CHAPTER 1

INTRODUCTION

1.1 Overview and background

In recent decades, the new technology and instrumentation used to measurement of chemicals, biological samples and materials that involve with environmental science, life science, agriculture, industrial processes, production and quality control of pharmaceutics etc., which reveal a great demand for good analytical results. According to those demands it is necessary to improve analytical techniques through automated in order to obtain a high throughput capability, reproducibility, cost effectiveness, simplicity, accuracy and friendliness with environment management. Analytical methods are well established for environmental monitoring. However, a paradoxical situation has emerged because most of the analytical methodologies employed to investigate environmental problems generate chemical wastes, resulting in an environmental impact. In some circumstances, the chemicals employed are even more toxic than the species being monitored and chemical wastes disposal are high cost. As a consequence, the work of some analytical chemists has been focused on the development of methodologies that are automatic, less harmful to humans and to the environment. In the development of a new analytical procedure, the amount and toxicity of the wastes are as important as any other analytical features. The strong trend of the last decade, Flow injection analysis (FIA) has great potential for miniaturization of almost every system. It is called micro-flow analysis (μFA) system or lab-on-a-chip. It concerns about the development of new analytical methods that reduce the use and generation of toxic substances in all steps of analytical

procedure. This technique is rapid, easy to handle, portable, high sensitive and very low waste production. Moreover, the application of microfluidic technology to drug discovery, biology, physic, nanotechnology and medicine is a growing area of interest. In addition, the microdevices are now used in everyday life such as accelerometers that are display in current cars (airbag trigger system) [1]. Typical microfluidic devices require sample and reagent about 10 µL. The motivating of microfluidics in the analytical chemistry field has never terminated rising since the beginning development of the miniaturized total chemical analysis system. The opportunity to work in a small scale is one of the key challenges to creating any new microfluidic devices.

1.2 Principle of flow injection analysis (FIA) [2]

The original concept of FIA is explained by Ruzicka and Hansen in 1975 [2]. It is a rapidly growing analytical technique that is applied to many fields of analytical chemistry. FIA is based on a well-defined injection of a solution sample into a continuous flowing carrier stream of reagent with a constant flow rate. The injected sample reacts with the reagent in the mixing coil forming a product zone which is transported towards to a detector that continuously records the absorbance or any other physical parameters as it continuously changes due to the passage of the sample material through the flow cell [3]. The change will be relative to the analyte concentration under controlled experimental conditions being reserved equal both for samples and standards. The simplest FIA (Figure 1.1) analyzer consists of a pump, which is used to drive the carrier or reagent stream through a narrow tube of the FIA manifold. An injection port or valve which provides a precise volume of sample

solution is injected into the carrier stream in a reproducible manner. A reactor (frequently a mixing coil) in which the sample zone disperses and reacts with the component of carrier stream, forming a chemical product that is sensed by a flow through detector where the FIA signals are recorded.

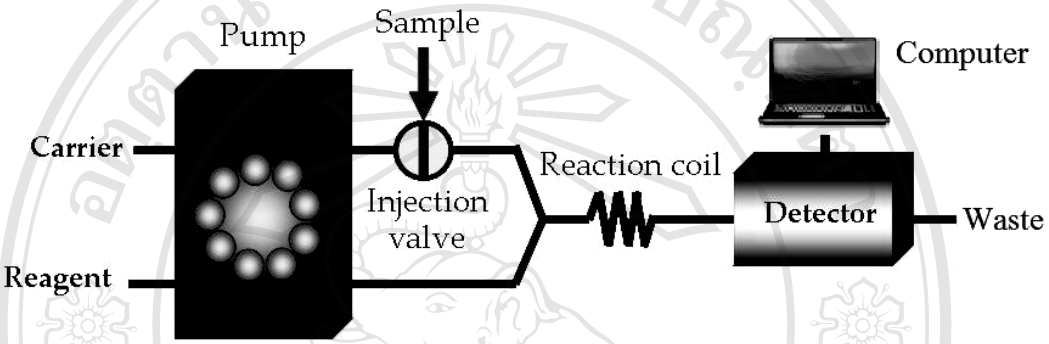


Figure 1.1 The basic components of the FIA system

A typical recorder output is a peak and afterward recorded as a function of time (Figure 1.2). The height (H), width (W), or area (A) of which is directly relational to the concentration of the analyte present in the samples. The time consumed between the sample injection (S) and the highest of peak is the residence time (T). Commonly, the residence time (T) is in the range of 5-20 s and the injected sample volumes may be between 1-200 μL per sampling series. This makes FIA a simple, automated microchemical technique, high sampling rate, a minimum sample and reagent consumption. In addition, good sensitivity, reproducibility and high sample throughput are considered to be the main advantages of the flow injection method.

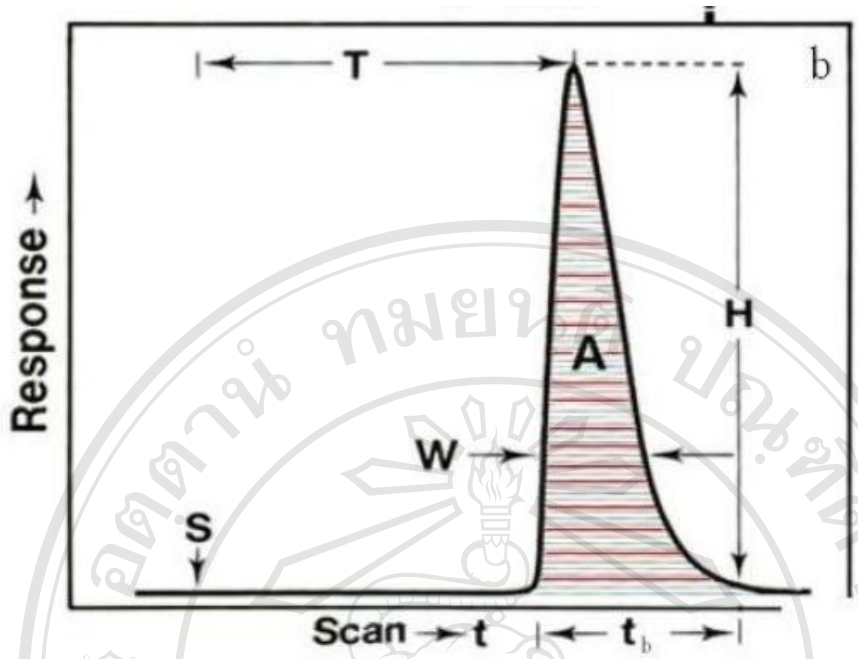


Figure 1.2 The FIA gram; S : time of injection, H : the peak height, W : the peak width at a selected level, A : the peak area and T : the residence time corresponding to the peak height measurement [3]

Moreover, dispersion process [2, 4, 5] and FIA instrumentation [6, 7, 8, 9, 10, 11, 12, 13] are defined in appendix A.

1.3 Micro flow analysis [14]

The micro total analytical system (μTAS) was first introduced at the Transducer 1989 Conference [15], also called “lab-on-a-chip” (LOC) [16]. It involves the miniaturization of all functions found in chemical analysis including sampling, removal of matrix interference, preconcentration process, pumps, valves, reaction chambers and detectors. The amazing prospect of the μTAS method is the suggestion that the complete chemical measurement laboratory might be miniaturized onto a few

square centimeters in size (Figure 1.3). μTAS has many advantages which compare with conventional analytical methods. The main advantage is to reduce the reagent consumption significantly leading to low waste generation. Various biological and chemicals are expensive and chemical waste is not easy to manage including high cost to eliminate. LOC can reduce cost, toxicity and disposal risks from using chemical. Moreover, the work with microfluidic technology provides higher sensitivity, cost effectiveness per test, shorter time to analysis and less laboratory area to setup component. There are many types of materials which can be fabricated microdevices including several polymers, glass, silicon, or combinations of these materials with many fabrication techniques. Until today, there are many applications of LOC for chemical and biological analysis as shown in Table 1.1.

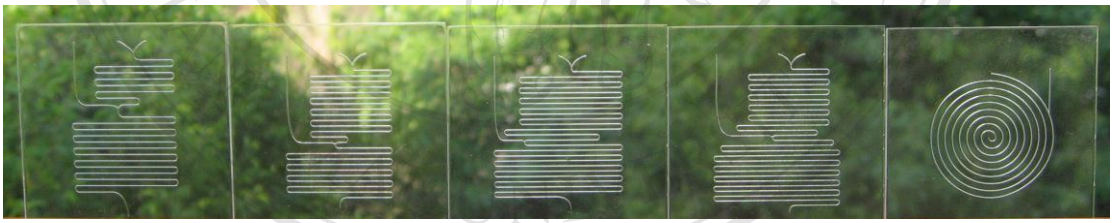


Figure 1.3 The lab-on-a-chip patterns

Table 1.1 The applications of lab-on-a-chip analysis in various samples

Year	Topics	Detection mode	Analyte	Reference
1999	Further studies into the development of a micro-FIA (μFIA) system based on electroosmotic flow for the determination of phosphate as orthophosphate	Spectrophotometry	Phosphate	[17]
1999	Micromachined electrophoresis chips with thick-film electrochemical detectors	Electrochemistry	Nitroaromatic explosives	[18]
2000	Capillary electrophoresis chips with thick-film amperometric detector: separation and detection of hydrazine compound	Amperometry	Hydrazine	[19]
2000	Micromachined separation chips with a precolumn reactor and end-column electrochemical detector	Amperometry	Amino acids	[20]
2000	Capillary electrophoresis microchips with thick-film amperometric detector: separation and detection of phenolic compounds	Amperometry	Phenolic compounds	[21]
2000	Micromachined electrophoresis chips with electrochemical detectors for analysis of explosive compounds in soil and groundwater	Amperometry	Nitroaromatic explosives	[22]
2000	Tris(2,2-bipyridyl)ruthenium (II) chemiluminescence in a microflow injection system for codeine determination	Chemiluminescence	Codeine	[23]
2001	Microfabricated plastic chips by hot embossing methods and their applications for DNA separation and detection	Fluorescence	DNA	[24]
2001	Capillary electrophoresis microchips for separation and detection of organophosphate nerve agents	Amperometry	Organophosphate	[25]
2001	The development of an on-chip micro-flow injection analysis of nitrate with a cadmium reductor	Spectrophotometry	Nitrate	[26]
2001	Immobilization and detection of DNA on microfluidic chips	Fluorescence	DNA	[27]
2002	Capillary electrophoresis–electrochemistry microfluidic system for the determination of organic peroxides	Amperometry	Organic peroxides	[28]

