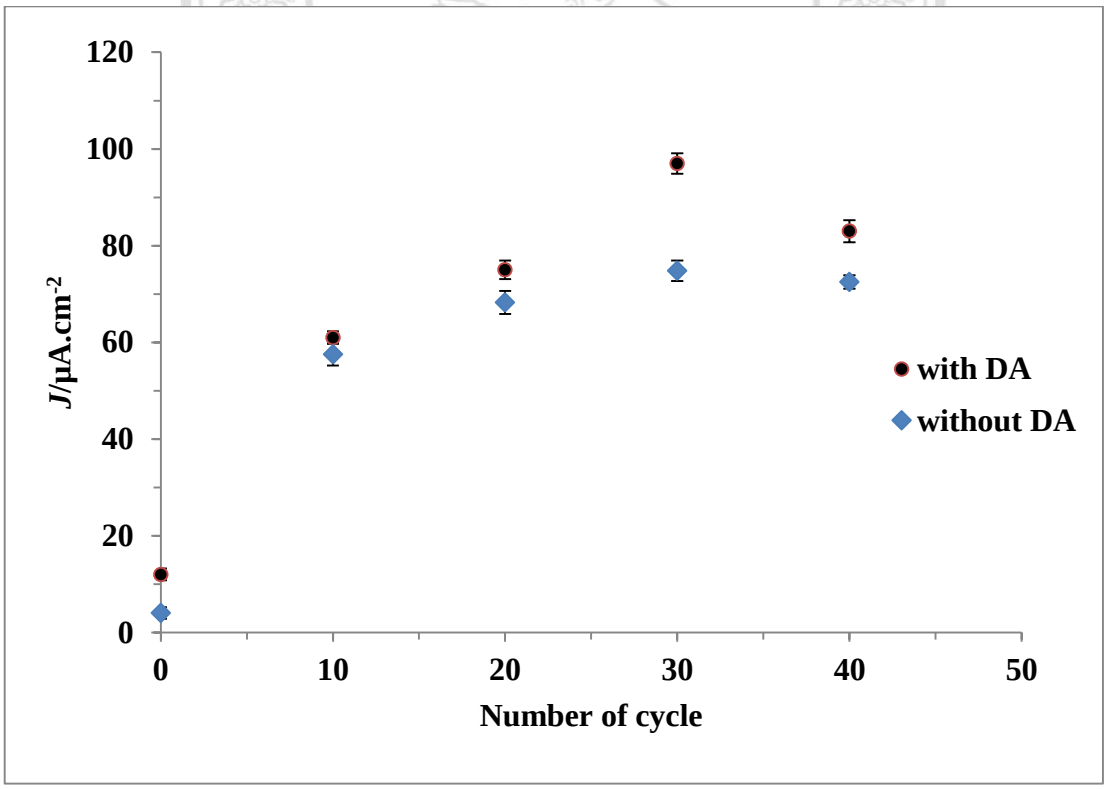


Figure 3.8 Effect of the electropolymerisation cycles with 1 mM DA and without DA in PBS pH 7.4

3.2.2.4 Effect of concentration of template

The effect of the template concentration during the film electrodeposition was shown in Figure 3.12. The response of the MIPANI-GP-electrode to DA increase with the increase of the template concentration between 0 to 1.0 mM. When the template concentration was higher than 1.0 mM, the electro-oxidation current almost had no change. At this stage the amount of DA arrived at the MIPANI-GP-electrode did not change. Based on the results, the optimal template concentration of was chosen as 1.0 mM.

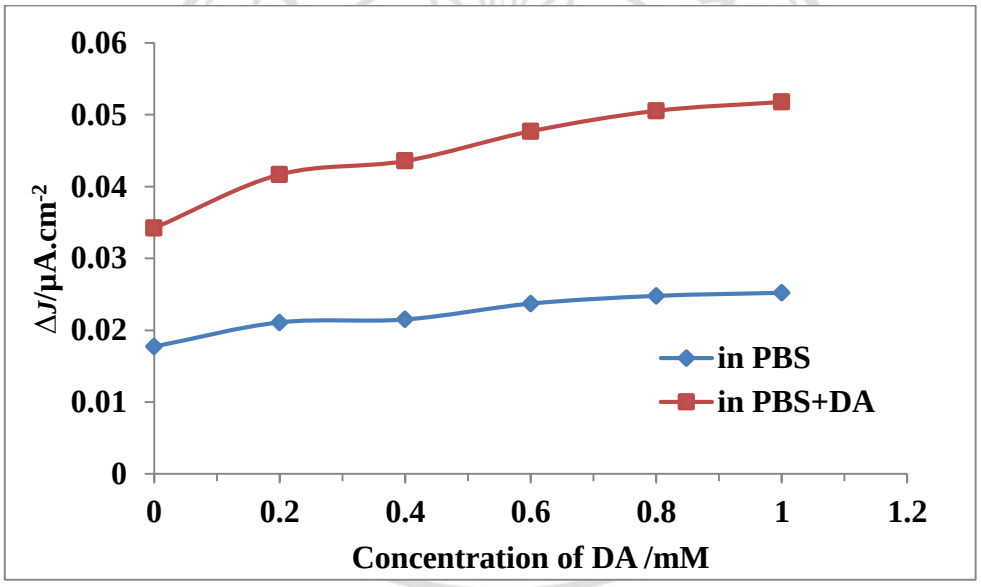


Figure 3.9 effect of the template concentration to MIP response

3.2.3 Characterisation of MIPANI-GP-modified SPCEs

The effect of scan rate on the MIPANI-GP modified electrodes was investigated. As shown in Figure 3.10 (a), both peak currents and peak-to-peak separation increase while the scan rate increases from 10 to 250 mVs^{-1}. It can be found that both the anodic peak current (I_{pa}) and cathodic peak current (I_{pc}) are proportional to the scan rate, and can be expressed as: I_{pc} (μA) = -90.987+270.74υ (R^2=0.9931), I_{pa} (μA) = 123.78-320.73υ (R^2=0.9921) (where, υ is the scan rate in the unit of mVs^{-1}), suggesting that a type surface controlled electrochemical behaviour (Figure 3.10 (b).

