CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Parabens

Paraben is a common name of p-hydroxybenzoic acid. Its structure comprises of benzene ring and alkyl ester at para position to the hydroxyl group. The general chemical structure of paraben is shown in Figure 2.1. Most of these compounds are generally obtained from synthesis, and some of them are found naturally in various vegetable sources [5, 33, 34]. Anti-microbial activities are their property; therefore, parabens are widely used as preservatives in beverages, food, pharmaceutical and cosmetic products. In addition, they also have numerous favorable properties such as biodegradability, good stability, efficacy in wider pH range, non-volatility, low cost and resistance to hydrolysis. An antimicrobial activity of parabens increases with the increasing length of its alkyl chain, while its water solubility decreases. Two or more parabens are often used together to achieve synergistic effect [1-7].

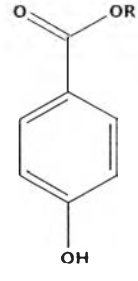

Figure 2.1 General chemical structure of a paraben, R = alkyl chains that are CH_3 for methyl paraben, C_2H_5 for ethyl paraben, C_3H_7 propyl paraben and C_4H_9 for butyl and isobutyl paraben [35]

2.2 Chromatography

Chromatography is a separation technique of mixed components of samples. The principle of chromatography is based on the partition of two immiscible phases; stationary phase and mobile phase. The stationary phase is statically contained in the column and the mobile phase is the moving phase under the applied forces

such as pressure or gravity. The analytes are distributed into the stationary phase by a suitable mobile phase. The chromatography system should be performed in a close system to achieve equilibrium; the velocity of the mobile phase and the stationary phase are optimized to accomplish rapidly equilibration. Under these conditions, the distribution coefficient (K); which is normally presented as the ratio of the analyte concentration in the stationary phase (C_s) to that of the mobile phase (C_m), can be described by equation 2.1 [36, 37].

$$K = \frac{C_s}{C_m} \qquad (2.1)$$

K depends on the structure of analytes, temperature, the nature of stationary phase and mobile phase. The principle of the separation is the different distribution coefficients of the analytes; if the two compounds have the same distribution coefficient, they will not be separated. The distribution of the analytes between the stationary phase and the mobile phase will result into the chromatogram which is the signal from the detector (Figure 2.2).

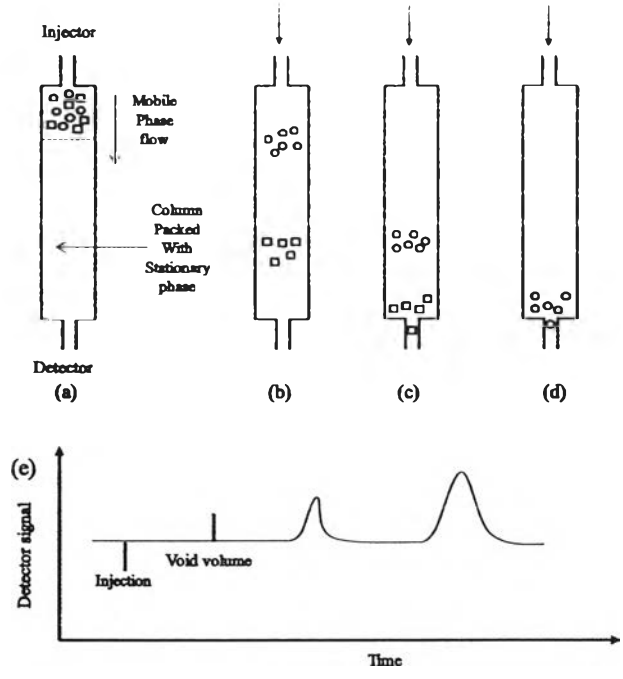

Figure 2.2 Chromatographic separations on a column. (a) Introduction of the sample. (b) Elution of unretained components at column void volume. (c) Elution of more weakly retained compound. (d) Elution of more strongly retained component. (e) Chromatogram recorded by detector at the end of the column [36]

The stationary phase can be a solid or liquid spread as a thin film in the wall of the column or over an inert support solid. The mobile phase can be a gas, a liquid, or a supercritical fluid and it is also called in term of the "eluent". The classification of chromatography relies on the stationary phase and the mobile phase. Using the mobile phase as gas or liquid can classify into several methods of chromatography as illustrated in Table 2.1.

Table 2.1 Methods of chromatography [36]

Mobile phase	Stationary phase	Chromatographic method	Abbreviation
gas	Liquid	Gas-liquid chromatography	GLC
	Bonded Liquid	Gas-liquid chromatography	GLC
	Solid	Gas-solid chromatography	GSC
Liquid	Liquid	Liquid-liquid chromatography	LLC
	Bonded Liquid	High-performance liquid chromatography (reversed-phase)	HPLC
	Solid	High-performance liquid chromatography (normal-phase)	HPLC
Supercritical fluid	Bonded Liquid	Supercritical fluid chromatography	SFC

2.2.1 High-Performance Liquid Chromatography (HPLC)

Liquid Chromatography (LC) is one of methods in chromatography using liquid as the mobile phase. The separation occurs due to the different interaction of each component between the two phases. High-performance liquid chromatography (HPLC) is the term used to describe a liquid chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase. The most popular classification of liquid chromatography is based on the mechanisms of interaction between the analytes and the stationary phase. The classification of liquid chromatography is divided into five mechanisms: adsorption, partition, size exclusion, affinity and ion exchange as shown in Figure 2.3 [38].

