

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization of G/PVP/PANI nanocomposite-modified SPCE

4.1.1 Physical characterization of the modified SPCE

Firstly, the scanning electron microscopy (SEM) was used for physical characterization of G/PVP/PANI modified SPCE. Without the modification, uncontrolled agglomeration of graphene is common due to attractive van der Waals forces that cause inhomogeneous graphene. Therefore, PANI and PVP were used to increase the dispersibility of graphene in solution. The SEM image in Figure 4.1 shows the homogeneous and uniform distribution surface morphology of G/PVP/PANI nanocomposite-modified SPCE. Moreover, the transmission electron microscopy (TEM) image in Figure 4.2 also exhibits a good dispersion of graphene in solution without an agglomeration. These graphics reveal that the modification of screen-printed carbon electrode by G/PVP/PANI nanocomposite using electrospraying was a suitable technique to modify the SPCE with simplicity, homogeneity of droplet and inexpensiveness.

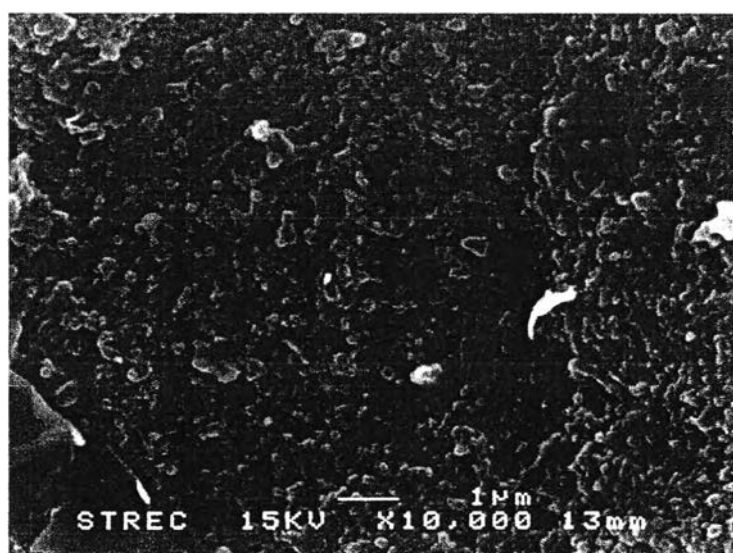


Figure 4.1 SEM image of G/PVP/PANI nanocomposite-modified SPCE

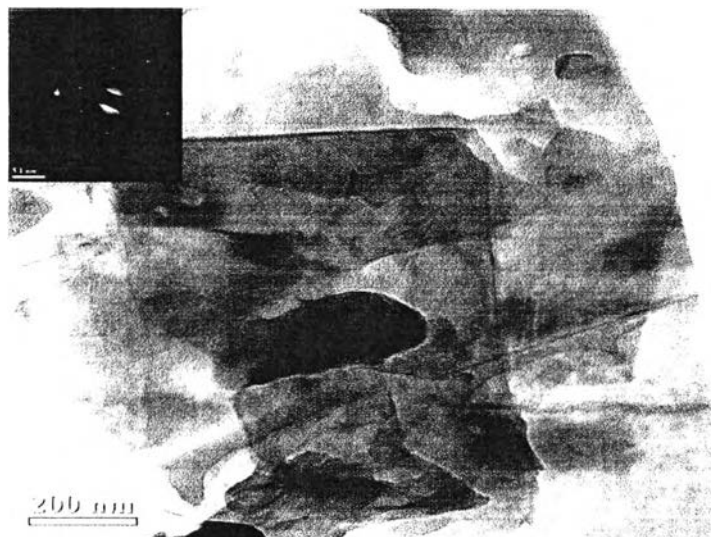


Figure 4.2 TEM image of G/PVP/PANI nanocomposite-modified SPCE with the electron diffraction pattern of graphene

4.1.2 Chemical characterization of the modified SPCE

The electrochemical characterization of the modified electrode can be described by electron transfer process; the cyclic voltammtry of ferri/ferrocyanide redox couple was investigated. The potential was scanned from -0.6 V to +1.0 V using 1 mM of ferri/ferrocyanide in 0.5 M KCl with scan rate 100 mV s^{-1} at G/PVP/PANI nanocomposite-modified SPCE. The results were compared between G/PVP/PANI nanocomposite-modified and unmodified SPCE. The cyclic voltammogram (Figure 4.3) of ferri/ferrocyanide at G/PVP/PANI nanocomposite-modified SPCE exhibited a well-defined peak shape and the response current obtained from G/PVP/PANI nanocomposite-modified SPCE was distinctly higher than those of the unmodified SPCE. In addition, due to smaller ΔE_p of ferri/ferrocyanide at G/PVP/PANI nanocomposite-modified SPCE which is caused by nanocomposite of graphene, it can be assumed that nanocomposite of graphene act as an accelerator of the electron transfer kinetic of the system.

To study the mass transfer process of G/PVP/PANI nanocomposite-modified SPCE, various scan rates of ferr./ferrocyanide were examined. From the results in Figure 4.4, the anodic and cathodic peak current increased linearly with the

square root of scan rate in the range of 20 to 100 mV s^{-1} (linear regression $r > 0.99$). It was therefore confirmed that the mass transfer process of G/PVP/PANI nanocomposite-modified SPCE was controlled by the diffusion process corresponding with the Randles-Sevcik equation as shown below.

$$I = kn^{3/2}FA D^{1/2} v^{1/2} \quad (4.1)$$

where $k = 2.96 \times 10^5 \text{ (C mol}^{-1} \text{ v}^{1/2})$, n is the number of transferred electron, F is Faraday's constant ($96,485 \text{ C mol}^{-1}$), A is the electrode surface area (cm^2), D is the diffusion coefficient of $[\text{Fe}(\text{CN})_6]^{3,4-}$ which is $6.70 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$, and v is the scan rate (mV s^{-1}) [63].

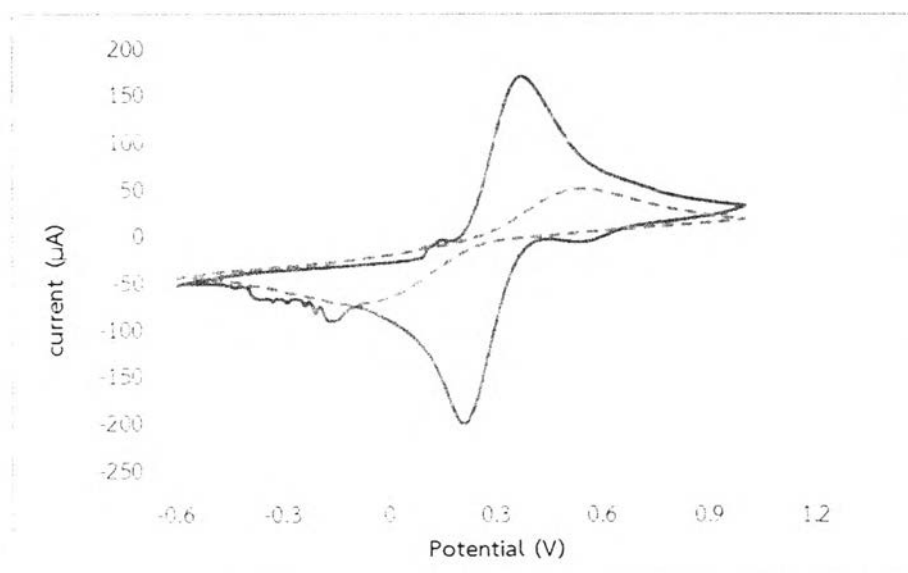


Figure 4.3 Cyclic voltammogram of 1mM ferri/ferrocyanide in 0.5 M KCl at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs Ag/AgCl