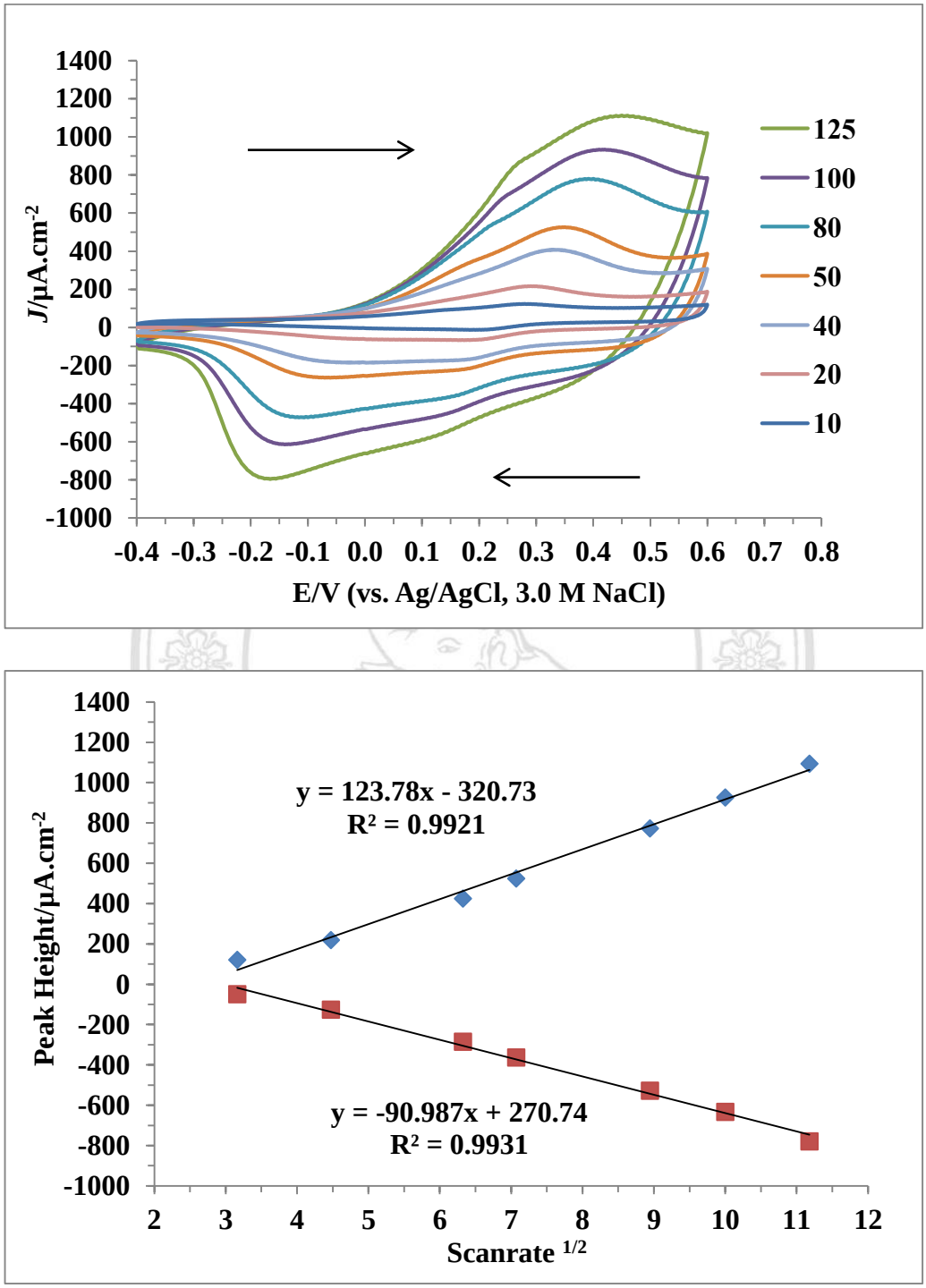


Figure 3.10 (a) CVs using 0.5 mM $[Fe(CN)_6]^{3-/4-}$ in 1.0 M KNO_3 with the MIPANI-GP electrode at the scan rates of 10, 20, 40, 50, 80, 100, 125 and 250 $mV.s^{-1}$ (b)Plot of peak height vs. square root of scan rate for increasing scan rates (10 $mV.s^{-1}$ to 50 mVs^{-}

3.2.3.1 Raman and morphological studies of electrodes

Raman spectroscopy provides a non-destructive technique to study the binding nature of various carbon materials such as graphite GP. Raman spectroscopy (excited by 532 nm laser) has been used to characterise all modified SPCE. Further characterizing the reduction of GPO, Raman spectroscopy was employed to study the structure, defect levels and crystallinity of the as synthesised graphene nano sheets and are compared with the SPCE.

Figure 3.11a. demonstates Raman spectra from the extracted GP and graphite. It can be seen that D, G and 2D bands at about 1330, 1550, 2650 cm^{-1}, respectively. GP and graphite are considerably different. The G peak which arises from the in-plane vibration of sp^2 bonds, of GP is relatively broader than that of graphite. In addition, the GP shows relatively strong D and 2D bands compared to graphite, indicating partially disordered crystal structure of GP sheets with larger number of edge-plane and zone boundary defects, respectively. Moreover, 2D band of GP is relatively broad compared to the G band, confirming that synthesized structure is few-layer GP.

The main Raman lines of PANI (MIPANI-GP and nonMIPANI-GP SPCE electrodes) are situated at 1170, 1214, 1374, 1486, and 1570 cm^{-1}. The C-H bending (Q)-A_g mode, C-N stretch. +ring def. (B) + C-H bending (B), C-C stretch. (Q) + C-H bending (B), C=N stretch. +C-H bending (B) and C-C stretch. (B) + C=C stretch. (Q), vibration mode (B= benzene ring, Q= quinoid ring), respectively [12]. In the results of raman spectra in Figure 3.11b, it was confirmed that PANI molecule were trapped onto the GP-SPCE with the non-MIPANI and MIPANI- GP SPCEs.