Table 1.1 (continued)

Year	Topics	Detection mode	Analyte	Reference
2002	Chemiluminescence biosensor chip based on a microreactor using carrier air flow for determination of uric acid in human serum	Chemiluminescence	Uric acid	[29]
2003	CO_2 laser microfabrication of an integrated polymer microfluidic manifold for the determination of phosphorus	Spectrophotometry	Phosphorus	[30]
2003	The determination of phosphorus in a microfluidic manifold demonstrating long-term reagent lifetime and chemical stability utilising a colorimetric method	Spectophotometry	Phosphorus	[31]
2003	Chemiluminescence microfluidic system sensor on a chip for determination of glucose in human serum with immobilized reagents	Chemiluminescence	Glucose	[32]
2004	Fluorometric determination of sulfite and nitrite in aqueous samples using a novel detection unit of a microfluidic device	Fluorescence	Nitrite	[33]
2004	On-chip micro-xow polystyrene bead-based immunoassay for quantitative detection of tacrolimus (FK506)	Fluorescence	Tacrolimus	[34]
2004	Application of on-chip cell cultures for the detection of allergic response	Fluorescence	Allergic	[35]
2004	In-channel dual-electrode amperometric detection in electrophoretic chips with a palladium film decoupler	Amperometry	Catecholamines	[36]
2004	Electric chips for rapid detection and quantification of nucleic acids	Potentiometry	Nucleic acids	[37]
2004	Droplet-based microfluidic lab-on-a-chip for glucose detection	Spectrophotometry	Glucose	[38]
2005	Chemiluminescence microflow injection analysis system on a chip for the determination of uric acid without enzyme	Chemiluminescence	Uric acid	[39]
2005	Microfluidic chip with electrochemiluminescence detection using 2-(2-aminoethyl)-1-methylpyrrolidine labeling	Electrochemilumine-scence	Avidin, biotin	[40]
2005	Fabrication of miniature Clark oxygen sensor integrated with microstructure	Electrochemistry	Oxygen	[41]

Table 1.1 (continued)

Year	Topics	Detection mode	Analyte	Reference
2006	Chemiluminescence microfluidic chip fabricated in PMMA for determination of benzoyl peroxide in flour	Chemiluminescence	Benzoyl peroxide	[42]
2006	Microfluidic pH-sensing chips integrated with pneumatic fluid-control devices	Electrochemistry	-	[43]
2006	Miniaturized one-chip electrochemical sensing device integrated with a dialysis membrane and double thin-layer flow channels for measuring blood samples	Electrochemistry	Lactate	[44]
2006	Microflow injection system based on a multicommutation technique for nitrite determination in wastewaters	Spectrophotometry (photodiode)	Nitrite	[45]
2007	Microfluidic chip integrated with amperometric detector array for *in situ* estimating oxygen consumption characteristics of single bovine embryos	Amperometry	Oxygen	[46]
2007	On-chip microfluidic biosensor for bacterial detection and identification	Electrochemistry	Bacteria	[47]
2007	Integrated microfluidic gas sensor for detection of volatile organic compounds in water	Electrochemistry	Volatile organic compounds	[48]
2007	Greener analytical method for the determination of copper(II) in wastewater by micro flow system with optical sensor	Spectrophotometry	Copper	[49]
2007	A simple and green analytical method for determination of iron based on micro flow analysis	Spectrophotometry	Iron	[50]
2008	Improved microfluidic chip-based sequential-injection trapped-droplet array liquid–liquid extraction system for determination of aluminium	Chemiluminescence	Aluminium	[51]
2008	A microchip-based flow injection-amperometry system with mercaptopropionic acid modified electroless gold microelectrode for the selective determination of dopamine	Amperometry	Dopamine	[52]

Table 1.1 (continued)

Year	Topics	Detection mode	Analyte	Reference
2008	Environmentally friendly disposable sensors with microfabricated on-chip planar bismuth electrode for *in situ* heavy metal ions measurement	Chemiluminescence	Lead, cadmium	[53]
2009	A prototype microfluidic chip using fluorescent yeast for detection of toxic compounds	Fluorescence	Toxic compounds	[54]
2009	Rapid detection of formaldehyde concentration in food on a polydimethylsiloxane (PDMS) microfluidic chip	Fluorescence	Formaldehyde	[55]
2009	Flow injection based microfluidic device with carbon nanotube electrode for rapid salbutamol detection	Electrochemistry	Sulbutamol	[56]
2009	Portable microfluidic system for determination of urinary creatinine	Spectrophotometry	Urinary creatinine	[57]
2009	The use of ‘‘lab-on-a-chip’’ microfluidic SDS electrophoresis technology for the separation and quantification of milk proteins	Fluorescence	Milk proteins	[58]
2010	Microfluidic formation of single cell array for parallel analysis of Ca^{2+} release-activated Ca^{2+} (CRAC) channel activation and inhibition	Fluorescence	Calcium	[59]
2010	Fast cholesterol detection using flow injection microfluidic device with functionalized carbon nanotubes based electrochemical sensor	Chonoamperometry	Cholesterol	[60]
2010	Microfluidic chip-based aptasensor for amplified electrochemical detection of human thrombin	Voltammetry	Human thrombin	[61]
2010	Determination of glucose on free enzyme-based poly(dimethylsiloxane) microchip	Amperometry	Glucose	[62]
2010	In-channel modification of biosensor electrodes integrated on a polycarbonate microfluidic chip for micro flow-injection amperometric determination of glucose	Amperometry	Glucose	[63]
2011	A microfluidic chip platform with electrochemical carbon nanotube electrodes for pre-clinical evaluation of antibiotics nanocapsules	Cyclic voltammetry	Antibiotics nanocapsules	[64]

Table 1.1 (continued)

Year	Topics	Detection mode	Analyte	Reference
2011	Separation and detection of urinary proteins by microfluidic chip integrated with contactless conductivity detector	Conductimetry	Urinary proteins	[65]
2011	The development and analysis of plasma microfluidic devices	Dielectric barrier discharges	Plasma	[66]
2011	Low-cost polymer microfluidic device for on-chip extraction of bacterial DNA	Fluorescence	Bacterial DNA	[67]
2011	A microfluidic affinity sensor for the detection of cocaine	Fluorescence	Cocaine	[68]
2011	A polymer lab chip sensor with microfabricated planar silver electrode forcontinuous and on-site heavy metal measurement	Voltammetry	Heavy metals	[69]
2011	An automated microfluidic colourimetric sensor applied in situ to determine nitrite concentration	Spectrophotometry	Nitrite	[70]
2011	Poly(dimethylsiloxane) cross-linked carbon paste electrodes for microfluidic electrochemical sensing	Electrochemistry	Catecholamines, thiols, dopamine	[71]
2011	Ultra-sensitive microfibre absorption detection in a microfluidic chip	Spectrophotometry	Bovine serum albumin	[72]
2012	Fabrication of tunable microreactor with enzyme modified magnetic nanoparticles for microfluidic electrochemical detection of glucose	Amperometry	Glucose	[73]
2012	Sensitive label-free oligonucleotide-based microfluidic detection of mercury (II) ion by using exonuclease I	Fluorescence	Mercury	[74]
2012	A new rhodamine derivative bearing benzothiazole and thiocarbonyl moieties as a highly selective fluorescent and colorimetric chemodosimeter for Hg^{2+}	Fluorescence	Mercury	[75]
2012	Cobalt hexacyanoferrate modified multi-walled carbon nanotubes/graphite composite electrode as electrochemical sensor on microfluidic chip	Amperometry	Hydrazine	[76]

A few years ago microfluidic technology was developed as a multipurpose and choice to the completely working. However, when macroscopic conventional systems are miniaturized, specific problems get up when examining sample handling. One of the largest problems is producing representative and reproducibility of small samples. These need to be operated through the sub-systems of the μTAS, i.e. from the sampling unit to the microfluidic unit and the detector device. UV-Visible spectrophotometry as detection mode is the most simple, universal and inexpensive. Nevertheless, chips have small path-lengths greatly reducing detection limit of analyte and sensitivity with optical detection. Therefore, there are few applications of spectrophotometric absorbance detection in LOC analysis systems [26, 31, 45]. Furthermore, light alignment of the fiber optics with the small liquid channel is difficult to setup. Others used designs have been presented to increase the path length by passing the light along the length of the microchannel rather than across it [77]. In addition, the practicality of combining projection microscope and a spectrophotometer for outside absorbance measurements on disposable PDMS chips was to increase the sensitivity of a UV–Vis spectrophotometer [78].