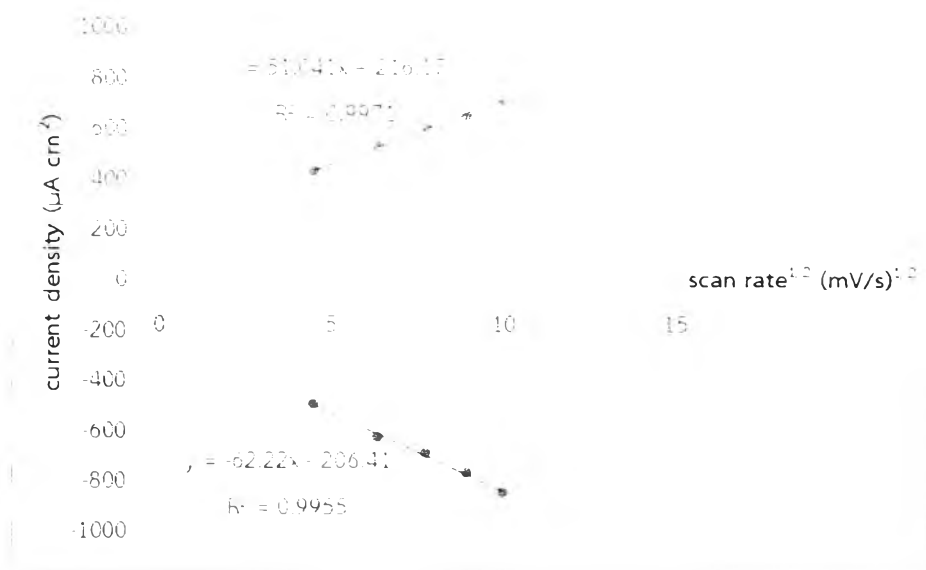


Figure 4.4 The relationship between square root of scan rate and peak current density measured from 20 to 100 mV s^{-1}

4.2 Cyclic voltammetry study

The electrochemical behavior of five parabens was first investigated by cyclic voltammetry (CV). CV was measured by a potentiostat with three-electrode system; a G/PVP/PANI nanocomposite-modified SPCE or unmodified electrode as working electrode, silver/silver chloride (Ag/AgCl) with a salt bridge as reference electrode, and platinum wire as counter electrode. The electrochemical characteristics of five parabens ($10 \mu\text{g mL}^{-1}$) were investigated at G/PVP/PANI nanocomposite-modified SPCE and unmodified SPCE using 0.05 M phosphate buffer (pH 6) as the supporting electrolyte. The cyclic voltammograms of MP, EP, PP, BP and IBP were shown in Figure 4.5, 4.6, 4.7, 4.8 and 4.9, respectively. The results revealed that all five parabens presented the anodic peaks with the irreversible reaction, and the peak currents of each paraben obtained by the modified SPCE were approximately between 3-8 times higher than those using the unmodified electrode. Furthermore, the five parabens were oxidized at a similar anodic peak potential of about +1.0 V vs Ag/AgCl.

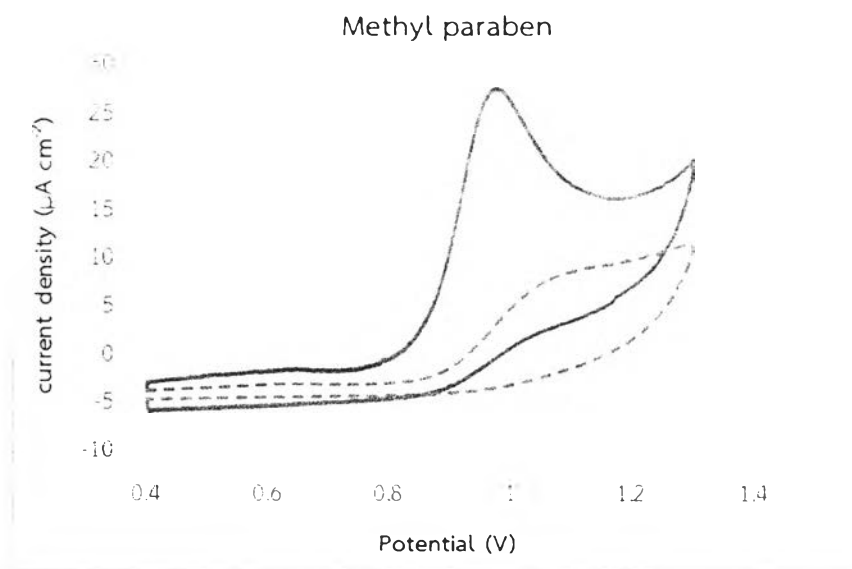


Figure 4.5 Cyclic voltammogram of MP ($10 \mu\text{g mL}^{-1}$) in 0.05 M phosphate buffer (pH 6) at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs. Ag/AgCl with scan rate 100 mV s^{-1}

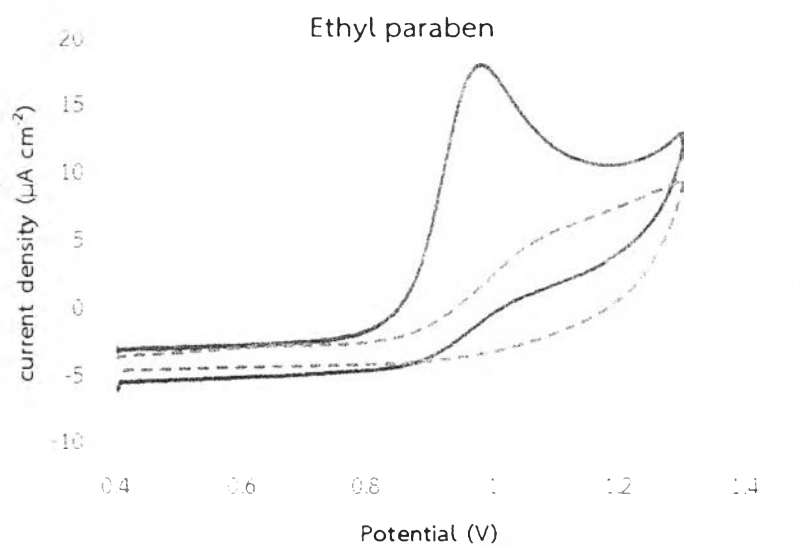


Figure 4.6 Cyclic voltammogram of EP ($10 \mu\text{g mL}^{-1}$) in 0.05 M phosphate buffer (pH 6) at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs. Ag/AgCl with scan rate 100 mV s^{-1}

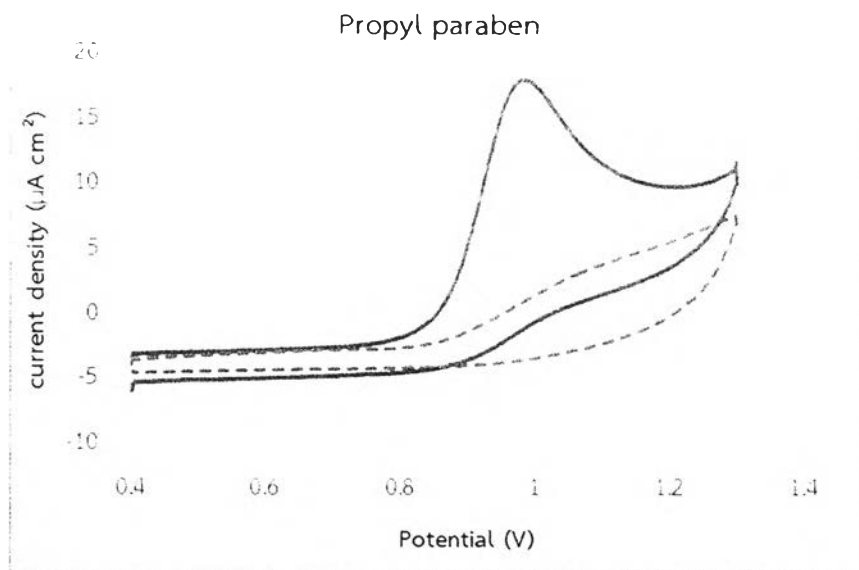


Figure 4.7 Cyclic voltammogram of PP ($10 \mu\text{g mL}^{-1}$) in 0.05 M phosphate buffer (pH 6) at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs. Ag/AgCl with scan rate 100 mV s^{-1}

