CHAPTER 3

RESULTS AND DISCUSSION

3.1 EC-SPR measurement for electropolymerization of 2ABA

The P2ABA thin film was grown on the working electrode by electropolymerization of 50 mM 2ABA monomer in 0.5 M H_2SO_4 by cycling the potential between –0.2 and 0.1 V vs. Ag/AgCl for 10 cycles at a scan rate of 20 mV/s. During cyclic voltammetry scan, the first oxidation peak was observed at about 0.85 V corresponds to the oxidation of 2ABA monomer to form P2ABA film as shown in Figure 3.1. The dedoping peak at about 0.4 V in the cathodic scan and doping peak at about 0.5 V in the anodic scan of the second cycle correspond to the electron transfer to formation of redox couple during the oxidation of P2ABA on electrochemical sensor. The SPR angular curves taken by scanning an incident angle range of deionized water before and after electropolymerization are shown in Figure 3.2. The SPR curve was shifted to the higher dip angle after electropolymerization indicating the P2ABA was deposited on the gold electrode [99]. The thickness of the film was calculated by Fresnel calculation (Winspall software version 3.02) and by curve fitting to obtained SPR curves. The thickness of deposited P2ABA film was estimated to be 10 nm.

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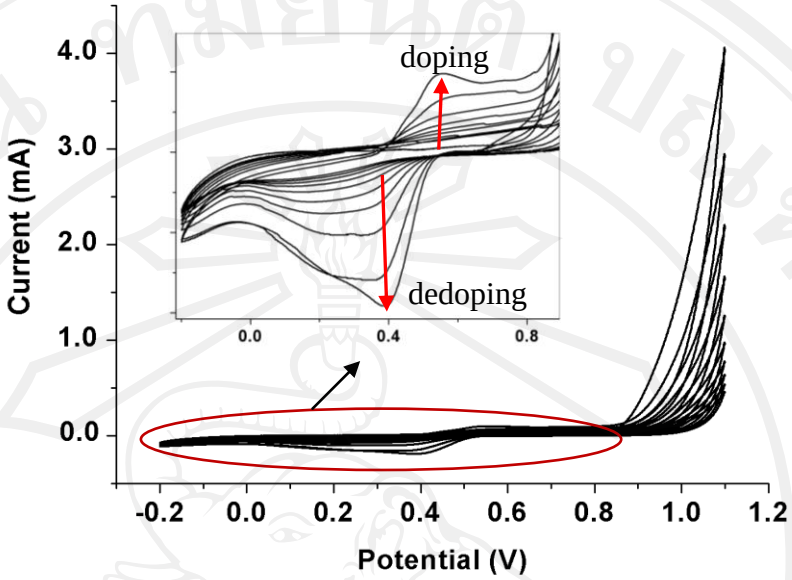

Figure 3.1 Cyclic voltammograms of 50 mM 2ABA monomer on gold electrode by cycling the potential between –0.2 and 0.1 V vs. Ag/AgCl for 10 cycles at a scan rate of 20 mV/s to form P2ABA film.

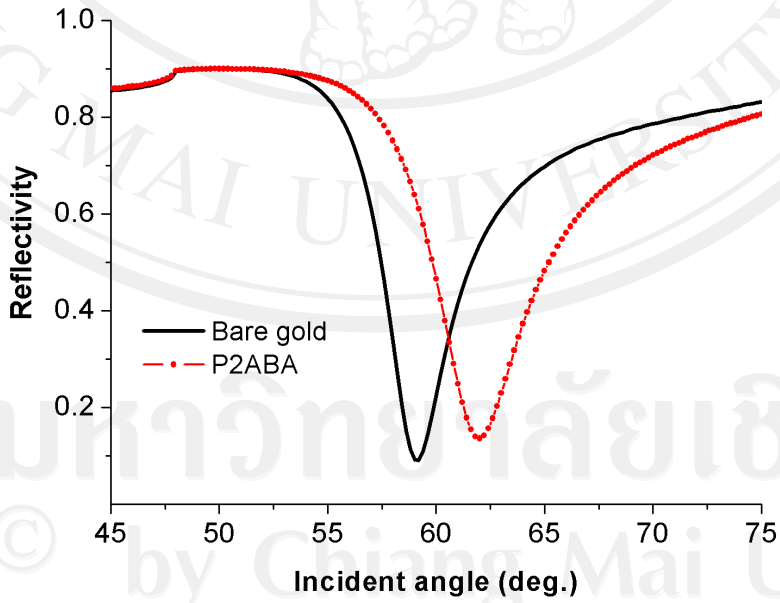

Figure 3.2 Angular-reflectivity curves before and after electropolymerization of P2ABA thin film on gold-coated high reflective index glass substrate.

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3.2 Detection of adrenaline on the P2ABA thin film

Polyaniline (PANI) film was deprotonation and loss in electroactivity at pH higher than 4 [24]. Electroactivity of P2ABA was studied in PBS supporting electrolyte solution (pH 7.4) by cyclic voltammetry, which indicated that the electrochemical signal can be enhanced event of specific reaction with adrenaline [23, 100]. The reaction of adrenaline with the P2ABA film was shown in Figure 3.3.

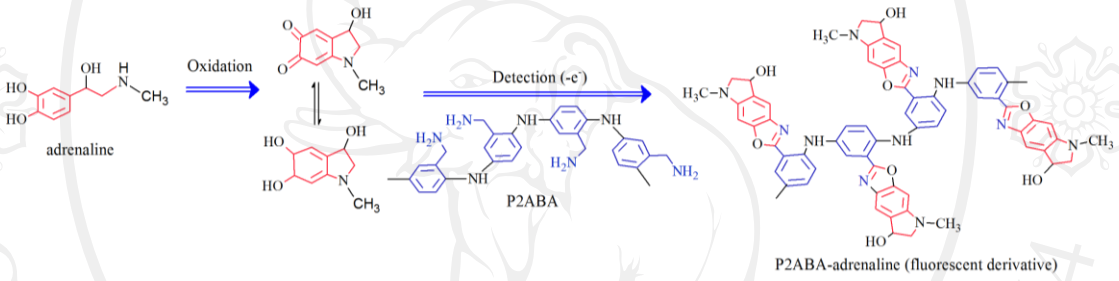

Figure 3.3 The specific reaction of adrenaline to P2ABA film.

The SPR responses of P2ABA and PANI thin film on the detection of adrenaline as observed in the reflectivity change at a constant potential of 0.5 V (doped-state of P2ABA film) and an open circuit are shown in Figure 3.4 (a). SPR reflectivity response on the injection 1 mM adrenaline into P2ABA and PANI thin film at a constant applied potential of 0.5 V, which corresponds to the doped-state of the P2ABA and PANI film. The SPR reflectivity change in PANI was smaller than the reflectivity in P2ABA because of PANI has only physical adsorption. After completion of the reaction, the reflectivity of PANI returns to the original because of non-specific adsorption and the reflectivity of P2ABA continue to increase because of the specific P2ABA–adrenaline reaction can occur at an open circuit Figure 3.4 (b) confirm the reaction after completion of the reaction after end constant applied

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potential of 0.5 V. Figure 3.5 shows the SPR responses during the detection of adrenaline in comparison with UA and AA of P2ABA film at a constant applied potential of 0.5 V. The reflectivity change in adrenaline was obviously higher than those of the UA and AA. The SPR reflectivity change was observed after injection of 1 mM adrenaline at an applied potential of 0.5 V. SPR reflectivity increase rapidly and gradually increase due to the specific adsorption and physical adsorption between adrenaline with benzylamine in P2ABA structure [88, 101].

