CHAPTER II

THEORY AND LITERATURE SURVEY

The capillary electrophoresis microchip forms the heart of the system for measuring inorganic ions. The design of the microchip and the optimization of the separation conditions are critical to reach the goal of analyzing complex samples. In this chapter, the fundamentals of capillary electrophoretic separations are discussed followed by an overview of the microchip design parameters and their influence on the separation performance. In the second part of this chapter, the aspects of on-chip electrochemical detection, amperometry, are treated. The microchip systems are obtained to optimize the sensitivity and dynamic range. The discussions in this chapter the basis of the microchip used in the subsequent chapters.

2.1 Fundamentals of capillary electrophoresis

2.1.1 Migration of ions in electric fields

The separation of ions by means of electrophoresis is based on the migration of charged species through an electrolyte solution under the influence of an externally applied electric field. All particles in a stream of buffer moving under the influence of an electric field are carried along by the EOF. This is not immediately advantageous when a chemical separation is desired because the flow itself does not cause separation of molecules in the solution. However, the presence of the electric field attracts and/or repels the charged analytes in the buffer, and this phenomenon is known as electrophoresis. The concentration of analytes residing in the buffer solution is usually much lower than that in the BGE and, therefore, the EOF properties of the system coexist with this electrophoretic phenomenon.

Fundamental to any CE platform that utilizes a mobile carrier solution, an externally applied field produces two independent and distinguishable flow parameters in response to electroosmosis and electrophoresis: the bulk migration of the buffer solution itself, the electroosmotic mobility, (μ_{EOF}) was described , and the

migration of charged analyte molecules suspended in the buffer solution, or electrophoretic mobility (μ_{EP}), both of which are established in response to the field. (Figure 2.1) The migration velocity, v, of solute molecules is proportional to the applied field, E, and the electrophoretic mobility, μ_{EP}, as follows :

$$v = \mu_{EP} \cdot E \qquad \text{(Equation 2.1)}$$

The electrophoretic mobility depends on the radius, r, and charge, q, of the molecule, and, assuming a constant velocity where the electric force, F_E, is balanced by the countering friction force, F_f, such that $F_E = F_f$

$$F_e = q \cdot E \qquad \text{(Equation 2.2)}$$

$$F_f = 6 \cdot \pi \cdot \eta \cdot r \cdot v \qquad \text{(Equation 2.3)}$$

Finally, μ_{EP} can be represented in (Equation 2.4)

$$\mu_{EP} = \frac{v}{E} = \frac{q}{6 \cdot \pi \cdot \eta \cdot r} \qquad \text{(Equation 2.4)}$$

Many analytes will partially dissociate in a solvent, and consequently will have a resulting charge. Positively charged analytes will move faster than the bulk flow because they are attracted to the cathode, resulting in a positive μ_{EP}. Conversely, migration of the anions will be retarded by the electric field because they are attracted to the anode, and the μ_{EP} is negative.

While the anions are attracted to the anode, they do not actually move toward it, but simply resist the effects of the EOF. The total mobility, and corresponding flow of the analytes migrating in the channel is the sum of the electroosmotic and electrophoretic flows; however, the predominating value is the EOF, which is directed towards the cathode. The sum of the two mobilities yields μ_{APP}, which is the apparent mobility of the analyte:

$$\mu_{APP} = \mu_{EOF} \pm \mu_{EP} \qquad \text{(Equation 2.5)}$$

Since neutral molecules have no net charge when dissolved in the saline buffer, and are therefore essentially oblivious to the presence of the electric field, their movement in response to an applied field is entirely due to the bulk flow, or EOF, of the buffer solution. The value of μ_{EP} is zero for these molecules. This detail is traditionally used to quantify the native electroosmotic flow in a capillary system because in this case, μ_{EOF} will be equal to μ_{APP}. Accordingly, the migration of charged analytes will be either enhanced or retarded by the presence of the electric field and will therefore have a non-zero μ_{EP} value. This produces a value for μ_{APP} that is either slightly larger or smaller than μ_{EOF}, depending on the charge of the particular species (Figure 2.1).

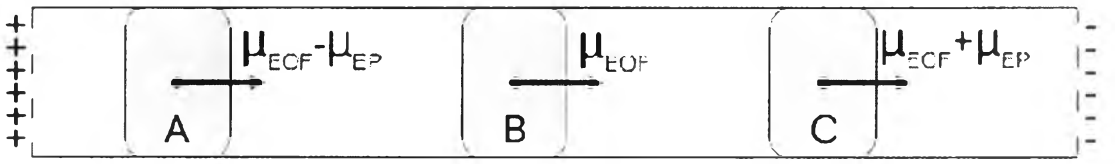

Figure 2.1 Representation of effective mobilities for (A) negatively charged species, (B) neutral species, and (C) positively charged species (Adapted from Baker).

Separation by size does occur to a small extent in CE; however, this effect is negligible for small analytes, whose hydrated molecular shell is subjected to very little drag while moving under the influence of the electric field. For extremely large molecules, proteins for example, where the charge-to-size ratio is considerably different from smaller molecules, this drag force would become significant and have to be considered. The typical order of elution of positive, negative, and neutral molecules in the BGE is outlined in Figure 2.2.

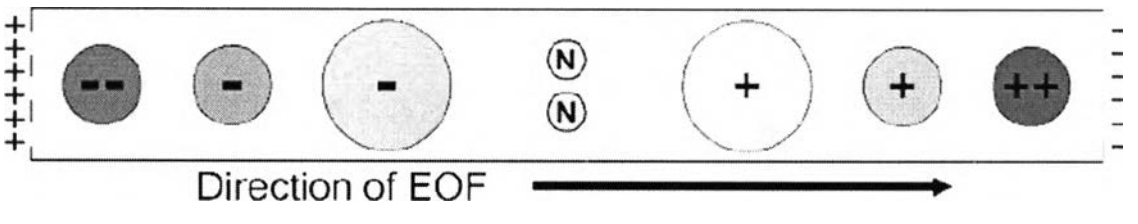

Figure 2.2 Representation of charged (positive and negative) and neutral species distribution in an electrophoretic separation, including representation for separation by size (Adapted from Baker).

Other factors will affect both the magnitude and, in some cases, direction of the EOF (pH, concentration, temperature, consistency of the zeta potential, viscosity, etc). Increasing the basicity of the BGE generally increases the EOF, due to an increase in the dissociation of hydrogen ions from the walls of the capillary. This situation makes more negatively charged sites available on the capillary wall, which results in an enhanced EOF. Temperature variations, most commonly associated with Joule heating from the large voltages applied to the channels, induce flow-field distortions that adversely affect the plug flow in the channels by increasing longitudinal diffusion.

Certain capillary wall treatments can completely mask the silanol groups and create a region of anionic molecules at the BGE wall interface, which establishes a reverse EOF condition (19). CE as described above is a term for what is more specifically known as capillary zone electrophoresis (CZE).

2.1.2 Electroosmotic flow

The onset of an electroosmotic flow (EOF) is an important phenomenon in CE. Many materials, including glass, develop a charge at the surface when they come into contact with a protic solvent. For glass, the surface charge develops from the protonation or deprotonation of silanol groups (pKa~3.5). For polymers without any proton donating or accepting groups, traces of residual unreacted material in the bulk or charged substances adsorbed to the surface can result in a surface charge.

Electroosmosis is the process of inducing ionized liquid motion adjacent to a stationary charged surface under an applied external electric field. In microchannel systems, the liquid flows under the influence of external electric fields. An influential factor of the flow behavior is the zeta potential because it regulates the ion mobility, which is in turn an important factor on the velocity of the analyte. The zeta potential is directly proportional to the applied field and the channel material. This flow is governed by a slightly modified the Navier-Stokes equation that accounts for the potential distribution in the diffuse-layer, which is in turn governed by the Poisson-Boltzmann equation.

$$\mu\left[\frac{\partial V}{\partial t} + (V \bullet \nabla)V\right] = -\nabla p + \mu\nabla^2 V - \rho_f \underbrace{E}_{\text{Potential distribution term}}$$

(Equation 2.6)

In microscales, electroosmosis is a good alternative for controlling the flow in micro fluidic systems for biological and chemical analysis because it alleviates difficulties associated with the large pressure gradients required in the traditional pressure driven flows. It alse eliminates the moving mechanical components such as valves, switches and gates.

In microchannels, the flow moves with low Reynolds numbers and laminar flow. Consequently, species mixing is a slow, diffusion-dominated phenomenon that requires long mixing channels and large retention times to attain a homogeneous solution. When an electric field is applied across the channel, the ions in the double layer move towards the electrode of opposite polarity. This creates motion of the fluid near the walls that transfers via viscous forces into convective motion of the bulk fluid. The velocity profile is laminar and parabolic except when close to the walls of the channel.

There are two significant disadvantages of electroosmotic flow. First, it is affected by the surface properties. Second, it requires high voltages to obtain appreciable velocities.

Selecting the proper material to construct the channels is also a great challenge because it controls the formation of the electrical double layer (EDL) in which the excess of positive ions flow and induce the fluid motion. This section, where the excess of positive ions flow, is also known as the Debye length and it is equivalent to the distance from the wall where the electroosmotic potential energy is equal to the thermal energy. The EDL has two distinctive sections, as shown in Figure 2.3. The first is the Stern plane, which is the immobile layer of ions next to the solid surface. Its function is to stabilize the charge of the wall. The second section is the diffusion layer, which is characterized by the zeta potential (ζ) and governed by the Poisson-Boltzmann equation.