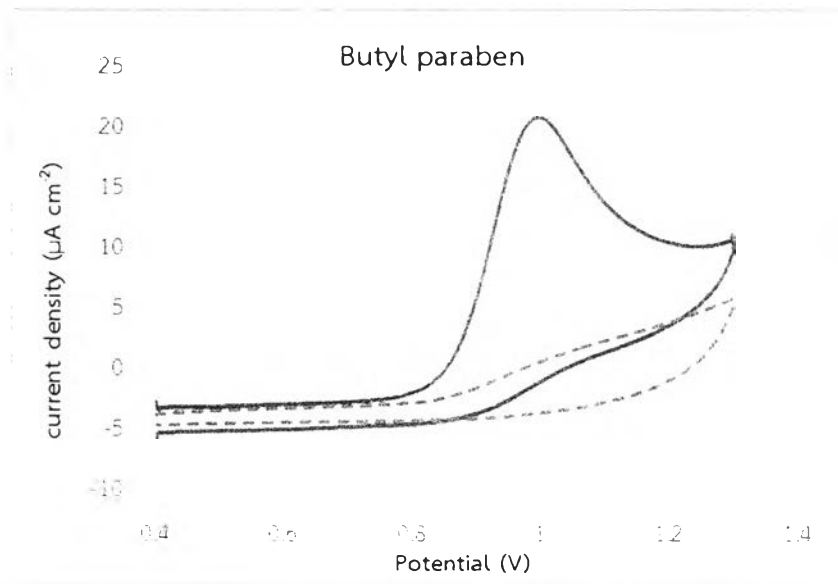


Figure 4.8 Cyclic voltammogram of BP ($10 \mu\text{g mL}^{-1}$) in 0.05 M phosphate buffer (pH 6) at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs. Ag/AgCl with scan rate 100 mV s^{-1}



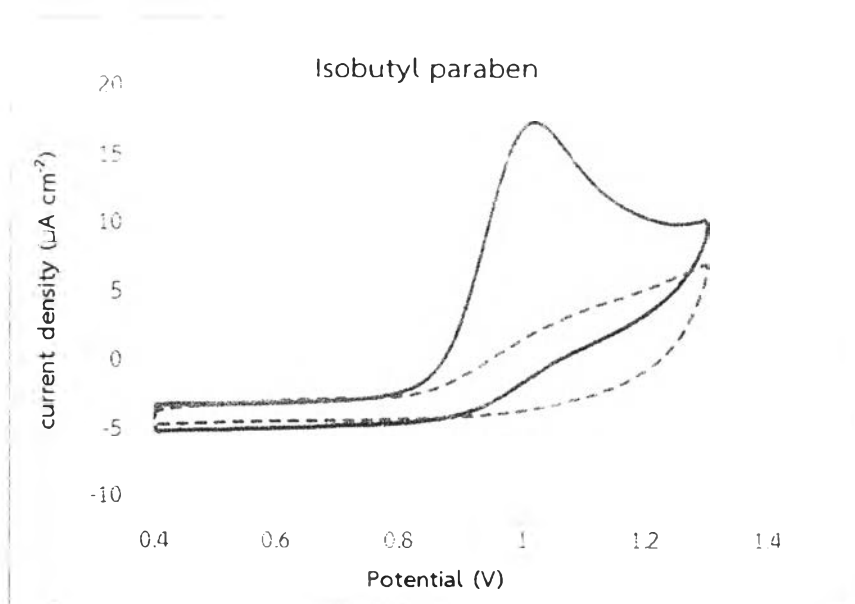


Figure 4.9 Cyclic voltammogram of IBP ($10 \mu\text{g mL}^{-1}$) in 0.05 M phosphate buffer (pH 6) at G/PVP/PANI nanocomposite-modified SPCE (—) and unmodified SPCE (---) vs. Ag/AgCl with scan rate 100 mV s^{-1}

4.3 HPLC-ECD

Firstly, the comparison of unmodified SPCE and G/PVP/PANI nanocomposite-modified SPCE was investigated for the simultaneous determination of five parabens (MP, EP, PP, BP and IBP) using HPLC with 0.05 M phosphate buffer pH 5:acetonitrile (60:40, %v/v) as mobile phase, a flow rate of 1.5 mL min^{-1} and an injection volume of $10 \mu\text{L}$, coupled with amperometric detection with an applied potential of $+1.2 \text{ V}$ vs Ag/AgCl at room temperature ($\sim 25 \text{ }^\circ\text{C}$). According to the chromatograms obtained in Figure 4.10, it was found that the five studied parabens can be completely separated and detected, even BP and IBP which are isomeric compounds. The peak currents obtained from using the G/PVP/PANI nanocomposite-modified SPCE as the working electrode were approximately 2-3 times higher than those of the unmodified SPCE.

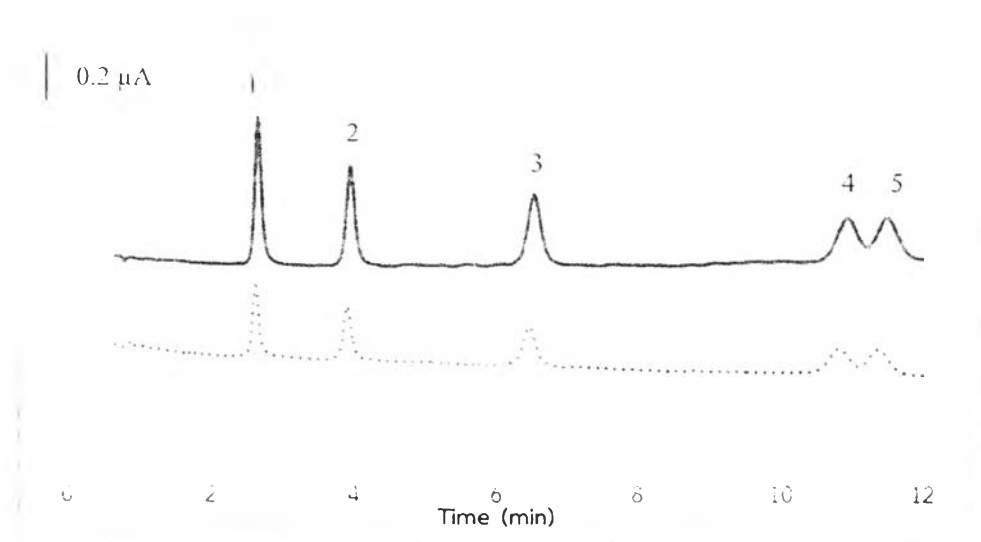


Figure 4.10 Typical chromatograms of standard mixture of $10 \mu\text{g mL}^{-1}$ five parabens, MP (1), EP (2), PP (3), IBP (4) and BP (5), by amperometry using the G/PVP/PANI nanocomposite-modified SPCE (—) and the unmodified SPCE (.....); the mobile phase of 0.05 M phosphate buffer solution (pH 5):acetonitrile (60:40, %v/v), the injection volume of $10 \mu\text{L}$, and the flow rate of 1.5 mL min^{-1} .