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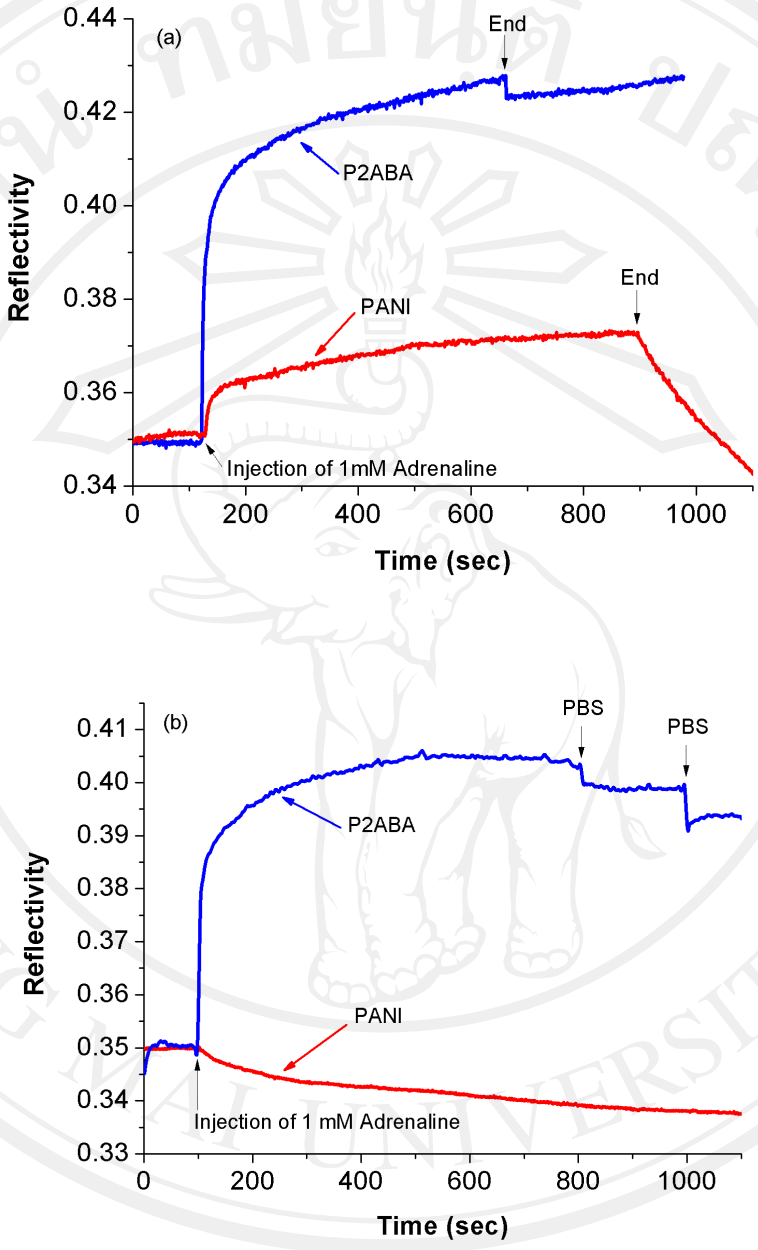

Figure 3.4 SPR reflectivity responses upon injection of 1 mM adrenaline into P2ABA and PANI thin film at (a) constant potentials of 0.5 V and (b) an open circuit potential.

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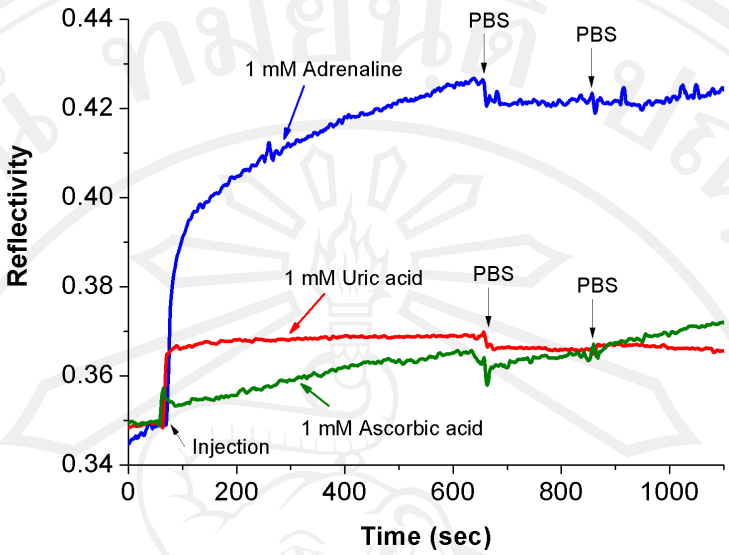

Figure 3.5 SPR reflectivity response after injection 1 mM each of adrenaline, UA and AA into P2ABA thin film at a constant applied potential of 0.5 V.

EC-SPR response of adrenaline in the presence of UA and AA was also studied, the current and reflectivity changes in the UA and AA were observed to be smaller than that of adrenaline because UA and AA did not has a catechol group in the structure to react with benzylamine to form fluorescence derivative structure [102]. Furthermore, the experiment at –0.2 V and open circuit (dedoped and neutral state range of P2ABA in PBS solution) were found that the selectivity of adrenaline detection over UA and AA were increased. Figure 3.6 (a) shows the reflectivity changes after injection of 1 mM adrenaline in the UA and AA at –0.2 V. Figure 3.6 (b) shows the good efficiency of P2ABA thin film during the measurement 1 mM each of adrenaline, AA and UA in PBS solution at an open circuit. Since adrenaline easily oxidizes to react with benzylamine site and offered the electron to P2ABA, the P2ABA becomes the dedoped state [6, 23]. Moreover, the P2ABA film has an amino-

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functionalized group, which can attract the negative ion from adrenaline [6]. Adrenaline cannot be oxidized with P2ABA at −0.2 V. Therefore, the reflectivity change is much less than that at 0.5 V, indicating that only a small amount of adrenaline can react with P2ABA at −0.2 V (Figure 3.7). It should be noted that the injected adrenaline is a mixture of both oxidized and non-oxidized forms. Because there is virtually no change upon PBS rinsing after the adsorption of adrenalin, it again supports the notion that the adsorption is due to a specific chemical reaction. Therefore, EC-SPR response of adrenaline in the presence of UA and AA at –0.2 V and an open circuit was observed in terms of better selectivity than that of 0.5 V.

