CHAPTER 2

EXPERIMENTAL

2.1 Chemicals

All chemicals were analytical reagent are shown in Table 2.1

Table 2.1 Chemical, purity, molecular formula, molecular weight and company

Chemical	**Purity**	**Molecular formula**	**Molecular weight(unit or Dalton)**	**Company**
2-Aminobenzylamine	98%	$C_7H_{10}N_2$	122.17	Tokyo Kasei Kogyo Co, Ltd
Adrenaline	99%	$C_9H_{13}NO_3$	183.21	Sigma-Aldrich
L-ascorbic acid	99%	$C_6H_8O_6$	176.12	Tokyo Kasei Kogyo Co, Ltd
Uric acid	99%	$C_5H4N_4O_3$	168.11	Sigma-Aldrich
Sulfuric acid	98%	H_2SO_4	98.08	Kanto Chemical
Phosphate buffer saline (Sigma tablets)				Sigma-Aldrich
Zinc oxide nanoparticles		ZnO	81.38	Prepared follow: Liewhiran[94].

2.2 Instruments

1. Electrochemical set- a three electrode cell driven by HZ-5000 potentiostat (Hokuto Denko Ltd., Japan)

2. SPR- a home-built SPR system using Kretschmann optical configuration

3. UV-vis spectrophotometer- a V-650 UV-VIS Spectrophotometer (JASCO International Co., Ltd., Japan)

4. Thermal evaporation- a home-built thermal evaporation sources with resistive molybdenum boats.

5. Quartz crystal microbalance with dissipation (QCM-D)-a Q-Sense D300 microbalance material analyzer (qsense, Gothenburg, Sweden)

6. Atomic force microscope (AFM)- a scanning probe microscope SPM-9600 (SHIMADZU, Japan)

7. Fourier transforms infrared spectroscopy attenuated total reflectance (FTIR/ATR)- a nicolet 6700 FI-IR spectrometer (Thermo scientific, USA)

Cyclic voltammetry and amperometry were performed using a potentiostat HZ-5000 (Hokuto Denko Ltd., Japan) interface with a conventional three electrodes cell. The reference electrode was a Ag/AgCl (3 MNaCl, BAS Inc.) aqueous electrode, a platinum wire served as counter electrode and the gold film used both excitation surface plasmon and used as working electrode. The thickness of gold film about 47 nm was chosen for optimum excitation of the surface plasmon vacuum evaporated onto a glass substrate (with an adhesion layer of 3 nm chromiumfor ensure mechanical stability of the gold film during electrochemical experiment) previously evaporated on glass substrate. The surface area of the gold electrode was 0.785 cm^2. A triangular S-LAH66 prism was also used. The Au/glass substrates were clamped against a Teflon cell with an O-ring, providing a liquid-tight seal. The Teflon cell was then mounted onto a two-axis goniometer to enable investigation by SPR. Surface plasmon resonance spectroscopy (SPR) setup combines the three electrode electrochemical cell with a Kretschmann configuration for the excitation of surface plasmon. The excitation source is a He–Ne laser with λ = 632.8 nm as shown in Figure 2.1. Kinetic measurements were performed to monitor both the P2ABA thin film grown on gold film surface and the oxidation/reduction, doping/dedoping properties of the deposited P2ABA, P2ABA/ZnO nanoparticles composites and P2ABA/SWNTs composites thin film and the some biomolecule-sensing via reflectivity changes as a function of time. Angular measurements were performed by scanning an incident angle range of deionized water before and after electropolymerization of 2ABA monomer and/or P2ABA/ZnO nanoparticles composites, P2ABA/SWNTs composites thin film.

The biomolecules sensing were investigated via reflectivity changes (at a fixed incident angle lower than the dip angle) as a function of time. EC-SPR is a powerful technique to study the reaction of some biomolecules on P2ABA, P2ABA/ZnO nanoparticles composites and P2ABA/SWNTs composites thin film and compared to the response detection of the interference.