4.3.1 Ratio of mobile phase

The mixture of five parabens; MP, EP, PP, IBP and BP was separated by HPLC with C18 packed column in an isocratic system for the simultaneous determination of five parabens. The ratio of mobile phase between 0.05 M phosphate buffer pH 6 and acetonitrile (ACN) and acetonitrile was examined by varying percentages of acetonitrile in the range of 35-55% (%v/v). The chromatograms obtained were shown in Figure 4.11. It was found that the separation time was longer than 15 min when the percentage of acetonitrile was lower than 40%. Faster separation was achieved by increasing the percentage of acetonitrile, however, the peaks of the isomeric compounds of BP and IBP were not resolved when the percentage of acetonitrile was above 45%. Therefore, the ratio between 0.05 M phosphate buffer (pH 6) and acetonitrile of 60:40 (%v/v) was chosen as optimal for the mobile phase for further experiments.

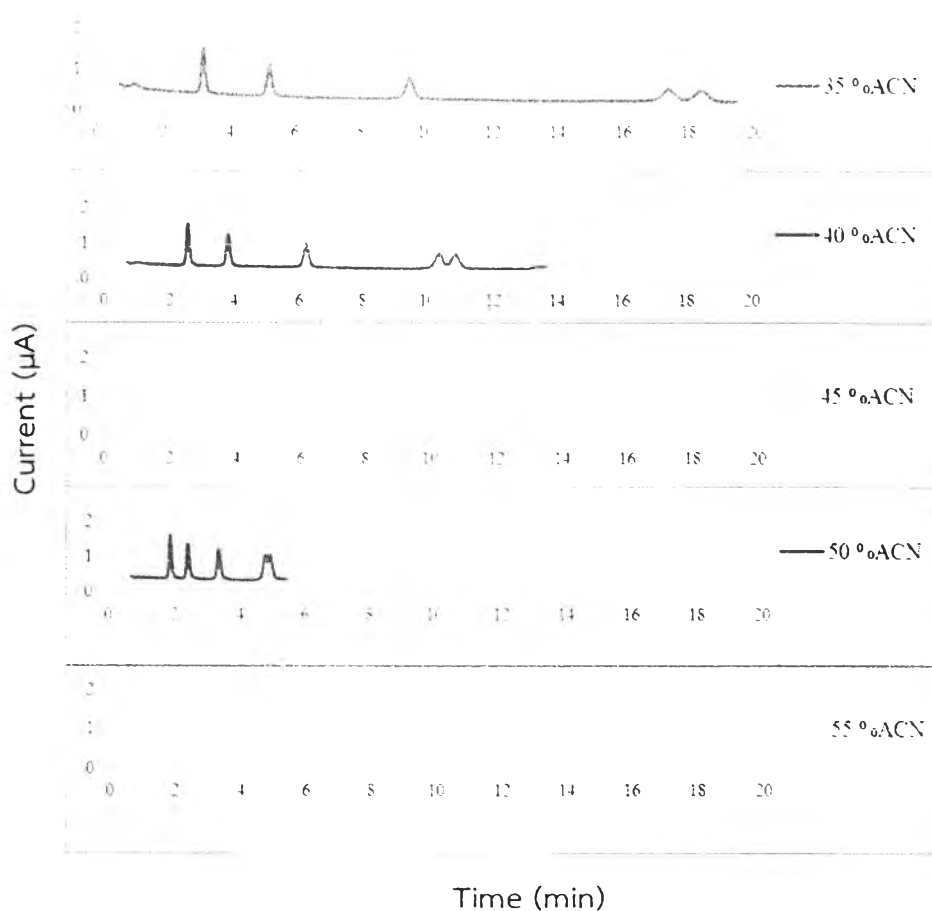


Figure 4.11 HPLC-ECD chromatograms of a standard mixture of five parabens at $10 \mu\text{g mL}^{-1}$ in a mobile phase 0.05 M phosphate buffer solution (pH 6):acetonitrile at various ratios.

4.3.2 The optimization of pH

After the completed separation of parabens in column, the effect of pH was then studied by amperometric detection. The pH values of phosphate buffer solution were studied in the range of 4-8 which were lower than the pK_a (approximately 8) of all parabens. The use of pH higher than pK_a value was not executed as it would adversely affect the silica column C18. Figure 4.12 displayed the relationship between the currents of five parabens and pH. From the obtained result, phosphate buffer solution pH 6 was an appropriate value for paraben

detection at the G/PVP/PANI nanocomposite-modified SPCE. The pH value has an influence to the redox form of paraben that could affect the electrochemical detection.

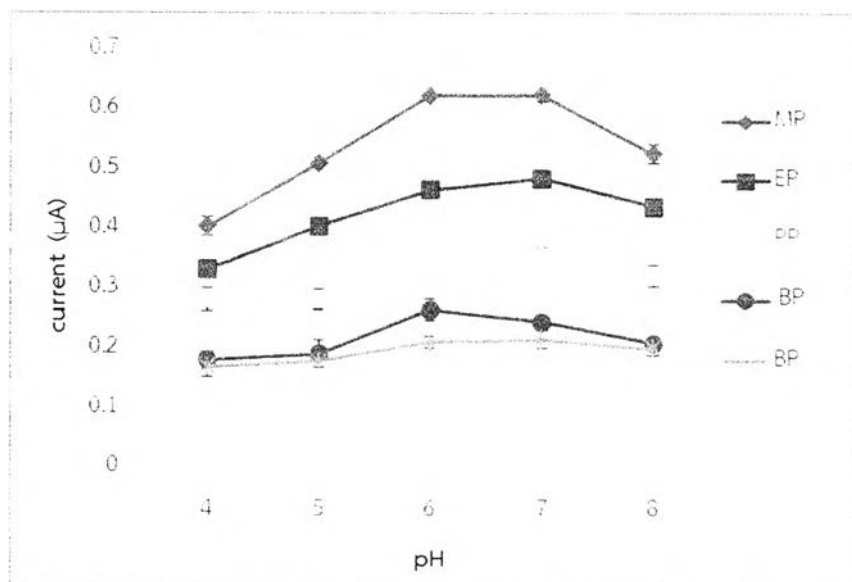


Figure 4.12 The effect of pH of $10 \mu\text{g mL}^{-1}$ parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v)

4.3.3 The Optimization of detection potential

Hydrodynamic voltammetry was utilized for the optimization of applied potential to determine five parabens at G/PVP/PANI nanocomposite-modified SPCE coupled with HPLC. The applied potential was in the range of +1.0 to +1.4 V vs. Ag/AgCl. The ratios of oxidation peak currents and background currents (S/B) of five parabens continually increased when higher potentials were applied; however, the S/B significantly decreased when the potentials above +1.2 V were applied partly due to the increasing background currents (shown in Figure 4.13). Therefore, the detection potential of +1.2 V vs Ag/AgCl was clearly the optimal applied potential and hence was adopted for the simultaneous determination of five parabens with amperometric detection.