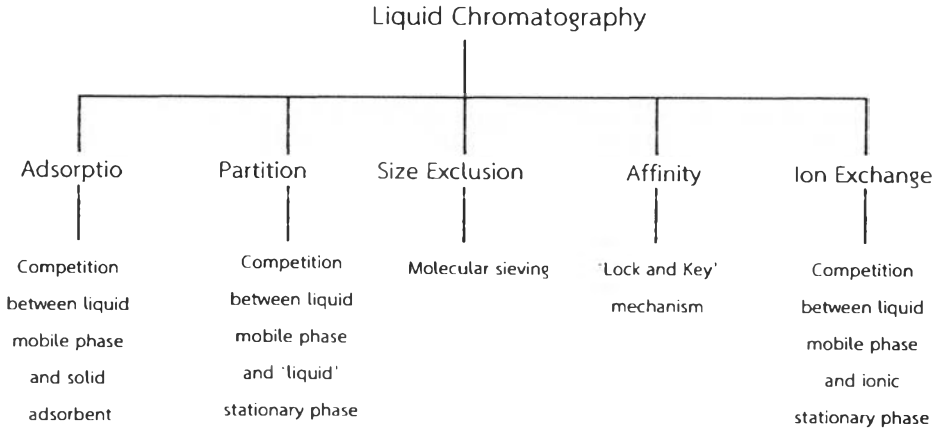

Figure 2.3 Classification of liquid chromatography according to the interaction mechanisms [38]

2.2.2 Instrument for HPLC system

An instrument of HPLC system includes four major parts, as follow:

- a pump (to deliver the mobile phase)
- an injector (to provide sample introduction)
- a column, stationary phase (to separate the components)
- a detector (to determine the analytes)

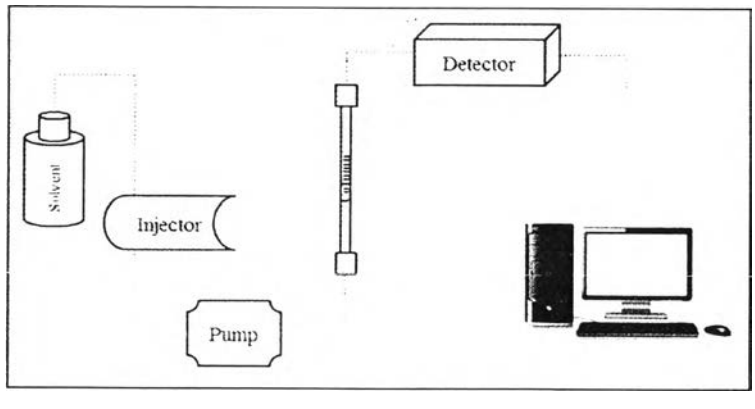

Figure 2.4 Schematic of instrument for HPLC system

All components of instrumentation are connected with miniature tubings to minimize band broadening. The pump is employed to force the liquid mobile phase through the column and into the detector. The injector is used to inject the sample into the flowing stream. The analytes are separated by the column, and then detected by the detector. All of the components are controlled by a computer which collects, stores, and analyzes the signal from the detector [39].

2.2.3 Ultrahigh-Performance Liquid Chromatography (UHPLC)

UHPLC or UPLC (Ultra performance liquid chromatography, waters coorperations) or UFLC (Ultra-fast liquid chromatography, Shimadzu) is a HPLC equipment with low-volume capabilities and very high-pressure (P > 400 bar) as compared to the commercial HPLC instruments which employed a maximum pressure of 400 bar. UHPLC was produced to support the particles which were smaller than 2 μm of stationary phase (while the particles of HPLC column are 3 to 5 μm) [39, 40].

Figure 2.5 UFLC instrument (Shimadzu, LC-20AD XR UFLC)

Van Deemter equation shows that the efficiency increases with using the smaller particles size but it lead to the higher backpressure. Figure 2.6 shows Van Deemter plot for the evolution of particle sizes over the last three decades.

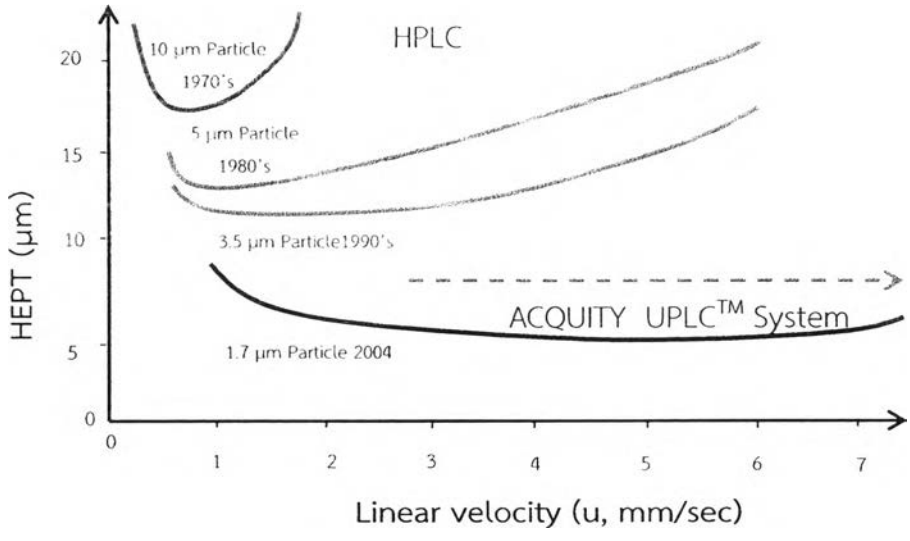

Figure 2.6 Schematic of Van deemter plot over the last three decades [41]

2.3 Electrochemistry

Electrochemistry is a branch of chemistry that study of changes in chemical solution that causes electrons moving; this movement is called electricity. In electrochemistry, a reaction of the movements of electrons from one element to another is well known as a redox reaction or oxidiation-reduction reaction. Redox reaction arises in electrochemical cells. There are two types of electrochemical cells: galvanic (voltaic) cell and electrolytic cell. Galvanic cell; the redox reaction spontaneously occurs and the energy from redox reaction is converted to electrical energy (Figure 2.7 a). Electrolytic cell; the redox reaction non-spontaneously occurs which energy is applied by an external source (Figure 2.7 b) [42, 43].