Poisson-Boltzmann Equation $\nabla^2\psi = \kappa^2\psi$ (Equation 2.7)

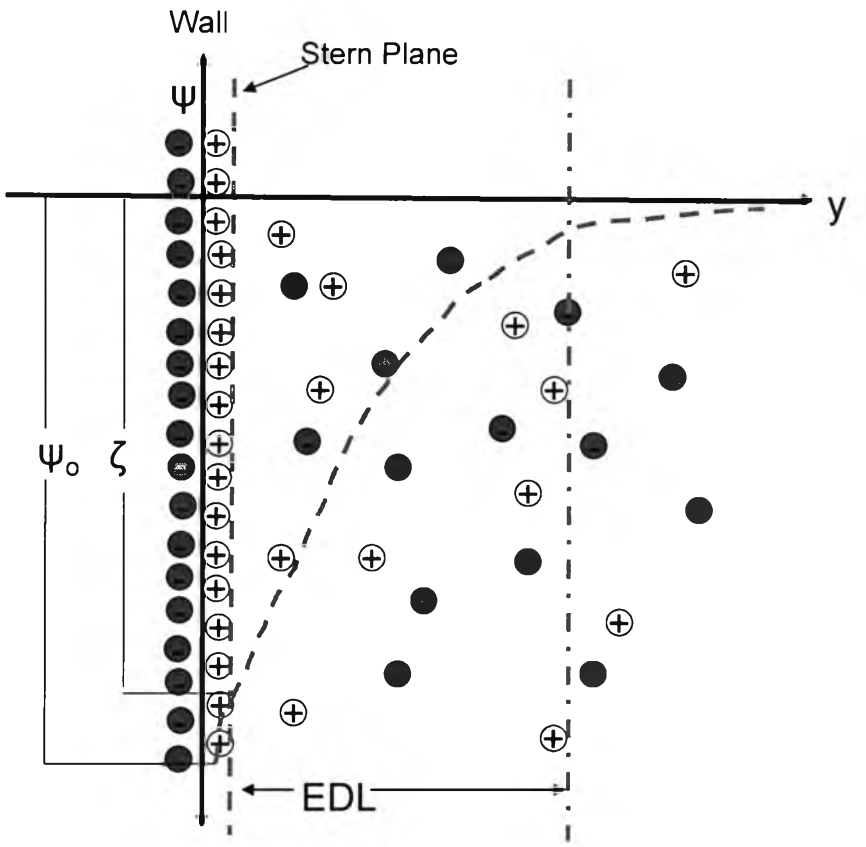

Figure 2.3 Double layer potential. This scheme shows the electric double layer formed next to the negative charged solid surface. The EDL is due to the surface potential ψ_o, applied to the wall of the channel.

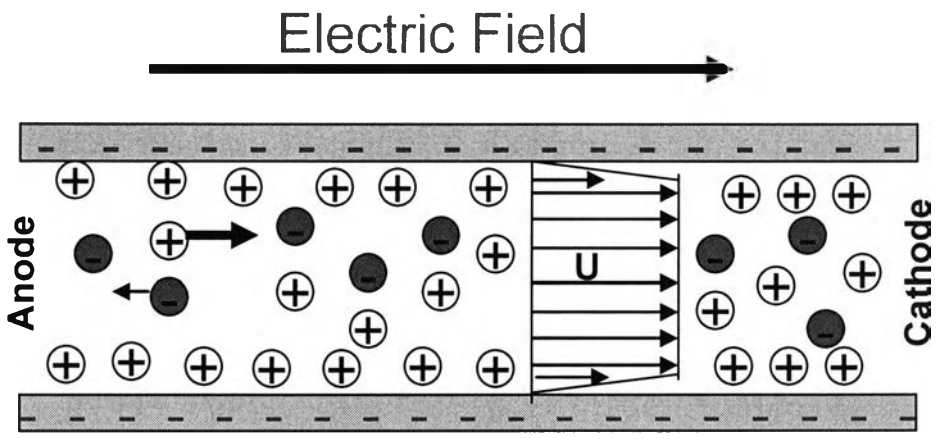

Figure 2.4 Electroosmotic velocity profile in microchannels. This is a schematic representation of the fluid flow inside a symmetric channel with constant electric potential at the walls.

The rest of the fluid is dragged along in the capillary, creating a bulk flow of liquid in it. The flow profile will not be parabolic as in hydrodynamic flow, where drag from stationary surfaces slows the liquid at the walls. Instead the flow profile is flat. Flat flow profile gives less band broadening than a parabolic flow profile from hydrodynamic flow, see Figure 2.5

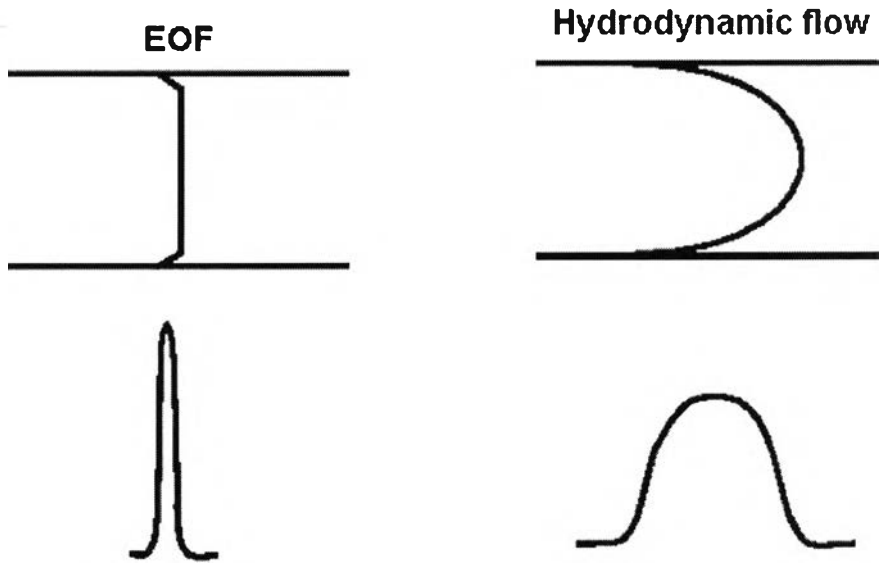

Figure 2.5 Flow profiles and the effect on peak shapes for electroosmotic flow and hydrodynamic flow.

2.1.3 Capillary electrophoresis separation modes

The capillaries used in most traditional CE systems are made from glass, most commonly in the form of fused silica. The surface chemistry of this material consists of silanol (≡Si-OH) groups (Figure 2.6), which deprotonate when in contact with a polar solution (pH > 3), resulting in a small negative surface charge.

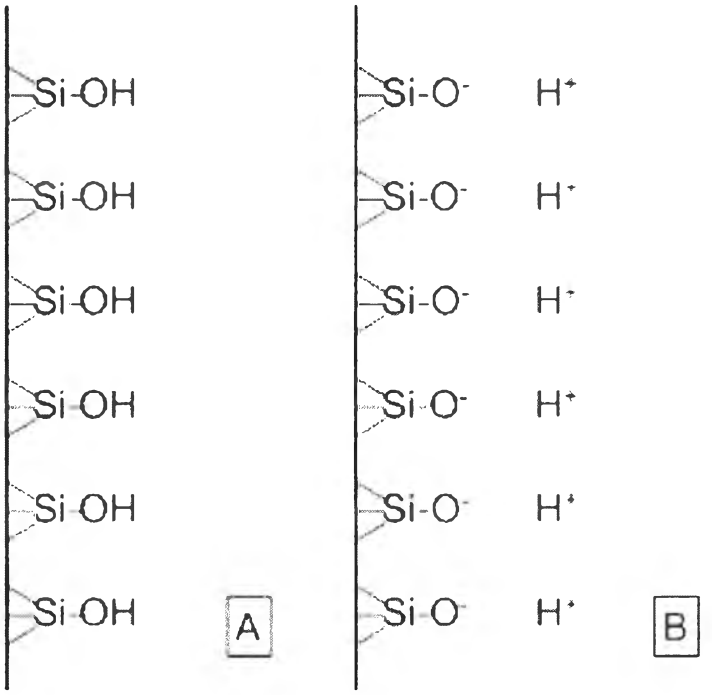

Figure 2.6 A) Schematic of silanol groups on the surface of fused silica; B) deprotonation of the silanol groups at pH > 3 in an aqueous environment.

Different types of separations by means of capillary electrophoresis can be distinguished. For inorganic ions, moving boundary electrophoresis (MBE), isotachophoresis (ITP) and capillary zone electrophoresis (CZE) are the most important separation modes. MBE and ITP are often used in combination with CZE. In a CZE separation, a defined sample plug is introduced into one end of a capillary that is filled with background electrolyte (BGE) (Figure 2.7A). Upon applying a potential difference between the ends of the capillary, the cationic species migrate towards the cathode, and the anionic species to the anode. The analytes separate into zones with different mobilities, which are detected at the end of the capillary (Figure 2.7B). In ITP, two different BGEs are used on both sides of the sample plug (Figure 2.7E). The terminating electrolyte (TE) contains a BGE co-ion (an ion with the same charge sign as the analyte) that has a lower mobility than the ions in the sample.

The leading electrolyte (LE) contains a BGE co-ion that has a higher mobility than the ions in the sample. Under these conditions an uninterrupted train of analyte zones form (Figure 2.7F). The difference with CZE is that the zones are not separated from each other by regions with BGE. In MBE, the sample is not introduced as a plug, but is permanently present at the inlet of the capillary (Figure 2.7C). Only a partial separation of the analytes occurs since, apart from the leading analyte zone, all other zones contain a mixture of analytes. The actual separations in this thesis are all performed under CZE conditions.