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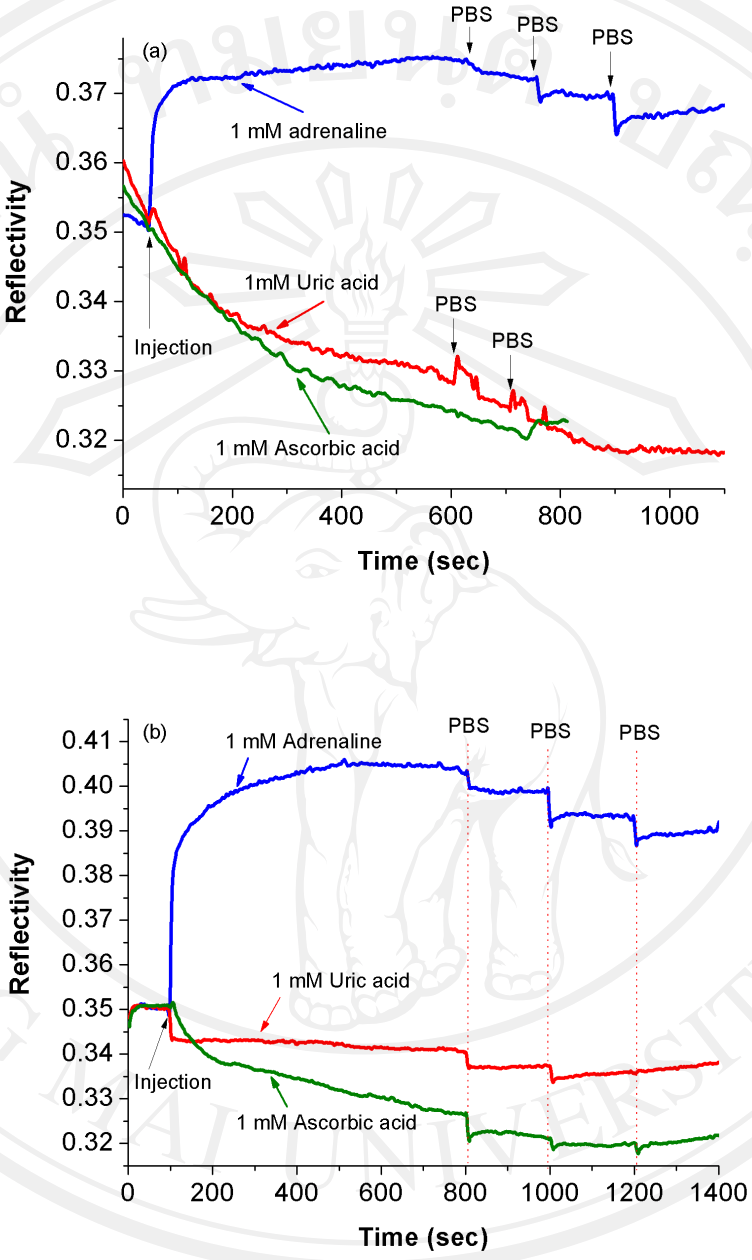

Figure 3.6 The SPR reflectivity result of P2ABA film after reaction with 1 mM each of adrenaline, UA and AA at constant applied potentials of (a) –0.2 V and (b) an open circuit potential.

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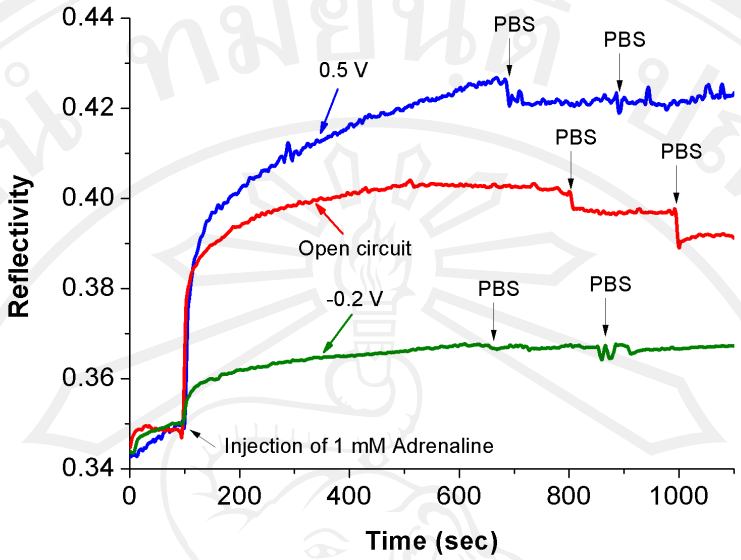

Figure 3.7 SPR reflectivity responses upon injection of 1 mM adrenaline into P2ABA thin film at −0.2, open circuit potential, and 0.5 V.

Figure 3.8 shows plots of the changes in reflectivity as a function of adrenaline concentration was observed at open circuit potential. In these plots, the relationship is linear. The Langmuir-Freundlich model instead of a simple Langmuir model was used for fitting the kinetic curves [107, 108], as the binding reaction of benzylamine with adrenaline occurs not only at the surface but also inside the P2ABA film. The detection limit of SPR reflectivity response upon injection of adrenaline into P2ABA thin film was determined to be 10 pM.

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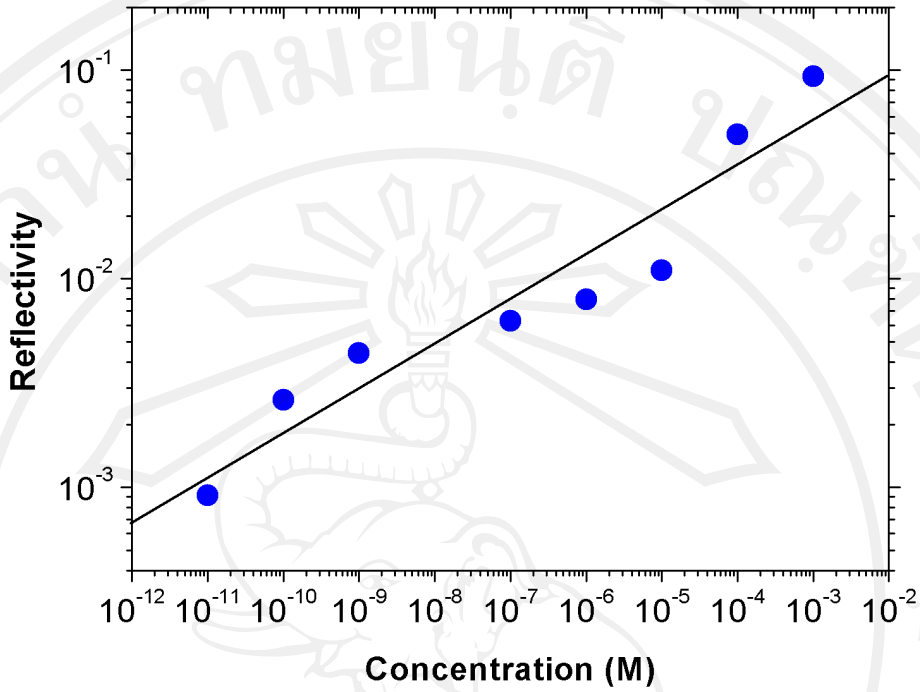

Figure 3.8 Calibrated double-logarithmic plots of the change of the reflectivity as a function of adrenaline concentration.

3.3 QCM-D measurement

To study the reaction of adrenaline with the P2ABA thin film, QCM-D of P2ABA thin films was studied at an open circuit. Figure 3.9 shows the frequency and dissipation change of 15 MHz quartz crystal microbalance upon injection of 1 mM, 100 µM, 10 µM, and 1 µM adrenaline, the frequency rapidly decreased when injection of adrenaline and gradually decreased, which suggested that the specific reaction from benzylamine site in P2ABA with adrenaline was a physical adsorption.

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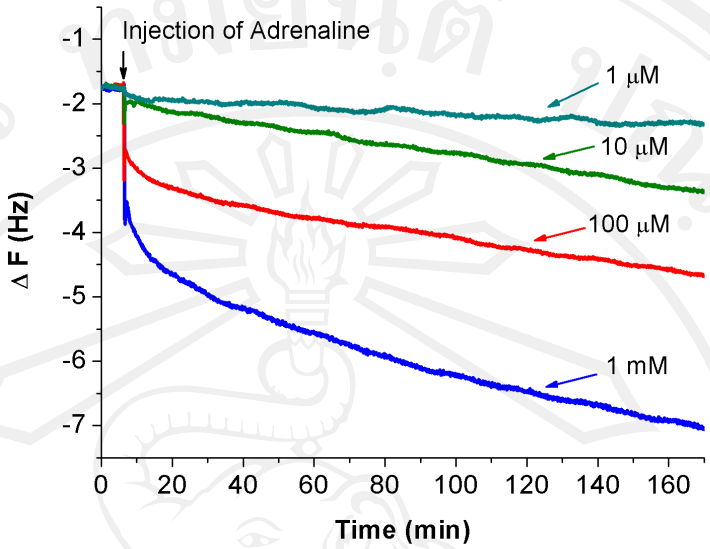

Figure 3.9 QCM-D response upon injection of 1 mM, 100 µM, 10 µM, and 1 µM adrenaline into P2ABA thin film at an open circuit potential.