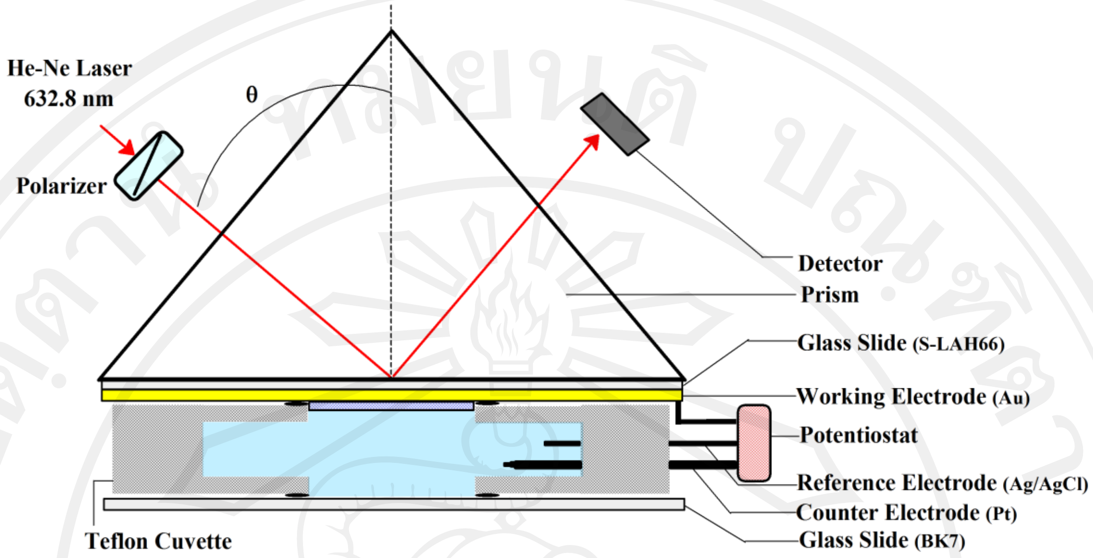

Figure 2.1EC-SPR instrument setup**.**

2.2.1 Thermal evaporator

Evaporation is a common method of thin film deposition. The source material is evaporated in vacuum. The vacuum allows vapor particles to travel directly to the target (substrate), where they condense back to a solid state. Evaporation is used in micro-fabrication. The thermal evaporation process is shown in Figure 2.2.