Microfluidic devices are commonly created from glass, polymer, silicon and plastics with various fabrication techniques. Channels of typical dimensions range about 200 μm in width and depth and liquid volume between 0.01-10 μL that are created onto many materials and this is enough to provide laminar flow process. Laminar flow allows the parallel flow of many layers of liquid that can enable the design of separation channel and detection chips based on laminar liquid diffusion process. Analytical chemistry is the most of many LOC applications. The first system

is presented in 1992 [79] for separation of fluorescein/calcein with capillary electrophoresis chip using laser induced fluorescence detection and the result displayed perfectly signals. Next, there are many works that are reported with similar systems [80, 81, 82] to promote the LOC.

Methods for transporting liquid in microchannel network commonly employed electroosmotic, pressure driven and flow driven. Moreover, the alternative pumping methods are presently under inspection. To achieve better flow control, valving and mixing of solutions are required. LOC devices are commonly used for capillary electrophoresis, drug development, high throughput screening and biological assays in various industry sectors like pharmaceutical, analytical chemistry and medical. Currently, there are many benefits from the new technology of LOC instead of traditional techniques.

1.3.1 Materials and fabrication technique of chips [83, 84, 85, 86]

Various materials including silicon, quartz, glass, metals and polymers have been employed to construct microdevices with different fabrication techniques as shown in Table 1.2. Silicon and glass were the first widely used materials for creation of chips. A newest platform use polymeric material instead of silica and glass, but glass provides some advantages over polymer such as chemical resistance. Significant considerations in material selection include chemical compatibility, easiness and reproducibility of fabrication, whether or not the material promotes electroosmotic flow (EOF) with the solvents of interest and compatibility with detection methods.

Glass is a favorite choice since it grants EOF. Glass chips are attractive because they can be fast integrated because of their small size sheet and robustness.

Glass is a perfect material with optically transparent and easy to clean commonly. It enables visual inspection and optical detection can be setup within the microfluidic channel or micro reactor. Its very good chemical resistances as well as its high thermal solidity make it compatible with many operating conditions and applications. Moreover, the properties of glass are good EOF as well as its rigidity and good mechanical stability. For micro reactor channel applications, reagents mix by diffusion process without turbulence flow. There are many types of glass for LOC. For example, foturan glasses are interesting materials for LOC system due to they can completely photosensitive and hence do not require a photoresist layer for lithography fabricating technique [96, 97]. In addition, photosensitive glasses also keep added advantage over fused silica in that heat treatment may be achieved to smoothness surfaces of microchannels through transformation to a glass [98], but they are expensive and their fabrication process is difficult. In biological applications involving UV-Vis analysis, glasses can offer even further benefits with higher design flexibility since most silicate glasses have a very low absorption in the UV light range and no self-fluorescence, when compared to polymers [99, 100]. In general, Borosilicate glass substrates are normally available as substrates for supporting polymer microfluidic structures. The etching process, through-hole in glass substrates can be generated by drilling and powder blasting [101]. However, the high cost involved in machining glass is the limit usage as disposable and commercial devices.

Silicon is a semiconductor as microelectronic devices. It suffers no plastic deformation or creep except beneath very extreme temperatures well beyond relevance to microfluidic applications. Silicon has a high melting temperature (about

1,400°C) matching to many metals and relatively low coefficient for thermal expansion. Silicon possesses suitable material properties for miniaturized devices due to there are many advantages including good mechanical strength, high electrical and thermal conductivities. Additionally it exhibits high absorptivity at UV-Visible region and transparency at infrared (IR) region. The precision of dimensions of silicon devices is always greater than that in glass or polymer devices. Photolithography and wet etching fabrication techniques for silicon microdevice enable virtually any dimension with good reproducibility. The smooth of surface depends on the creation techniques. For example, plasma etching roughness is of 100 nm root mean square, whereas crystal-plane-dependent wet etching results in the order of magnitude better values about 10 nm [102]. The surface of silicon can be employed electrochemical etching to polish the surface porous for on-chip chromatographic applications. Although sometime spectrophotometric chip prefers to use glass or polymers over silicon because silicon is opacity in visible wavelengths that can be a problem for an external detection.

Polymerization techniques in a microfluidic device have significantly facilitated to fabrication of chips and components. Polymers are popular materials for microfluidic components such as polydimethylsiloxane (PDMS), polycarbonate (PC), polymethyl methacrylate (PMMA) and cyclic olefin copolymer (COC). The properties of polymer depend on the type of bonds existing between the chains of monomers. The selection of polymer for chip fabrication should consider their chemical and physical properties. The glass transition temperature (T_g), melting temperature (T_m) and thermal expansion coefficient are the important parameters for

accomplished fabrication of polymer chip with various fabrication techniques. Moreover, the important properties of a polymer that suitable for microfluidic device include the solidity, surface charge, analyte adsorption, electroosmotic flow mobility, chemical resistance and optical properties that are essential for both the fabrication method and successful application to the chip systems. Thermoplastic materials such as PMMA, COC are soluble in organic solvents and they can be melted when contact to heat. Elastomers (e.g. PDMS) are cross-linked molecules which can be easily strained to high prolongations. However, thermosetting polymers cannot be soluble in organic solvents such as epoxy, rubber. Furthermore, there are various choices of different polymer materials in microdevice manufacturing. For instance, polyetheretherketone (PEEK) resists temperature, PMMA and PC are optical transparent materials. In the case of COC, it is a new popular polymer which gives excellent optical transparency, low water absorption and non-toxicity. In addition, it has high heat resistance, the T_g is 138ºC which is much higher than that of PMMA and thus high separation voltages can be applied to COC microfluidic chips to obtain high separation efficiency [105]. The advantages of polymers microdevices are low cost to fabrication in large numbers with various patterns, easy to bonding of open microchannel. Several fabrication techniques are available for polymer chips such as injection molding, hot-embossing, plasma etching, casting, soft lithography and laser ablation.

Fabrication techniques are depending on the choice of materials, size of channel pattern of chip and availability of machine. Various techniques are presented such as photolithography, hot embossing, powder blasting, injection molding and

laser ablation [106, 107]. However, there are two favor techniques. Firstly, for glass, metals, polymer and silicon materials, photolithographic fabrication and wet etching of channel networks is accomplished as shown in Figure 1.4. The channel pattern is designed with suitable drawing software (such as CorelDraw, Photoshop and AutoCAD) and printed a film negative of the desired final size which is then prepared to form the optical mask. Commercially supplied borosilicate glass photolithographic plates (thickness 3 mm) coated with a thin film of metal etch mask layer (normally chromium) plus an upper layer of positive photoresist (0.5–2.0 mm thickness) are used for channel network fabrication. The pattern of the required network of interconnecting channels is transferred from the optical mask to the top of the photoresist layer. After UV light exposure, the photoresist is developed and removed, together with the chromium layer with some solvents, to reveal the zone of glass to be etched. The channels are then etched using a mixture of 1% HF and 5% NH_4F, resulting in an etch rate of 0.3–0.5 mm min^{-1} [110]. During the etching process it is important that the system is well vibrated to ensure consistent supply of etchant to the surface plus removal of etches fragments. The base plate containing the etched microchannel network has to be then sealed by bonding to a blank plate containing pre-drilled holes by using machine [111] which acts as reservoirs for reagents, sample and products. The upper plate is aligned with the channel geometry, the holes of solution in/out and then thermally bonded to the base plate (glass to silicon, glass to glass usually between 500-600 °C about 3 h) [108, 109]. Thermal bonding is assisted by placing a weighting block of non-sticking quartz of high softening temperature on the higher plate. Some parameters that effect to the rate of etching such as the strength

of substrates, the concentration of acid and temperature are considered. In addition, lithography techniques can be further divided into photolithography, electron lithography, X-ray lithography and ion lithography [112]. The creation of microchannel in two-dimensional of substrates and etching process has to be controlled with professional operation which is limitation of photolithographic technique.

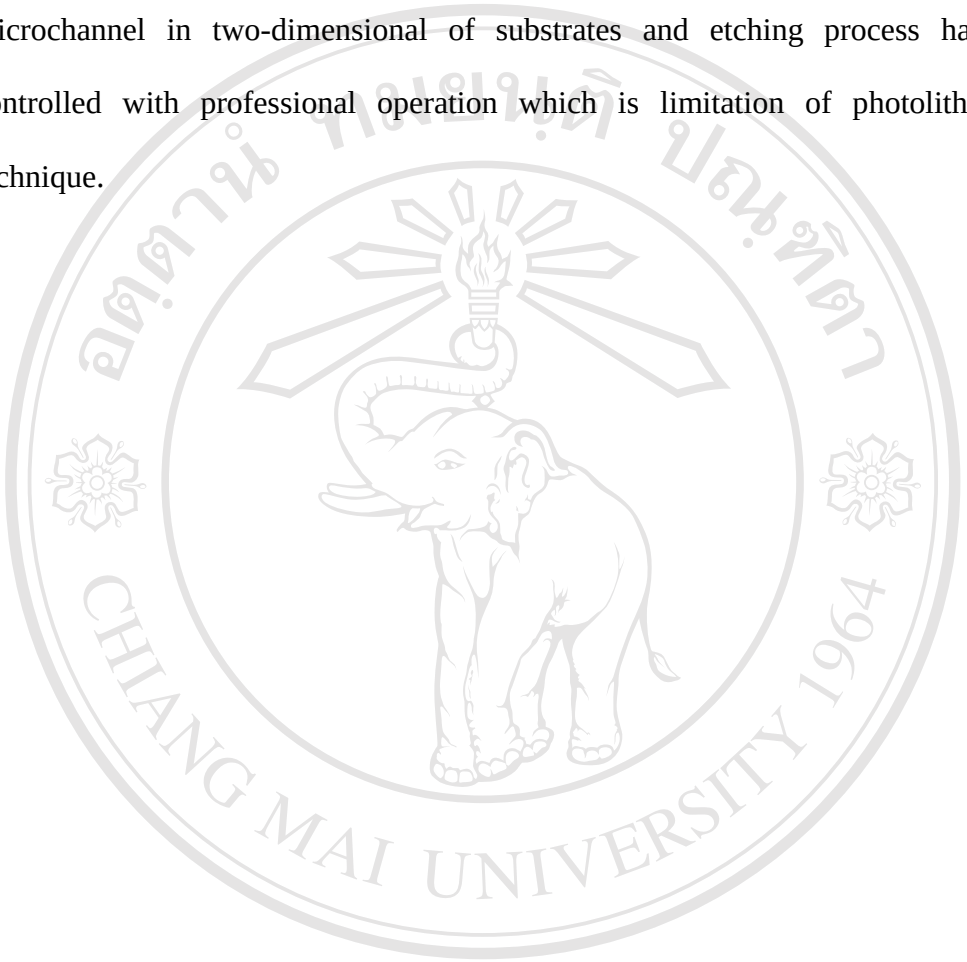


Table 1.2 Summary of materials and techniques for fabrication of microfluidic devices