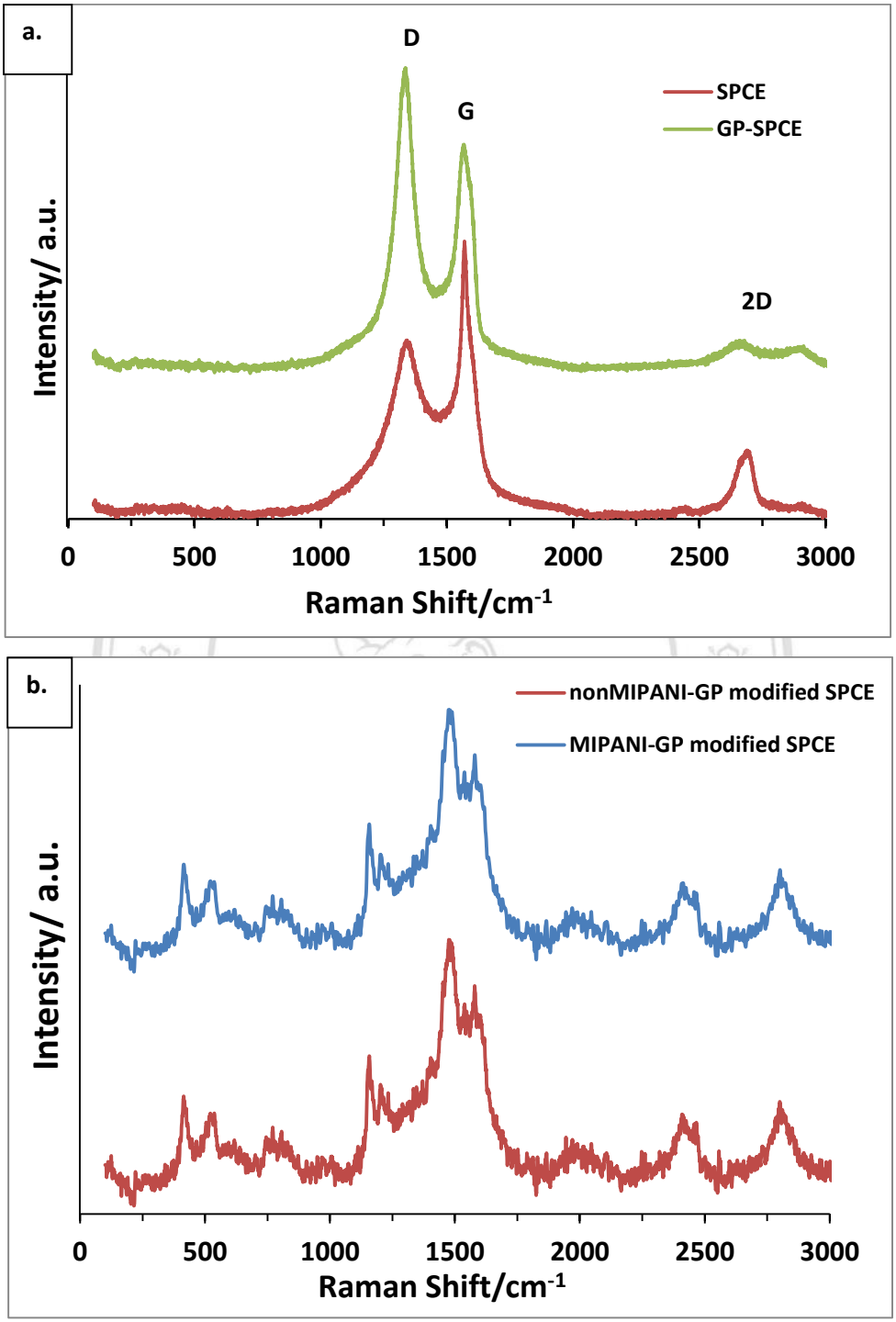
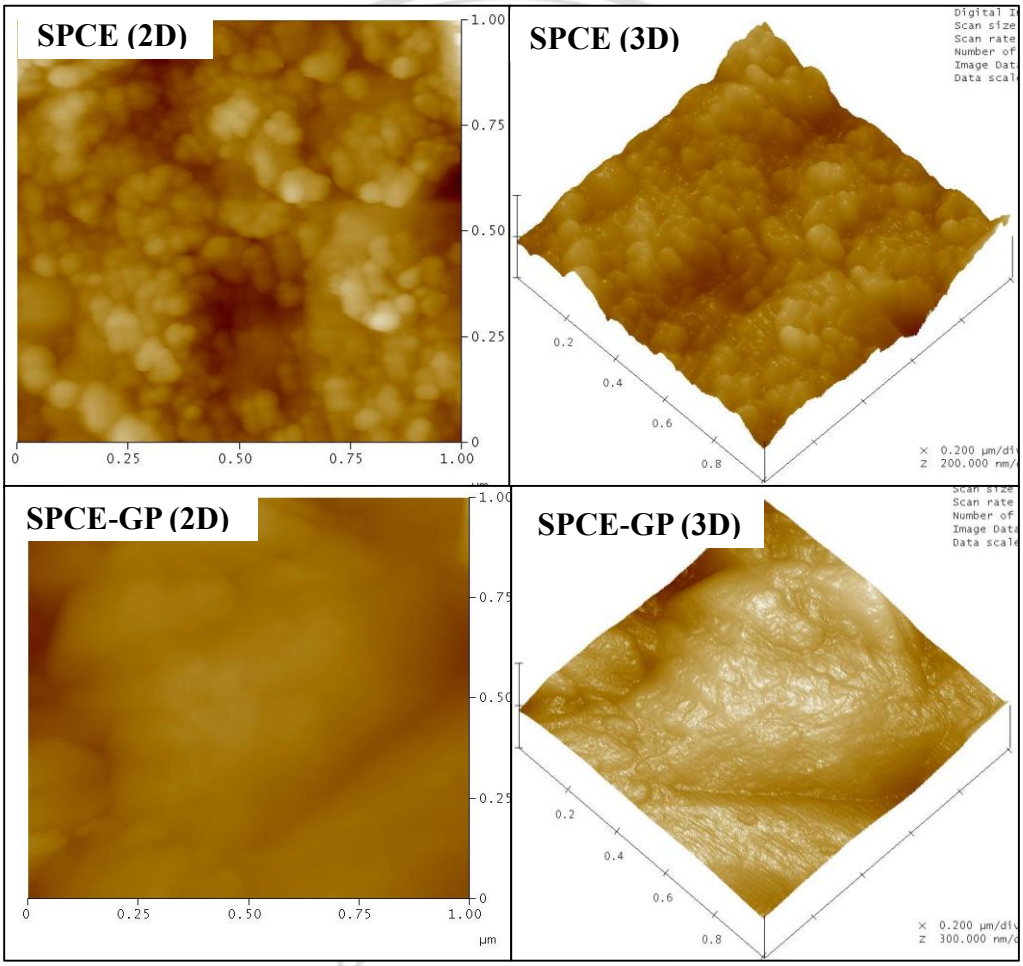