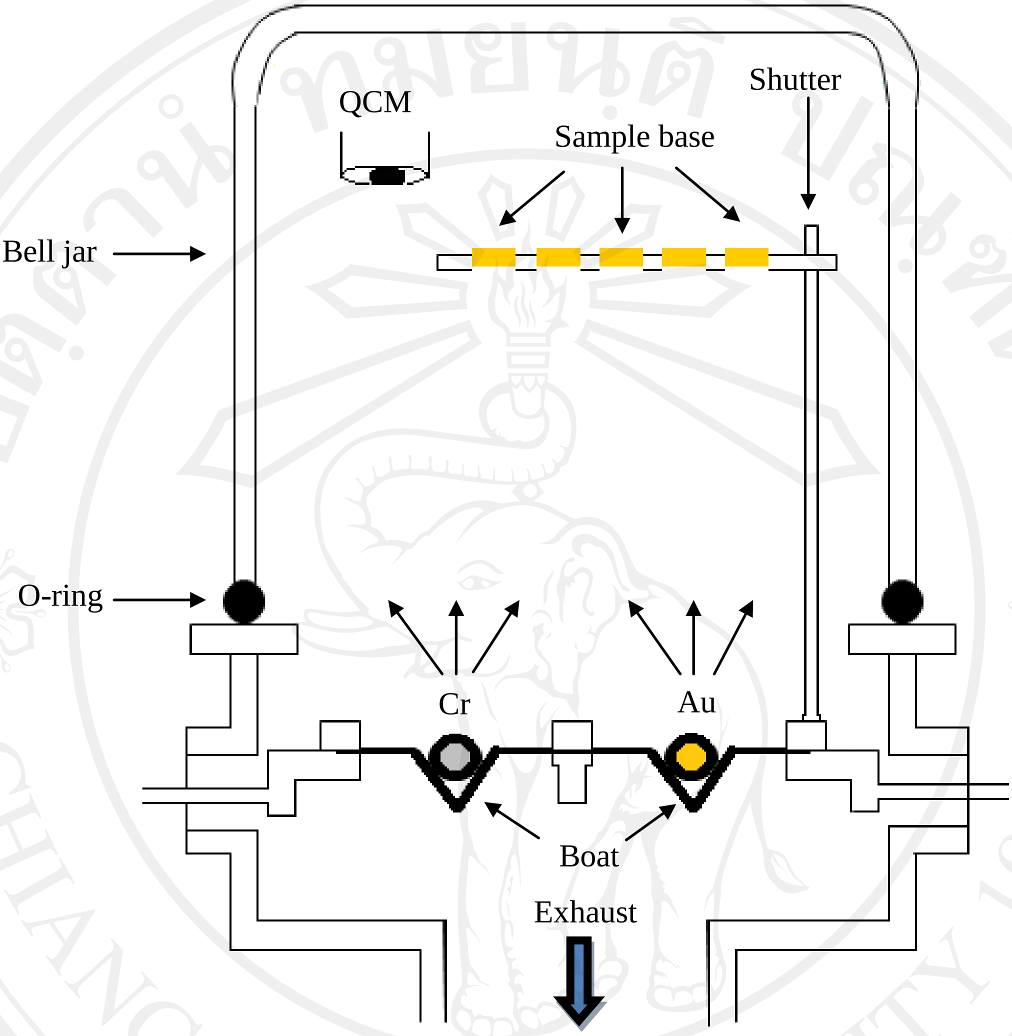

Figure 2.2 Schematic diagram showing vacuum evaporation device.

2.3 Electropolymerization of 2ABA on gold electrode

The gold-coated glass substrate and QCM electrode were washed with 0.5M sulfuric acid and deionized water before thin film formation. 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 as shown in Figure 2.3. The thickness of P2ABA thin film on the gold electrode was calculated by Winspall software version 3.02 (Winspall program, MPIP, Germany). The P2ABA thin film thickness was estimated to be 10 nm. Finally, the P2ABA thin film was rinsed thoroughly with 0.5 M H_2SO_4 and deionized water respectively before use. Polyaniline and their derivative were poor solubility in various solvents and its loss of electroactivity in neutral solution [9]. The reason for the better performance of the P2ABA film may be due to the film can be electroactive in neutral solution, the electronic properties and the self–doping effect from the functional group. Electroactivity of P2ABA was confirmed from cyclic voltammetry in PBS solution, indicated that the signal can be enhanced with event potential and probe sensitive P2ABA thin film based electrochemical sensors.

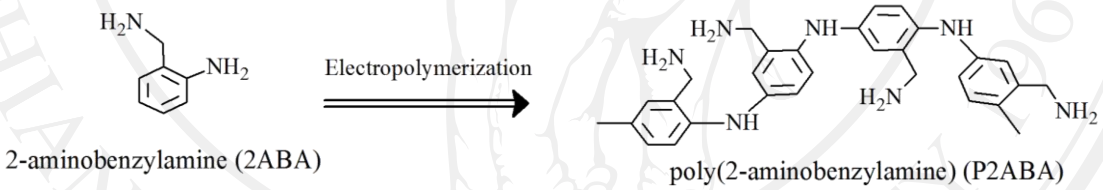

Figure 2.3 Immobilization of P2ABA thin film on gold film electrode by electropolymerization of 50 mM 2ABA monomer in 0.5 M H_2SO_4 followed by cycling the potential between –0.2 and 0.1 V vs. Ag/AgCl for 10 cycles at a scan rate 20 mV/s.