Year	Material	Fabrication technique	Sealing method	Advantage	Reference
1999	Silicon-glass	Plasma etching	Anodic bonding	Strong connection at narrow hole	[87]
2000	Borosilicate glass	Photolithography	Thermal bonding	homogeneous channel, compatible with various material	[23]
2001	PDMS	Photocopying	Adhesive bonding	Low temperature, high bond strength, low	[88]
2004	PMMA	CO_2 laser	Thermal bonding	Simple, fast, ability to modified surface	[89]
2004	PMMA	Injection molding	Thermal bonding	Good dimension control, short cycle time	[90]
2006	PMMA-polyaniline	CNC machine	Microwave welding	Without any structural distortion	[91]
2007	PMMA	Imprinting	Solvent bonding	Simple, low temperature, low cost	[92]
2009	COC	Hot embossing (imprinting)	Thermal bonding	Reproducible imprints at room temperature	[93]
2010	Foturan glass	Femtosecond laser	Thermal bonding	Non photoresist layer for patterning	[94]
2010	PC	Hot embossing	Thermal bonding	Low cost of volume production	[95]

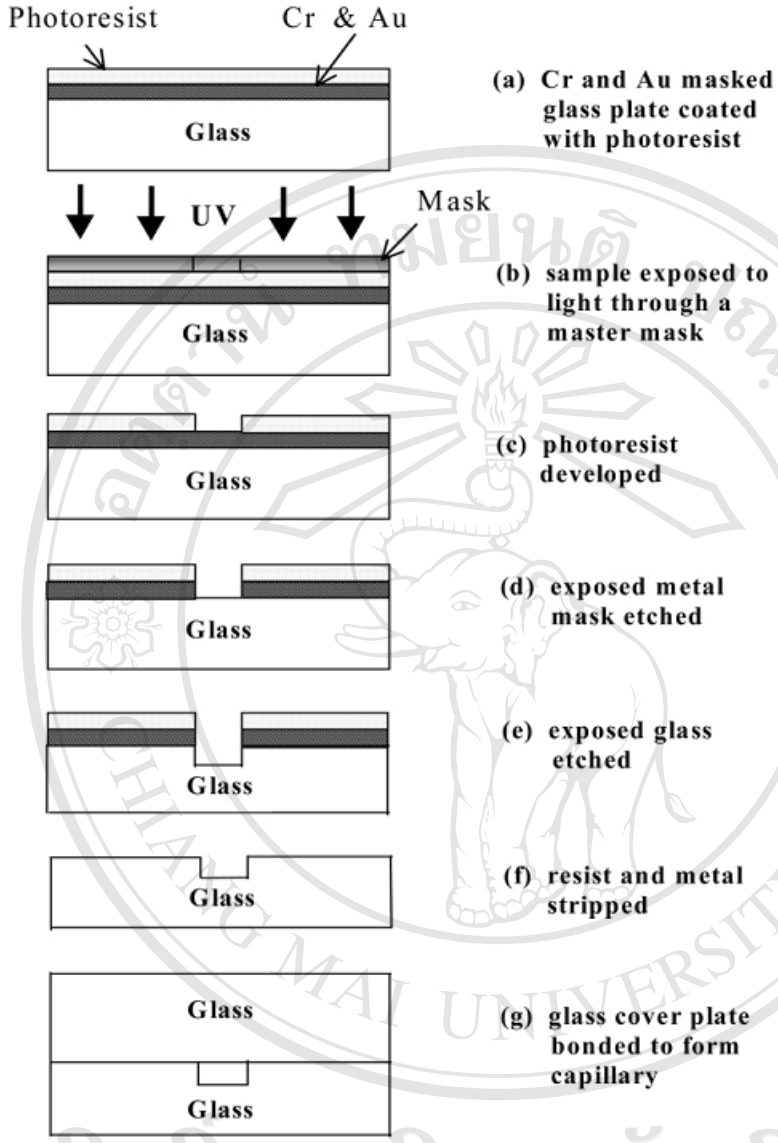


Figure 1.4 Process of photolithographic fabrication [83]

Secondly, laser ablation is one of the popular techniques for the fast fabrication of microchannel networks. It also offers an attractive method for engrave creation and has been introduced by Schwarz et al. [113] for the fabrication of microchannel networks. Laser beam can be employed directly write to silicon, glass

or polymers etc. For example, long spiral-shaped micro fluidic channels on glass plate have been fabricated by femtosecond laser direct writing [114]. Laser ablation is involves the employ of high power laser pulses to break chemical bonds in the solid material and then eject degraded particle fragments from the ablation volume. Laser ablation mechanism is a complex blending of photochemical and photothermal processes [115]. When the laser beam is focused onto a solid target, the first step in laser ablation process is the absorption of laser energy by the target material [116]. This is occurred by linear or nonlinear processes. For opaque materials, linear absorption mechanism is occurred at long pulse widths modulator with low intensity. For transparent materials, the absorption is nonlinear process through laser beam induced optical breakdown. Then the material sheet in the focus region is heated to melting temperature that depending on laser power and pulse width, subsequently to vaporization temperature. In the high power operation, a shock wave is generated and small particles are ejected from the material thus creating a small channel or cutting materials. After ablation process, it can be seen very small particulates on the surface material. Moreover, other decomposition products such as CO_2(g) and CO(g) are happen during operation laser machine. The light source in laser machine may range from the deep ultraviolet to the infrared region spectrum. There are several types of laser, but the most common sources used for microdevice fabrication are carbon dioxide (CO_2) laser, ArF and KrF excimer lasers and femtosecond laser. ArF (193 nm) and KrF (248 nm) excimer lasers operate in the UV range at frequencies between 10–10^4 Hz [117]. Moreover, excimer lasers have been processed successfully in a variety of materials including polymers, glass, metals and semiconductors. Excimer

lasers [118] and femtosecond lasers (800 nm) [119] have advantage over CO_2 laser (1060 nm) by ejected material particles without small heat-affected-zone that leading to thermal damage and distortion of microchannel shape. Excimer lasers have the advantage of create smaller microchannel when comparison to the CO_2-laser due to their smaller wavelength. However, UV-lasers are difficult to handing gas quality, discharge conditions, homogeneous beam profile and their machines are more expensive than CO_2 lasers machines. For CO_2 laser, it operates in the range of the infrared spectrum region with a wide range of output powers. For instance, the mechanism of CO_2 laser for create PMMA microchannel, wherever the focused laser beam expose to the PMMA surface, the temperature of the exposed spot will increase so quickly that the focused area of PMMA will first melt, then decompose (Figure 1.5) and vaporize, creating a channel in the PMMA substrate. The depth and the width of microchannel are depend on many factors including thermal diffusion of substrate materials, scanning speed of laser beam or X-Y stages, the laser power, pulse rate and number of passes made across the channel. Generally, the patterns of microchannels are square, rectangle and Gaussian shaped of cross section profile.

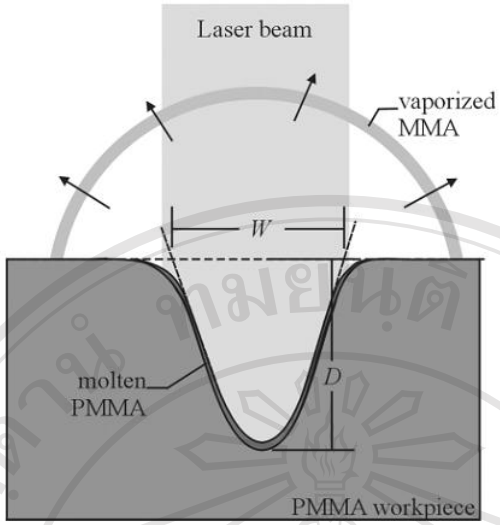


Figure 1.5 Schematic diagram of the laser micromachining process on PMMA [120]

Normally, all laser machine systems consist of two primary components which are a pulsed laser source and a mode of stage moving control. Pulsed laser device may be bought from various shops such as optical companies, online retailing and directly from laser manufacturers. The second part is the stage control that controls the moving of substrate in X-Y dimension or the scanning of laser beam with three dimensions to a substrate. Commonly, the laser output is focused on a stationary point and the substrate is moved in the desired pattern with respect to this site [121] or moving the focused beam relative to an immovable substrate produce the pattern chip. Simple lines, complex lines and spirals shapes can be creating with laser ablation technique on a variety of different materials that is reviewed in previous section. Commonly, the computer numerical control (CNC) machine can be coupled with laser device. It is always used to control the direction of platform and the firing of the laser power with program commands.