The mass change was calculated by Sauerbrey equation [103]:

$$\Delta m = -C\Delta f \qquad 3.1$$

where Δm is = mass change, C is = 17.7 ng $cm^{-2}s^{-1}$ and Δf is = frequency change (n is over tone number = 1) . The mass change of 93.0, 53.0, 28.7 and 10.5 ng cm^{-2} can be obtained for 1 mM, 100 µM, 10 µM, and 1 µM adrenaline, respectively. The experimental detection limit was determined to be 100 pM at an open circuit potential. The mass changes after injection of UA (mass change 10 ng cm^2) and AA (mass not change) were observed to be smaller than the mass change in adrenaline due to non-specific reaction with P2ABA thin film [6] as shown in Figure 3.10 and support the SPR result at an open circuit potential.

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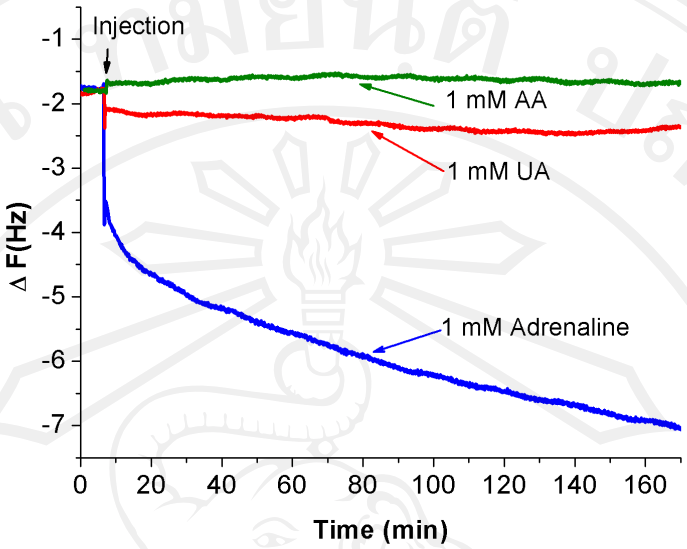

Figure 3.10 QCM-D response upon injection of 1 mM adrenaline into P2ABA thin film compared with UA and AA at an open circuit potential**.**

3.4 UV-vis absorption properties of P2ABA thin film before and after adsorption of adrenaline

UV-vis absorption spectra of each material in PBS solution and after injection of 1 mM each of adrenaline, UA and AA. The UV-vis spectrum of P2ABA thin film without the reaction with 1 mM adrenaline, UA and AA was obtained after applying the constant potential of 0.5 V for 20 min in PBS solution (pH 7.4) as shown in Figure 3.11 (a) comparing with the UV-vis spectrum of P2ABA thin film after the reaction with 1 mM adrenaline, UA and AA at 0.5 V for 20 min as shown in Figure 3.11 (b).

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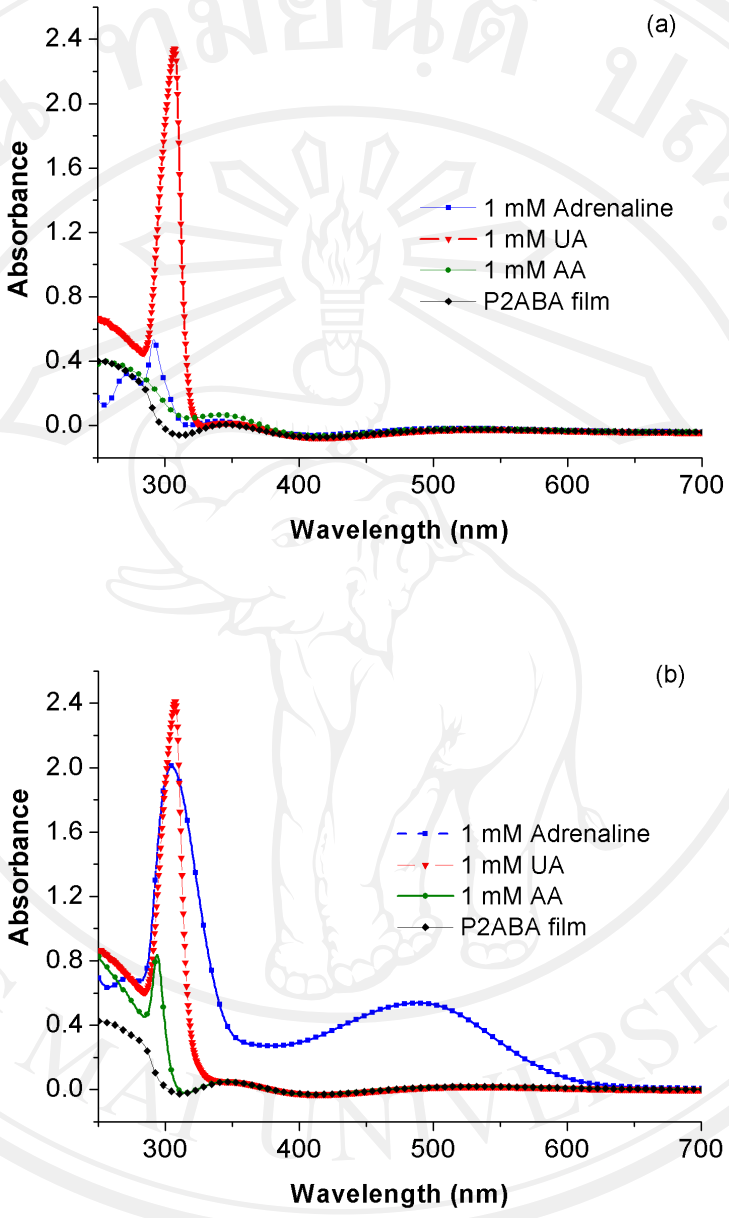

Figure 3.11 (a) UV-vis spectrum of each material in PBS solution and (b) UV-vis spectrum of P2ABA after the reaction with 1mM each of adrenaline, UA and AA at a constant applied potential of 0.5 V.

The new peak appeared at 490 nm after injection of 1mM adrenaline should be due to the specific reaction with benzylamine site in P2ABA structure. The maximum

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peak at 301 nm was a main peak of adrenochrome [104]. A mechanism for this reaction to produce fluorescent derivative, it may possible due to the P2ABA thin film was remain in the doped state in the PBS solution and oxidized adrenaline by accepting the electron from adrenaline, then the P2ABA become the dedoped state [6].

3.5 Atomic force microscopy (AFM) analysis

The AFM is a very high-resolution scanning probe microscopes, which demonstrated resolution in fractions of a nanometer, more than 1000 times better resolution than the optical diffraction limit. AFM was used to examine the specific reaction, the surface topography of binding reaction of adrenaline to P2ABA film at a several applied constant potentials as shown in Figure 3.12. The roughness of P2ABA film (7.617 nm) for the binding reaction of adrenaline at a constant potentials of –0.2 V, open circuit and 0.5 V were 4.106, 2.111 and 1.448 nm, respectively. The roughness decreased with increasing the constant applied potential, suggesting that P2ABA-surface has a rough topography and space filling the specific reaction site. P2ABA film became smooth surface after the binding reaction with adrenaline. This result coresponds with the SPR reflectivity upon injection of 1 mM adrenaline into P2ABA film at a different applied constant potential in Figure 3.7.