Figure 3.11 Raman spectra of SPCE and SPCE-GP (a), non-MIPANI-GP, MIPANI-GP electrodes (b).

The morphology of the electrode was characterised with AFM. As shown from the image of AFM (Figure 3.12), SPCE and SPCE-GP shows average roughness of around 18.672 nm and 9.523 nm. From AFM analysis in Figures 3.15 and 3.16, the average roughness (R_a) of the PANI grafted on the GP-SPCE surface with nonMIP and MIP were determined to be about 26.816 nm and 31.952 nm, respectively, indicating that GP and PANI had been anchored on to SPCE and improved the surface effect of the electrode interface.



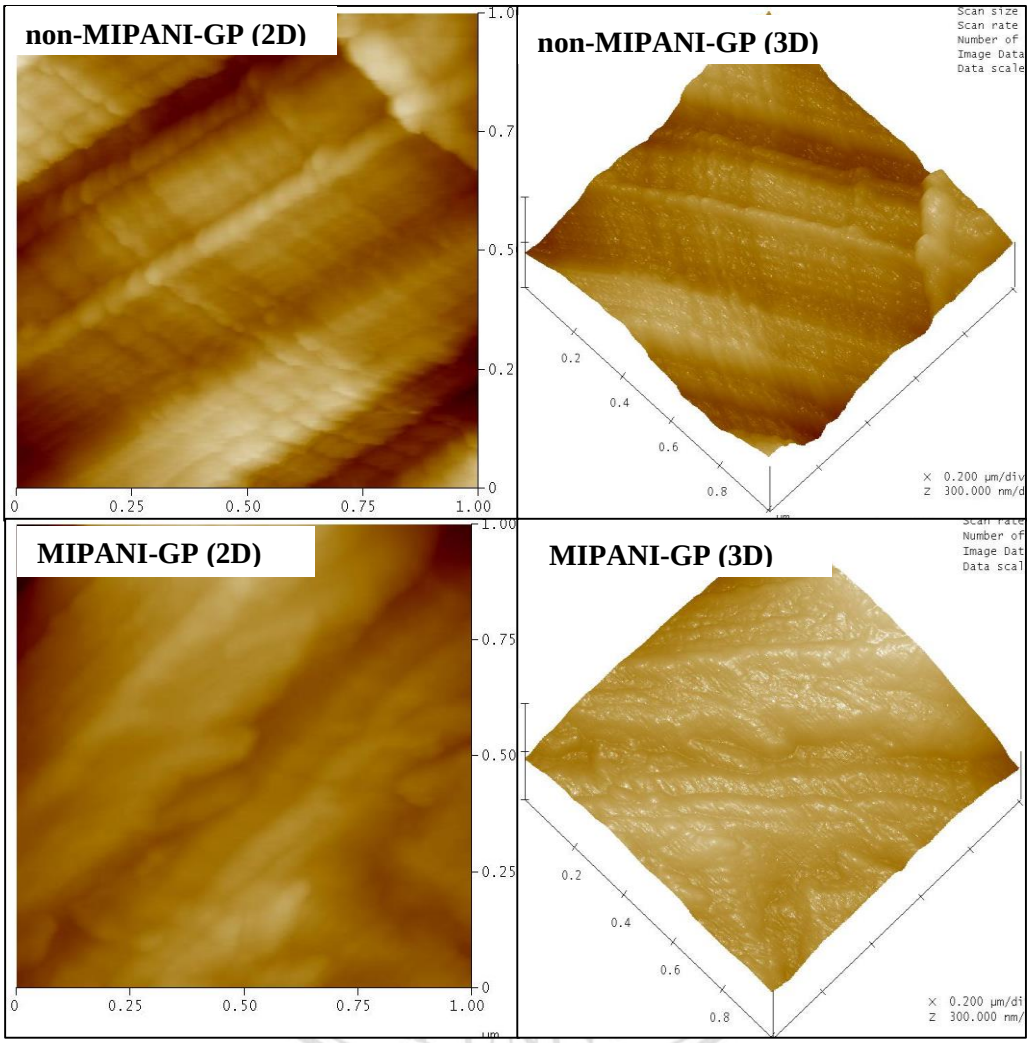


Figure 3.12 AFM images of two and three dimensional of (a.) SPCE, (b.) SPCE-GP, (c.) non-MIPANI-GP, (d.) MIPANI-GP electrode.

3.3 Determination of DA using MIPANI-GP-modified SPCE

3.3.1 Effect of pH

The pH of the medium has a significant influence on the peak current and peak potential of the electrochemical oxidation of DA on the polymeric film. Figure 3.13 shows the effect of the DPV peak current and potential of DA by the pH, in the range of 6 to 8. The DA signal shifted to more cathodic potential as the pH increases. The peak current of DA oxidation increased and shifted to more positive potentials with an increase in pH. The highest current value was obtained at pH value of 7.4, with a 0.1 M PBS and 0.1 mM DA in 0.1 M PBS. Therefore, the pH of PBS buffer was chosen to be 7.4.