2.4 Fabrication of the P2ABA/SWNT composite thin film

The deposited P2ABA film (thickness ~10 nm) on gold-coated high reflective index glass substrate electrode was washed 3 times with 0.5 M sulfuric acid and DI water before assembled carboxylated SWNTs. The carboxylated SWNTs were prepared by follow-up Zhang et al.[95]. The 40 mg of SWNTs and 50 ml deionized water were addedto a flask and dispersed with the aid of an ultrasonic water bath for 60 min at room temperature. Then 0.45 g potassium persulfate (KPS) was added to the flask and the pH of the reaction system was adjusted to 13 by adding concentrated KOH solution. The flask equipped with a reflux condenser and a magnetic stir bar was kept at 85^{o}C with vigorous mixing for 3 hr., and then cooled down to room temperature naturally. The contents of the flask were carefully diluted with water and dispersed with ultrasonic water bath, followed by a centrifugation at 3000 rpm for 20 min. The centrifugation was expected to separate the catalyst and bundled SWNTs from the solution. The supernatant solution was collected and filtered through a hydrophilic polytetrafluoroethylene membrane (47 mm/0.2 mm, from Millipore) and washed with DI water. Finally, the carboxylated SWNTs powder were dried overnight at 85^{o} C. The details of the carboxylated SWNTs have been reported in the literature [95]. The 0.5 mL suspension of 1, 0.1 and 0.01%wt of carboxylated SWNTs in DI water were assembled on the P2ABA film for 20 min. First, SWNTs were assembled on the Au/P2ABA surface to form Au/P2ABA/SWNTs film because of the reaction between the –COOH in SWNTs and $–NH_2$ within P2ABA structure (–NH–CO covalent bond) [106]. P2ABA was electropolymerized from 2ABA monomers. The deposition process was investigated by EC-SPR technique. Finally, the Au/P2ABA/SWNTs was rinsed with DI water before use. The detection of optical and electrochemical signals from the reaction of uric acid was reported by EC-SPR technique as shown in Figure 2.4. The RawSWNTs electrode (Au/P2ABA/RawSWNTs) was prepared by using original SWNTs for comparison purpose.