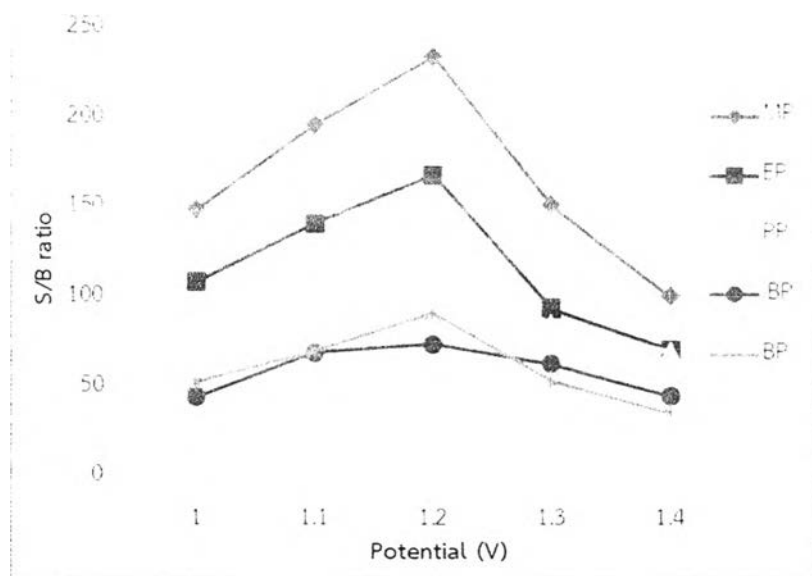


Figure 4.13 Hydrodynamic voltammogram signal-to-background ratios at G/PVP/PANI nanocomposite-modified SPCE of $10 \mu\text{g mL}^{-1}$ parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v)

4.3.4 The Optimization of injection volume

The injection loop of the instrument was selectable in the range of 0.1 to 50 μL . Therefore, the injection volume was investigated. The injection volumes of 10, 20, 30, 40 and 50 μL were studied; the mobile phase was 0.05 M phosphate buffer solution pH 6 and acetonitrile (60:40, %v/v) at the flow rate of 1.5 mL min^{-1} with the amperometric detection at the potential 1.2 V vs. Ag/AgCl. As a result in Figure 4.14, it was found that the current response increased linearly with the larger injection volume. Figure 4.15, displayed the chromatograms comparison between the injection volume of 10 μL and 50 μL ; it was shown that with the injection volume of 50 μL , the chromatogram is still sharp without band broadening and much higher sensitivity was attained. Hence, the injection volume of 50 μL was selected for the following experiments.

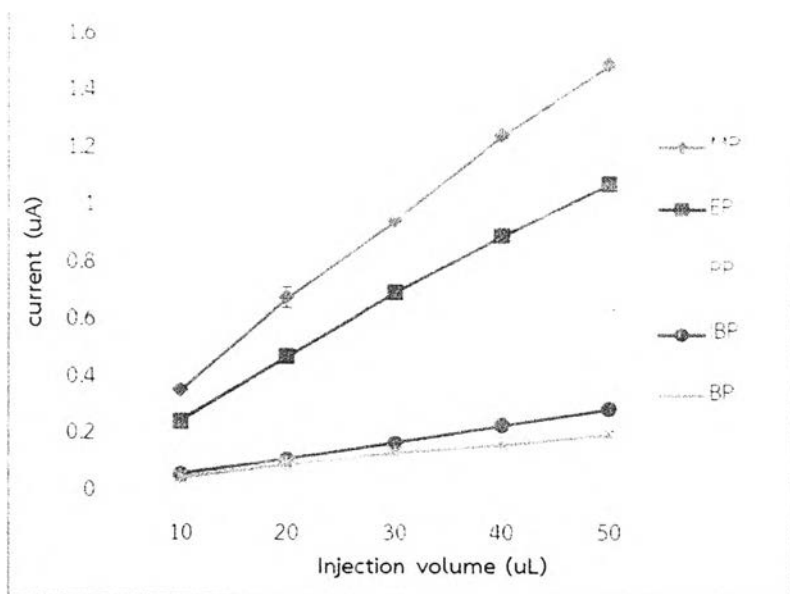


Figure 4.14 The effect of injection volume at G/PVP/PANI nanocomposite-modified SPCE of $10 \mu\text{g mL}^{-1}$ parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v)

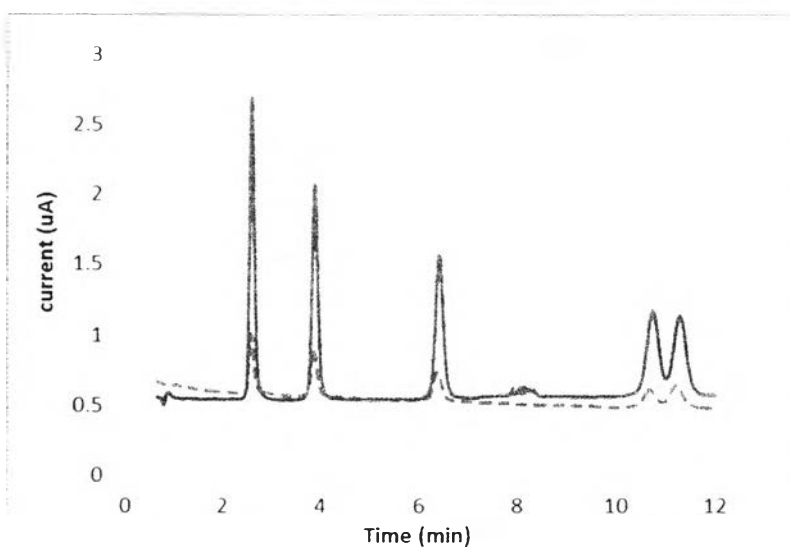


Figure 4.15 The chromatogram of $10 \mu\text{g mL}^{-1}$ parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v) comparing the injection volume between 50 μL (—) and 10 μL (---).

4.3.5 The Optimal conditions for HPLC-ECD

The optimal conditions for simultaneous determination of five parabens at G/PVP/PANI nanocomposite-modified SPCE coupled with HPLC were summarized in Table 4.1.

Table 4.1 The optimal condition for HPLC-ECD

Parameter	Optimal condition
Column	Luna 5 μm C18 column (15 mm \times 4.6 mm i.d.)
Mobile phase	0.05 M phosphate buffer pH 6:ACN (60:40, v/v)
Flow rate	1.5 mL min ⁻¹
Injection volume	50 μL
Temperature	room temperature (~ 25 °C)
Amperometric detection	1.2 V vs. Ag/AgCl
Working electrode	G/PVP/PANI nanocomposite-modified SPCE

4.4 Analytical performance

4.4.1 Calibration curve and Linear range

The calibration curves and linear range of this system were obtained under the optimized conditions. The chromatograms of five parabens at various concentrations were shown in Figure 4.16. The calibration curves were obtained from a plot between peak areas and various concentrations MP, EP, PP, IBP and BP as illustrated in Figure 4.17, 4.18, 4.19, 4.20 and 4.21, respectively. The calibration curves of five parabens were linear in similarly equal range of 0.1 and 30 $\mu\text{g mL}^{-1}$ with the correlation coefficient (R^2) of higher than 0.99 ($n=3$).