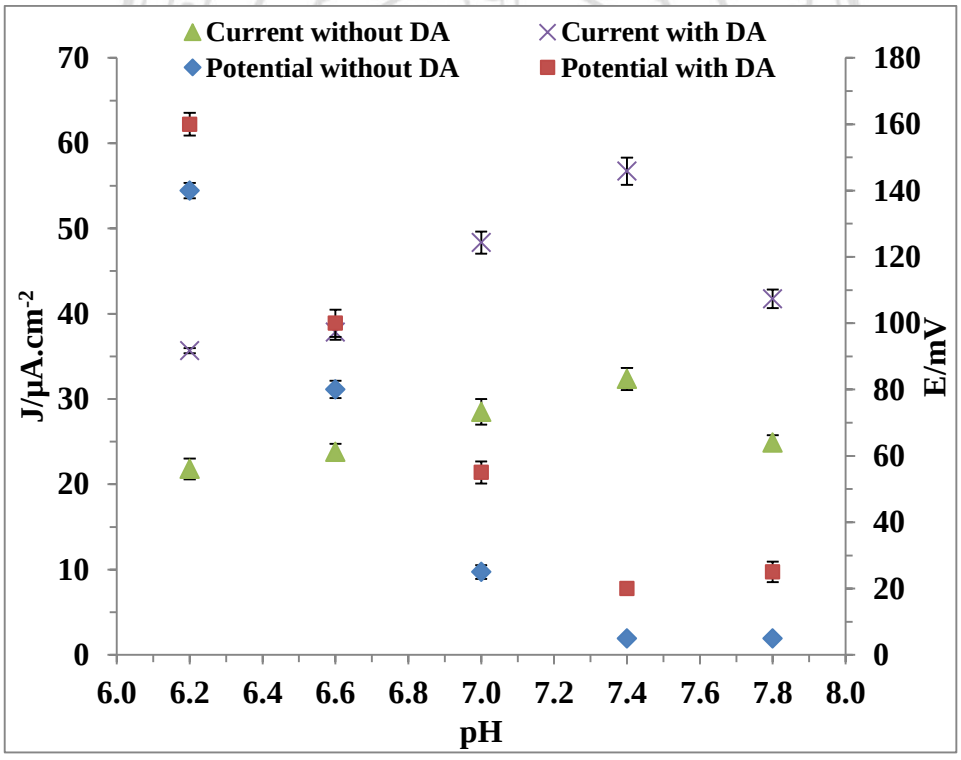


Figure 3.13 Effect of the pH on the MIPANI modified GP response in the range of 6-8 with 0.1 mM DA and without DA in 0.1 M PBS.

3.3.2 Effect of temperature

The temperature of the medium has a significant influence on the peak current and peak potential of the electrochemical oxidation of DA on the polymeric film. Figure 3.14 shows the effect on the DPV peak current and potential of DA by the temperature, in the range of 20 to 40 ˚C. The DA signal shifted to more cathodic potential as the temperature decreases. The peak current of DA oxidation decreased and shifted to more positive potentials with increase in temperature. The highest current difference between the current and potential of MIPANI electrode for DA was obtain by applying 25 ˚C. Therefore the optimum temperature of the template was found to be 25 ˚C.

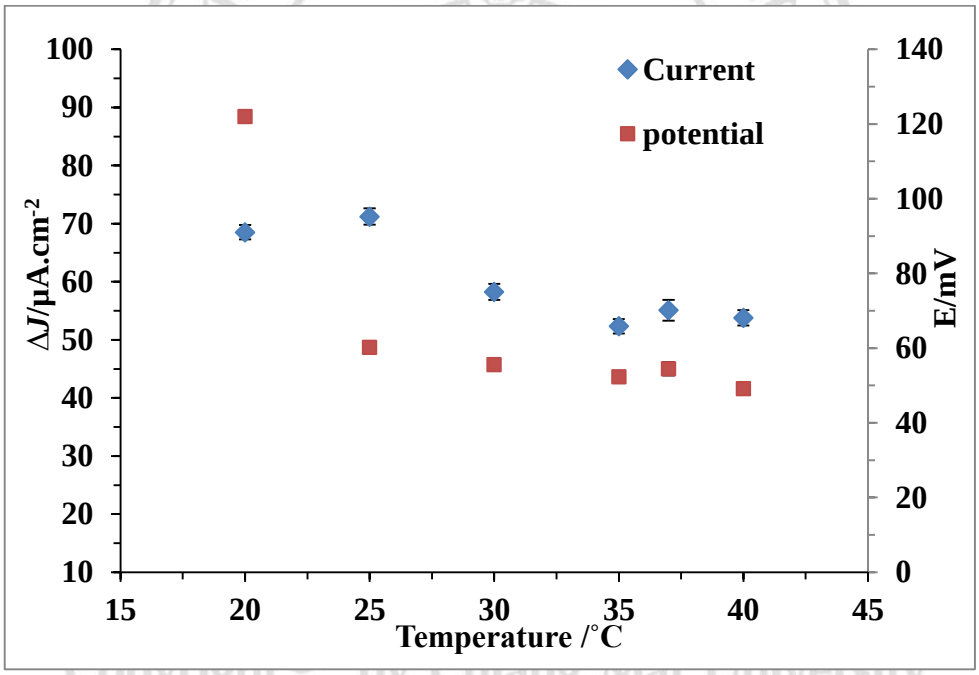


Figure 3.14 effect of the temperature to MIP response

3.3.3 Analytical performance of the proposed sensor

3.3.3.1 Response studies with varying DA concentration

DPV can be useful tools for rapid and sensitive detection of analytes. To investigate the sensing response of MIPANI GP electrode, a series of DPV have been recorded with varying DA concentration in the range of

0.001-400 µM at pH 7.4 Figure 3.15 shows uniform and continuous increase in the peak DPV current with increase in DA concentration. A calibration curve of DA at -0.02 V showed that the MIP electrode can detect DA with linearity upto 400 µM and correlation coefficient (R^2) of 0.9949 and 0.9986 at low and high concentrations of DA, respectively. The MIPANI GP electrode have good sensitivity of 28.80 µA.cm^{-2}.µM and 0.47 µA.cm^{-2}.µM at low and high concentrations of DA, respectively, and a low detection limit of 190 pM, n = 3µM (Figure 3.16 (a) and (b)).