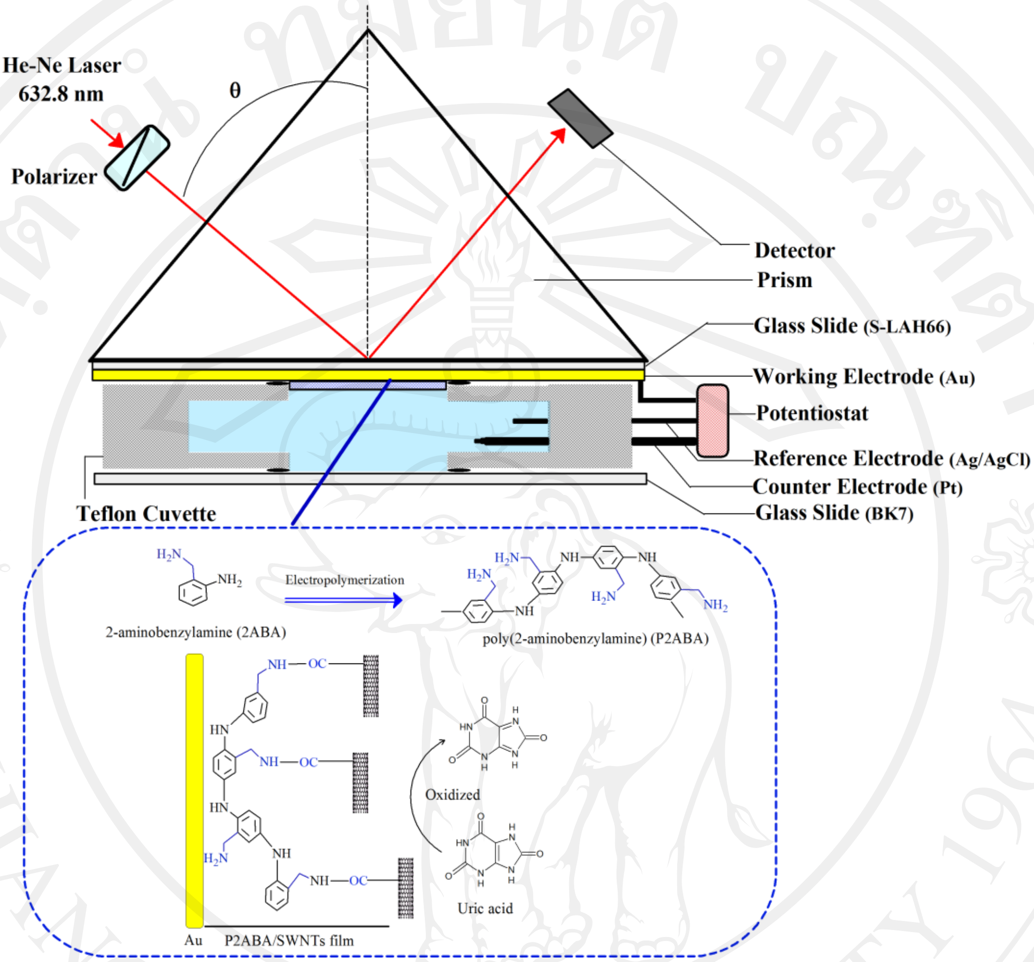

Figure 2.4 EC-SPR setup in the Kretschmann configuration for using surface plasmon resonance and immobilization of P2ABA/SWNTs composites thin film on gold film electrode and the PABA/SWNTs composites thin film reaction with uric acid.

2.5 Fabrication of the P2ABA/ZnO nanoparticles composites thin film

The deposited P2ABA film (thickness ~10 nm) on gold-coated high reflective index glass substrate electrode was washed 3 times with 0.5 M sulfuric acid and DI water before assembled ZnO nanoparticles composites. The ZnO nanoparticles were prepared by following up the literature reported by Liewhiranet al. [94]. Precursor solutions (0.5 M) were prepared by dissolving an appropriate amount of zinc naphthenate (Strem, 10 wt% Zn) in toluene/acetonitrile mixtures with ratio of 80/20 vol%. In a typical run, the as prepared precursor mixture was fed into a nozzle at a constant feed rate of 5 ml/min using a syringe pump (Inotech). At the end of the nozzle, the precursor solution was dispersed by 5 ml/min oxygen forming a spray with a pressure drop at the capillary tip kept constant at 1.5 bars by adjusting the orifice gap area. This flame conditions will be depicted as 5/5 flame. The spray was ignited by supporting flamelets fed with oxygen (2.4 L/min) and methane (1.13 L/min) which are positioned in a ring around the nozzle outlet. The flame height was 10 cm and showed a yellowish-orange flame appearance. After evaporation and combustion of precursor droplets, particles are formed by nucleation, condensation, coagulation and coalescence. Finally, the nanoparticles were collected on a glass microfibre filters (Whatmann GF/A, 25.7 cm indiameter) with the aid of a vacuum pump. The flame made (5/5) ZnO as-prepared nanopowders were designated as P0. The 0.5 mL suspension of 1, 0.1 and 0.01 %wt of ZnO nanoparticles in DI water were assembled on the P2ABA film for 20 minas shown in Figure 2.5. P2ABA was electropolymerized from 2ABA monomers. ZnO nanoparticles can form H–bonding through –NH group within P2ABA at neutral medium (pH~7) [96]. The deposition process was investigated by EC-SPR technique. Finally, the Au/P2ABA/ZnO nanoparticles composite was rinsed with DI water before use.

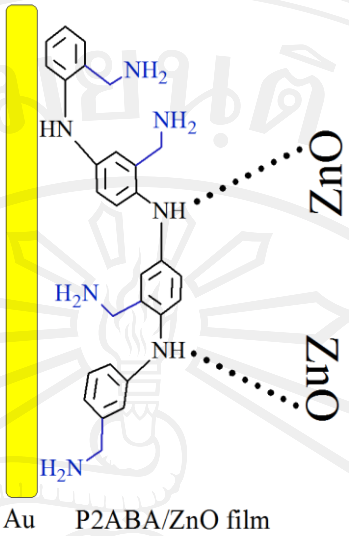

Figure 2.5 Immobilization of P2ABA thin film on gold film electrode and the structure of Au/PABA/ZnO electrode for the detection some biomolecules.

