CHAPTER 2

Materials and Methods

2.1 Materials

2.1.1 Chemicals

Chemicals	Purity (%)	Formula	Company	Country
Aniline	99	$C_6H_5NH_2$	Merck	Germany
Acetone	≥99.5	CH_3COCH_3	Lab Scan	Poland
L(+)-Ascobic acid	99	$C_6H_8O_6$	Merck	Germany
N,N-Dimethyl formamide	≥99	C_3H_7NO	Lab Scan	Poland
Di- Sodium hydrogen phosphate dihydrate	99	$Na_2HPO_4.2H_2O$	Scharlau	Spain
Sodium hydrogen phosphate dihydrate	98.5	$NaH_2PO_4.2H_2O$	Merck	Germany
Dopamine hydrochloride	99.5	$C_8H_{11}NO_2$	Sigma Aldrich	USA
Ethanol	95	C_2H_6O	Lab Scan	Poland
Gallic acid	98.8	$C_7H_6O_5$	Fluka	Switzerland
Glucose	99	$C_6H_{12}O_6$	Fluka	Switzerland
Graphite powder < 20 micron synthetic	-	C	Sigma-Aldrich	USA
Hydrazine hydrate	> 99.0	N_2H_4	Fluka	Switzerland
Hydrochloric acid	37	HCl	Lab Scan	Poland
Hydrogen peroxide	50	H_2O_2	AJAX	Australia
Nitric acid	65	HNO_3	Merck	Germany
Potassium fericyanide	98.5	K_3 [Fe (CN)]$_6$	Lab Scan	Poland
Potassium permanganate	99	$KMnO_4$	Carlo Erba	Italy

Chemicals	Purity (%)	Formula	Company	Country
Potassium nitrate	99	KNO_3	Lab Scan	Poland
Sodium chloride	99.5	$NaCl$	Carlo Erba	Italy
Sodium hydroxide	98.5	$NaOH$	Lab Scan	Poland
Sodium nitrate	99	$NaNO_3$	Carlo Erba	Italy
Sulfuric acid	96	H_2SO_4	Lab Scan	Poland
Uric acid	98.5	$C_5H_4O_3N_4$	Hopkin&Wiliums	U.S.A.

2.1.2 Apparatus and Instruments

Apparatus and Instruments	Company	Country
Centrifuge JA14-MC	Beckman	U.S.A.
Centrifuge Rotofix 32A	Hettich	England
µAutolab type II Potentiostat/Galvanostat	Metrohm	Netherlands
Raman Spectrometer (T64000)	Horiba Jobin Yvon	France
Hotplate & magnetic stirrer	PMC Industries, Inc., San Diego,	U.S.A.
Sonicator	Elmasonic	Germany
Silver/Silver Chloride electrode	-	-
Platinum electrode	-	-
Micropipete 10 µL, 100 µL, 1000 µL	Dragon Lab	China

2.2 Experiments

2.2.1 Preparation of chemically reduced GPO

A synthetic graphite powder (<20 μm, Aldrich) was used to prepare GPO. The GPO was synthesised by a chemical triple-exfoliation process which used the improved modified Hummers' method to oxidise graphite for the synthesis of GPO. This method makes use of the Hummers' reagent with addition amounts of $NaNO_3$ and $KMnO_4$. Concentrated H_2SO_4 and HNO_3 at the ratio of 3:2 (volume/volume) were added to the mixture of graphite and $NaNO_3$. The mixture was cooled down on an ice bath. $KMnO_4$ (3 folds of graphite) was added slowly to keep the reaction at low temperature. The solution was stirred for overnight and then added with H_2O_2. The reaction mixture was centrifuged (9000 rpm for 25 min), and the supernatant was decanted away. The remaining solid material was then washed with of deionised (DI) water and centrifuged again, and this process was repeated until the pH becomes natural. Finally, GP sheet is formed by conventional reduction with hydrazine.

2.2.2 Preparation of Phosphate buffer saline (PBS)

0.1 M PBS pH 7.4 was prepared by dissolving 0.61 g of $NaH_2PO_4.2H_2O$, 1.08 g of $Na_2HPO_4.2H_2O$, 8.00 g of NaCl and 0.20g of KCl in 800 mL of DI water, and the pH was adjusted to 7.4 with NaOH. Then the volumn was brought up to 1000 mL with DI water.