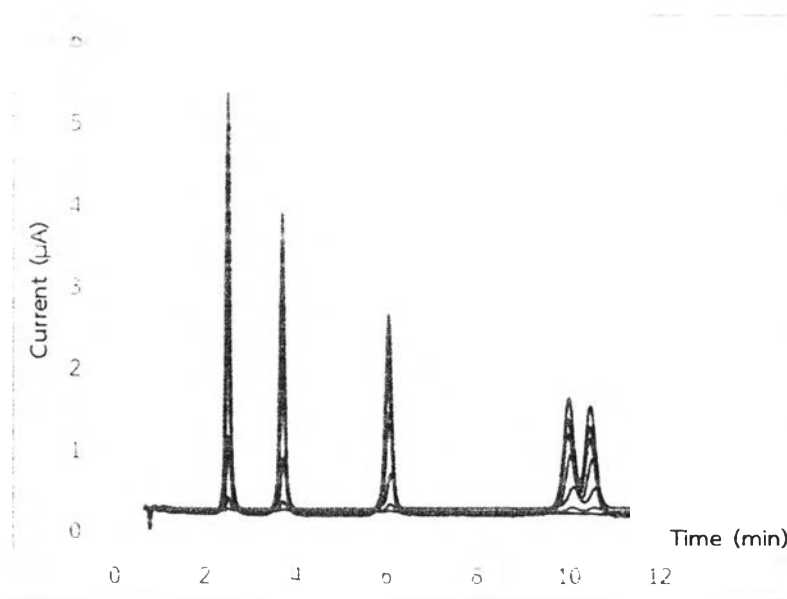


Figure 4.16 the chromatogram of five parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v) at various concentrations in the range of 0.1-30 $\mu\text{g mL}^{-1}$. The detection potential was +1.2 V vs Ag/AgCl using G/PVP/PANI nanocomposite-modified SPCE.

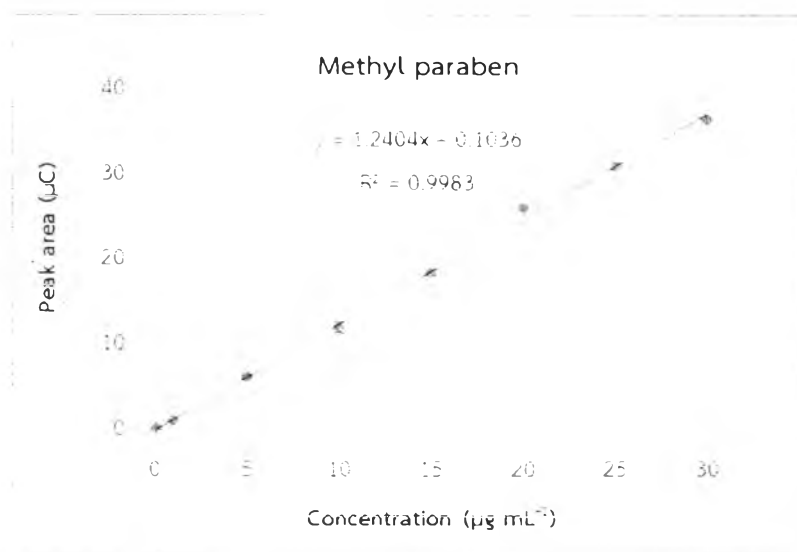


Figure 4.17 The calibration curve of MP by HPLC-ECD using G/PVP/PANI nanocomposite-modified SPCE, concentration in the range of 0.1 to 30 $\mu\text{g mL}^{-2}$.

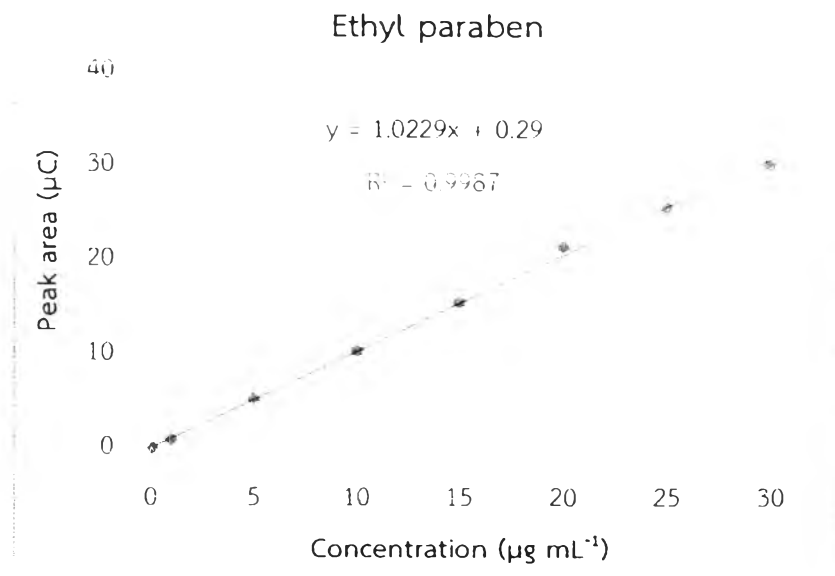


Figure 4.18 The calibration curve of EP by HPLC-ECD using G/PVP/PANI nanocomposite-modified SPCE, concentration in the range of 0.1 to 30 μg mL⁻¹.

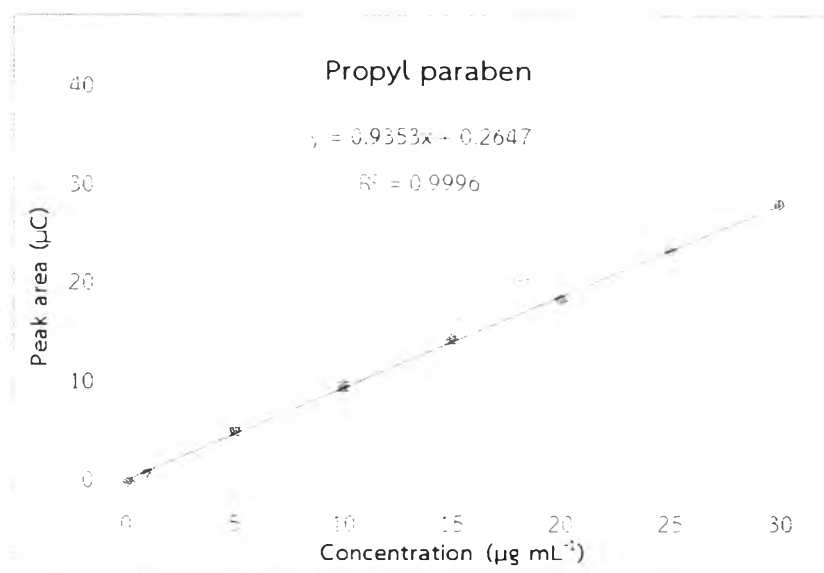


Figure 4.19 The calibration curve of PP by HPLC-ECD using G/PVP/PANI nanocomposite-modified SPCE, concentration in the range of 0.1 to 30 μg mL⁻¹.

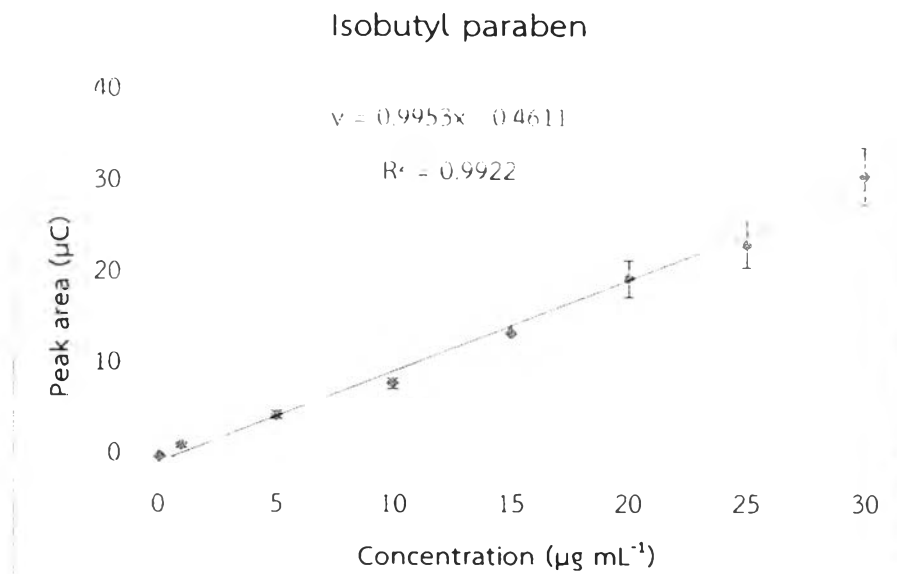


Figure 4.20 The calibration curve of IBP by HPLC-ECD using G/PVP/PANI nanocomposite-modified SPCE, concentration in the range of 0.1 to 30 $\mu\text{g mL}^{-1}$.