2.6 Characterization of the P2ABA thin films

2.6.1 Quartz crystal microbalance with dissipation (QCM-D)measurement

The QCM-D is a sensitive mass sensor for study molecular interaction and adsorption between P2ABA thin film and adrenaline. The interaction between the adrenaline and the benzylamine structure on the gold film surface was detected in real time by a change in the frequency. The mass changed on the quartz crystal surface was related to change in the oscillation frequency through the Sauerbrey relationship [82]. The Sauerbrey equation can be applied with the frequency change in the QCM setup. The obtained P2ABA thin film-modified gold coated QCM crystal were used to detect various concentrations of adrenaline (1–1000 µM/mL) at an open circuit potential in PBS solution. The QCM-D instrument is shown in Figure 2.6.

Figure 2.6 QCM-D instrument**.**

2.6.2 UV-vis absorption properties of P2ABA thin film

UV-vis absorption spectroscopy was performed using a V-650 UV-vis Spectrophotometer (JASCO International Co., Ltd., (Figure 2.7)) for studies absorption properties of P2ABA thin film before and after injection of adrenaline, uric acid (UA) and ascorbic acid (AA). An ITO-coated glass substrate was used as working electrode instead of gold thin film used in EC-SPR spectroscopy. The reaction of adrenaline, UA and AA were carried out in PBS solution for 20 min at a constant applied potential of 0.5 V, which corresponds to the oxidation potential of P2ABA film. The UV-vis spectrum of P2ABA film without the reaction with adrenaline, UA and AA were measured after applied constant potential of 0.5 V for 5 min in PBS solution to compared it with the UV-vis spectrum of P2ABA film after the reaction with adrenaline, UA and AA.

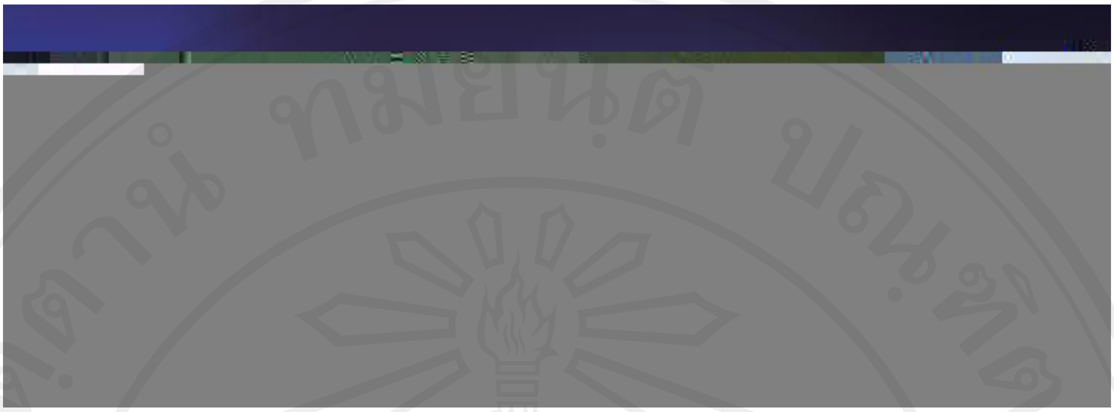

Figure 2.7 V-650 UV-vis Spectrophotometer.

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

FTIR is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An 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 dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared) and opened up new applications of infrared spectroscopy. FTIR/ATR instrument is shown in Figure 2.8. FTIR/ATR spectrum of P2ABA film without the reaction was measured after electropolymerized 2ABA on gold electrode and compared it with the FTIR/ATR spectrum of P2ABA film after the reaction with adrenaline.

Figure 2.8 FTIR/ATR instrument.

2.6.3.1 KBr Spectra determinations

The powder mixture of the original commercial samples and spectroscopic grade KBr in the ratio of 1:50 was finely ground in an agate mortar and pestle. Pellets were prepared using a handy press. The KBr spectra were recorded in absorbance.

2.6.3.2 ATR Spectra determinations

The surface modification step on P2ABA samples were determined by ATR measurement. Each prepared substrate was put on a ZnSe prism of a sample holder, and ATR spectra were recorded in absorbance. Figure 2.9 shows ATR measurement.