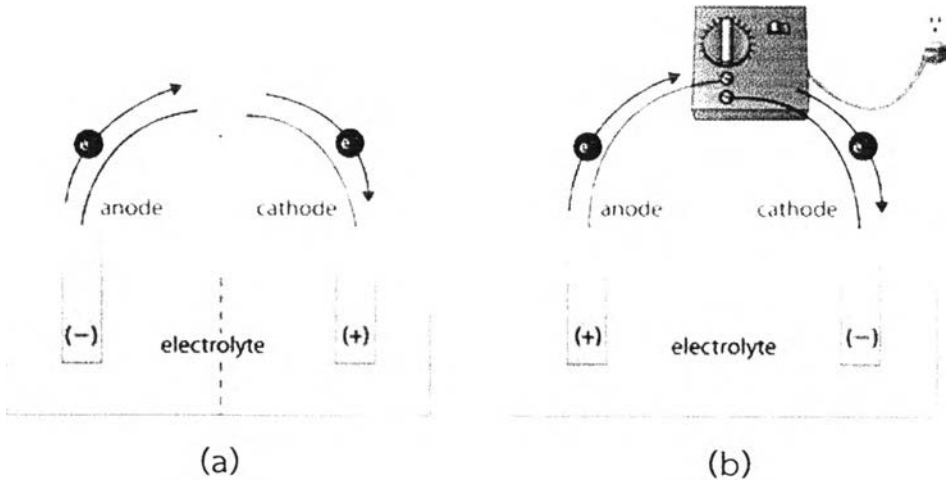

Figure 2.7 Electrochemical cell (a) galvanic cell (b) electrolytic cell [43]

2.3.1 Fundamental of electrochemistry

The redox reaction occurs only on the surface area of an electrode. There are four main factors that direct to the reaction rate and current at the electrodes: mass transfer to the electrode surface, kinetics of electron transfer, preceding and ensuring reactions and surface reactions (adsorption) as shown in Figure 2.8. A simple reaction is represented by:

$$O + ne \rightleftharpoons R \qquad (2.2)$$

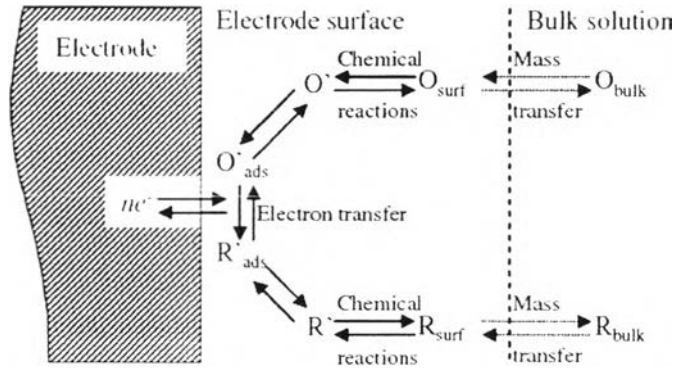

Figure 2.8 Process in electrode reaction [44]

O and R are the oxidized and reduced form of a redox reaction. For a system controlled by the laws of thermodynamics, the potential of the electrode can be used to determine the concentration of electroactive species by Nernst Equation:

$$E=E^{0}-\frac{2.3\,RT}{nF}\log\frac{C_O(0,t)}{C_R(0,t)} \qquad (2.3)$$

Where E^{0} is the standard potential for the redox reaction, Co (0,t) and C_R (0,t) are the concentration of the oxidized and reduced form, respectively. R is the universal gas constant (8.314 $JK^{-1}mol^{-1}$), T is the Kelvin temperature, n is number of electrons transfered in the redox reaction, and F is the Faraday constant (96,487 coulombs).

2.3.1.1 Mass transfer

Mass transfer process is one of the main fundamental electrochemistry to describe charge transfer on the surface area of the electrode [42, 45]. The mass transfer consists of 3 processes: migration, diffusion and convection.

2.3.1.1.1 Migration

The movement of ion under the electrical field in solution, positive charge will be attracted to negative charge and the negative charge will be also attracted to opposite way (Figure 2.9). The increased or decreased velocity of ion depends on the potential at the surface of electrode. Migration of all ion species in solution can take place; therefore, the current of ion from analyte might be suppressed. To solve this problem, the addition of a large concentration of electrolyte usually applies.

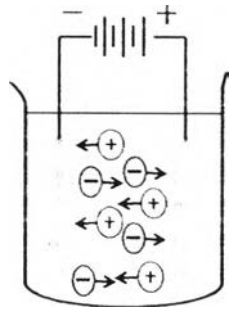

Figure 2.9 Migration of ion in solution

2.3.1.1.2 Diffusion

The movement of ion or molecule in solution occurs from the different concentration between two regions. Ion will move from a region of higher concentration to regions of lower concentration as shown in Figure 2.10.

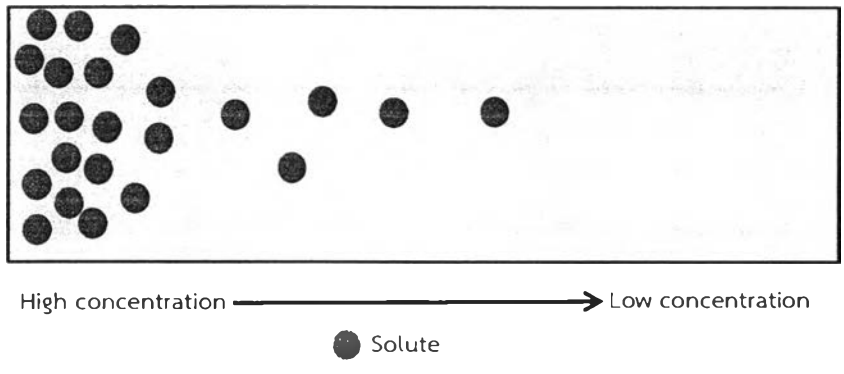

Figure 2.10 Diffusion from a different concentration

2.3.1.1.3 Convection

The movement of fluids is described by hydrodynamics. The convection is generated by the difference of temperature and density of solution from external mechanisms such as stirring, vibration, and flowing. The mass transfer is accelerated by the convection.