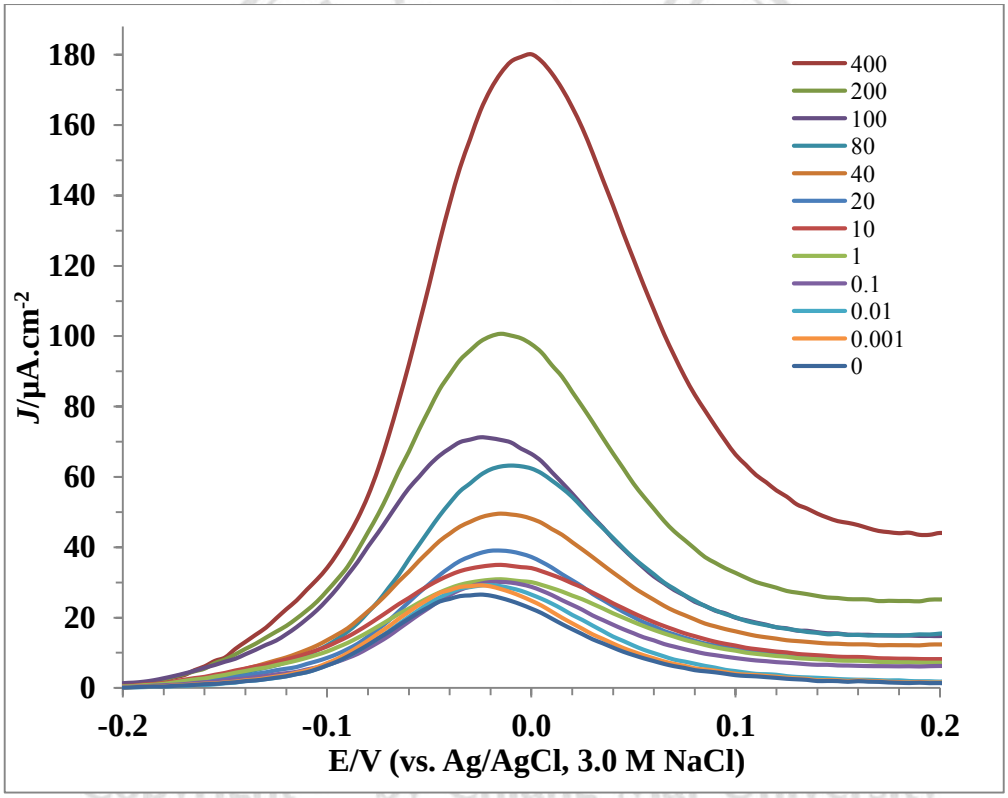
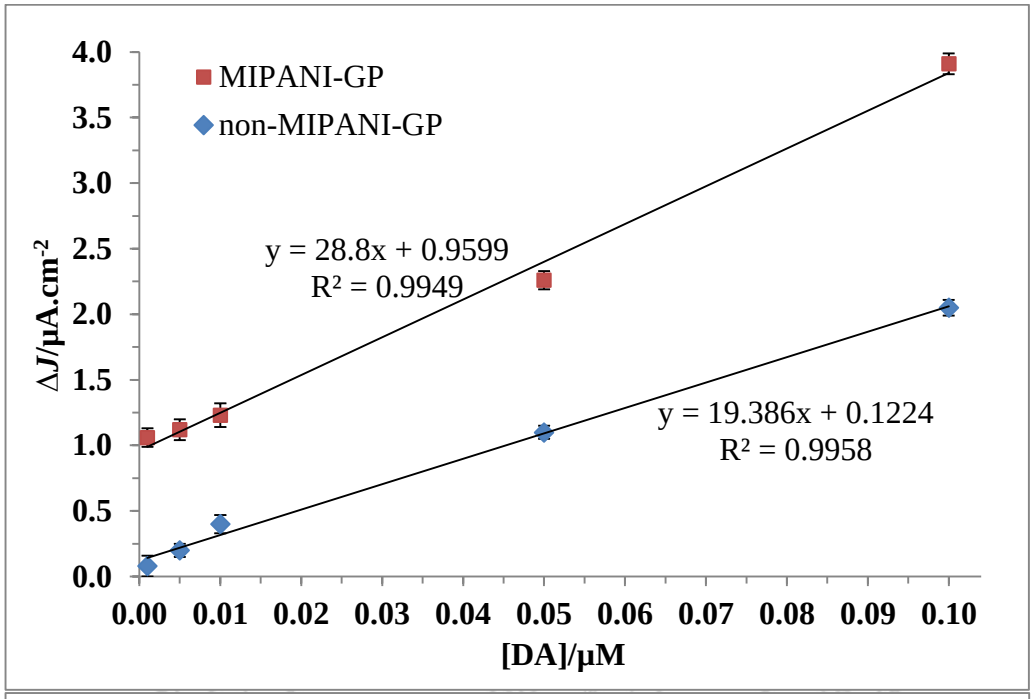


Figure 3.15 DPV of DA in the concentration range of 0.001 – 400 µM in 0.1 M PBS pH 7.4 measured on the MIPANI-GP-modified screen printed carbon electrode.

DPV technique was carried out in recording the response results to obtain the two linear ranges and limit of detection (LOD). The MIPANI-GP and non-MIPANI-GP sensor were incubated in different concentration of DA solution under the optimized conditions, respectively. The DPV results are presented in Figure 3.16. From Figure 3.16 we observed that the DPV

responses of the DA-imprinted sensor were linear to the concentrations of DA from 0.001 to 400 µM. The LOD was 0.190 nM (S/N=3).