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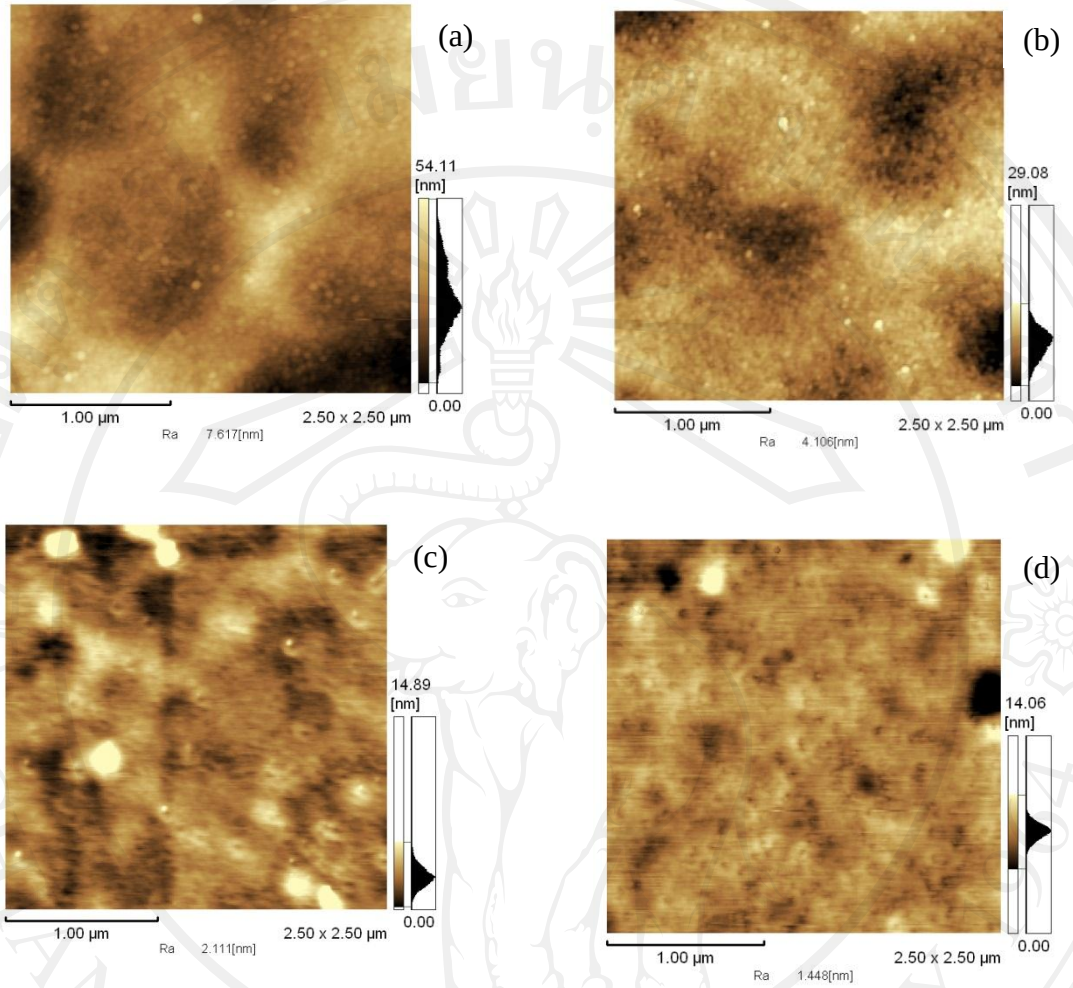

Figure 3.12 AFM images of P2ABA film (a) after binding reaction with adrenaline at constant applied potentials of (b) –0.2 V, (c) open circuit and (d) 0.5 V.

3.6 Fourier transforms infrared spectroscopy attenuated total reflectance (FTIR/ATR) analysis

FTIR/ATR is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. The FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. FTIR technique has made

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dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared) and opened up new applications of infrared spectroscopy. The P2ABA film was electropolymerization on the gold-coated high reflective index glass substrate to study the specific reaction. Figure 3.13 shows the FTIR/ATR spectra of bare gold and P2ABA film before the specific reaction. The board adsorption at 3300 cm^{-1} indicates the presence of –OH group. The absorption band in the 3000–2800 cm^{-1} region indicates the $–NH_2$ group. After the reaction of P2ABA with 1mM adrenaline, the adsorption at 3300 cm^{-1} and 3000–2800 cm^{-1} region disappeared. This suggests that the reaction of –OH group in adrenaline structure with the benzylamine structure in P2ABA, as shown in Figure 3.14. The adsorption spectra at 1710 cm^{-1} region disappeared after reaction with adrenaline, indicating that adrenaline was oxidized to adrenochrome and give electron to P2ABA. The C=O bond to form fluorescent derivative (see the reaction at Figure 3.3) and P2ABA became de-doped state. The detail of the specific reaction was waiting for more reference to confirm the reaction between adrenaline with P2ABA film.

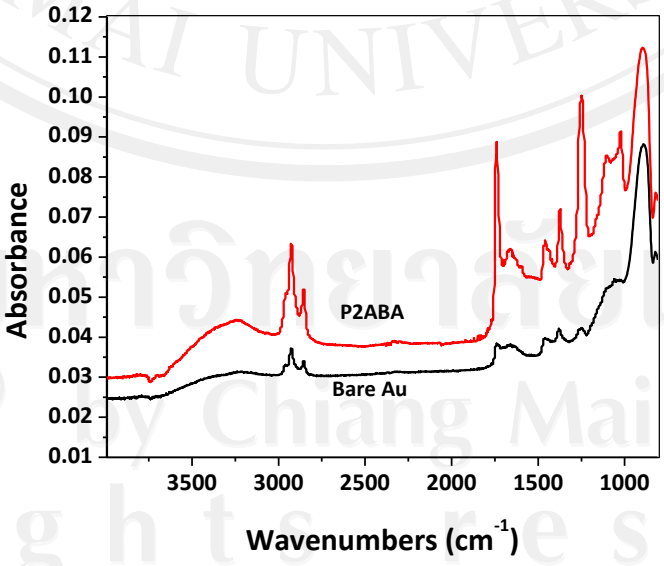

Figure 3.13 FTIR/ATR spectra of bare gold and P2ABA thin film.

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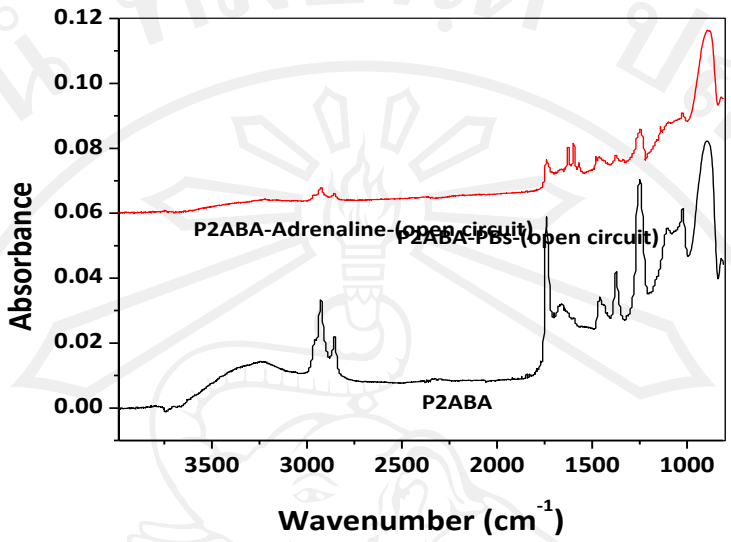

Figure 3.14 FTIR/ATR spectra of P2ABA thin film after the reaction with 1 mM adrenaline at an open circuit potential.