2.3.2 Voltammetry

Voltammetry is one of the electroanalytical methods which measure the current as a function of the applied potential. It is both quantitative and qualitative analysis method for molecular and ionic solution. This method consists of two or three electrodes, first, the working electrode where the redox reaction of the electroactive species in analyte solution can be indicated. Second, the reference electrode, its potential is constant which the varying potential of the working electrode is applied with certainly relative to. The third one is the counter (or auxiliary) electrode, used for the carrying of the current. All of the electrodes will be immersed into the analyte solution when the potential is applied. The potential is controlled between the working electrode and the reference electrode while the current flows between the counter electrode and the working electrode. The results from voltammetry are the signal of the current as a function of the applied potential which is called voltammogram. The most general waveforms is used in voltammetry are shown in Figure 2.11 [45-47].

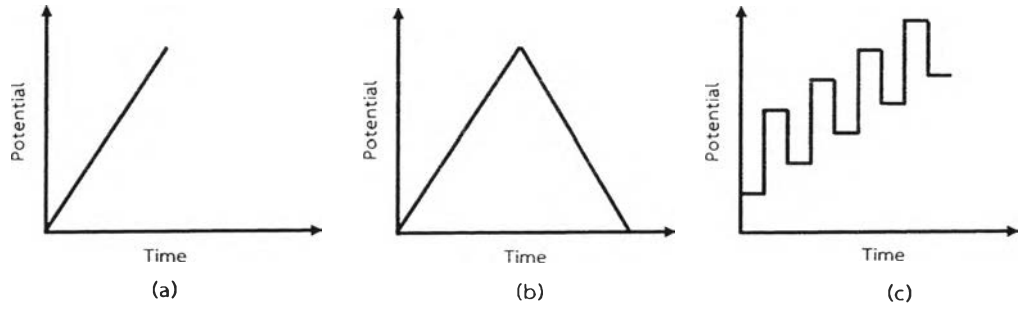

Figure 2.11 Potential-time waveforms often used in voltammetry (a) linear (b) triangular and (c) square for linear sweep voltammetry, cyclic voltammetry and square ware voltammetry technique, respectively [46].

2.3.2.1 Cyclic voltammetry

Cyclic voltammetry is one of the electroanalytical methods which have been often used for primary studies of electrochemical behavior of the system. It is a technique that at static working electrode (unstirred solution) was applied potential both forward and reverse directions as shown by the waveform in Figure 2.12 a. The potentiostat instrument is used to measure the current while the potential sweep is applied. The results of cyclic voltammetry are plotted between the current and potential as called cyclic voltammogram. Figure 2.12 b showed the cyclic voltammogram of a reversible redox couple. Clyclic voltammogram was characterized by peak potential (E_p) where E_{pa} and E_{pc} are anodic and cathodic peak potential, respectively. The i_p is the maximum current value where i_{pa} and i_{pc} are anodic and cathodic peak current, respectively [42].

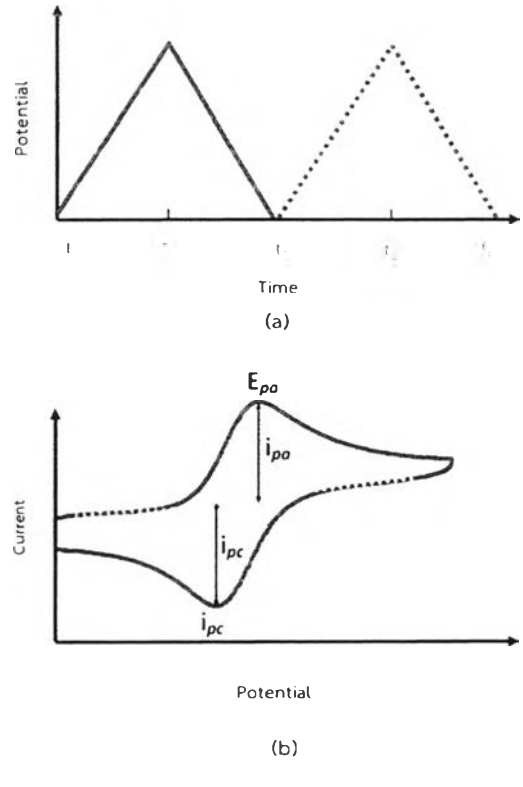

Figure 2.12 Waveform of cyclic voltammetry (a) and cyclicvoltammogram of reversible redox couple (b) [46]

For a reversible reaction, the ratio of i_{pa}/i_{pc} is approaching 1. This peak ratio can be impacted by chemical reaction coupled to the redox process. The formal potential for a reversible reaction is related to the peak potential (E_p) as follows:

$$E^0 = \frac{E_{pa}+E_{pc}}{2} \quad (2.4)$$

The peak current of reversible reaction is presented by Randles-Sevcik equation as shown below:

$$i_p = (2.69\times10^5)n^{3/2}ACD^{1/2}v^{1/2} \quad (2.5)$$

Where n is the number of transferred electron, A is the electrode surface area (cm^2), C is the concentration (mol cm^{-3}), D is the diffusion coefficient ($cm^2\ s^{-1}$), and v is the scan rate ($mV\ s^{-1}$). From the Randles-Sevcik equation, the peak current is proportional to the square root of the scan rate.

For an irreversible reaction; when the rate of mass transport increases, the reverse peak becomes disappeared. Generally, a shift of the peak potential with a scan rate can occur for the irreversible process.

2.3.2.2 Hydrodynamic voltammetry

Hydrodynamic voltammetry is different from normal voltammetry; the difference is the mass transfer process. In traditional voltammetry, the major mass transfer process on the electrode surface is diffusion process but in hydrodynamic voltammetry, the main mass transfer process is convection. The convection of the system can occur by movement of the electrode, or stirring, or flowing of the solution through the surface area of the working electrode. The advantage of convection is that the analyte can be brought into the surface area rapidly [46, 48].