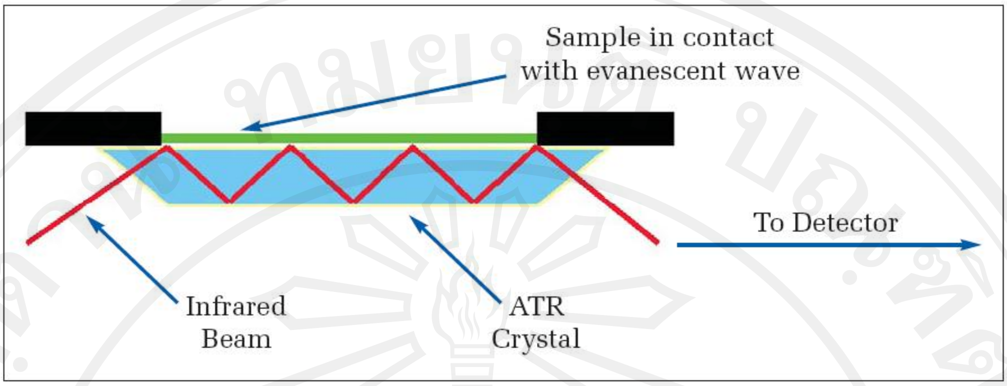

Figure 2.9 ATR measurement**.**

The advantages of FTIR/ATR spectroscopy technique are as follows:

- Faster sampling
- Improving sample-to-sample reproducibility
- Minimizing user-to-user spectral variation
- Higher quality spectral databases for more precise material verification and identification

2.6.4 Atomic Force Microscopy (AFM) Analysis

The AFM was invented by Binning et al. [97] in 1986. The AFM consists of a cantilever with a sharp tip (probe) at its end that is used to scan the specimen surface. The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nanometers. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to a deflection of the cantilever according to Hooke's law. Attractive or repulsive forces resulting from interactions between the tip and the surface will cause a positive or negative bending of the cantilever. The bending is detected by means of a laser beam, which is reflected from the back side of the cantilever as shown in Figure 2.10 [98]. The AFM is a very high resolution scanning probe microscopes, which demonstrated resolution of fractions of a nanometer, more than 1000 times better 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 were observed.

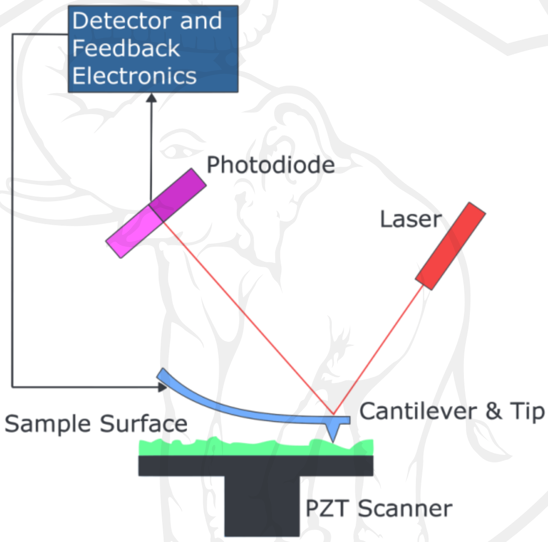

Figure 2.10 Blockdiagram of AFM [98].

2.7 Detection of adrenaline, UA and AA

2.7.1 Detection of adrenaline in the presence of UA and AA

SPR reflectivity changes after injection of 1 mM adrenaline in the presence of UA and AA at a constant applied potential of –0.2 V, open circuit and 0.5 V were performed by kinetic measurements of the binding reaction. The real time detection of optical and electrochemical signals from the reaction of adrenaline, UA and AA with P2ABA thin films were reported by EC-SPR spectroscopy.

2.7.2 Detection of UA and AA

The solutions of UA and AA were prepared daily by using PBS solution. The cyclic voltammetry was employed at the potential cycling between –0.2 and 0.9 V with a scan rate of 20 mV/s and the amperometry was performed to investigate the interaction of UA and AA with the P2ABA/SWNTs composites thin film. The real-time detection of optical and electrochemical signals from the reaction of UA and AA with P2ABA/SWNTs composites thin film was reported by EC-SPR.

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

2.7.4 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.