3.7 EC-SPR spectroscopy measurement of P2ABA/SWNTs composites thin film formation

Electropolymerization of 2ABA on the gold-coated high reflective index glass substrate was performed by cycling the potential between –0.2 and 1.1 V for 10 cycles at a scan rate 20 mV/s. During cyclic voltammetry scan, the first oxidation peak at about 0.85 V which is corresponding to the oxidation of monomer and P2ABA film. After the first oxidation, the dedoping peak at about 0.4 V in the cathodic scan and the doping peak at about 0.55 V in the anodic scan of the second cycle correspond to the electron transfer to the formation of redox couple during the oxidation of P2ABA on electrochemical sensor. SPR curves in Figure 3.15 were taken by scanning an incident angle range of deionized water before and after electropolymerization of 2ABA monomer. The SPR curves were shifted to the higher dip angle after electro-

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polymerization indicating that P2ABA was deposited on to gold film electrode. The suspension of 0.01%wt of carboxylated SWNTs in DI water was optimum for assembled on the P2ABA. The SWNTs can be dispersed in water without surfactants because incorporate the carboxylic group for their hydrophilic [105]. The SPR dip angle was shifted to a higher angle corresponding to the –NH–CO covalent bonding interactions between the –COOH in SWNTs structure and $–NH_2$ in P2ABA structure [106]. Figure 3.16 shows the cyclic voltammetry of P2ABA and P2ABA/SWCNT composite thin film in the PBS solution (pH 7.4) by potential cycling between –0.2 and 0.9 V at a scan rate of 20 mV/s for 2 cycles. The cyclic voltammetry measurement result, the redox current of P2ABA/SWNTs composite thin film was higher than P2ABA thin film because P2ABA/SWNTs composite thin film has more charge capacitance. The cyclic voltammetry at the second scan decrease because electroactivity of P2ABA can be shifted to neutral pH by doping P2ABA with anion [6, 9, 23, 41].

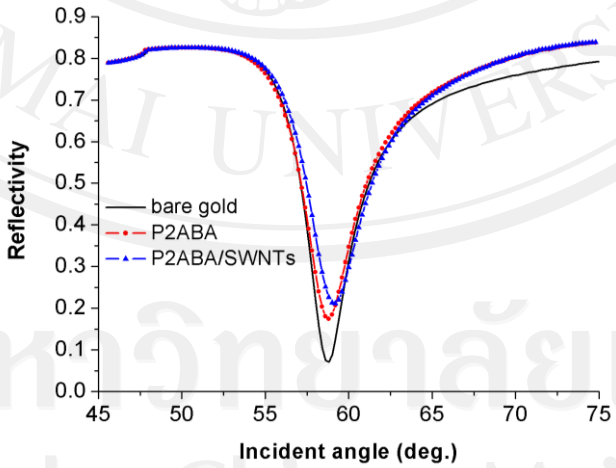

Figure 3.15 SPR angular reflectivity curves of bare gold, P2ABA (after electro-polymerization) and P2ABA/SWNTs composites thin film (after carboxylated SWNTs assembled on the P2ABA thin film).

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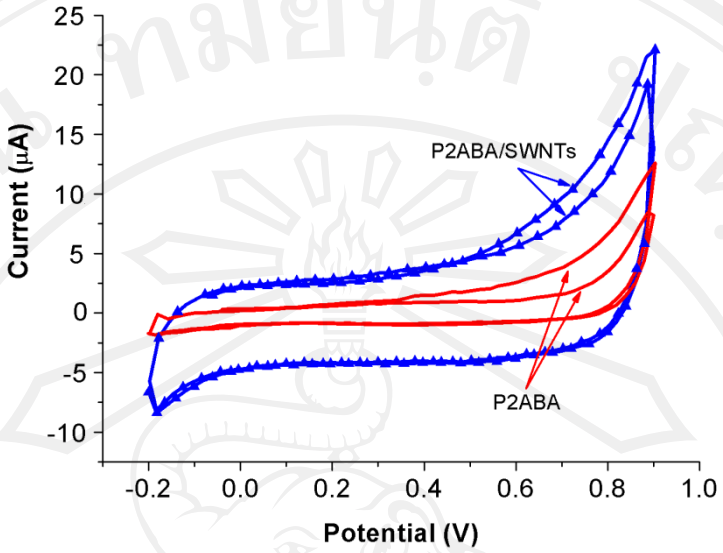

Figure 3.16 Cyclic voltammograms of P2ABA/SWNTs composites and P2ABA thin film in PBS solution.

3.8 Detection of UA by EC-SPR spectroscopy of P2ABA/SWNTs composites thin film

The EC-SPR responses on the determination of UA were observed in both the current and reflectivity responses at a constant applied potential of 0.5 V. Figure 3.17 shows the SPR reflectivity response changes upon injection of 1 mM UA compared with the response detection of AA. The reflectivity change in the AA addition was observed smaller than the reflectivity change in UA, which suggests that UA can effectively accumulate at the carboxylated SWNTs surface [106] and this method is possible to detect UA in urine. The SPR responses after finish the detection, the reflectivity rapidly decreases to the original. This indicates that UA does not accumulate at P2ABA/SWNTs surface because the SPR kinetic on the electrode surface was a reversible absorption. Figure 3.18 shows the current responses upon the injection of 1 mM UA and AA into carboxyated P2ABA/SWNTs composites thin

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film. The current initially increased rapidly following by decreased rapidly and gradually decreased, corresponding to the electrochemical double layer [6, 23] at P2ABA/SWNTs composites thin film and electrolyte interface and diffusion of UA.

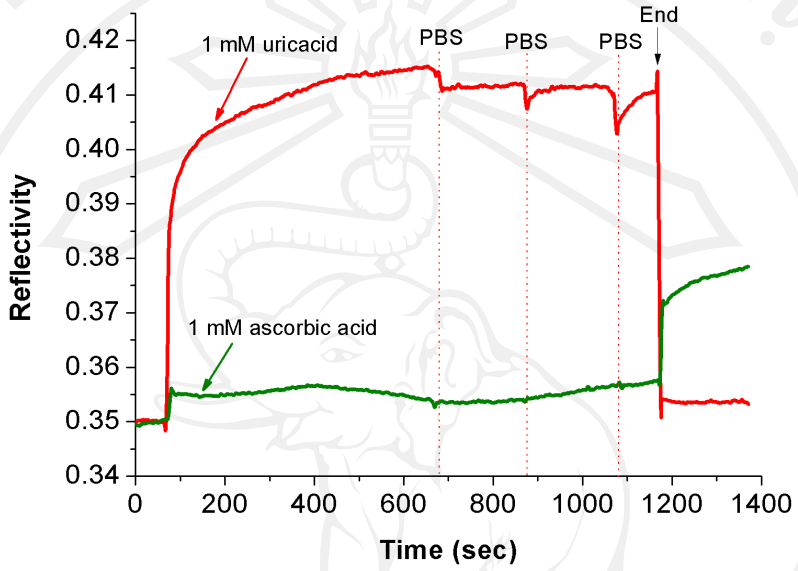

Figure 3.17 SPR reflectivity response upon injection of 1mM UA and AA into P2ABA/SWNTs composites thin film at constant applied potential of 0.5 V.

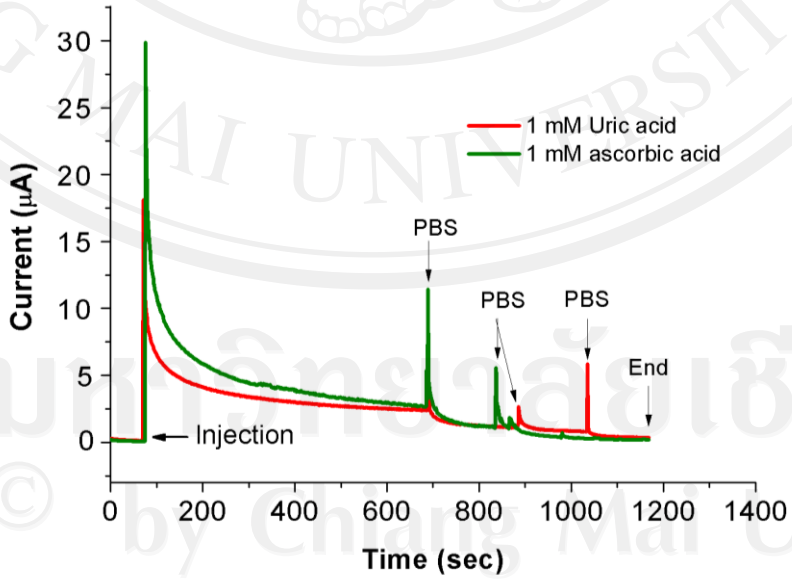

Figure 3.18 The current response upon injection of 1 mM UA and AA into P2ABA/SWNTs composites thin film at a constant applied potential of 0.5 V.