2.2.3 Apparatus and electrode

The CV and DPV measurements were performed with a μAutolab type II Potentiostat/Galvanostat (Metrohm Siam Ltd.). An electrochemical cell including non-MIPANI-GP-modified SPCE and MIPANI-GP-modified SPCE as working electrodes (d=0.1256 cm^{-2}), a Pt as counter electrode, and an Ag/AgCl as reference electrode. This system was used for the preparation of the polymer, the studies of interfering and the detection of DA template.

2.2.4 Preparation of imprinted polymer and non-imprinted polymer modified electrodes

The MIPANI was made by electropolymerisation of ANI on the surface of the GP-modified SPCE, using CV in the potential range between -0.2 to 0.9 V. Thirty cycles at 100 mVs^{-1} in a solution of 0.2 mM DA, 0.2 M of ANI, and in 1.0 M HCl were used (Figure 2.1). The non-MIPANI-GP modified electrode was also constructed under at the same method conditions, but without DA. The DA selective MIPANI-GP modified SPCE has been fabricated by the extraction of embedded DA moieties from the PANI films by over oxidation in a potential range of -0.9 to 0.9 V, which leads to a formation of DA selective recognition site in the electrode film.

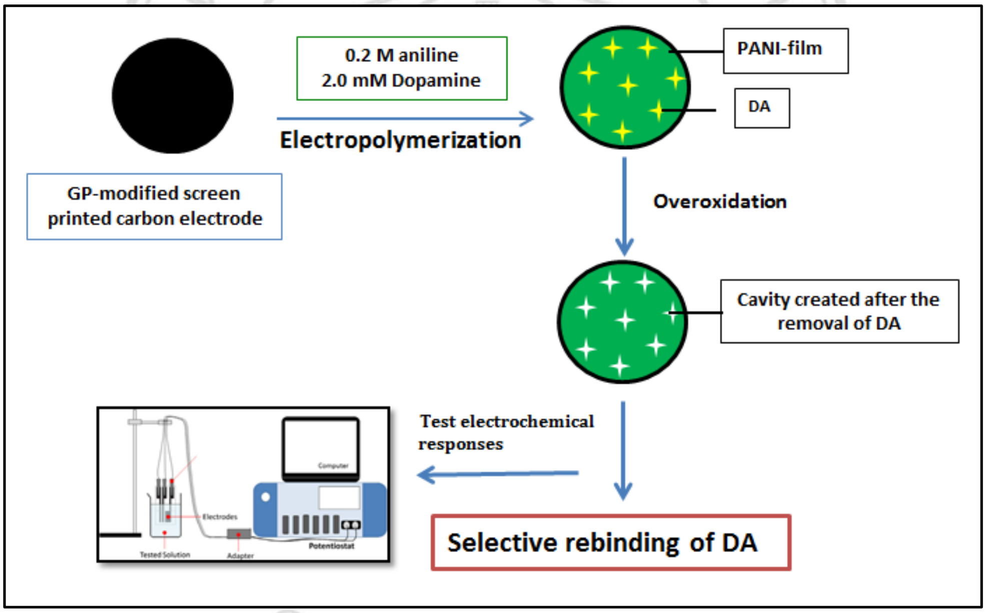


Figure 2.1 Preparation of imprinted polymer and non-imprinted polymer modified electrodes.

2.2.5 Calibration curve of DA assay

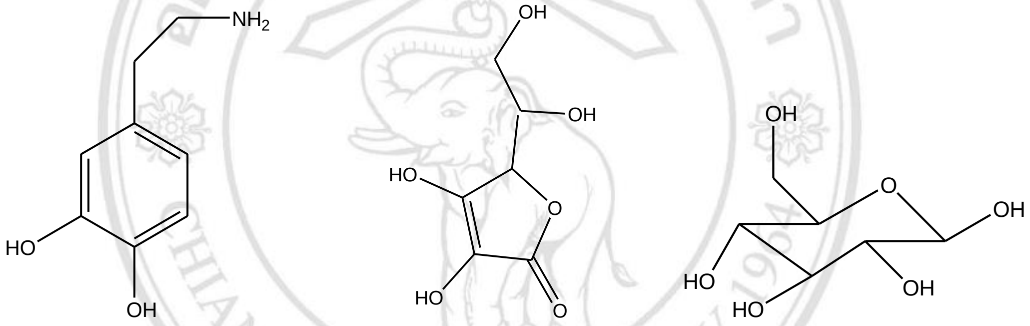
The optimum condition of the DPV detection system was used to construct the calibration curve from various concentrations of DA solutions in the range of 0 to 400 µM prepared in 0.1 M PBS buffer. Lowest detectable concentration and working range were estimated from this curve.