2.3.3 Amperometry

Amperometry is an electrochemical technique which uses a constant applied potential at the working electrode as shown in the waveform in Figure 2.13.

This technique is normally used in stirring or flowing system. The potential is fixed while the potentiostat instrument was carried out to detect the change in current signal. At fixed potential, the electroactive species undergo an oxidation or reduction at the working electrode. The amperometric current is a function of the number of molecules or ions in solution. Therefore, the current response is proportional to the concentration of the analyte [46, 49].

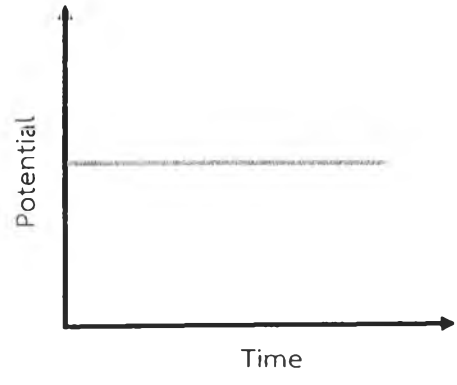

Figure 2.13 Waveform of ampermetry

2.3.4 Working electrode

Working electrode is the most important of all because the reaction of electroactive species of analyte occurs at the surface of this electrode. The materials used for construction of the electrode greatly impact the efficiency of electrochemical analysis. The factors that should be considered for the working electrode are conductivity, surface reproducibility and cost. Although there are many kinds of working electrode that were established, the most commonly used working electrode is carbon-based electrodes such as glassy carbon electrode, carbon paste electrode, boron doped-diamond electrode and screen printed-carbon electrode.

2.3.4.1 Screen printed-carbon electrode (SPCE)

Screen printing is a traditional technique over a thousand years ago. It was used in many fields such as textiles and advertising. The electrochemists

used this technique to construct electrode devices on many substrates. Recently, screen printed electrodes have been successfully applied in environmental analysis due to its inexpensiveness, simplicity, portability and reliability [50]. Silver ink was used as a conductive area and carbon ink or gold ink was used as a working area of the electrode. Gold is expensive, carbon ink therefore is a common material because it is inexpensive, easy to modify and chemically inert. Additionally, screen printed-carbon electrodes (SPCEs) is disposable electrode, therefore, SPCE has been applied in many researches. The examples of using SPCE as working electrode for electrochemical detection were:

Zhang *et al.* reported a screen-printed electrode modified with multi-walled carbon nanotubes and molecularly imprinted membrane using *in-situ* thermal polymerization techniques for rapid determination of ractopamine in pig urine by differential pulse voltammetry. Britton-Robinson (B-R) buffer at pH 7 was used as supporting electrolyte. Under the optimal condition, this novel sensor provided high sensitivity and selectivity for ractopamine detection with the limit of detection of 6 nM and the assay time was within 5 min [51].

Keawkim *et al.* proposed a bismuth film/crown ether/nafion modified sreen printed-carbon electrode for simultaneous determination of lead and cadmium in rice samples by sequential injection with anodic stripping voltammetry. Hydrochloric acid was used as supporting electrolyte, it was demonstrated that this new proposed method can improve the sensitivity with the limit of detection for lead and cadmium of 0.11 $\mu g\ L^{-1}$ and 0.27 $\mu g\ L^{-1}$, respectively [52].

Brugnera *et al.* reported screen printed-carbon electrode for determination of bisphenol A in river water and sewage samples by square wave voltammetry. In this work, cetyltrimethylammonium bromide (CTAB) was successfully applied for antifouling and pre-concentration agent. The experimental was carried out in B-R buffer solution with the detection limit of 5.1×10^{-8} M [53].

Karuwan *et al.* developed inkjet-printed graphene-poly (3,4-ethylenedioxythiophene):poly(styrene-sulfonate) (GP-PEDOT:PSS) electrode for electrochemical detection of salbutamol. Unoer the optimal condition, it was found

that salbutamol oxidation peak current of modified electrode was about 30 and 150 times higher than the unmodified electrode. This work was achieved for electrochemical sensing of salbutamol in pharmaceutical products with limit of detection of 1.25 μM [54].

From the previous works, SPCEs were often modified to improve the sensitivity and selectivity. Recently, nanomaterial is an attractive material employed to modify the working electrodes due to its large surface area. In this work, nanocomposite of graphene, PVP, and PANI was selected for SPCE modification.

2.3.4.2 Graphene

Graphene (G) is a monolayer, crystalline allotrope of carbon which is densely packed in a regular sp^2-bonded atom into a two dimensional honeycomb lattices. It has become a popular material since it was discovered in 2004 by Andre Geim and Kostantin Novoselov [55]. Graphene is a unique materal because of its mechanical, electrical and optical properties (Figure 2.14). Graphene also has the strong carbon/carbon bonding in the flat, aromatic structure, ¶ electrons and reactive sites for surface reactions [56].

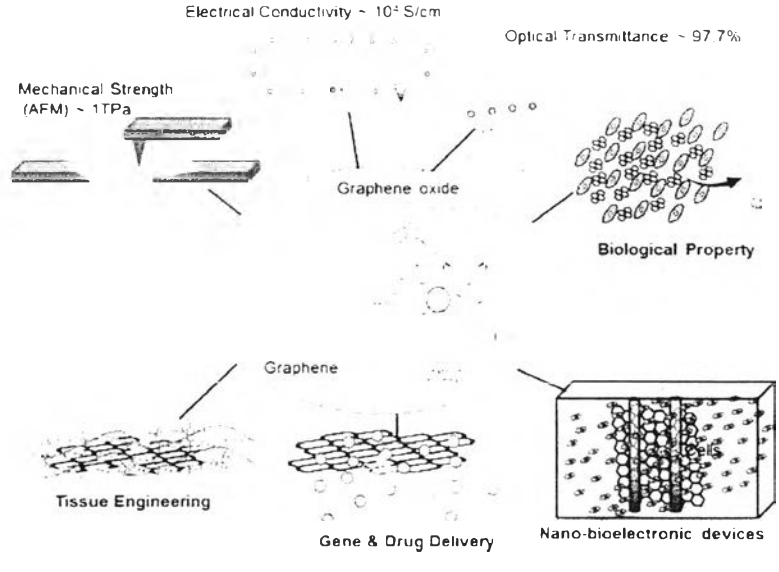

Figure 2.14 various application of graphene [56]