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3.9 Detection of UA by P2ABA/carboxylated SWNTs and P2ABA/Raw SWNTs composites thin film

Figure 3.19 shows SPR reflectivity response upon injection of 1 mM UA into P2ABA, P2ABA/RawSWNTs and P2ABA/COOH–SWNTs composite thin films. The reflectivity change in the P2ABA and P2ABA/RawSWNTs were smaller than the reflectivity changes in UA. Because P2ABA and RawSWNTs (bare SWNTs) did not have a –COOH group in the structure, therefore, UA cannot accumulate at the electrode surface. The result indicated that the hypothesis would be possible.

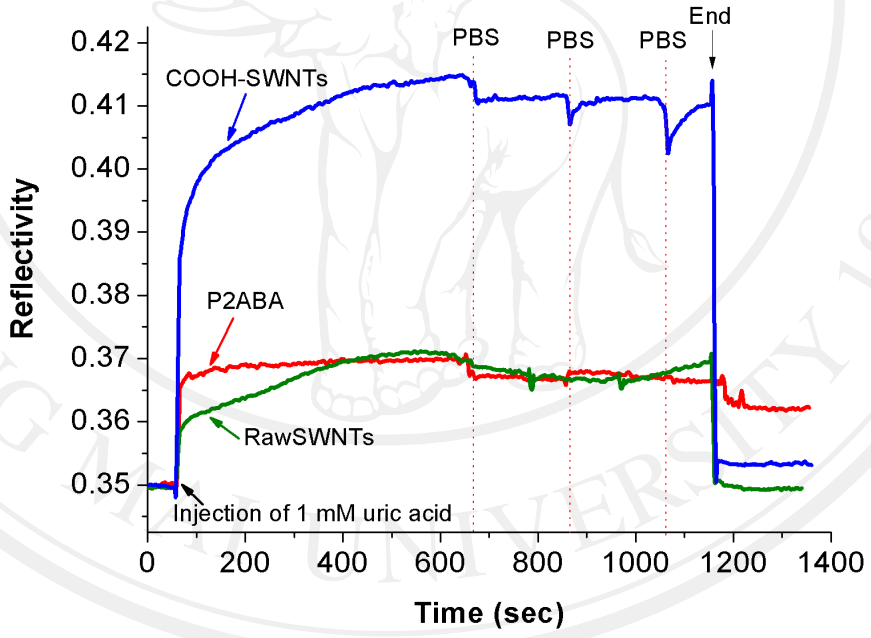

Figure 3.19 SPR reflectivity responses upon injection of 1 mM UA into P2ABA, P2ABA/RawSWNTs and P2ABA/COOH–SWNTs composite thin films.

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3.10 Detection of adrenaline on the P2ABA/ZnO nanoparticles composites thin film

Electropolymerization of 2ABA on the gold-coated high reflective index glass substrate was performed by cycling the potential between –0.2 and 1.1 V for 10 cycles at a scan rate 20 mV/s. SPR curves were taken by scanning an incident angle range of deionized water before and after electropolymerization of 2ABA monomer. The SPR curves were shifted to the higher dip angle after electropolymerization indicating that P2ABA was deposited on to gold film electrode. The suspension of 1, 0.1 and 0.01%wt of ZnO nanoparticles in DI water were used for assembling ZnO nanoparticles onto the Au/P2ABA electrode. The suspension of 0.01%wt of ZnO nanoparticles in DI water was optimum for assembled on the P2ABA. The SPR dip angle shifted to a higher angle corresponding to the H–bonding between the –NH in P2ABA structure and ZnO nanoparticles. SPR reflectivity changes after injection of 1 mM adrenaline in the presence of UA and AA at a constant applied potential of 0.5 V was found to show the selectivity of adrenaline detection over UA and AA as shown in Figure 3.20.

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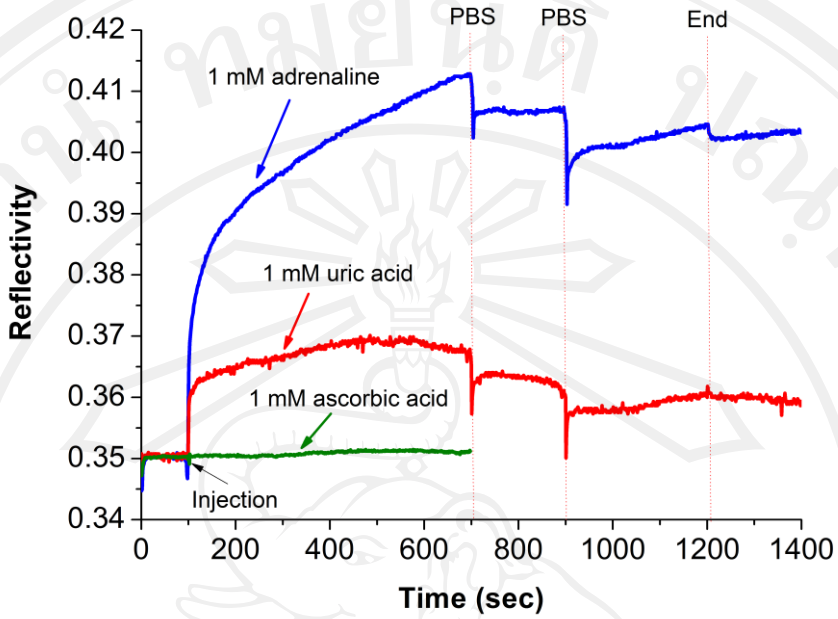

Figure 3.20 SPR reflectivity responses upon injection 1 mM each of adrenaline, UA and AA into P2ABA/ZnO nanoparticles composites thin film at a constant applied potential of 0.5 V.

However, EC-SPR response of adrenaline of P2ABA/ZnO nanoparticles composites thin film was observed to be smaller than P2ABA film as shown in Figure 3.21. The size of ZnO nanoparticles were bigger (10–20 nm) than the size of P2ABA film (10 nm) so ZnO nanoparticles formed H–bonding at amine site in P2ABA structure instead and blocked the physical absorption between P2ABA and ZnO nanoparticles layers. Therefore, the sensitivity of adrenaline sensor of Au/P2ABA/ZnO nanoparticles composites was lesser than Au/P2ABA electrode.