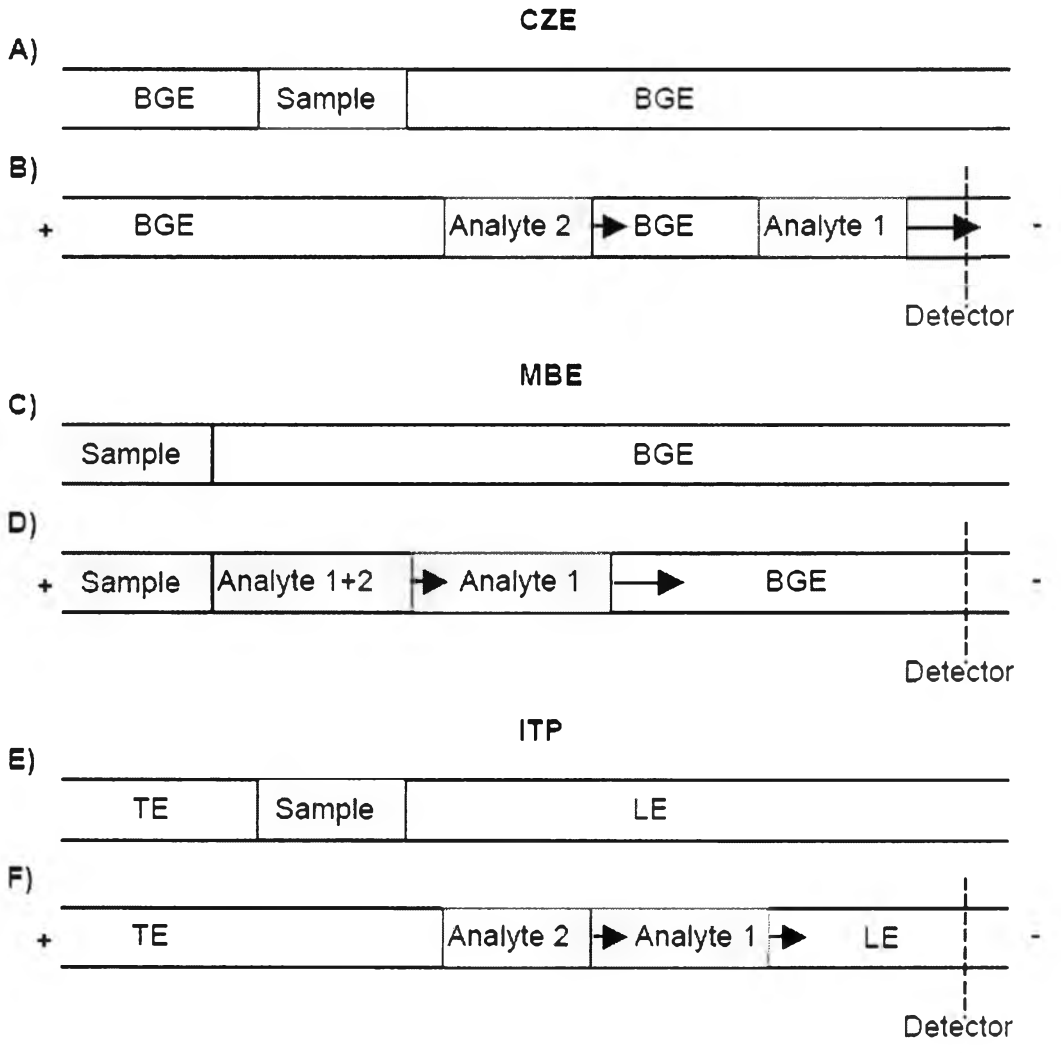

Figure 2.7 Schematic representation of different capillary electrophoresis separation modes. A, C, E; starting situations, B, D, F; separation into zones. See the text for a description.

In which a screen-printed carbon electrode was placed at the end of the channel. This approach simplifies the fabrication of the working electrode and also provides a convenient and sensitive means for the determination of metal ions by amperometry. Furthermore, by simply changing the electrode, the detection electrode, which is prone to contamination, can be easily removed or cleaned. It may provide a good portable device for screening, or simultaneously analyzing a complex system containing different metal species such as food. The optimization, characterization, and attractive performance characteristics of such a microchip CE, and its successful application to complex samples (such as vegetable juices) are reported in the following sections.

2.2. Microchip capillary electrophoresis

2.2.1. Microchip design and separation performance

Capillary electrophoresis may be carried out on miniaturised analytical devices. In this approach, a capillary column is replaced with a microchip with much shorter channel length and smaller dimensions, which offers dramatic decrease in analysis times and reagent consumption.

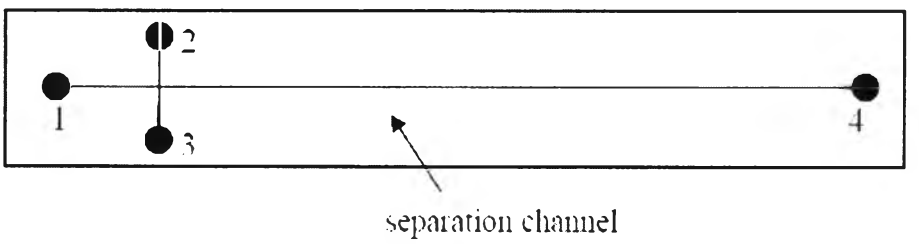

Figure 2.8 Layout of a microchip; 1:electrolyte inlet, 2: sample inlet, 3: sample outlet, 4: outlet

These planar devices with micrometer dimensions, hence the name microchips, are mainly produced by photolithographic and wet etching techniques readily available from the semiconductor industry. Non-insulating substrates such as glass, silicon, and more recently polymers are used. A typical fabrication process involves

metal film deposition, photolithography and etching of a CE channel with desired dimensions on a bottom plate. In the final step, a coverlid is then bonded on top. Fluid reservoirs usually plastic vials or pipet tips are glued on to the chips. They are connected to the microchannel through holes drilled into the cover plate. Platinum electrodes are placed into these reservoirs and connected to a high voltage power supply via relay.

A typical layout for a standard separation microdevice is seen in Figure 2.9. There are four reservoirs: a sample reservoir for introducing sample, a buffer reservoir for the mobile phase, a sample waste reservoir which sample flows to and finally a waste reservoir at the end of the separation channel which buffer runs to. Sometimes an extra buffer channel is added, which allows for real time changing of the mobile phase during runs. This is called gradient elution, where the percentage of organic modifier is changed most frequently to alter the selectivity of the column towards the analytes.

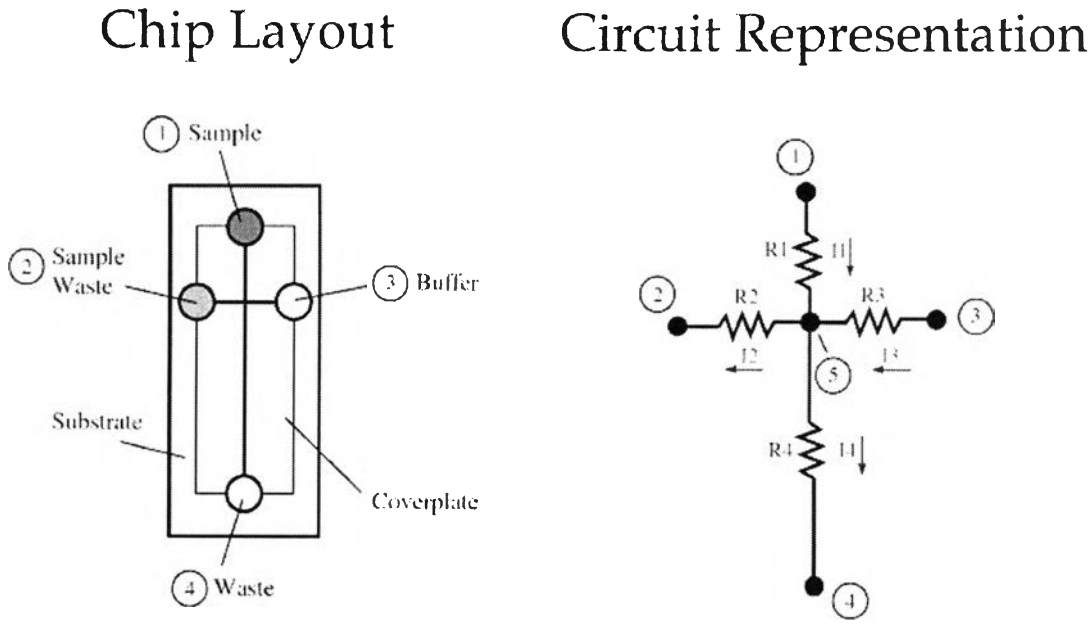

Figure 2.9 Microchip layout with a simple cross (left) and the electric circuit equivalent to the microchip (right) (20).

2.2.2 Sample loading and plug shaping

One of the differences between microchip CE and conventional CE is the method by which the sample plug is introduced into the capillary. In conventional instruments, one end of the capillary is immersed in the sample solution. The sample is driven into the capillary by applying a pressure pulse (hydrodynamic injection) or using EOF (electrokinetic injection). For a microchip separation, a very short sample plug (<1 mm) needs to be introduced. To permit this, microchip CE devices typically employ a column coupling configuration of channels (Figure 2.10A-H). In the cross injector design and double-T in Figure 2.10, A and B are among the earliest designs employed in microchip CE (21).

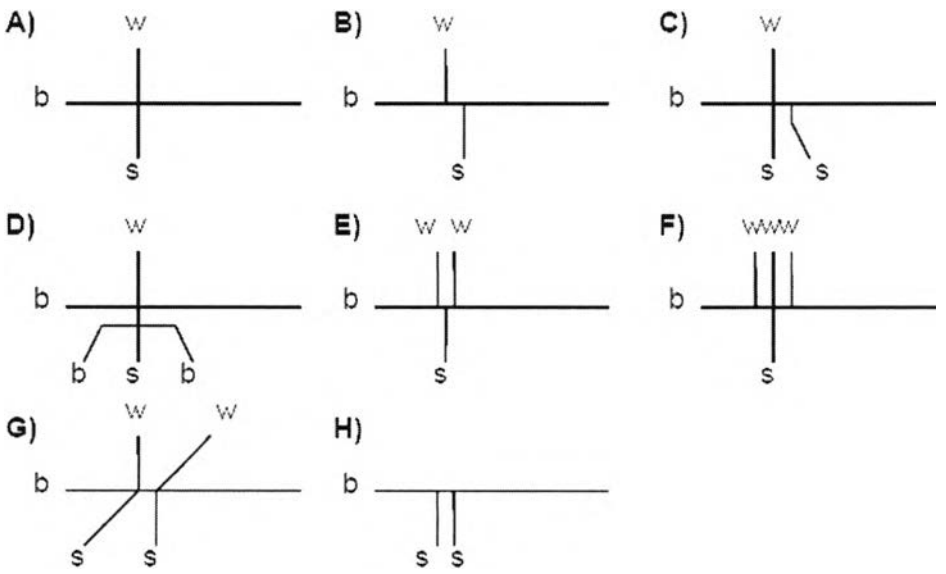

Figure 2.10 Examples of sample injector types. A) cross, B) double-T, C) double-L, D) double cross, E) triple-T, F) multi-T, G) stacking type, H) π-injector. Sample inlet s, waste w and BGE inlet B are indicated (20).

2.2.3 Sample injection in the microsystem

There are two main types of injection methods used in microchips: the gated injection and pinched injection described as follows.

2.2.3.1 Gated injection

Figure 2.11 illustrates the principle of the gated injection. The main waste reservoir on the end of the injection channel is set to ground at all times. In pre-injection and run mode the settings are the same. Voltages are set so that the mobile phase will flow from the buffer reservoir (B) to the waste reservoir (W), and the sample will run from the sample reservoir (S) to the sample waste reservoir (SW). To prevent any sample from "bleeding" into the separation channel, the voltage on B is set higher than the voltage on S so that some mobile phase will always run into the SW reservoir.