In addition, graphene has been adopted as a popular nanomaterial in electrochemistry because it exhibits many desirable electrochemical properties such as large surface area, high electrical conductivity and rapid electron transfer [26, 27]. There are many researchers who utilized graphene for the modification of working electrode surfaces to improve the sensitivity. The examples of using graphene as modifier are:

Fan *et al.* developed the nafion/TiO_2-graphene composite film modified glassy carbon electrode (GCE) for the determination of paracetamol in commercial tablet using differential pulse voltammetry. Nafion was used as solubilizing agent and antifouling coating. The nanocomposite of nafion/TiO_2-graphene suspension was dropped onto the freshly polished surface. The modified electrode presented the results with the detection limit of 2.1×10^{-7} M [57].

Liu *et al.* developed ionic liquid functionalized graphene sheet (IL-GS) loaded gold nanoparticles (AuNPs) nanocomposite-modified glassy carbon electrode for the electrochemical immunosensor to detect carcinoembryonic antigen (CEA) in clinical diagnostics. An anti-CEA can be attached to the IL-GS-Au modified electrode with the ultralow limit of detection of 0.1 fg mL^{-1} [58].

Li *et al.* reported the graphene-doped carbon paste electrode for the determination of ascorbic acid in vitamin C by differential pulse voltammetry. The experiment was performed in phosphate buffer solution; this modified electrode was capable of detecting ascorbic acid with the limit of detection of 7.0×10^{-8} M [59].

Pravin *et al.* reported the graphene paste electrode (GPE) for the determination of chlorpromazine in drug by differential pulse voltammetry. The graphene paste was packed into a piston-propelled GPE. This modified electrode was used successfully for chlorpromazine detection with the limit of detection of 6.0 nM [60].

2.3.4.3 Polyvinylpyrrolidone (PVP)

Polyvinylpyrrolidone (PVP) is a linear homopolymer of N-vinylpyrrolidone which is widely used in food and pharmaceutical science applications [61]. PVP is soluble in water and other polar solvents. In addition, PVP is inexpensive and biocompatible. Recently, it has been reported that PVP was successfully applied to stabilize the dispersion of graphene at high concentration in organic solvents [30]. The structure of PVP is shown in Figure 2.15.

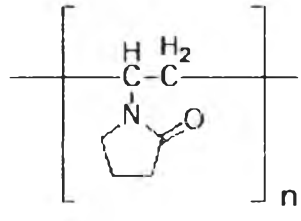

Figure 2.15 Structure of PVP [62]

2.3.4.4 Polyaniline

Conducting polymers, specifically polyaniline (PANI), poly (3,4-ethylenedioxythiophene) (PEDOT) and polypyrrole (PPy) also have been utilized for the modification of the electrode surface [63]. PANI is an attractive conducting polymer because of its excellent electrochemical properties, ease of synthesis and functionalization, high environmental stability, and low toxicity [29]. The structure of PANI is shown in Figure 2.16. In addition, PANI has often been used in electrochemical biosensor because PANI can improve the sensitivity and eliminate the electrode fouling [23].

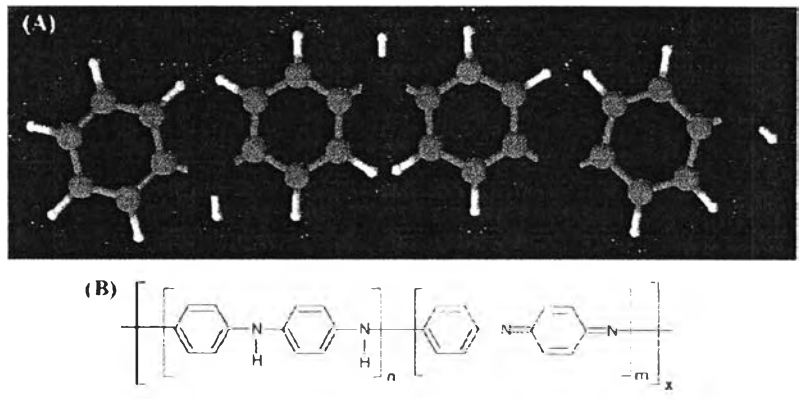

Figure 2.16 Structure of PANI (A) 3-dimensional (3D) and (B) 2-dimensional (2D) [23]

Recently, it has been reported that the doping of PANI onto graphene can improve the sensitivity:

Rodthongkum *et al.* developed graphene/polyaniline/ polystyrene (G/PANI/PS) nanofiber-modified screen-printed carbone electrode for the determination of dopamine in human serum and urine by square wave voltammetry. The current signal for redox reaction of ferri/ferrocyanide was 9 times higher than that of the unmodified electrode. The detection limit of as low as 0.05 nM was achieved with this modified electrode [63].

Xu *et al.* developed a graphene/polyaniline/gold nanoparticles (G/PANI/AuNPs) nanocomposite modified glassy carbon electrode for glucose biosensing using amperometry. This biosensor was successfully employed to detect glucose with the well- defined quasi-reversible redox peak and the limit of detection was 0.6 μM [64].

2.3.4.5 Electrospraying

Electrospraying technique has arisen as a useful and inexpensive method to produce 3D droplet-link nanostructure [31]. The electrospraying process was started by applying an external electric field. Then an equilibrium liquid suspension in a syringe is sprayed into aerosols. Due to an adequately strong electric field, the charges on the droplet surface will overwhelmed the surface tension to induce the formation of a liquid jet that is consequently

accelerated toward a ground collector. The electrospraying used to produce aerosols composed of sub-micrometer droplets with a narrow distribution [65, 66]. The equipment for electrospraying technique is shown in Figure 2.17.