1.3.2 Bonding techniques [84, 104]

Bonding or sealing techniques is one of the key challenges in fabricating of microfluidic devices that involve the sealing of the open tiny channels with a blank plate material. Microfluidic device may be making from glasses, silicon and polymers. There are a number of observations that has to be taken into account when selecting and applying an appropriate bonding method including bond strength, surface chemistry, optical properties, material compatibility, low dimension loss and homogeneousness of the channel sidewalls. There are several bonding techniques for fabrication of microfluidic chips as shown in Table 1.2.

First is thermal bonding that is suitable for glasses and polymers. During the thermal bonding process, substrates are heated to a temperature nearby the T_g of one or both of the substrate materials, while applying a pressure to higher contact forces of mating materials [104]. The merged temperature and pressure can produce sufficient flow of polymer at the boundary to accomplish intimate contact with diffusion of polymer chains between the surfaces leading to a strong bonding. The advantages of thermal bonding are high bond strengths, overall simplicity of the procedure and the microchannels retain homogeneous surface properties. For example, glass microchannnel are often applied to heat up to a suitable temperature in order to bond a cover plate about 550-680ºC for 12-18 h. This process is serial in nature and often time-consuming [117]. However, the high temperature makes this procedure incompatible for application with enclosing of metal and carbon electrodes. Low-temperature choices have been presented for bonding glass of microfluidic chips lower 100°C and even at room temperature [118], but there are other steps add

together. In case of sealing of polymer substrate, bonding of polymer microchannels and a cover plate material is much simpler than silicon to silicon or glass to glass channel and can often be manufactured using low temperature. The two pieces of PMMA substrates could be bond together with thermal in a conventional oven [123]. Moreover, thermal bonding of various thermoplastics for creating a chip has been widely studied including PC [124] and COC [125]. In addition, microchannel distortion may be occurred due to inappropriate temperature and pressure during bonding process. Thus, suitably controlling temperature, pressure and time are critical parameters to achieve high bond strength while limiting distortion of the fixed microchannels due to bulk polymer flow. Due to this reason, some work has preferred to use programmable heating control [126].

In some cases, temporary bonding can be useful for sealing of open microchannel. For example, a PDMS sheet can be used as a temporary plate to seal glass tiny channel and bond them onto a PDMS sheet to form open reservoirs for controlled injection and ejection of fluid samples [50], but in principle this technique can apply to use with a variety of materials due to elastomeric material (PDMS) have excellent adhesive force to a wide variety of substrate materials such as PMMA, glass and can be used to enclose microchannels with a non-permanent seal [127, 128].

Second is solvent bonding technique that uses a chemical to act as a solvent which is spread between substrates. For interconnections between organic solvents and polymers, when a polymer surface is solvated, polymer chains convert mobile and can readily diffuse across the solvated layer, leading to expansive intertwining of polymer chains between the surfaces and resulting in outstandingly strong bonds. The

application of solvent to the polymer substrates may be used in either liquid or vapor phase. In common, liquid phase is often performed using between polymer and solvent systems. Over solvent absorption on material surface could cause channel distortion during bonding that can also be avoided by using very short time for solvent exposure. PMMA is a good material choice for solvent bonding because it interacts with common organic solvents including methanol, ethanol, dichloromethane and isopropanol [129]. For example, solvent bonding of PMMA chips has also been performed using 2, 4-pentadione [130], acetonitrile [92], dimethyl sulfoxide (DMSO) and methanol [131]. In order to study the case of preventing unwanted distortion of bounded PMMA tiny channels during solvent bonding, ethylene dichloride and ethanol were employed as a mixture of solvent [132]. This solvent mixture keeps the property of boiling point below that of either separate component. The ethanol helped to prevent microchannel blockage, while the ethylene dichloride acted as a solvent bonding for the PMMA substrates. Due to the component of the solvent mixture which endures unchanged during evaporation step, the constant ratio of ethanol confirmed that channel distortion was avoided throughout the drying process. In addition, one approach was applied to control dose of solvent to the chip for preventing channel collapse even after stretched solvent exposure. In this method, open microchannels are filled with liquid wax [133] that is cooled to solidify the sacrificial material and then directly using acetonitrile to the surface of chip. These sacrificial materials avoid solvation of the microchannels walls and thus channel distortion can be virtually reduced. Furthermore, solvent bonding has significant method for sealing of COC chips. For example, the solvents deposition from the

vapor phase onto COC chips has been reported [134]. This work described 50% bond strength enrichment by treating the bonded chip with deep UV light at room temperature to promote the higher polymer chain mobility at the mating surfaces of chip after solvent exposure step.

Third is anodic bonding technique. It is an electrical together with thermal wafer bonding process without using intermediate layer. This bonding technique is mostly employed for connecting silicon-to-glass and metal-to-glass under electric fields. The requirements for anodic bonding are clean and smooth wafer surfaces and atomic link between the bonding substrates through a sufficiently powerful electrostatic field. Anodic bonding apparatus has a pair of electrodes setting two electrodes plates facing each other and two substrates to be bonded are positioned horizontally among the two electrodes. In bonding process, a voltage is applied to the two substrates through an anode electrode and a cathode electrode (Figure 1.6). Under a forceful of electric power with several voltages, surfaces are closely linked to each other with strong electrostatic force. Subsequently, anodic bonding lines are very strong below temperatures of 400 °C and a bond voltage of 500 to 1000 V [135]. When silicon and glass substrates are bonded, these two substrates are sited between two metal electrodes, the glass substrate is a cathode and the silicon substrate is an anode. When heated up to 400ºC and applying a DC potential (1 kV) to the electrodes as shown in Figure 1.16, Consequently, a large electrostatic force is generated between bonding surfaces due to the positive charges and the negative charges that occur from both substrates. Thereby the glass develops highly reactive with silicon surface and creates a solid chemical bond. Glasses appropriate for this purpose are

Corning 7740 (Pyrex), Corning 7750, Schott 8329 and Schott 8330 [112]. In addition, anodic bonding can be employed for bonding glass to glass in which the temperature does not exceed 400ºC [136]. The process of glass-to-glass bonding, it is the same to a conventional silicon-to-glass anodic bonding. Furthermore, bonding can only be fulfilled using an intermediate layer such as a titanium thin film interlayer [137]. Moreover, Silicon-to-silicon bonds can be made with the anodic method happening between the two silicon wafers applying a borosilicate glass intermediate layer [138]. The glass intermediate layer can be deposited by different techniques, such as spin-on glass and sputtering method. Anodic bonding techniques give a good bond, transparent bond at the end of the process and without loss of shape of microchannel.

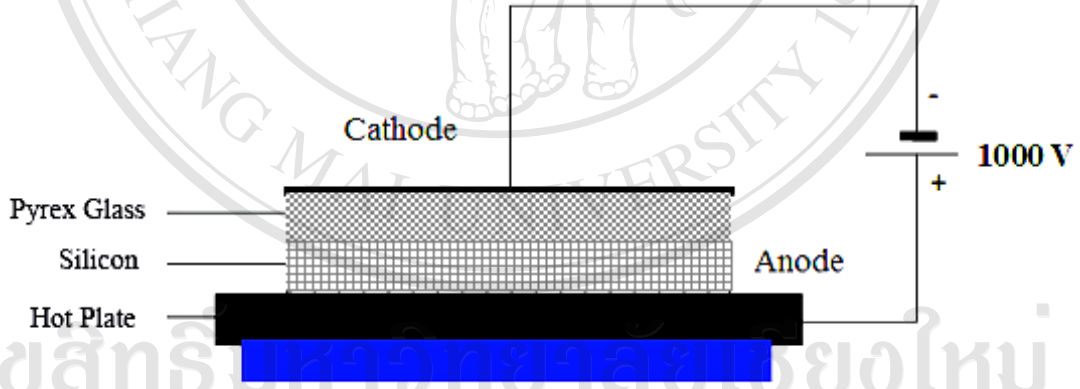


Figure 1.6 Glass to silicon bonding diagram

Fourth is adhesive bonding technique that describes a wafer bonding method with applying an intermediate layer to connect between substrates. Depending on substrate materials and applications, the intermediate layers often use glass, epoxies,

photoresists and various polymers. The advantages of adhesive method as an adhesive layer is the below temperature process demanded for polymer substrates and can be employed for all types of substrate material. However, a trouble is that small microchannels turn into broader during patterning what clogs the formation of an accurate intermediate layer with tight dimension control [139]. Adhesive bonding is fabricated by applying a thin layer of a high viscosity liquid adhesive material that forms a bond after curing by UV light radiation. UV-curing adhesives are usually produced from synthetic resins containing photosensitive to increase resin crosslinking on exposure to specific wavelengths of UV light. The characteristics of the UV epoxy resin are high adhesion, non-conducting, high transparency and low toxicity [140]. For examples, bonding using SU-8 as an adhesive layer was sandwiched between silicon and glass wafers [141]. After connection between substrates was completed, the bonding layer was exposed to UV light and the substrate packet was cured on a hotplate to finish the bonding process with temperature of 75 ºC. In this method the thickness of adhesive SU-8 layer was 50 µm. Next, UV epoxy resin for the bonding at room temperature of glass-to-glass plates. After introducing resin solution into the gap between the glass substrates, acetone is injected for cleaning the tiny channels, using pump to remove residual acetone and then UV lamp is shone on the device to cure epoxy resin. This bonding method can be accomplished within 40 min. Moreover, PDMS intermediate layer is usually employed to bond two PMMA substrates [142]. A thin layer of PDMS (10-25 µm) was spin-coated on PMMA blank substrate and then cured with the tiny channel PMMA

resulting bonding in PMMA-PDMS-PMMA chip. The PMMA substrates were bonded at low temperature without any global geometric distortion of channel.