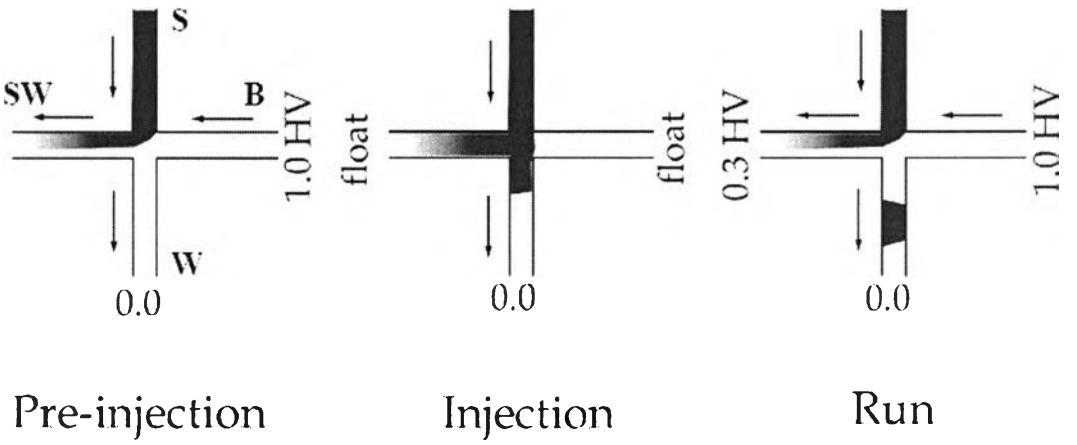

Figure 2.11 Principle of gated injection. Red stream is the sample, and white stream is the mobile phase. S: Sample, SW: Sample waste, B: Buffer, W: Waste (20).

In the injection mode, both B and SW are set to "float", which means that no current will run through either reservoir. As a result no fluid will flow to or from either reservoir. The voltages on S and W are the same as those in the run mode, so a "plug" of sample will flow into the separation channel as long as the voltages are in the injection settings. Typically, the injection mode is switched on for 0.1-1 seconds. The longer the settings are on the injection step, the longer the plug is injected into the column.

After the desired amount of analyte has been injected into the separation channel, the voltages are changed to the run mode. The settings in the run mode are the same as those in the pre-injection mode. The sample will flow from S to SW as before, and the fresh mobile phase will flow down the separation channel from B. The injected sample plug will flow down the separation channel, where individual components will be separated and finally detected.

Gated injection is very simple to perform and it allows for injection of as long a plug as thought necessary for injection into the separation channel. That a new sample plug can be injected into the separation channel at any time is another advantage of the gated injection. This means that a new sample can even be injected into the separation channel before the previous sample reaches the detector.

One drawback to the gated injection method is a bias in the injection. The species with the highest electrokinetic velocity are injected at a larger extent than those with lower electrokinetic velocities. This means that in regular EOF, a sample plug injected into the cation sample plug will be slightly longer than that for neutral species and the anion sample plug will be slightly shorter than for neutrals. This will lower the limit of detection somewhat for negatively charged species, but the effect is usually not large enough to be significant. If the gated injection is also used to make a calibration curve for the species, this bias will even out since the same bias will apply for the calibration and sample injection.

That flows are steered by applying electrical potentials to different points on the chip is an advantage of electrokinetic injection, which does not require mechanical components. Overall, the simple cross and double-T designs are still the most commonly used types owing to their simplicity. For all experiments in this thesis, a simple cross design is used.

2.2.3.2 Pinched injection

Figure 2.12 illustrates the principles of the pinched injection. Here, the sample is placed on one of the side-arms of the cross, the mobile phase is placed in the top reservoir, and the sample waste is placed on the other side-arm. The waste reservoir needs to be filled with fresh mobile phase. In the "load position", sample

flows from S towards SW, over the cross. Mobile phase flows from both B and W to the SW to prevent bleeding of the sample into the main channel. The sample stream is "pinched" together by the two mobile phase streams.

In the "run position", the voltages are switched so that the mobile phase will flow from B to the cross and towards all three reservoirs. Most of the flow is directed toward the separation column to drive the sample plug forward, but some mobile phase flow will also be directed towards S and SW to avoid bleeding.

The size of the sample plug is determined by the geometry of the injection cross, so that its length can never be more than the width of the channels at the injection cross. However, the sample plug can be made smaller. By increasing the buffer flow from B and W, the pinched sample stream at the junction becomes narrower, and a shorter plug is injected.

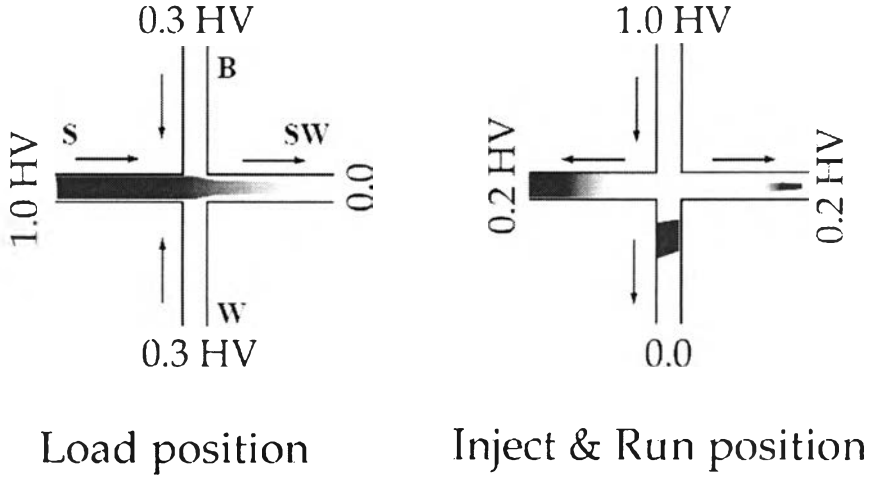

Figure 2.12 Principles of pinched injection. Red stream is the sample, and white stream is the mobile phase. S: Sample, SW: Sample waste, B: Buffer, W: Waste. (20)

As long as the loading step in the pinched injection is given sufficient time, there is no bias in the sample injected, as in the gated injection. A drawback of using the pinched injection is that after each run, the microchip needs to be switched to the "load position" and allowed to equilibrate before a new run is possible. Another disadvantage of the pinched injection is that the "load position" has a backflow of

mobile phase from the W reservoir, which will contain some analytes after a few runs. Therefore, the separation channel will fill up with analytes during this backflow. Since the sample volumes in the channels are very small, a sufficiently large volume of the reservoirs will dilute the analytes so much so that they should be undetectable when they run back through the detector from the waste reservoir. Care should be taken by regularly changing the buffer in waste reservoirs.

2.2.4 Dimension of the microfluidic channels

The two main factors defining the microchip are the length of the separation channel and the length of the sample plug. In order to compare different designs, a measure to describe the performance is necessary. Following the definitions used in chromatographic separations, the terms, number of theoretical plates, plate height and resolution are used.

The plate number N is defined by the spatial variance of a zone σ^2 (m^2) after migrating a distance L (m):

$$N = \frac{L^2}{\sigma^2} \qquad \text{(Equation 2.8)}$$

In analogy, the plate height H (m) is given by:

$$H = \frac{L}{N} = \frac{\sigma^2}{L} \qquad \text{(Equation 2.9)}$$

In the ideal case, the broadening of a zone is the result of only molecular diffusion in the time interval t (s) before the analyte zone reaches the detector. The spatial variance resulting from the diffusion of an initial infinitely small zone with a diffusion coefficient D (m^2/s) is provided by the Einstein equation:

$$\sigma^2_{diff} = 2Dt \qquad \text{(Equation 2.10)}$$

Combining equations 2.5, 2.12 and 2.14 yields the plate number under ideal conditions:

$$N = \frac{\mu V}{2D} \qquad \text{(Equation 2.11)}$$

This equation forms the basis of microchip CE. It shows that the separation efficiency is independent of the separation channel length under condition that the diffusion solely determines the spatial variance. The only experimentally accessible parameter is the applied voltage *V*.

The diffusion constant and electrophoretic mobility both involve movement through the medium and can be converted into one another:

$$D = \frac{\mu RT}{zF} \qquad \text{(Equation 2.12)}$$

Equations 2.11 and 2.12 demonstrate that the plate number is also not affected by the mobility or diffusion constant, since they cancel each other out.

A more useful parameter to characterize the separation performance is the resolution *(Rs)* between two analyte peaks (see Figure 2.5) that are separated by a distance of Δx (m):

$$R_s = \frac{\Delta x}{4\sigma} = 1.18\frac{\Delta x}{w_{1/2}^{peak1} + w_{1/2}^{peak2}} \qquad \text{(Equation 2.13)}$$

To calculate the resolution from experimental data, it is easier to measure the width of the peak at half height, $w_{1/2}$, instead of the variance or the width at the baseline (w_1, w_2 in Figure 2.13).

The equations for plate number, plate height and resolution all indicate that the only way to increase the separating performance is to either increase the separation voltage or reduce the EOF. In reality, diffusion is only one of many potential sources of dispersion.

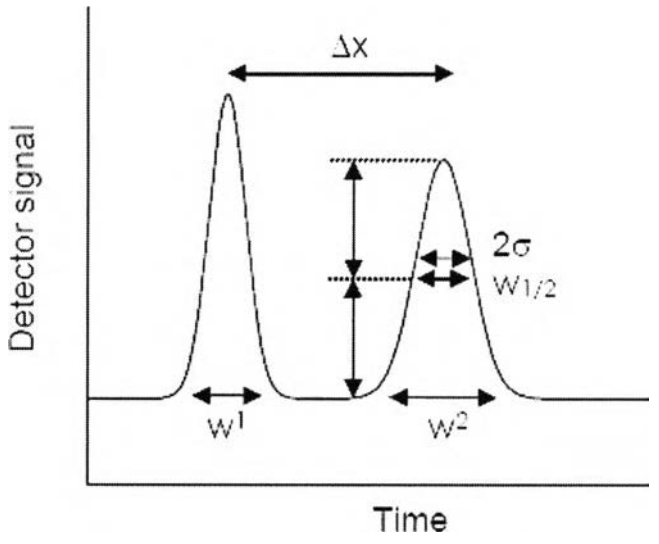

Figure 2.13 Representation of the resolution in separation science.