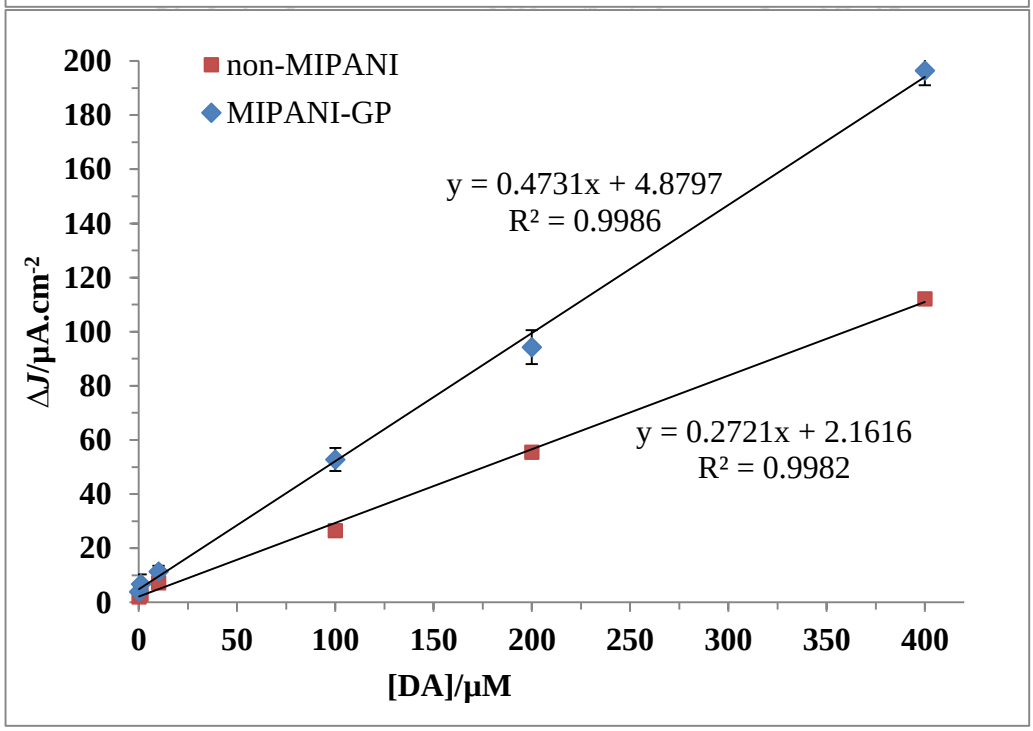


Figure 3.16 (a) Calibration curve of 0.001-0.1 µM in 0.1 M PBS pH 7.4 measured on the MIPANI and non MIPANI-GP-modified SPCEs. (b.) Calibration curve of 0.1-400

μM in 0.1 M PBS pH 7.4 measured on the MIPANI and non-MIPANI GP-modified SPCEs.

3.3.3.2 Imprinting factor calculation

The response studies of non-MIPANI-GP SPCEs have been carried out as a function of the DA concentration. The observed results do not show systemetic variation in the response of non-MIPANI-GP SPCEs as a function of DA concentration. Imprinting factor, which indicates the ability of the molecularly imprinted polymer to recognise the patterned molecule and provides the information relating to the binding efficiency of MIP with the analyte, can be calculated using Equation.1

Imprinted factor IF= $\frac{\Delta I(MIP)}{\Delta I(NIP)}$ (Eq. 1)

Thus; IF= $\frac{52.76}{26.50}$ = 1.99

where, $\Delta I(MIP)$ refers to the change in peak current with respect to MIPANI-GP SPCE with 100 μM concentration of DA and $\Delta I(NIP)$ to that of nonMIPANI-GP SPCE with the same concentration of DA. The imprinting factor for the MIPANI-GP SPCE has been calculated as 1.99.

3.3.3.3 Specificity and selectivity studies

For a MIPANI-GP sensor, the selective recognition to template molecule is a very valuable factor. To evaluate the selectivity of the DA-imprinted sensor, we introduced some species in the detection process such as AA, UA, GA and Glu, which were similar to DA in structure and acted as interfering substances at the concentration of interferences 1000 folds more than DA concentration. The detection results of the DA-imprinted sensor and non-imprinted sensor were shown in Figure 3.17. The current response toward DA was higher compared to those of the other four structure analogs, and the response of imprinted sensor was stronger than that of the

non-imprinted sensor, which may be attributed to the binding sites in the DA-imprinted sensor that were complementary with DA in size and shape. All of the above results confirmed that the imprinted sensor showed high selectively toward DA.

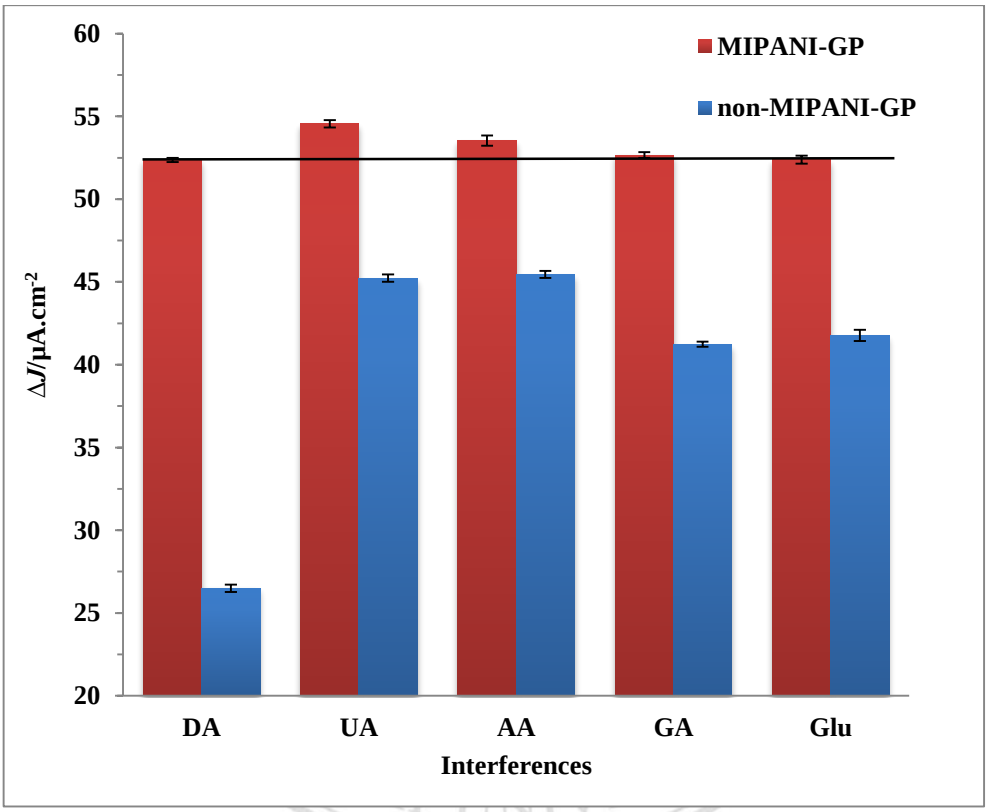


Figure 3.17 Comparison plots show changes in current of DA-MIPANI GP electrode with different interferences and DA.