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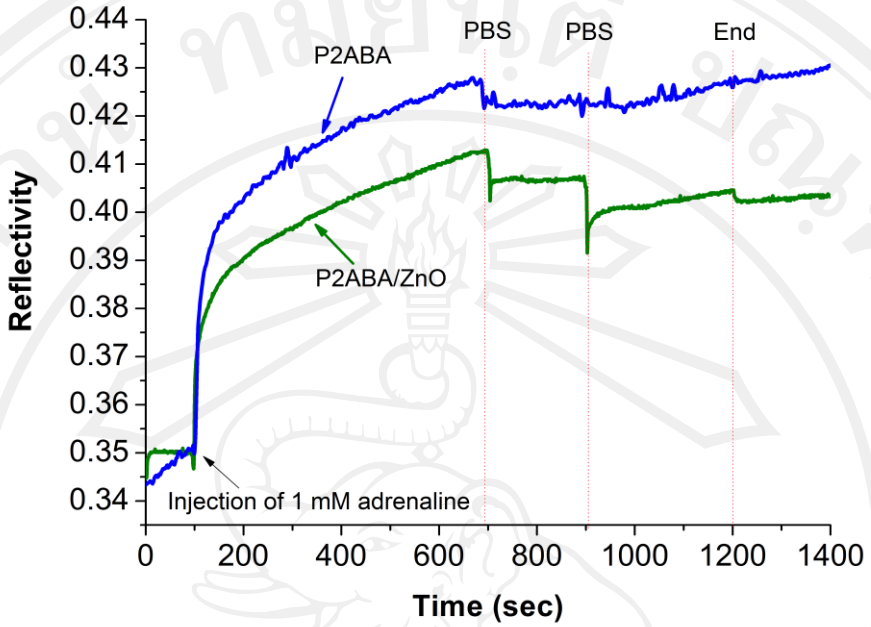

Figure 3.21 SPR reflectivity response upon injection of 1 mM adrenaline into P2ABA/ZnO nanoparticles composites and P2ABA thin film at a constant applied potential of 0.5 V.

3.11 Detection of UA on the P2ABA/ZnO nanoparticles composites thin film

P2ABA/ZnO nanoparticles composites thin film was employed to detect UA in the presence of AA. Figure 3.22 shows the SPR reflectivity response upon injection of 1 mM UA and AA into P2ABA/ZnO nanoparticles composites thin film at a constant applied potential of 0.5 V. It was found that P2ABA/ZnO nanoparticles composites thin film showed higher sensitivity towards UA than that of AA.

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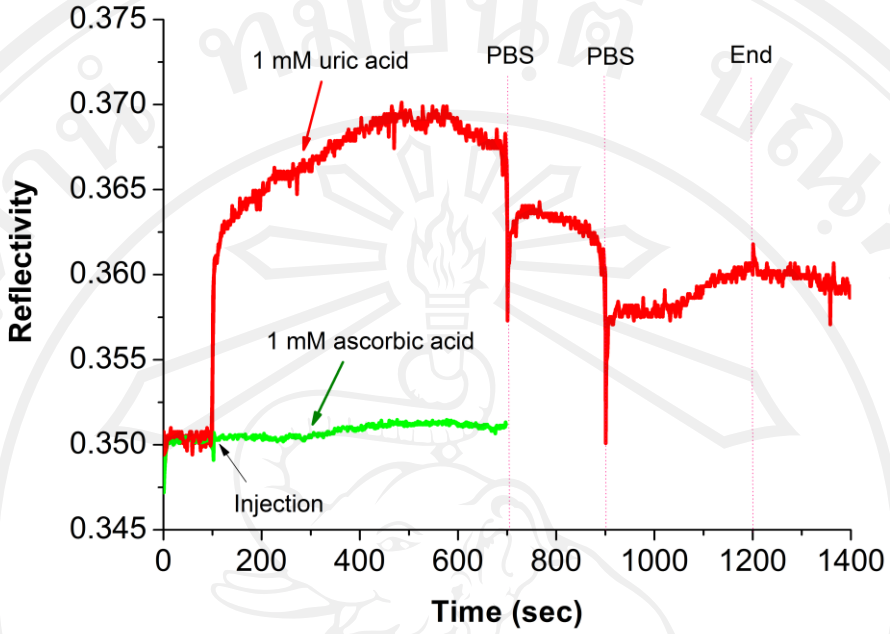

Figure 3.22 SPR reflectivity response upon injection of 1 mM UA and AA into P2ABA/ZnO nanoparticles composites thin film at a constant applied potential of 0.5 V.

3.12 Comparison of the P2ABA/ZnO nanoparticles composite and P2ABA/SWNTs composite thin films on the detection of UA

P2ABA/ZnO nanoparticles composite and P2ABA/SWNTs composite thin films were employed for the detection of UA. SPR reflectivity and current responses upon injection of 1 mM UA into P2ABA/ZnO nanoparticles composite and P2ABA/SWNTs composite thin films were measured at a constant applied potential of 0.5 V as shown in Figures 3.23 (a) and 3.23 (b). P2ABA/SWNTs composites thin film gave higher SPR and current response than P2ABA/ZnO nanoparticles composites thin film, therefore it can be concluded that P2ABA/SWNTs composites thin film was found to be a better biosensor for the detection of UA.

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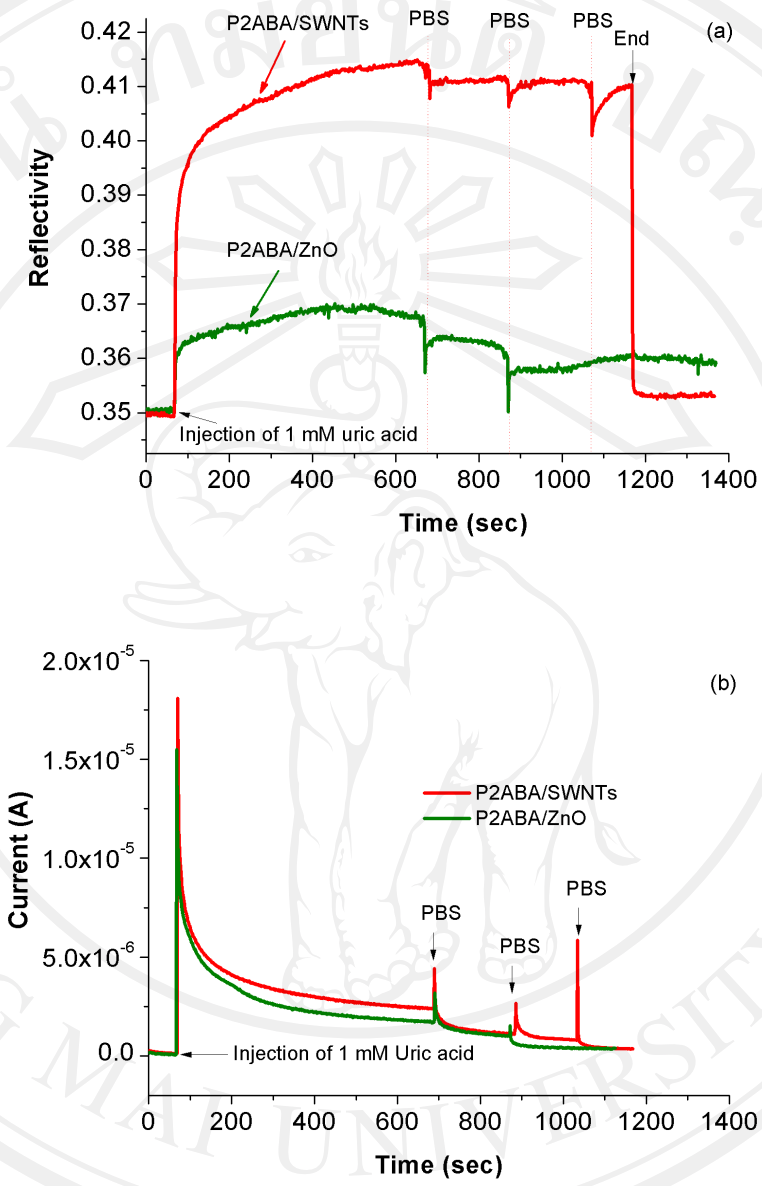

Figure 3.23 (a) SPR reflectivity and (b) current responses upon injection of 1 mM UA into P2ABA/ZnO nanoparticles composite and P2ABA/SWNTs composite thin films at a constant applied potential of 0.5 V.

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