Also, the length of the initial sample plug and the size of the detection area are not infinitely small. When these effects are taken into account it is possible to define a set of design rules for CE microchips that will provide the best performance.

For conventional CE separations, fused silica capillaries ranging in length from a few decimeters up to a meter are used. It is possible to incorporate such a length onto a microchip by folding up the channel. However, the turns that are introduced are an additional source of zone broadening (22). Although the dispersion can be kept to a minimum, following a couple of design rules (e.g. optimizing the turn radius and using narrow channels in the turns), long channels are seldom used. One of the reasons is that the migration time increases due the longer migration distance, while the migration velocity decreases due to the lower electrical field strength at the same voltage. This diminishes the benefits of microchip CE as a faster alternative to conventional systems. The separation channels on microchips are therefore typically shorter than 10 cm. The width and depth of the channel also affect the performance, but in a more indirect manner. A hydrostatic pressure difference will cause a hydrodynamic flow during the separation. The flow profile has a parabolic shape, which produces additional dispersion of the zones. A pressure difference is avoided by filling all fluid compartments to the same height. However, since the channels are typically very short, the low hydrodynamic resistance can cause problems even for small pressure differences.

2.2.5 Electromigration dispersion

Electromigration dispersion (EMD) is, together with the diffusion, the most important source of band broadening. It originates from the fact that the analyte zones change the local electrical conductivity and hence the local electrical field strength. The migration velocity of a species is therefore a function of its concentration. As a result, the zones develop a triangular shape shown schematically in Figure 2.7. The larger the difference in mobility of the analyte ion and the BGE co-ion, the higher the analyte concentration in the sample. In general, the BGE will be optimized to minimize dispersion by selecting a co-ion with a mobility close to that of the analytes. However, for conductivity detection a large difference in mobilities is required for optimum sensitivity, which is discussed in section 2.4.2. Electromigration dispersion is therefore an important contributor to the peak broadening.

2.2.6 Detection methods for inorganic ions

A particular advantage of microchip technology is integration of the separation column and detection, which minimizes the dispersion ensuing from the dead volume. For conventional systems, optical detection is still the favored method because it is applicable to most organic substances. Inorganic ions are best measured using conductivity detection, since many of these species cannot be detected directly with optical methods. Conductivity detection also suits the need for non-selective detection that is suitable for a universal point-of-care testing platform.

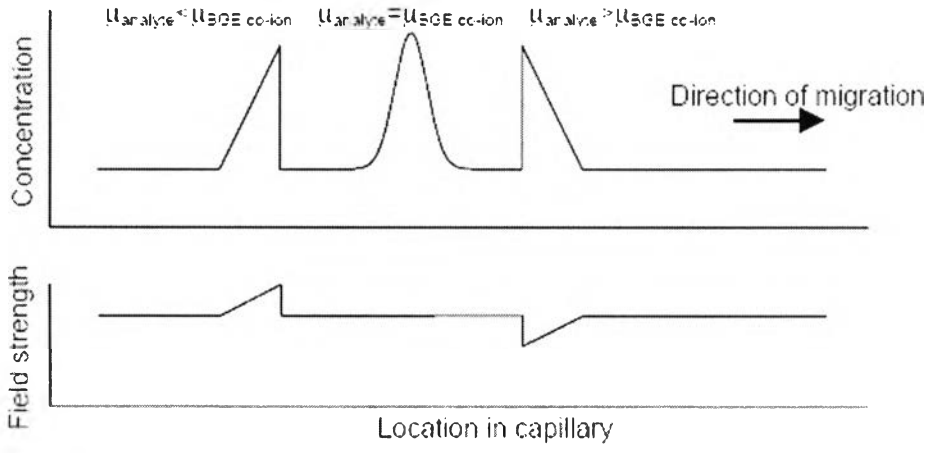

Figure 2.14 Concentration distribution and local electrical field strength caused by electromigration dispersion and diffusion for three analytes with different mobilities.

2.3 Detection methods compatible with capillary electrophoresis

A large variety of detection principles have been applied for inorganic ion analysis with CE. A selection of detection methods is presented in Table 2.1. For conventional CE, the optical methods, UV/Vis and fluorescence are the standard, since most applications are developed for organic compounds. With optical methods, the concentration can be measured without having to make contact with the solution.

This prevents problems arising from the high-voltage used. In addition fluorescence is one of the most sensitive detection methods, while UV/Vis can be used without analyte labeling and it adds an amount of selectivity by selection of the wavelength.

Table 2.1 Detection methods for inorganic cations.

Detection method	*Detection limits* *Mass (mol)* *Conc. (mol L⁻¹)*	*Primary advantages*	*Primary drawbacks*
Indirect optical			
UV–Vis Absorption	10^{-12}–10^{-14}	Universal	Relatively low
indirect by BGE	10^{-4}–10^{-6}		sensitivity
Indirect by	10^{-13}–10^{-15}	Sensitive	Requires
complexation	10^{-6}–10^{-8}		derivatization
Fluorescence	10^{-15}–10^{-18}	Sensitive, universal	Limited number of
indirect by BGE	10^{-6}–10^{-9}		suitable fluorophores
Indirect by	10^{-14}–10^{-17}	Sensitive, selective	Requires
complexation	10^{-5}–10^{-7}		derivatization
Electrochemical			
Conductivity	10^{-14}–10^{-16}	Universal	Maintenance of
	10^{-4}–10^{-8}		electrodes
Potentiometry	10^{-13}–10^{-15}	Universal	Limited number of
	10^{-3}–10^{-8}		suitable ionophores
Amperometry	10^{-18}–10^{-20}	Sensitive, selective	Maintenance of
	10^{-5}–10^{-11}		electrodes
Direct optical			
ICP–OES	10^{-13}–10^{-15}	Selective	Sensitivity
	10^{-3}–10^{-8}		
Mass Spectrometry			
ESI–MS	10^{-16}–10^{-17}	Selective	Limited choice of
	10^{-8}–10^{-9}		buffer, sensitivity
ICP–MS	10^{-13}–10^{-15}	Sensitive, selective	Limited number of
	10^{-3}–10^{-8}		detectable elements
TOF–MS	10^{-13}–10^{-15}	Number of detectable	Sensitivity
	10^{-3}–10^{-8}	elements	
Radioactivity	10^{-16}–10^{-20}	Selective	Requires long count
	10^{-9}–10^{-13}		times
X-ray fluorescence	10^{-13}–10^{-15}	Selective, number of	Complex set-up and
	10^{-3}–10^{-8}	detectable elements	alignment
Photothermal	10^{-13}–10^{-15}	Sensitive, selective	Complex set-up and
	10^{-6}–10^{-7}		alignment

The use of diode array detectors can help with identifying compounds. Optical detection can be integrated into microfluidic chips with the use of optical fibers and light emitting diodes. However, many inorganic ions cannot be detected directly by optical methods. Indirect optical detection of such species is possible using absorbing species in the BGE (23). The analyte displaces the absorbing species resulting in negative peaks in the signal.

Amperometric detection of redox active substances and potentiometric detection require no complex optics, which make these methods attractive for use in small portable instruments. Despite the very low detection limits that can be achieved with amperometric detection, these methods are not used very often. Interference by the electrical field required for the separation and the need for a stable reference electrode necessitate the use of special detectors. Using the high voltage electrode as a pseudo reference electrode eliminates the need for a real reference electrode, but the positioning of the working electrode and stability of the electrical field remain critical. Conductivity detection is a more straightforward choice, since all ionic species can be detected and the conductivity can be measured even from outside the capillary. On the other hand, compared to many other techniques it is challenging to achieve extremely low detection limits.

2.4 Background electrolyte

The main function of the BGE is to provide an electrically conductive medium that has buffering properties. Microchips generally have small fluid compartments, which can result in a rapid change of pH due to the electrolysis products accumulating in a restricted volume (24). Changes in the pH alter the effective mobility of weak electrolytes and the mobility of the EOF. The species selected for the BGE should therefore exhibit excellent buffering capacity. For applications where the BGE enables the indirect detection of analytes, also the concentration of at least one of its constituents has to be measurable. In the case of conductivity detection, the main consideration for the selection of the BGE is the sensitivity, which is influenced by the mobility of the BGE ions.

The BGE concentration needs to be optimized for minimal electromigration dispersion (EMD). At low BGE concentrations the EMD is excessive, while at high concentrations the joule heating causes additional dispersion. A sufficiently high BGE also provides the conditions for sample stacking (25). This is a process that occurs at the start of the separation when the ions migrate out of the sample matrix and into the BGE, resulting in a compression of the sample plug. Under most circumstances the sample solution has a lower conductivity than BGE solution. As a

result the electrical field strength is higher in the sample plug than in the remainder of the capillary (Figure 2.15A). The analytes quickly migrate out of the sample plug and slow down as they enter the BGE.

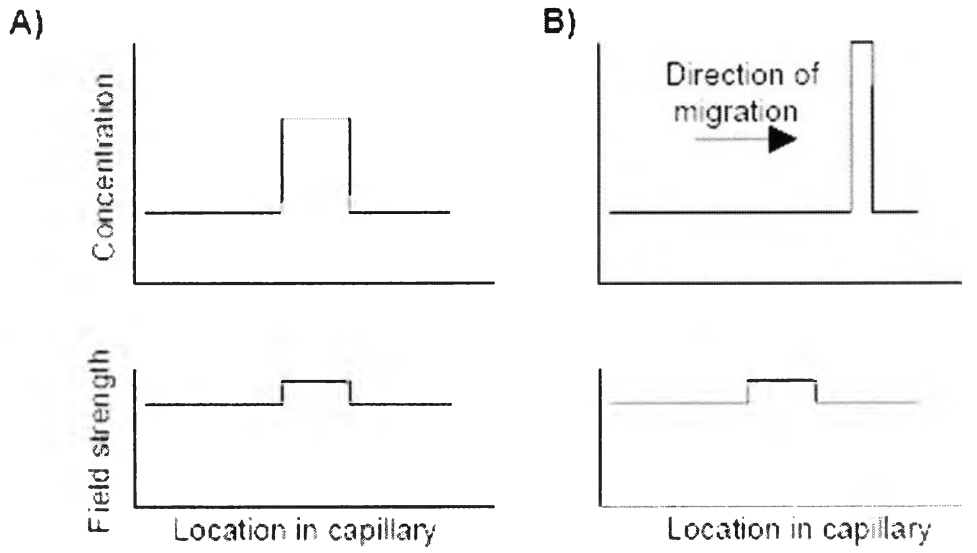

Figure 2.15 Schematic representation of the stacking of a sample plug. A) Starting conditions, B) after stacking (25).