In addition, microwave welding technique can be employed for bonding of PMMA substrates [91]. Polyaniline (PA) microchannels of 200 μm and 400 μm widths have been completely sealed using a microwave power of 300 W for 15 s resulting in PMMA-PA-PMMA chip. This technique offers an alternative for sealing polymer-based microfluidic devices, without any shape distortion of microchannel and displays the ability of microwave technology for welding and bonding applications in polymer microfluidic devices.

1.3.3 Propelling system [14]

The liquid propelling system, it is an elementary unit in all flow injection analysis systems. For μFA system is a technique based on transport of small volume of reagent and sample, highly reproducible timing, a feature that demands pulse less and reproducible flow rate in liquid propelling. In particular, the pumping mechanism should guarantee a reliable and homogeneous flow rate, autonomously from temperature, pressure and viscosity, these parameters may change as the examined reaction proceeds. Moreover, to optimize the fluid flow rate, dead volumes due to the use of connectors and tubing that leading to occur back pressure, should be avoided [143]. The requirement to drive microliter of a liquid volume very precisely, accurately and low pulse into a manifold has provided an inspiring work. Continuous flow micropumps are based on a direct modification of non-mechanical or mechanical energy into a continuous liquid movement [144]. Various types of fluid control methods have been reported in μFA system. For examples, syringe pump, micro

peristaltic pump, piezoelectric pumping, gas pressure, electrostatic micro pump and electroosmotic flow (EOF), all of their advantages and disadvantages should be taken into consideration. However, electroosmosis and hydraulic pressure are commonly employed in μFA system.

1.3.3.1 Syringe pump

Accurate syringe pump (Figure 1.7) is one of the propelling systems selected to be employed in the μFA system due to it offers pulse-free and highly precise flow rate. Besides, this pump can aspirate and dispense liquid in level of micro liter volume [145]. However, it is relatively expensive, requires priming before work and has a limited of reservoir volume. In addition, it does not provide a flow that is smooth sufficient for many microfluidic applications and cannot be simply applied into complex microfluidic system. The components of syringe pump are a motor-driven screw driver syringe, syringe with various volume and the check valves. Flow rate of the solution can be veered by changing motor speed.

Figure 1.7 Syringe pumps

1.3.3.2 Micro peristaltic pump

The peristaltic pump is a highly beneficial propulsion device that is no doubt employed most frequently, not only in FIA, but also using in other continuous flow analysis systems. In μFA system peristaltic pump should be miniaturized into a small scale (micro peristaltic pump) with tiny internal diameter tubing to transport a small volume of liquid in the system, it also decreases back pressure and dead volume of solution. Micro peristaltic pump is relating to simple and quick devices for delivering reagent solutions within a microreactor in a controlled approach, this method is very appropriate for finished time resolved chemical reactions on-chip, without the need of flow sensors. This pump consists of a small motor-driven wheel with marginally positioned rollers and a compression cam (or band) which is pressed against the rollers. One or several pump tubes are affixed so that they rest on a minimum of the rollers at all times (Figure 1.8). Micro peristaltic pump has been widely and successfully applied in continuous microflow analysis system.

Figure 1.8 Micro peristaltic pump (series 100, Williamson Manufacturing Co Ltd, USA)

1.3.3.3 Piezoelectric pump [14]

Piezoelectric actuations are widely employed in micro pumps. A piezoelectric pump is an attractive device to be used as a micro pump for little flow rates of solution. Commonly, this pump is made from a silicon wafer containing channels that are enclosed by a glass plate and a piezoelectric element layer (e.g. ZnO) and inter digital transducers (IDTs) cover the glass plate (Figure 1.9). Electric potential is applied to one IDT forming a convex/concave outline, generating mechanical stress in the piezoelectric layer. This creates a curvy acoustic wave that plays as a peristaltic pump that transports the liquid and with the purpose of guide the flow of the pumped liquid, on the silica wafer are created two check valves. These are situated at the inlet and the out let of the pump chamber. At this point the piezoelectric actuator is switched off, the solution flows from the inlet into the pump chamber. The solution is driven to the outlet when the actuator is switched on. Since an intermissive voltage is applied to the actuator, transporting of fluid is achievable. This type of pump can simply convert blocked and often produces pulsing during flow of liquid. However, this method desires a difference in flow resistance between inlet and outlet way for diverse flow directions. Such a behavior is realized with the passive cantilever valves, which show a large flow rate in forward direction and low leakage rates in reverse operation. Recently, the development of piezoelectric micro pumps has changed on the way to using low-cost, optically transparent materials such as PDMS and PMMA, instead of micromachining glass or silicon substrates [146, 147].

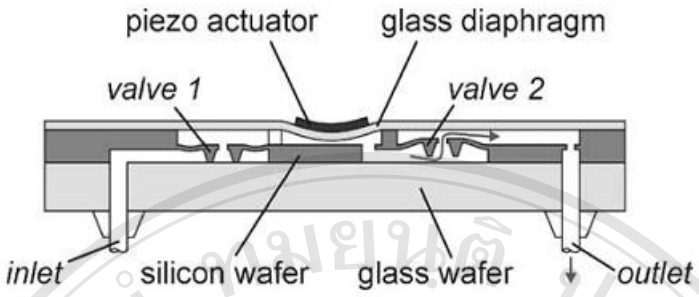


Figure 1.9 Piezoelectric micro pump [148]

1.3.3.4 Electroosmotic flow [14]

Electroosmotic flow (EOF) is pumping of liquids through the micro-fluidic system with voltages applied to electrodes located in the reservoirs, which involves ion transport by an electrifying field. EOF comes about in device with channel wall made from materials that are charged under experimental states [149]. The liquid consist of the double layer closely to the charged surface will not be neutral, but rather will comprise a higher than bulk solutions concentration of counter-ions, resulting in a charged liquid. It gives several meaningful advantages over hydrodynamic based pumping methods. It can be easily miniaturized without mechanical moving portions are concerned and the required voltage sequences can be readily managed with automatic computer control. The key parameters that suggest the performance of electroosmotic pumps are the magnitude of the applied electric field and applied voltage, the cross section of the structure in which flow is produced, the surface charge density of the solid surface area that is in contact with the working liquid and ion density and pH of the working liquid [150]. For a glass micro reactor, the channel wall fluid interface commonly has a negative charge, climbing from ionization of

silanol groups, which are stationary. This stationary surface charge attracts a diffuse layer of mobile, contrary charged counter-ions in the fluid close to the channel wall. Figure 1.10 shown application of an electric field through the channel length causes the nanometer thick film of mobile cations to transfer towards the more negative electrode, which draws all the mediating solution in the bulk of the channel with it. An important highlight of EOF is that the solution EOF velocity is steady across the channel except in the nanometer thick zones of the diffuse layer of counter ions very close to the wall. It should be highlighted, that underneath EOF control, charged solutes transfer with an electrophoretic velocity in adding to the EOF of the solvent.

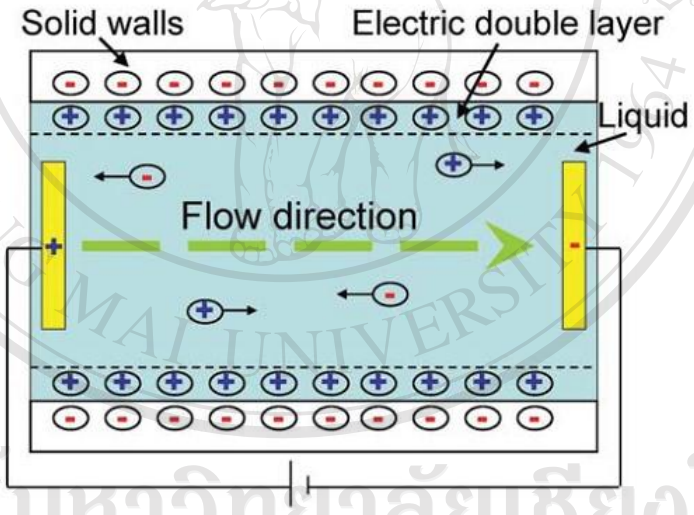


Figure 1.10 Electroosmotic flow [151]

1.3.4 Sample introduction to the microflow analysis system [152, 153, 154, 155]

A very important solution handing function is capability to dispense very defined small volumes of solutions, repeatedly, on request and very reproducibly [153]. There is two-step process that involved sample introduction techniques. First is a gated-valve injection that the sample and reagent streams make a turn at the straight-cross intersection. For injection, the analyte flow stream is deviated shortly into the separation channel. The main advantages of the gated-valve technique are the capability to vary the size of the dispensed sample zone and the fact that the flow stream in the separation channel is continuing, next for a pinched-valve injection, the sample is focused through the intersection by constant flow rate of reagent from the nearby dispensing and reagent promote microchannels. To manage the sample, an electric field is applied through the dispensing channel. By synchronous application of pullback voltages to the sample and sample-waste reservoirs, make sure samples can be dispensed with this technique. For this reason, pinched-valve injections have been chosen over gated-valve injections of samples in LOC applications. The injection part is the important device to provide the necessary requirement to introduce the accurate and well-reproducible sample volumes into the carrier stream of the microfluidic analysis system without any trouble**.** Most conventional FIA injectors (e.g. rotary valve) are clearly too large of volume for practical employ in μFA because of the demands of injecting very small volumes of solution. Many of the injection techniques employed in μFA were initially improved from techniques that used in conventional FIA and CE systems. There are some exemptions that seem to be

adjustable to μFA systems including electrokinetic mobility (electrophoretic mobility or electroosmotic flow) [111, 156], pressure injection [157], miniaturized valves [158, 159] and selection valve with zero dead volume. To succeed better flow control, valving and mixing of solutions are necessary.