2.2.6 Fabrication stability of DA-MIPANI-GP modified SPCE

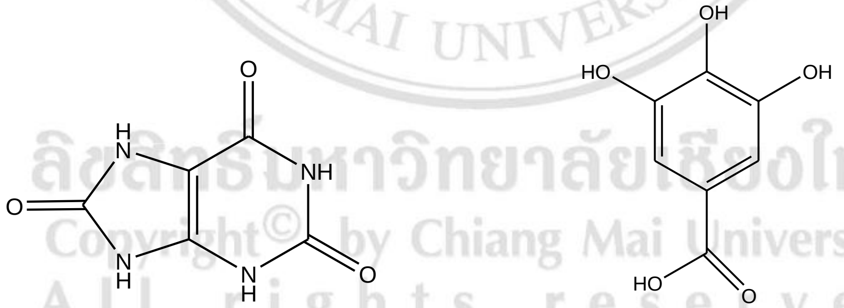
The stability of molecularly imprinted DA-MIPANI-GP-modified SPCE has been investigated by comparing DPV response of different electrodes prepared under similar experimental conditions. To study the reusability of these DA-MIPANI-GP-modified SPCE electrodes were tested with different DA concentrations with the time interval of 2 days (1st, 3th, 5th and 7th day) and after one month.

2.2.7 Selectivity of the MIPANI-GP-modified SPCE

In order to investigate the effect of interferences of the DA-MIPANI-GP-modified SPCE electrode, we have selected UA, AA and GA, Glu and all mixers with the concentration of interferences 1000 folds more than DA concentration (in the presence of 0.2 mM DA).



Dopamine Ascorbic acid D-Glucose



Uric acid Gallic acid

Figure 2.2 The structures of dopamine, ascorbic acid, D-glucose, uric acid and gallic acid.

2.2.8 Testing of Accuracy

The accuracy of the system on the analysis of real sample was demonstrated by the combined DA solution and urine samples to gain the final added concentrations of DA are 10, 20 and 40 μM. The percentages of difference were calculated from the results obtained from the standard curve as compared to the expected values from the calculation. All urine samples were the first urine of the day, collected directly into a sterile container.

2.3 Characterisation

2.3.1 Atomic force microscopy (AFM)

The surface morphology of all electrodes were characterised with atomic force microscopy.

2.3.2 Raman Spectroscopy

The vibrational, rotational and other low-frequency modes of electrode were characterised with Raman spectroscopy (Jobin-Yvon Horiba form France, model: T64000 triple monochromators, with laser excitation at 532 nm.).

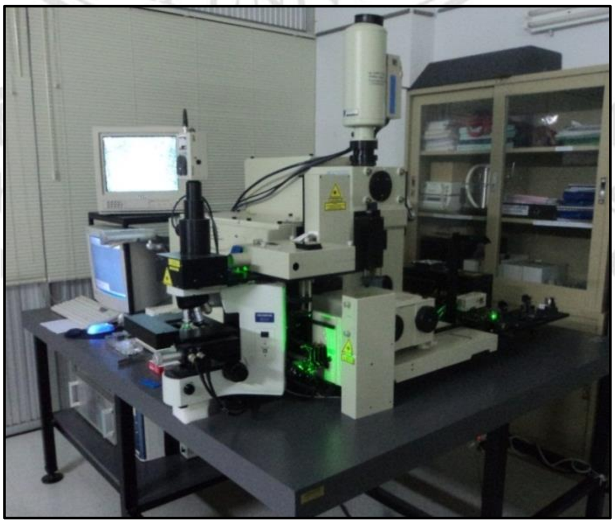


Figure 2.3 Raman spectrometer