The analyte zone therefore is compressed into a smaller volume with an increased concentration (Figure 2.15B). The ions in the original sample plug are displaced by ions from the BGE, but at a lower concentration. This resulting zone is detected as a negative peak in amperometric detection. Stacking typically is used to enable the injection of a large sample plug with a low analyte concentration, which is then automatically concentrated. The opposite process is used to dilute samples with a high ionic strength. In chapter 4 simulations are performed to optimize the BGE concentration. A BGE which is often cited for electrochemical detection is an equimolar mixture of 2-(Nmorpholino) ethanesulfonic acid (MES) and histidine (His). These substances have an almost identical pKa, which means that both are buffering when put into solution together.

Furthermore they have a low electrophoretic mobility which makes them suitable for sensitive conductivity detection. This BGE composition is therefore used in this work as the starting point for optimization of the microchip system.

2.5 Electroanalytical Chemistry

2.5.1 Voltammetry

Voltammetry comprises a group of the electroanalytical methods in which information about the analyte is derived from the measurement of current as a function of applied potential. It is based on the measurment of a current that develops in an electrochemical cell under conditions of complete concentration of polarization of working electrode. In the presence of the electroactive (reducible or oxidizable) species, a current will be recorded when the applied potential becomes sufficiently negative or positive for it to electrolyze. The recording result is called a voltammogram. The potential excitation signal is imposed on an electrochemical cell containing an electrode. Three waveforms of most common excitation signals used in voltammetry are shown in Figure 2.16. The classical voltammetric excitation signal is a linear scan shown in Figure 2.16a. The potential applied to the cell of this excitation increases linearly as a function of time. The two pulse excitation signals are shown in Figure 2.16b and 2.16c. The current responses of the pulse type are measured at various times during the lifetime of these pulses.

Voltammetry is widely used for the fundamental studies of oxidation and reduction processes in various media, adsorption process on electrode surfaces, and electron transfer mechanisms at electrode surfaces. In the mid-1960s, several major modifications of classical voltammetric techniques were developed that enhanced the sensitivity and selectivity of the method (26-28).

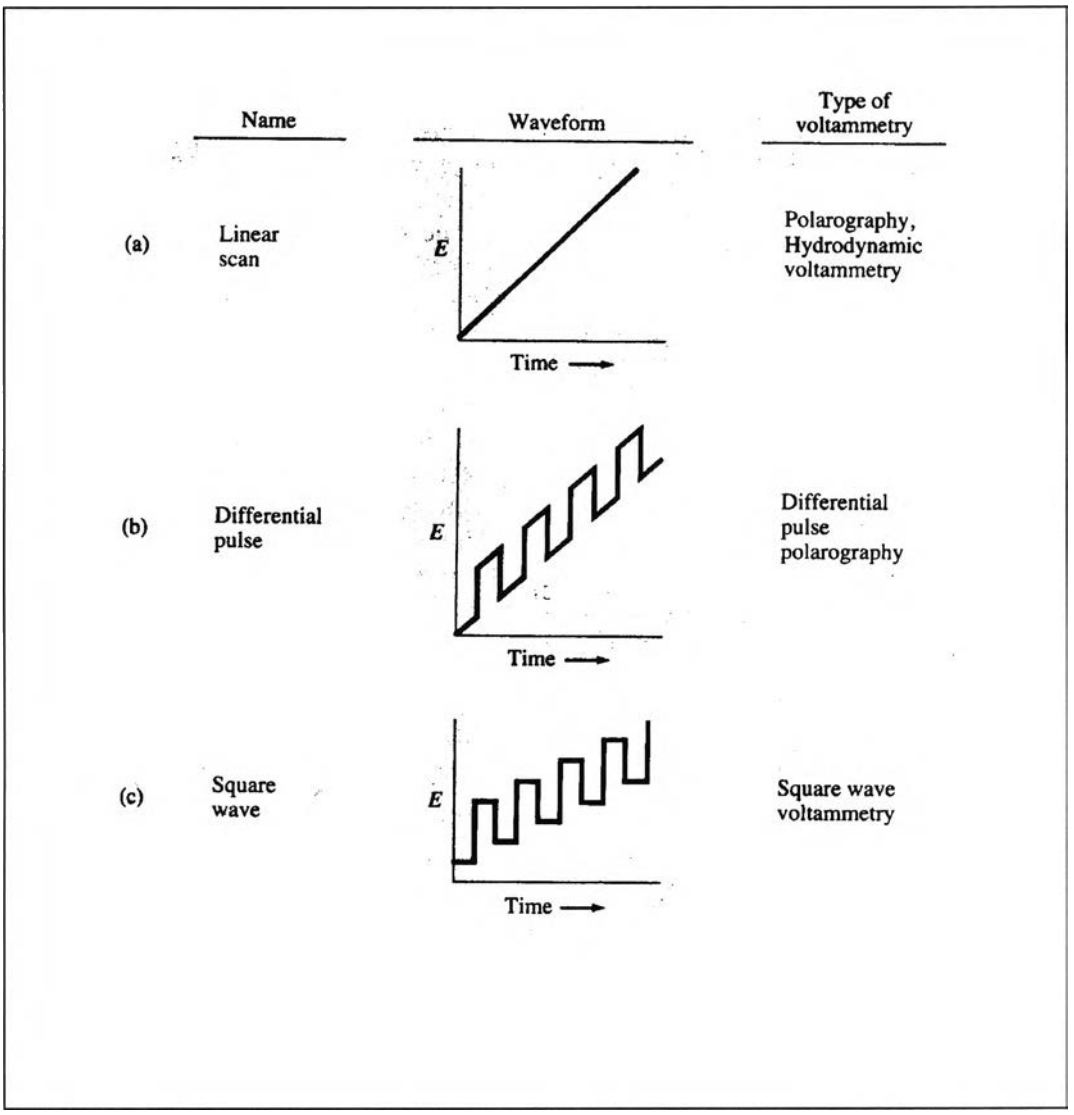

Figure 2.16 Typical excitation signals for voltammetry (27)

2.5.1.1 Cyclic voltammetry

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of cyclic voltammetry result from its ability to rapidly provide considerable information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reaction or adsorption processes. Cyclic voltammetry is often the first experiment performed in an electroanalytical study. In particular, it offers a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media upon the redox process (29,30).

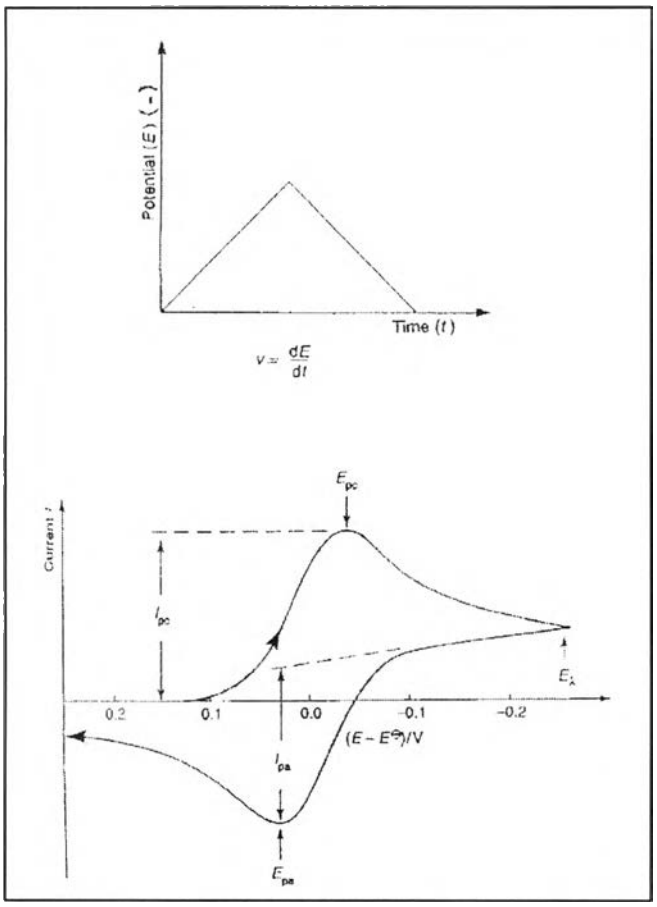

Figure 2.17 Schematic of (a) a potential wave form used in cyclic voltammetry, and (b) a cyclic voltammogram (31)

Cyclic voltammetry consists of scanning linearly the potential of a stationary working electrode (in an unstirred solution) using a triangular potential waveform (Figure 2.17a). The triangular waveform produces the forward and then the reverse scan. Depending on the information sought, single or multiple cycles can be used. During the potential sweep, the potentiostat measures the current resulting from applied potential. The resulting plot of current versus potential (i-E plot) is termed a cyclic voltammogram (Figure 2.17b). The significant parameters in cyclic voltammogram are the cathodic peak potential (E_{pc}), the anodic peak potential (E_{pa}), the cathodic peak current (i_{pc}), and the anodic peak current (i_{pa}). The cyclic voltammogram is a complicated, time-dependent function of a large number of physical and chemical parameters.

2.5.1.2 Amperometry

Amperometry is one of the controlled-potential electrochemical techniques. A simple potential-time waveform is shown in Figure 2.18. It is normally carried out in stirred or flowing solutions or at working electrode. The potential of a chosen working electrode with respect to a reference electrode is set at a fixed potential to detect the change in current response. At this potential, the electroactive species undergo an oxidation or reduction at the electrode (29,30,32,33).