1.3.4.1 Electrokinetic injection [14, 160]

Electrokinetic principles are widely applied to transfer solutions in microchannels. For ionic species, charges or dipoles in solution can earn electrokinetic force from the applied electric field and transfer along the electric field. When they move in the solution, a drawing force is generated through friction of the particles with solution environs them and induces a liquid flow on glass chips with a smooth plug flow profile in a narrow channel. The flow profile power fully depends on fluid conductivity, magnitude of the electrical field applied and pressure produced. There are two modes of injection in microfluidic system as time-based and discrete volume-based injections [161]. Time-based injections related applying an electric field to transport the sample solution continuously at a known flow rate that mean to volume for a given period of time into the micro reaction channel of the μFA. This technique has several advantages including ease of control, variation of injected sample volume and continuing and/or sequential injection for on-line analysis. However, disadvantages of this technique are the feasibility of suffering from uncontrolled dispersion leading to injection volumes being irreproducible except the flow rate is held in reserve constant and biasing of the sample that is injected. In addition, although the electrokinetic pumping can transfer solution, but they are not good for mixing liquids in the tiny channels due to low-Reynolds-number flow

conditions [160]. Next is a discrete volume-based injection of sample that concerns filling a channel length with a fixed volume of sample. This technique makes available a very reproducible and definite injection volume of sample because dispersion is decreased by discrete injection. The introduction of a sample into a geometrically defined volumetric hole, it is potential to have a repeatable injection that is free of flow rate and simply automated. The simplicity of continuous and sequential injection unit for on-line detections is close to that of time-based injections of sample and has much higher reproducibility. Moreover, when compared to glass, common polymers have lower charge density. It was reported that the electroosmotic mobility of the laser ablated PMMA chip is 2.47×10^{-4} cm^2 V^{-1} s^{-1} [126]. This comparison with the original atmospheric molded PMMA chip is 2.37×10^{-4} cm^2 V^{-1} s^{-1} [162], while the value is smaller than that obtained by the electroosmotic mobility of the glass chips that is recorded to be 4.46×10^{-4} cm^2 V^{-1} s^{-1}[162].

1.3.4.2 Pressure injection [14]

Pressure-driven flow is normally employed in microfluidic systems. The velocity profile of flow is typically parabolic similar across the liquid–liquid interface in a micro reactor. The parabolic velocity profile could cause a substantial difference in the period of time of diffusive move between the diffusion near the top and bottom walls and in the center of the channel [163]. The variance of diffusion process with the location may influence the detection of mixing intensity and molecular properties as well. The movement of liquids under the control of a pressure pumping mechanism has many drawbacks in μFA. Due to the very low width and length of the microchannels, pumping mechanisms generally create high back pressure and causes

band broadening of signal. Pressure injection is based on a pressure variety is introduced sample solution to the reservoir using positive pressure (piston-type) or by suction (vacuum) pressure, which drives the sample solution into the microchannel of the μFA . The advantage of this approach over the electrokinetic injection is the deficiency of preference among different ion mobilities, resulting in a dependable representation of the sample. The volume of sample injected must be calculated, but it depends on sample viscosity, injection time and pressure difference, so the injection precision is difficult to control. Atmospheric experimental conditions and elevation of solution would affect the pressure differences across the reservoirs and therefore the repeatability of the injection.

1.3.4.3 Microvalves [14, 164]

Microvalves are often one of the most significant components for the miniaturization of a completely microfluidic system. It can be generally categorized into two groups as active and passive microvalves. There are several types of active microvalves such as electrokinetic microvalves, thermopeneumatic microvalves and rotary microvalves. Sometimes, passive microvalves are considered as a part of micropumps in many applications, in this case, membrane microvalves, in-line polymerized gel microvalves and hydrophobic microvalves. Each type of microvalve has a different component and operating functions. For example, the miniaturization of the micro rotary injection valve is alternative mode to introduce sample solution onto the microfluidic system. Micro rotary valves inject a fixed, accurately and reproducible volume of liquid solution from an injection loop. It has not been employed on a microfluidic system, but display potential for decreasing the size of

certain applications. Varying the sample volume of the injection loop can easily change volumes using small tubing. Unfortunately, low volumes of solution are difficult to achieve and are generally too large for practical use in μFA that demands very small volumes. A commercial microinjection valve (Valco, Switzerland) with an internal loop of 20 nL was accomplished. This shows the present possibilities for miniaturizing conventional flow injection techniques.

1.3.4.4 Selection valve with zero dead volume

The conventional selection valve is miniaturized to solve dead volume as it is applied in the microfluidic system which is more simply than miniaturization of the micro rotary injection valve. The selection valve has to allow random access of the ports. The common port is linked to the pump. Other ports are linked to reagent solutions, standard solution, samples, carrier solution and the chips. The 10 port multi-position valve is by far the most widely employed for LOC. This sample injection valve is precise, reproducible volume of sample and it can integrate to automatic system. The flow rate of the solution is control the sample volume and switching time to the sample position. These can be controlled by manual or manage with software computer. Disadvantage of this valve is the tubing internal diameter employed for impel sample should be very small to eliminate dead volume and it is suitable for sample volume more than 500 nL due to the problem of dead volume will occur in the system.

1.3.5 Liquid mixing [165]

The objective is to obtain at a homogeneous mixture of two solutions in a short time as possible. Liquid flow can be defined as functioning in one of two flow

rules: turbulent or laminar. The Reynolds number (Re) quantitatively characterizes liquid flow; Re above around 2300 shows turbulent flow process, while Re below that value displays laminar flow process for water in a smooth circular channel. Re is directly proportionate to channel diameter and as Re values <1 are common in microfluidic systems, indicating the normal appearance of laminar flow in microchannels. A normal result of laminar flow in microfluidic channels is that multiple streams can flow side-by-side without turbulent flow mixing only diffusional mixing occurs, which leads to an advantage in some applications, but problem for states is required rapid mixing of liquids in tiny channels of a chip.

1.3.6 Detection system

The detection part is sensing unit of the μFA systems, which agree to continuous monitoring of a given characteristic of the sample or product and furnish qualitative and quantitative data of the analyte. Nearly all detection techniques utilized in laboratory practice are suitable in LOC analysis. Many detection systems, which could be adjusted for microflow through detection, may be applied as detectors for μFA such as UV-Vis spectrometer, IR spectrometer, AAS, ICP, fluorimeter, nephelometer, radiometer, photomultiplier tubes, thermal lens microscopy, mass spectrometer and various electrochemical detectors. These detectors should be adapted for detecting in the microfluidic system. For example, UV-Vis spectrometer should be modified with a small fiber optic probe to connect light source and detection unit for the microfluidic system. However, in analytical chemistry, optical detection and electrochemical detection are commonly applied.

UV-Vis absorbance detection is one of the most fundamental aspects employed detection method in µFA system. UV-Vis wavelength range between 180 and 800 nm light absorption is classically due to an electronic transition to a higher energy state. UV detection through an UV detector is also a feasible method of detection in LOC analysis. The tiny dimensions of microfluidic channels limit the path length accessible for absorbance measurements leading to decreasing the sensitivity of absorbance measurements on-chip. Therefore, most work in the field of absorbance detection with microfluidic systems turns around the exploration for ways to increase the obtainable path length for increasing sensitivity. In the case of plastic microfluidic devices, special care must be taken in selecting an appropriate material that must be transparent to light, specifically in wavelength regions equivalent to the sampling wavelength [165]. Currently, the development of a wide range of intense light emitting diodes (LED) and photodiodes that can be well coupled directly to microfluidic devices to provide a miniaturized technique of on-chip detection can easily be realized [166]. For a common fiber optics-based UV absorption system, combining optical fiber optics into the chip segments is a simple line minimizing. The chip was positioned between the ends of lines of two optical fibers facing one another. The top fiber optic was connected to a LED light source and the bottom fiber optic collected the transmitted light and conducted it into a charge-coupled device (CCD) array detector such as USB4000 and USB2000 spectrometer. The USB4000 spectrometers [167] are miniature fiber optic UV-VIS-NIR detection devices for low light level measurements. They are small package and portable with the size of 63.34 mm x 89.10 mm x 34.37 mm and weighing only 180 g. These spectrometers admit

light energy transmitted throughout a single-strand optical fiber optic and disperse it via a located grating across the CCD array. It is offers to a 200-850 nm wavelength range. The USB4000-UV-VIS involved programmable microcontroller to supply flexibility in controlling the spectrometer and accessories. Moreover, it can be employed LEDs as light source. LEDs are robust, low cost, low power consumption, very efficient in terms of energy conversion, small size and they cover an increasingly broad spectral range from UV to near infrared [168]. They have small outside shape dimensions often less than 10 mm x 10 mm and longer life time up to 50,000 hours. LEDs range from a narrow spectral band emitting light of a single color, such as red, yellow, green, or blue, to a broader spectral band light of white color with a different distribution of radiant intensity and spectra and shades depending on color mixing and package design [169]. In addition, LEDs with white color are a blend of all visible wavelengths about 400-800 nm. Thoroughly with the clear blue color as peak wavelength range 455–490 nm, there are other wavelengths, including green color as 515–570 nm, yellow color as 570–600 nm and red color as 625–720 nm that are components of white light.