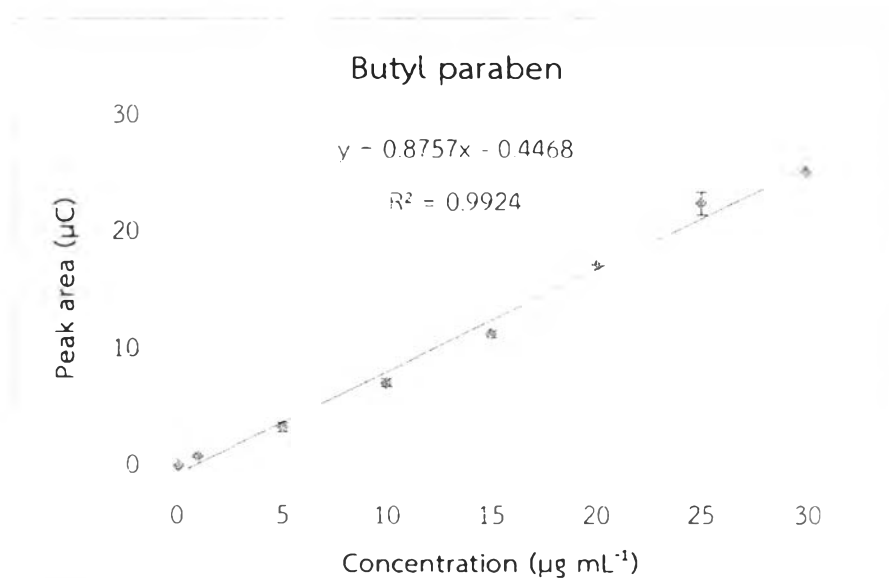


Figure 4.21 The calibration curve of BP by HPLC-ECD using G/PVP/PANI nanocomposite-modified SPCE, concentration in the range of 0.1 to 30 $\mu\text{g mL}^{-1}$.

4.4.2 The limit of detection (LOD) and the limit of quantitation (LOQ)

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from $3S_b/S$ and $10S_b/S$ respectively, where S_b is the standard deviation of blank measurement ($n=10$) and S is the slope of calibration curve. LOD and LOQ of five parabens were found in the range of 0.01 to 0.03 $\mu\text{g mL}^{-1}$ and 0.04 to 0.10 $\mu\text{g mL}^{-1}$, respectively. Summaries of information are listed in Table 4.2.

Table 4.2 Summary of analytical performance of the proposed method (HPLC-ECD) using G/PVP/PANI nanocomposite-modified SPCE for the simultaneous determination of five parabens ($n=3$); linear range, slope and correlation coefficient (R^2), limits of detection (LOD) and limits of quantitation (LOQ).

Analyte	Linear range ($\mu\text{g mL}^{-1}$)	Slope ($\mu\text{C mL } \mu\text{g}^{-1}$)	R^2	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)
MP	0.1-30	1.2404	0.9983	0.01	0.04
EP	0.1-30	1.0229	0.9987	0.01	0.04
PP	0.1-30	0.9353	0.9996	0.01	0.04
IBP	0.1-30	0.9953	0.9922	0.02	0.06
BP	0.1-30	0.8757	0.9924	0.03	0.10

4.4.3 Reproducibility and precision

The intra-day precision of 30 measurements was evaluated to ascertain the stability of this working electrode (shown in Figure 4.22). It was found that the obtained peak areas did not significantly change in repeated measurements with the relative standard deviation (RSD) in the range of 3.3 to 4.7% for all five parabens. In addition, the inter-day precision was also investigated for 3 days using the same electrode. It was found that the sensitivity significantly decreased in the

second and the third day, as compared with the peak area obtained in the first day; as demonstrated in Figure 4.23. Therefore, the replacement of the G/PVP/PANI nanocomposite-modified SPCE on a day-to-day basis was highly recommended. The reproducibility of G/PV/PANI nanocomposite-modified SPCE was also investigated using 10 different electrodes. It was found that the RSD was obtained in the range of 3.42 to 7.2% for five parabens which is acceptable for the AOAC recommended value.

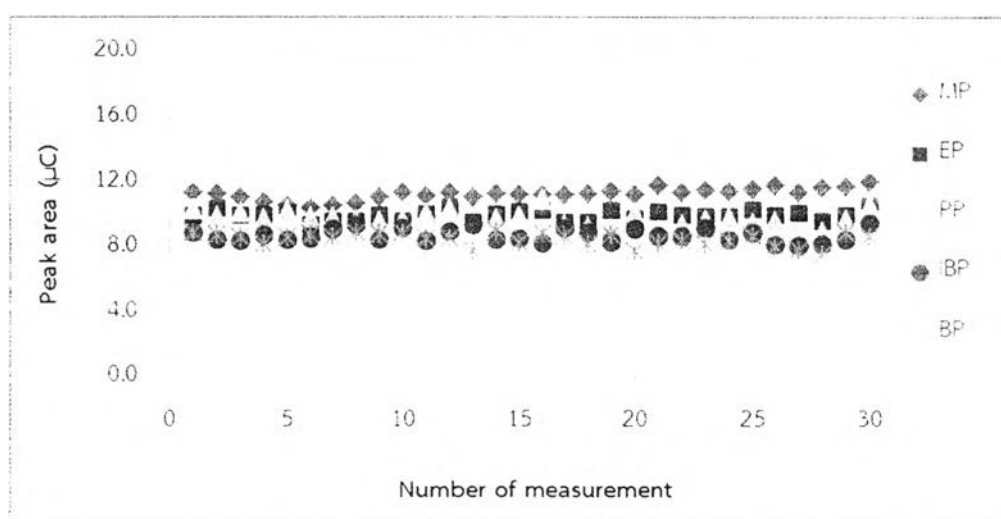


Figure 4.22 The intra-day study of 30 measurements to approve the stability of G/PVP/PANI nanocomposite-modified SPCE; $10 \mu\text{g mL}^{-1}$ parabens in 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v)