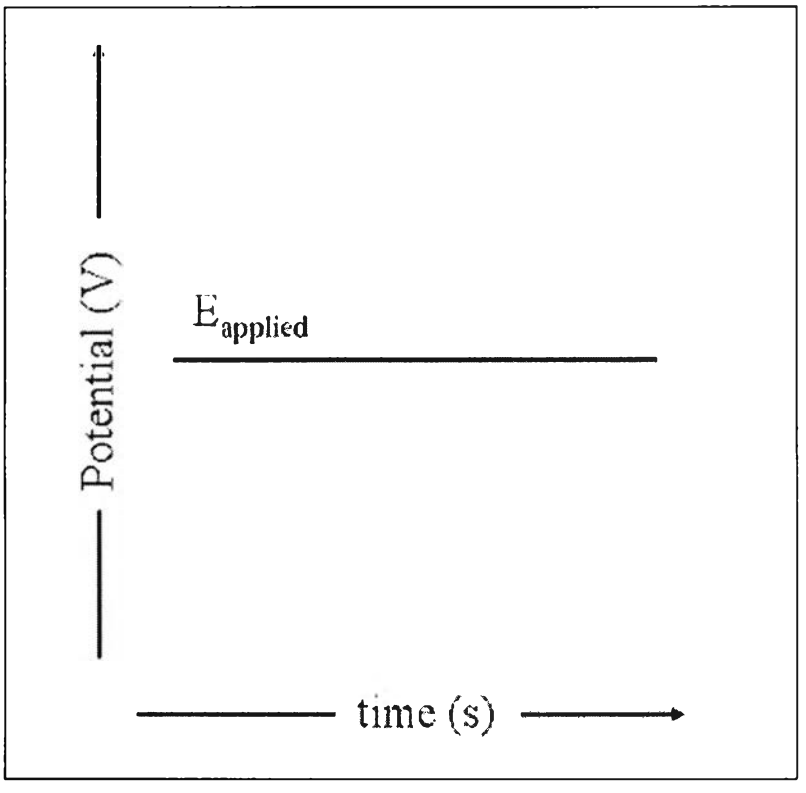

Figure 2.18 A typical waveform employed in amperometry (27)

The amperometric current is a function of the number of the molecules or ions that have been removed by the reaction at the electrode. Hence, the resultant amperometeric signal is directly proportion to the concentration of the analyte.

2.6 Sample Preparation

Sample preparation is a technique used to clean-up a sample before analyzing and/or to concentrate a sample to improve its detection (34). In carrying out this process properly, four critical criterias must be concerned, namely

2.6.1 Sample Concentration

Frequently, the component of interest is present in level too low a for detection. Sample preparation can make the component to become concentrated to an adequate level for measurement.

2.6.2 Contaminations

The presence of interfering matrix elements can mask the analysis of the component of interest. Sample preparation can remove excess contaminants to yield clean, informative chromatograms.

2.6.3 In Solution

For most analyses (HPLC, GC, Spectrophotometer, etc.), the sample must be properly prepared in solution for subsequent analysis. Classification of extraction methods depend on the type of samples and their preparations are as follow : solid, liquid or gas.

2.6.4 Liquid Samples

Liquid samples are much easier to prepare for analytical measurement relative to volatile compounds or solids, because dissolution or an extraction step may not be involved. Often, dilution in a compatible solvent is all that is required. The major considerations for liquid samples are the matrix interferences, the concentration of analytes, and compatibility with the analytical technique.

2.6.4.1 Dilution

Sample is diluted with solvent that is compatible with analytical measurement technique to avoid chromatographic column overload or to be in linear range of detector or spectrophotometer. Solvent should be compatible with analytical measurement technique; solvent should not be too strong for HPLC mobile phase conditions so that injection causes unacceptable band broadening.

2.6.4.2 Evaporation

Liquid is removed by gentle heating at atmospheric pressure with flowing air or inert gas or under vacuum. Cautions should be taken in that the samples must not be overheated or evaporated too quickly and it is favorably performed under inert gas such as nitrogen. The schematic of a typical evaporator is shown in Figure 2.19.

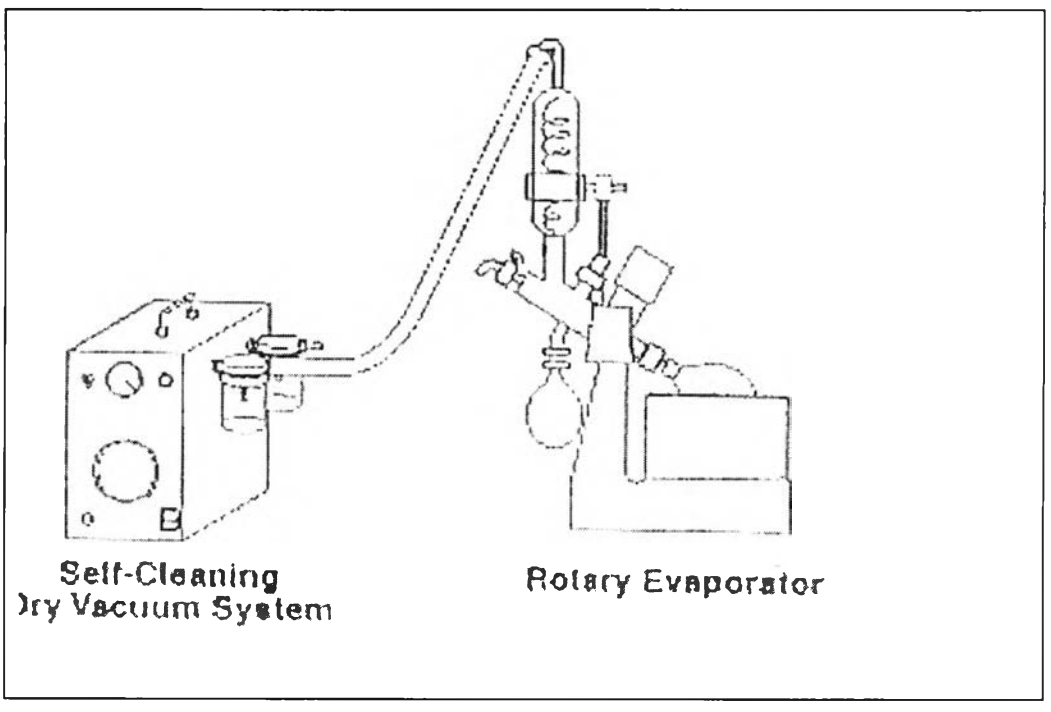

Figure 2.19 Instrument of evaporator (34)

2.6.4.3 Distillation

Sample is heated to boiling point of solvent and volatile analytes are concentrated in vapor phase, condensed, and collected; steam distillation involves boiling with water or purging with steam and collecting distillate. Mainly for samples that can be volatilized; sample can decompose if heated too high; vacuum distillation can be used for nonvolatile compounds. The instrument of distillation as shown in Figure 2.20

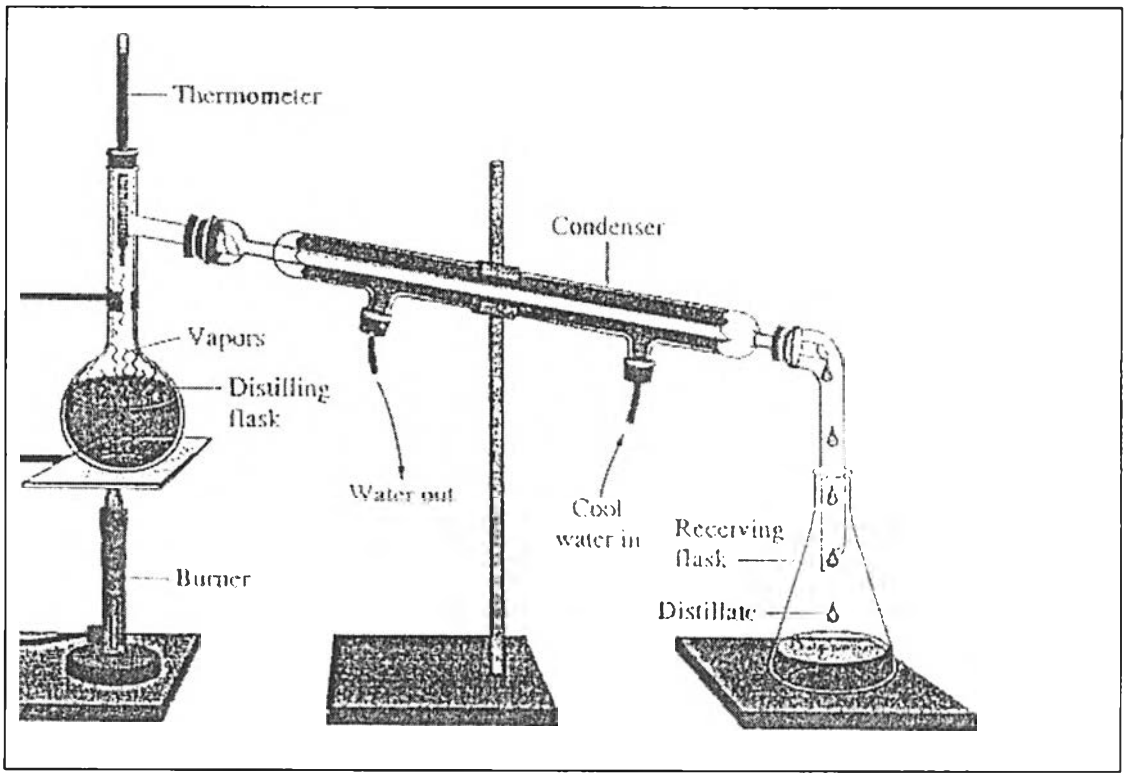

Figure 2.20 Instrument of distillation (34)

2.6.4.4 Centrifugation

Sample is placed in tapered centrifuge tube and spun at high force, liquid is decanted. Quantitatively removing solid sample from tube sometimes presents practical problem; ultracentrifuge normally not used for simple particulate removal. The instrument of distillation is shown in Figure 2.21