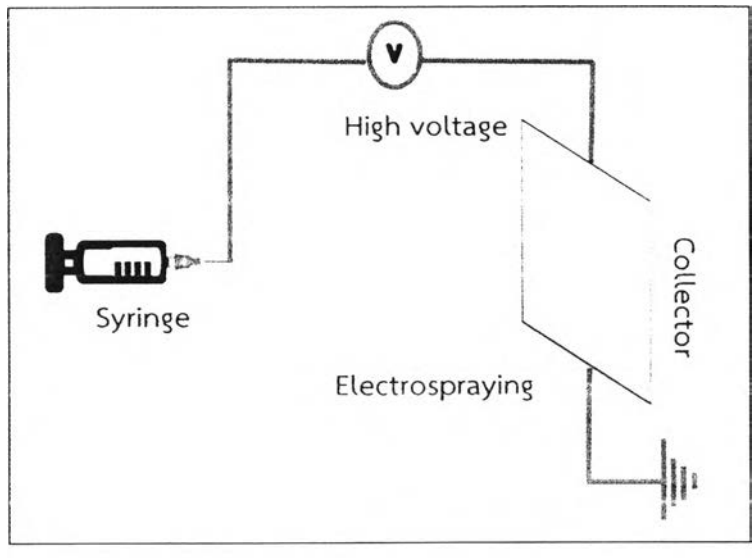

Figure 2.17 Scheme of electrospraying equipement

2.4 Literature reviews

2.4.1 Conventional detection methods for the determination of parabens

Various analytical methods have been utilized for the determination of parabens, for instance, UV-spectroscopy coupled with high performance liquid chromatography (HPLC), flame ionization detection (FID) in gas chromatography (GC), mass spectrometry (MS), and chemiluminescence detection. There are many researches that have been reported using these detections, especially, UV detection is the most common detection to determine parabens.

In 1999, Noguera-Orti *et al.* developed a simple and rapid micelle liquid chromatography coupled with UV detection at wavelength 280 nm for the determination of methyl paraben, ethyl paraben, propyl paraben and butyl paraben in cosmetic products. In this research, sodium dodecyl sulphate (SDS) was used as

micellar solution and the separation was carried out by a micellar mobile phase containing 0.1 M SDS, 2.5% n-propanol, 10 mM phosphate (pH 3), with C18 column. The limit of detection (LOD) was in the range of 0.03 to 0.3 ng which is lower than the regulated level [67].

In 2005, Zhang *et al.* developed a new method for the simultaneous determination of four parabens in wash-off cosmetic products and foods by high-performance liquid chromatography (HPLC) coupled with chemiluminescence detection. This research reported the high selectivity and sensitivity of the method by increasing the chemiluminescence intensity of cerium (IV)-rhodamine 6G chemiluminescence reaction with parabens. The separation was achieved by C8 column, and an isocratic elution with a mobile phase of methanol and water (60:40, v/v) within 8.5 min. The limit of detection was in the range of 1.9×10^{-9} to 5.3×10^{-3} g mL^{-1} [68].

In the same year, Saad *et al.* reported the simultaneous determination of preservatives which were benzoic acid (BA), sorbic acid (SA), methyl paraben (MP) and propyl paraben (PP) in foodstuffs using high-performance liquid chromatography. The separation was carried out by C18 column with the mobile phase of methanol-acetate buffer (pH 4.4) (35:65, v/v), after which it was changed to methanol-acetate buffer (pH 4.4) (50:50, v/v), and detected with a UV detection at 254 nm. Under the optimal conditions, all preservatives were detected in less than 23 min with the limit of detection for BA, SA, MP and PP of 0.5, 0.1, 0.3, and 0.1 mg L^{-1}, respectively [15].

In 2007, Sheng *et al.* reported the determination of seven phthalates and four parabens in cosmetic products using high-performance liquid chromatography coupled with diode array detection (HPLC-DAD) and using gas chromatography-mass spectrometry (GC-MS). For HPLC-DAD method, the separation was performed by C8 column at an oven temperature of 30 °C with a gradient elution system. All analytes were detected by DAD at 230 nm. For GC-MS method, the experiment was carried out by helium as carrier gas and the analytes were detected with electron impact ionization and selected-ion detection modes. These techniques were accomplished with the limit of detection in the range of 10.0 to 100.0 μg kg^{-1} and 20.0 to 200.0 μg kg^{-1} for phthalates and parabens, respectively [69].

In 2008, Han *et al.* developed a convenient and automated method for on-line pretreatment and determination of three parabens in cosmetic products by using a combination of flow injection analysis (FIA), solid-phase extraction (SPE) and micellar electrokinetic chromatography (MEKC). All the analytes were loaded onto a C8 column at 0.6 mL min^{-1} for 60 s and eluted with a mixed eluent of 40% (v/v) 10 mmol L^{-1} sodium tatraborate buffer (pH 9.3) and 60% (v/v) ethanol at 0.75 mL min^{-1}. The MEKC separation was achieved with a running buffer of 20 mmol L^{-1} sodium tetraborate (pH 9.3) containing 100 mmol L^{-1} sodium dodecyl sulfate (SDS) at 15 kV. The limits of detection were in the range of 0.07 to 0.1 g mL^{-1} [70].

In 2010, Darias *et al.* reported the sample preparation of solid-phase microextraction (SPME) coupled to gas chromatography with flame ionization detection. The functionalized polymeric ionic liquid has been used as coating in SPCE for the sensitive determination of polycyclic aromatic hydrocarbons which are parabens (butyl paraben and benzyl praben), and alkylphenols (4-tert butylphenol, 4-tert-octylphenol, 4-octylphenol, 4-cumylphenol, 4-n-nonphylphenol and bisphenol-A) in water. The separation was performed by GC column under the temperature program and the carrier gas was nitrogen. This proposed method was successfully used with the average recoveries of higher than 96.1% from deionized waters sample and higher than 76.7% from bottled drinking water samples [18].