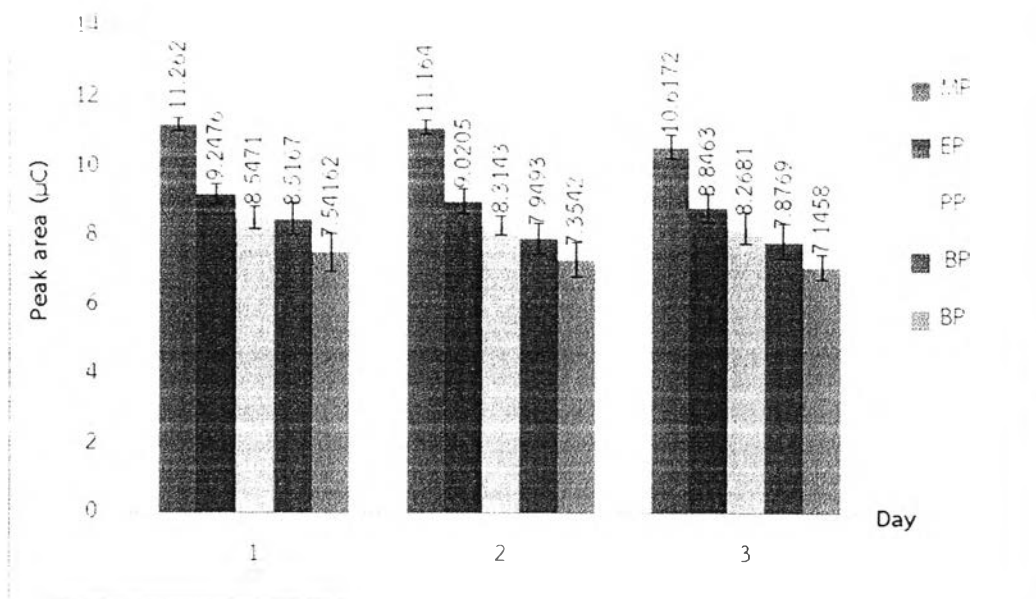


Figure 4.23 The comparison of the peak area from inter-day on the same electrode of G/PVP/PANI nanocomposite-modified SPCE

4.5 Application in real samples

4.5.1 The simultaneous determination of five parabens in real samples

Eventually, the proposed method using the G/PVP/PANI nanocomposite-modified SPCE was applied for the simultaneous detection of five parabens in real samples, i.e. a soft drink and a cosmetic product. The preparation of all samples was done by extraction method using methanol as a solvent. This method was very simple with high recovery. The chromatograms of real samples were shown in Figure 4.24. The chromatogram in Figure 4.24 (A) of the soft drink sample apparently showed no sign of any paraben. Therefore, the soft drink sample was spiked with parabens at a concentration of $5 \mu\text{g mL}^{-1}$ to determine the recovery by the standard addition method and the typical chromatogram of spiked samples is shown in Figure 4.24 (B). For cosmetic product, the chromatogram in Figure 4.24 (C) reveals peaks of MP and PP at the retention times of 2.6 and 6.4 min, respectively. The remaining peak at retention time of 5.7 min is an unknown electrochemically active specie in the cosmetic sample. The summarized result of the simultaneous

determination of five parabens in real samples by the proposed method (HPLC-ECD) using G/PVP/PANI nanocomposite-modified SPCE is shown in Table 4.3.

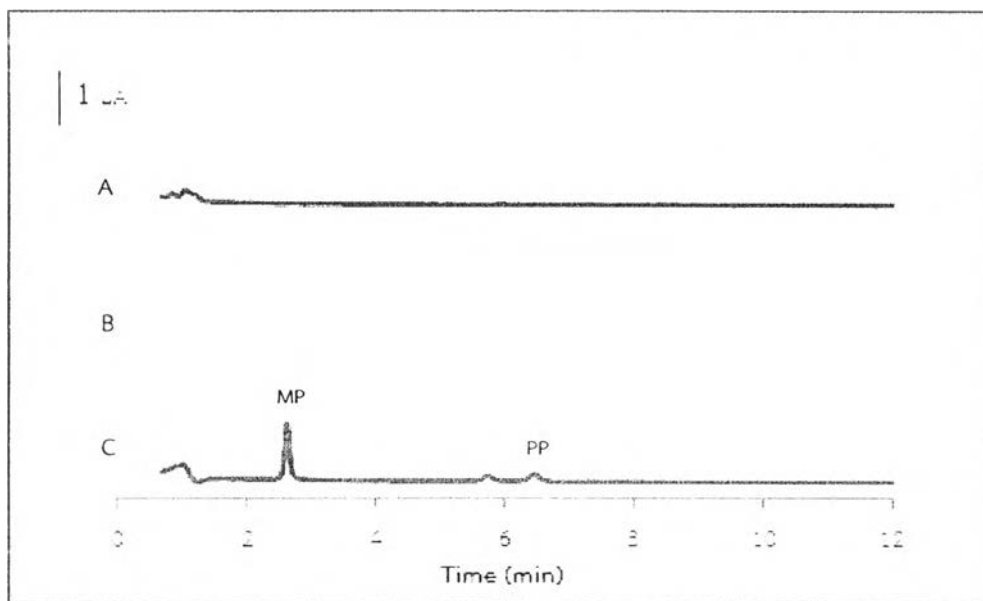


Figure 4.24 Chromatograms of five parabens in real samples, a soft drink sample (A), a soft drink sample was spiked with parabens at a concentration of $5 \mu\text{g mL}^{-1}$ (B) and a cosmetic product sample (C), in the mobile phase of 0.05 M phosphate buffer solution (pH 6):acetonitrile (60:40, %v/v).

Table 4.3 The simultaneous determination of five parabens in real samples (n=3)

Sample	Analyte	concentration of parabens found ($\mu\text{g mL}^{-1}$)	
		ECD	UV
soft drink	MP	ND	ND
	EP	ND	ND
	PP	ND	ND
	IBP	ND	ND
	BP	ND	ND
cosmetic	MP	498.67 \pm 16.62	504.00 \pm 5.25
	EP	-	-
	PP	134.60 \pm 16.62	134.75 \pm 11.81
	IBP	-	-
	BP	-	-

4.5.2 Accuracy

The accuracy of the developed method was determined from the recovery and also by comparison with the traditional detection method, HPLC-UV. Since there is no sign of any paraben in the soft drink sample, parabens were spiked into the soft drink sample at a concentration of $5 \mu\text{g mL}^{-1}$ to determine the percentage of recovery by the standard addition method. The recoveries were in the range of 90.4-105.0% for five parabens which is an acceptable value under AOAC recommendation. For cosmetic product, the peaks of MP and PP were evident. The results of this present method (HPLC-ECD) using the G/PVPV/PANI nanocomposite-modified SPCE, were then compared to the traditional method, HPLC-UV. The

student's t-test was used to evaluate the two different methods. The calculated t-values were smaller than the t-critical value, indicating that there is no significant difference between the proposed method and the HPLC-UV method at a confidence level of 95%. The summary of real samples analyses is shown in Table 4.4





Table 4.4 Summarized results of the simultaneous determination of five parabens in real samples by the proposed method (HPLC-ECD) using the G/PVP/PANI nanocomposite-modified SPCE, compared with the traditional standard method (HPLC-UV).

Sample	Analyte	Spiked ($\mu\text{g mL}^{-1}$)	found ($\mu\text{g mL}^{-1}$)		%Recovery		t-value ($t_{\text{critical}} = 2.3060$)
			ECD	UV	ECD	UV	
soft drink	MP	-	ND	ND	-	-	-
	EP	-	ND	ND	-	-	-
	PP	-	ND	ND	-	-	-
	IBP	-	ND	ND	-	-	-
	BP	-	ND	ND	-	-	-
soft drink	MP	5	4.52 \pm 0.31	4.78 \pm 0.28	90.4	95.6	1.39
	EP	5	5.10 \pm 0.39	4.82 \pm 0.28	102.0	96.4	1.30
	PP	5	5.13 \pm 0.32	4.87 \pm 0.27	102.6	97.4	1.39
	IBP	5	5.25 \pm 0.32	4.91 \pm 0.24	105.0	98.2	1.90
	BP	5	4.92 \pm 0.42	4.90 \pm 0.25	98.4	98.0	0.09
cosmetic	MP	-	498.67 \pm 16.62	504.00 \pm 5.25	-	-	0.68
	EP	-	-	-	-	-	-
	PP	-	134.60 \pm 16.62	134.75 \pm 11.81	-	-	0.02
	IBP	-	-	-	-	-	-
	BP	-	-	-	-	-	-