3.3.3.4 Stability and reusability of the modified electrode

The stability and reusability of DA-MIPANI electrode has been investigated by comparing DPV response of electrodes prepared under similar experimental conditions at different time periods. Negligible decrease in response was found even after seven times of usage of the DA-MIPANI GP-modified electrode. The sensing results obtained with same DA-MIPANI GP-modified electrode with different DA concentrations within the time interval of 2 days (1^{st}, 3^{th}, 5^{th} and 7^{th} days) have been shown

in table 1. The current response decreased by about 15 % after 1 month, compared to its initial point, which indicated a good stability.

Table 1 The stability and reusability of DA-MIPANI electrode

Day	Concentration of dopamine (μM)	Current (μA)	% Recovery
1	10	35.03	100
	20	39.01	100
	40	49.54	100
3	10	34.01	97.09
	20	38.25	98.05
	40	49.42	99.76
5	10	33.36	95.23
	20	37.51	96.15
	40	46.95	94.77
7	10	33.05	94.35
	20	37.16	95.26
	40	46.73	94.33
30	10	29.86	85.25
	20	33.20	85.12
	40	42.31	85.42

3.3.4 Determination of DA in real samples

The determination of DA in urine sample (n=3)

To assess the applicability of the proposed system in biological model, the urine samples containing spiked DA ware analysed on three independently prepared electrodes. Three replicates of samples were detected and averaged. The DA in urine samples was assayed by the standard addition method and calculated the concentrations by extrapolation. The analytical results are summarised in table 2 the recovery range from 101.5 to 103.9 % and the RSD (n=3) was under 4.0%, which indicated that this system is highly accurate in urine sample.

Table 2 the determination of DA in urine sample

Sample	Amount of added DA (μM)	Amount of found DA (average) (μM)	% Recovery	% RSD
Blank (PBS buffer)	0	-	-	2.0
Std. DA	10.00	10.00	100.0	2.1
	20.00	20.00	100.0	2.3
	40.00	40.00	100.0	3.1
Urine 1	0.00	2.11	-	2.4
	10.00	10.24	102.4	2.5
	20.00	20.47	102.4	2.2
	40.00	40.87	102.2	2.1
Urine 2	0.00	2.04	-	3.4
	10.00	10.23	102.3	2.1
	20.00	20.41	102.1	2.5
	40.00	40.76	101.9	2.3
Urine 3	0.00	3.80	-	1.9
	10.00	10.39	103.9	2.4
	20.00	20.71	103.6	2.3
	40.00	40.89	102.2	2.4
Urine 4	0.00	2.11	-	2.1
	10.00	10.22	102.2	3.0
	20.00	20.54	102.7	2.6
	40.00	40.87	102.2	2.8
Urine 5	0.00	3.32	-	2.3
	10.00	10.36	103.6	2.1
	20.00	20.65	103.3	2.5
	40.00	41.23	103.1	2.1

Table 2 (Continued)

Sample	Amount of added DA (µM)	Amount of found DA (average) (µM)	% Recovery	% RSD
Urine 6	0.00	2.27	-	2.7
	10.00	10.21	102.1	2.5
	20.00	20.54	102.7	2.3
	40.00	40.61	101.5	2.8
Urine 7	0.00	2.41	-	2.6
	10.00	10.25	102.5	3.1
	20.00	20.56	102.8	2.4
	40.00	40.9	102.4	2.6
Urine 10	0.00	3.03	-	2.7
	10.00	10.30	103.0	2.4
	20.00	20.73	103.7	2.6
	40.00	41.34	103.4	2.4

3.3.5 Comparison of the proposed electrode to other modified electrodes for DA determination

Table 3 shows a performance comparison between the proposed imprinted polymer electrodes, modified electrode report in literature. It is evident that the DA-MIPANI-GP-SPCE has better DA detection performances with a relatively wide concentration range and low detection limit compared to the others.

Table 3 shows Comparison of the proposed electrode to other modified electrodes for DA determination

Modification of electrode		**Method**	**Linear range**	**Detection limit (µM)**	**R^2**	**References**
Electrode type	**Modification methode**					
SPCE	SDS/carbonized PANI loaded GP fiber	DPV	0.5–100	0.07	0.9929	15
inkjet printed	Nafion/MWCNT chip	DPV	0.1 -10	0.2	0.999	16
GCE	GSCR-MIPs	LSV	0.1-830	0.1	0.9950	17
GCE	PANI/AgCl	DPV	0.7-6.0	0.054	0.9958	18
glass covered/ITO	PANI-PA/CG	CV	10 - 230	0.1	0.998	19
CPE	SDS-modified CPE	DPV	0.5–800	0.05	0.9996	8
GCE	MWNTs-MIPs	DPV	0.625-100	0.06	0.9978	24
SPCE	MIPANI-GP	DPV	0.001-0.1 0.1-400	0.00019	0.9949 0.9986	This work

Abbreviations: MWNTs-MIPs, multiwalled carbon nanotubes-molecularly imprinted polymers; OPA, o-aminophenol; GSCR-MIPs, graphene sheets/Congo red-molecular imprinted polymers; rGO–PpPD hybrids ,reduced graphene oxide–poly(p-phenylenediamine); GCE, glassy carbon electrode ; CPE, carbon paste electrode; SDS, sodium dodecyl sulfate; PANI-PA/CG ,polyaniline phosphonic acid /cashew gum; MIPANI, molecularlyimprinted polyaniline; GP, graphene; ITO, indium tin oxide; SPCE, screen printed carbonelectrode;DPV, differential puls voltammetry; CV, cyclic voltammetry; LSV, linear sweep voltammograms.