In 2012, Cha *et al.* developed a simple and sensitive high-performance liquid chromatography (HPLC) for the determination of 19 preservatives including methyl paraben, ethyl paraben, propyl paraben, butyl paraben and isobutyl paraben in cosmetic matrices. The detection method was a UV photodiode array detector and the separation was accomplished with a C18 column and methanol, 0.05 mol L^{-1} ammonium acetate buffer and water as the mobile phase under the gradient elution conditions. All the preservatives were separated within 55 min and the recoveries obtained were in the range of 94.9% to 102.8% with the relative standard deviations of less than 3.2% [71].

In 2013, Youngvises *et al.* developed a greener liquid chromatography using a guard column with micellar mobile phase for the separation of some pharmaceuticals and determination of four common parabens. The objective of this

research was focused on using less toxic reagents by employing a short guard column: C18 guard cartridge of 12.5×4.6 mm id and 5 µm particles size in combination with sodium dodecyl sulphate (SDS) as micellar solution. All parabens were detected by UV detector at 254 nm with the limit of detection in the range of 0.04 to 0.10 µ mol L^{-1}. This work is not only a greener method but also shorter in the analysis time than a conventional liquid chromatography [72].

2.4.2 Electrochemical detection method for the determination of parabens

Electrochemical detection (ECD) is an alternative and very attractive detection method for the determination of parabens because of its low cost, simplicity, fast analysis, portability and high sensitivity. There are several working electrodes that have been reported for the determination of parabens.

In 1997, Kang *et al.* reported a simultaneous determination of methyl paraben (MP), propyl paraben (PP) and thimerosal (TMS) in pharmaceutical products by high-performance liquid chromatography coupled with electrochemical detection. All three preservatives were separated by C18 column with a mixed mobile phase of methanol and aqueous 0.02 M phosphoric acid (59:41, v/v). They were detected by amperometric method at glassy carbon electrode with a potential of +1.25 V *vs* Ag/AgCl. The limit of detection for a 20 µL injection of MP, PP and TMS was 1, 2, and 5 ng, respectively [2].

In 2008, Radovan *et al.* studied the electrochemical sensing for investigation of three common parabens in both hydro-alcoholic and aqueous media. The total parabens were detected at a boron-doped diamond working electrode, a platinum foil counter electrode and a saturated calomel electrode as reference. The cyclic voltammetry and chronoamperometry were used to establish the calibration curve with very good linearity of R^2 between 0.990 and 0.998 [73].

In 2010, Wang *et al.* developed a selective, sensitive, rapid and reliable method based on molecularly imprinted polymers (MIPs) with dual templates to determine total content of parabens in cosmetics. The sensor proved to be applicable for all kinds of parabens due to the similar structure of parabens.

All parabens were detected at the working electrode of MIPs film: methacrylic acid as a functional monomer and tripropylene glycol diacrylate as a cross-linker, modified on glassy carbon electrode. The square wave voltammetry was carried out in 0.2 M phosphate buffered saline aqueous solution (pH6.5). Scan potential from +0.2 V to +1.2 V at scan rate of 10 mV s^{-1} were used. Square wave amplitude was 25 mV with the frequency of 15 Hz. The limit of detection achieved were 0.4 µM for methyl paraben and ethyl paraben, and 0.2 µM for other parabens [20].

In the same year, Chu *et al.* developed a sensitive method for the determination of four parabens in soy sauce samples by capillary zone electrophoresis with amperometric detection. All four parabens were separated within 16 min at the separation voltage of 16 kV in 80 mmol L^{-1} borax running buffer (pH 9.94). And then, they were detected with the three electrodes consisting of a carbon-disk as working electrode, a platinum counter electrode and a saturated calomel electrode as reference electrode. The limit of detection was accomplished in the range of 5.7×10^{-8} to 4.4×10^{-8} g mL^{-1} [74].

In 2011, Martins *et al.* reported the determination of parabens in shampoo using high performance liquid chromatography with amperometric detection. Three common parabens were successfully separated by C8 column with a mobile phase of 0.025 mol L^{-1} disodium phosphate (pH 7) and acetonitrile (40:60, v/v). All parabens were detected in the thin layer flow cell which included a boron-doped diamond electrode, stainless steel and platinum as working, reference and counter electrode, respectively. This method was successfully used with the limit of detection of 0.01 % (w/w) [21].

In 2012, Luo *et al.* developed a novel voltammetric sensor using multi-wall carbon nanotubes (MWNTs) coupled with nafion modified glassy carbon electrode (GCE) for the determination of methyl paraben (MP). The investigation of MP was carried out in the phosphate buffer solution (pH 6.5). MWNTs and nafion were able to increase the sensitivity as the peak current of MP at modified electrode was higher than the unmodified GCE. Moreover, the oxidation peak potential was decreased. The modified electrode with good repeatability and the limit of detection was 1×10^{-8} M was successfully developed in this research [22].

In 2013, Chuto *et al.* reported a rapid separation for the determination of three parabens by ultra-performance liquid chromatography coupled with electrochemical detection. This research was accomplished with the separation of three common parabens within 2 min by C18 monolithic column and a mobile phase of 0.05 M phosphate buffer (pH 5) and acetonitrile (25:75, v/v). In addition, all parabens were detected by amperometric detection at boron-doped diamond electrode with a detection potential of +1.5 V *vs* Ag/AgCl. Under the optimal conditions, the limit of detection of 0.03 mg L^{-1} was achieved [75].

In this work, five common parabens, i.e. methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP) and isobutyl paraben (IBP) were simultaneously determined by high-performance liquid chromatography (HPLC) coupled with electrochemical detection (ECD) using the G/PVP/PANI nanocomposite-modified screen printed-carbon electrode.