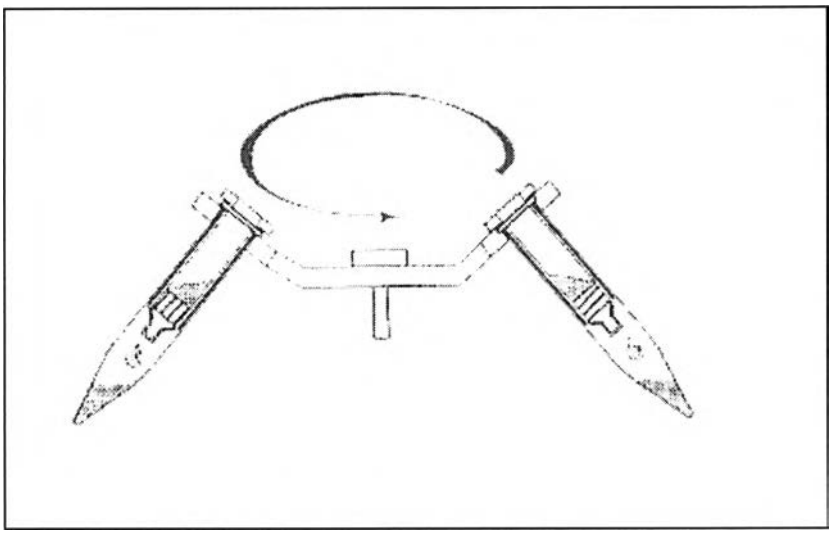

Figure 2.21 Instrument of centrifugation (34)

2.7 Literature surveys

Microfluidic analytical systems fabricated on silica, glass microchips have undergone an explosive growth during the past decade. Capillary electrophoresis (CE) microchips have received much attention because of their high degree of integration, portability, minimal solvent/ reagent consumption, high performance, and speed. They hold considerable promise for applications such as environmental monitoring, biomedical and pharmaceutical analysis, clinical diagnostics, and forensic investigations (5,35-37). Many microfluidic systems were produced on glass substrates using standard photolithographic techniques (1,38). Microchip CE has been investigated for several years to analyze various pollutants. Microchip CE in its various modes of operation offers advantages from the viewpoint of cost, analysis times, and environmental impact in addition to high resolving power, high separation efficiency, and unique selectivity. Optical, electrochemical, and mass spectrometric

detection modes have become routine for microfluidic chips. Electrochemistry (EC) offers great promise for such microsystems, with features that include remarkable sensitivity, inherent miniaturization of both the detector and control instrumentation, independence of sample turbidity or optical path length, low cost, minimal power demands, and high compatibility (39-43). Many of these separation and detection systems offer effective alternatives to analytical methodologies currently in use for characterizing many environmental pollutants.

This thesis focuses on recent advances in the application of the microchip CE with electrochemical detection (ED) for analyzing environmental pollutants. This field of study has grown considerably in the past decade. Reports ranging from basic research to devices intended for onsite use have been published. The following sections will cover the system of the chips, ED, and application of microchip CE–ED to the analysis of metal ions.

2.7.1 Detection techniques

As with other analysis systems for pollutants, sensitive and selective detection techniques are also required for microchip CE. Most reports on microchip CE relied on laser-induced fluorescence (LIF) for detection (44,45). Mass spectrometry (MS) also received much attention to meet the requirements of proteomic analysis. However, both LIF and MS need sophisticated and expensive instrumentation. LIF typically requires pre- or post capillary derivatization of the sample with a fluorophore and is limited to fluorescent analytes and analyte derivatives (46,47). Commercially available MS systems are not inherently portable and are more costly and less sensitive than LIF. Recently, electrochemical detection (ED) has attracted considerable interest for an electrophoretic microchip system (48-50). It offers great promise for microchip CE systems, with features that include high sensitivity, inherent miniaturization of both the detection and control instrumentation, low cost and power demands, and high compatibility with microfabrication technology. In principle, ED can be classified into three general modes, conductrometry, potentiometry, and amperometry. However, only conductimetry and amperometry have been commonly used for the detection of microchip CE. Both detection modes have also been applied for monitoring environmental pollutants.

2.7.1.1 Electrochemical detection

The main challenge in coupling CE and ED has been the conflict between the high voltages used in the electrophoretic separation and the detection potential used. However, this drawback does not apply at the micro scale. Three strategies have been employed for ED: end-channel; in-channel; and, off-channel.

In the end-channel ED, the electrode is placed just outside the separation channel; that involves alignment of the electrode. Separation voltage has minimal influence on the potential applied in the electrochemical detector because most of the voltage drop is across the channel.

For in-channel ED, the electrode is placed directly in the separation channel, and that involves placing the working electrode directly within the separation channel using an isolated potentiostat.

Off-channel ED involves using a decoupler to ground the separation voltage before it reaches the detector. Electrode placement in off-channel detection is similar to that in in-channel detection, but the separation voltage is isolated from the amperometric current using a decoupler. Conceptually speaking, the decoupler effectively shunts the separation voltage to ground and a field-free region is created where analytes are pushed past the electrode by the electroosmotic flow (EOF) generated prior to the decoupler. Since no decoupler is necessary, end-channel configurations offer the following advantages: simplicity; ruggedness; and feasibility for electrode replacement; and, microfabrication facilities are not strictly required. However, the main drawbacks are alignment of the electrode with the outlet of the channel and the loss of separation efficiency, due to the distance between the end of the channel and the working electrode. This separation distance is also crucial for the signal-to-noise ratio obtained and can lead to a complete loss of the analytical current. In both in-channel and off-channel configurations, the analytes migrate over the electrode while they are still confined to the channel, thus eliminating the band broadening often observed with end-channel alignments. However, in these two configurations, microfabrication facilities are usually needed and, in addition, the nature of on-chip miniaturized electrodes limits the ability to modify the electrode surfaces and clean their surfaces periodically.

2.7.1.1.1 Amperometric detection

Amperometry is the most widely reported EC detection method for chip-based separations (51-59). It is accomplished by applying a constant potential to the working electrode and measuring the resulting current that is proportional to the concentration of analytes oxidized or reduced at the electrode surface. A band platinum detection electrode was fabricated just outside the exit of the separation channel using a photolithographlic process. In this flow-by design, the surface of the band platinum electrode was parallel to the flow direction. Wang's group described a planar screen-printed carbon line electrode for the microchip CE system (60). The detection electrode was mounted perpendicular to the flow direction, ca., 50 μm away from the capillary outlet. A flow-onto thick-film amperometric detector had been non-permanently mounted perpendicular to the channel outlet, allowing easy and fast replacement. The amperometric detection system was successfully employed to detect nitroaromatic explosives, phenols, nerve agents, etc. (41). The major limitation that has held back the routine application of amperometric detection for CE is the precise alignment between the separation channel and the working electrode (61). Another attractive route is to place the detector directly around the exit of the channel, based on electroless deposition and sputtering techniques.

The microfluid can flow through the ring detection electrode. Hilmi and Luong (62) described the use of electroless deposition for preparing on-chip gold electrodes. The electroless protocol allows deposition of the gold film directly onto the capillary outlet. This simple low-cost electroless preparation route obviates the need for photolithographic electrode fabrication or careful channel/electrode alignment. The detector performance had been characterized using a mixture of nitroarometric explosive compounds.

2.7.2 Inorganic and small organic ions

Ions are usually detected by CD after separation by microchip CE. Deng and Collins (63) reported an application example of microchip CE to separate and detect six toxic metal ions (Cd^{2+}, Pb^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Hg^{2+}) of environmental concern. Colorimetric metal complexation agent was added for transverse absorbance detection.

By combining metal chelation with SPE on a C18 silica gel microcolumn, the detection limits improved several hundred fold for the CE microchip measurements of toxic metal ions in water, ranging from 0.4 mg/L to 1.2 mg/L. Collins and Lu (64) developed a CE microchip with a red LED light source and a DAD detector for the sensitive and selective detection of uranium (VI). The Arsenazo III was introduced to the microchip to selectively react with lanthanide metal ions. Carbowax 20,000 was incorporated into the BGE to eliminate the EOF and prevent dye adsorption on the microchannel walls. The separation of uranium from four lanthanide metal ions was achieved within 2 min. A direct load was also investigated to inject a precomplexed metal ion mixture into the microchannel with a detection limit of 23 mg/L for uranium (VI) in the presence of seven lanthanide impurities (1.5 mg/L each). In addition, the analysis of inorganic arsenic species and selenium species using isotachaphoresis (ITP) chips with integrated conductivity electrodes has been reported. The first study (65) reported a rapid analysis of inorganic arsenic species within 600 s. Detection limits of 1.8 mg/L and 4.8 mg/L have been achieved for arsenic (V) and arsenic (III), respectively.

2.7.3 Metal ions

The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients. Therefore, in order to understand the toxicity of heavy metals in food, simple, sensitive and accurate detection methods are required.

For metal determination in food products, many analytical methods have been developed for single elemental determination including titrations (66), colorimetric analyses (67,68), UV-VIS spectrophotometers (69-71), flame and graphite furnace atomic absorption spectrometry (AAS) (72-75). The multi-elemental determination, ion chromatography (IC) (76-78), or inductively coupled plasma combined with atomic emission spectrometry (ICP-AES) (79,80), or mass spectrometry (ICP-MS) (81-83) have been described as well. Even though these methodologies are rapid and sensitive for the determination of trace amounts of metals, they require complicatde instrumentation, high capital and operational cost. In particular, it is difficult to make a fully portable analytical tool for screening, detecting, identifying, and quantitating metal ions.

Capillary electrophoresis (CE) has been widely applied to the separation and determination of different metal species because it is already proven as a fast and high resolution separation technique (11,49,84-87). The detection methods usually used in combination with the CE separation technique was ultraviolet light (UV), fluorescence or laser-induced fluorescence (LIF) detection. The lack of a strong chromophore for metal ions has certainly been one of the limitations in the analysis of most metals by these methods. In addition, these detection modes will suffer from the lack of sensitivity when a miniaturized device is used. Electrochemical detection (ED) offers high sensitivity and selectivity for metals that are easily oxidized or reduced. This technique is really suitable for and compatible with microfabrication technology that has been successfully employed in microchip CE.

Therefore, this work addresses the need for developing an inexpensive, field portable and sensitive sensor for metal ions, which could assist in fast screening and detecting contaminated food or waste materials on site. The detection of metal ions was accomplished using a microchip CE / amperometric detection system, in which a screen-printed carbon electrode was placed at the end of the channel. This approach simplifies the fabrication of the working electrode and also provides a convenient and sensitive means for the determination of metal ions by amperometry. Furthermore, by simply changing the electrode, the detection electrode, which is prone to contamination, can be easily removed or cleaned. The electrode may provide a good portable device for screening, or simultaneously analysing a complex system containing different metal species such as food. The optimization, characterization, and attractive performance characteristics of such a microchip CE, and its successful application to complex samples (such as vegetable juices) are reported in the following section.