Fluorescence is the most popular optical detection methods for microfluidics due to its excellent sensitivity and selectivity. LOC fluorescence system is typically detection of the emitted fluorescent light, which is generated by the using the lasers induced fluorescence molecules, with CCD cameras or photomultiplier tubes (PMT). The coherence and little deviation of a laser beam makes it easy to focus on very small detection areas of product and to achieve very high irradiation of light, following in one of the good detection limits of any LOC system [170, 171]. In

microfluidic systems can apply the addition of small-lenses and planar waveguides is necessary for upgrading the detection in sensing systems. For example, a glass chip is combined with small-lenses directly for the store of fluorescence light by melting a thin film of photoresist into a hemispherical shape [172]. In addition, for creation of a compact system, a pinhole, an interference filter and a photodetector were placed in close propinquity to the small-lens.

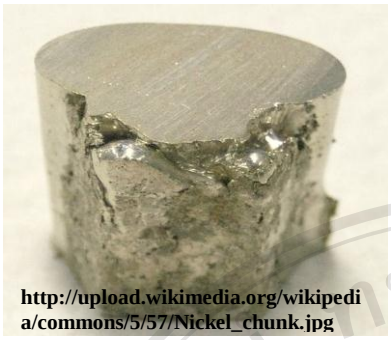
Chemiluminescence (CL) method for LOC has been widely used of emission detection, usually in the visible or near infrared region, due to high sensitivity, low detection limits and simple equipment when compared with other spectrophotometric techniques. The basic chemistry of CL examines is based on the reaction of radical oxidants with sign compounds to produce excited state species that emit chemiluminescence light. However, the limited number of chemiluminescence reagents, the effects of matrix interference and the expensive of PMT are the disadvantages of this technique. In addition, the mixing between reagent and analyte before detection is needed a complex chip pattern. The detector devices are integrated to LOC system such as photodiode and a miniaturized PMT [23]. For example, a silicone chip has been integrated a photodiode fabricated in the bottom of the channels etched for determination of glucose [173]. All apparatus and electrical connections were placed on the backside of the substrate to assist easy bonding of the microchannels by anodic bonding technique of a Pyrex glass sheet. Moreover, some of the most useful chemiluminescence reagents are luminol, acridinium ester, aryl oxalates, dioxetanes and tris(2,2′-bipyridyl)ruthenium(II) or $Ru(bpy)_3^{2+}$.

1.4 Nickel and zinc

1.4.1 Nickel [174, 175, 176, 177, 178, 179, 180, 181]

Nickel is one of the important elements in plants and animals (e.g. rat and chick). It is one of the transition elements of the periodic table with the chemical symbol Ni and an atomic number 28, the density of its at 20^{o}C is 8.908 g·cm^{-3}, melting point and boiling point of 1455 and 2913 ^{o}C respectively, electrical resistivity and thermal conductivity of 69.3 nΩ·m and 90.9 W·m^{-1}·K^{-1} respectively. It is a silver-white solid metal (Figure 1.11) with a slight golden tinge that takes a high polish. This metal has the most common of oxidation state +2. It is found in natural with in a wide variety of mineral (pentlandite, pyrrhotite, and garnierite). It also occurs as an impurity in ores of iron, copper, cobalt and other metals in the earth's crust.

Metallic nickel forms compounds with various anions such as sulfide, sulfate, carbonate, hydroxide, carboxylates, nitrate and halides. Several of these compounds are widely used in modern industry such as electroplating, electroforming, producing nickel alloys. Alloys are used in the making of brasses and bronzes coins, jewelry and metal items. In addition, Ni compounds are also used to color ceramics, to make alkaline batteries and as catalysts that increase the rate of chemical reactions. Other nickel compounds are used as coloring agents including dye mordant and electronic equipment. For example, the compound of nickel dimethylglyoxime is employed as a coloring agent in paints, cosmetics and many kinds of plastics.



http://upload.wikimedia.org/wikipedia/commons/5/57/Nickel_chunk.jpg

Figure 1.11 Nickel

The properties of nickel alloys are strength, ductility and resistance to corrosion and heat. About 65 % of the nickel consumption in the Western World is employed to make stainless steel [176]. The high consumption of nickel containing products inevitably leads to environmental pollution by nickel and its compounds at all stages of production, utilization and disposal [177]. The larger amount of all nickel compounds that are released to the environment which will be adsorbed by sediment and soil particles. Furthermore, small amounts of nickel are also present in ambient air about 6-20 $ng \cdot m^{-3}$, nickel in natural water contains low concentration ranges about 0.5-1.5 $\mu g \cdot L^{-1}$ and presence background level about 0.3 $\mu g \cdot L^{-1}$ in river water. Divalent nickel is the dominate form of nickel as soluble in waters. It is commonly accepted that Ni(II) concentrations level of lower than 0.1 $\mu g \cdot mL^{-1}$ in natural waters are innoxiously to aquatic organisms and irrigated plants [179].

Nickel and all compounds have not been displayed to be essential in human's body. Occupational exposure to human body arises in mining, refining, alloys and battery production, electroplating and welding. In addition, edible food containing

nickel which is the major source of exposure for most people, but drinking water contains small amounts of nickel. Inhalation from Ni has been displayed to provide rise to elevated levels of nickel in blood, urine and body tissues. The most common adverse health effect of nickel in human's body is an allergic reaction. Nickel allergies affecting pierced ears are often marked by itchy and red skin [174]. Furthermore, chronic exposure of nickel in the body can lead to lung fibrosis, cardiovascular and kidney diseases, the most serious concerns relate to nickel's carcinogenic activity [177]. Moreover, some nickel compounds can cause acute toxicity to human's body such as nickel carbonyl and nickel sulfate. For example, people who are exposed to nickel carbonyl by inhalation. Acute health effects normally result from short-term exposure to high level of nickel (>0.5 g) and they clear as a variety of clinical symptoms (nausea, vomiting, abdominal discomfort, diarrhea, visual disturbance, headache, giddiness, and cough) [181].

1.4.2 Determination of nickel

Nickel and its compounds are toxic and cause environmental problem when they contaminate in surroundings. Excessive nickel to human can cause nose cancer, lung cancer and leukemia in the plentiful nickel area. In addition, the effect of nickel in patients who eat foods rich in nickel, such as oats, nuts, beans and chocolate show that illness incidence increased [182, 183]. Therefore, considering the low concentration level of Ni(II) ion in environmental, biological and food samples, sensitive analytical techniques are essential. Several analytical techniques (as shown in Table 1.3) have been employed for analysis of Ni in various samples. Each of these proposed methods often propose advantages and disadvantages. The most commonly

used method to detect trace nickel concentrations in water samples and foods is graphite furnace atomic absorption spectrometry (GFAAS) [199]. This method has good sensitivity and reproducibility, nevertheless this method is unfortunately imperfect due to matrix interference in samples. Next, inductively coupled plasma mass spectrometry (ICP-MS) [185] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [195, 196], which display fast multi-elemental analysis, wide range of linearity and adequate detection limits for the determination of heavy elements present in environmental samples like river water, tap water, natural water and hair samples. However, there are limited because of interferences arising from polyatomic and matrix effects, together with the high rate of analysis and the expensive maintenance of these instruments. Next, inductively-coupled plasma optical emission (ICP-OES) [198, 208] provide multi-element trace metal analysis of environmental samples, good selectivity and sensitivity, but sometime the detection limits is not sufficient when the concentrations of metal are too low, therefore, it the preconcentration step of ultra trace elements and elimination salinity in seawater samples before their analysis are necessary. Moreover, this technique has the high cost of analysis and the expensive instrument. High-performance liquid chromatography (HPLC) [186, 204] has attained a reliable technique for measuring nickel ion concentrations and is accomplished of simultaneous quantitative determinations. However, this technique must use post-column derivatization with an appropriate complexing reagent, such as 8-hydroxyquinoline, before separation and detection, therefore, it involves multi-step procedures leading to time and reagent consuming. Next, electrothermal atomic absorption spectrometry (ETAAS) [209]

could be employed for determination of nickel that has usually enough limit of detection. On the other hand, there are some problems that occur at the low concentrations of nickel in biological and environmental samples about error from D_2 arc corrector followed by the high background signals for the determination of nickel. Flame atomic absorption spectrometry (FAAS) [187, 192, 207] which is widely used technique in most laboratories for nickel determination due to its simplicity and lower cost of instrument than GFAAS. However, matrix interferences and insufficient sensitivity are the limitations. Therefore, it demands the employ of a preconcentration step, such as ion-exchange, solid phase extraction, in order to reach a suitable level of sensitivity. Next, the chemiluminescence method (CL) [200] is interesting due to its very low detection limits and wide linear ranges can be accomplished for definite species with using special instrumentation that locates close to the detector and offers temperature control. When the CL-system is coupled with FIA, CL-based FIA methods offer a rapid, cheap, automatic and simple. Nevertheless, the choice of chemiluminescent reagents is a critical regard and the intensity of emissions varies sizably with the oxidant, employ of luminol in CL-systems with mixed oxidants is not forthright. In addition, interference with various metals is limitable of this technique. Next, electroanalytical methods [193, 206, 210] are the dominant techniques in analytical chemistry, especially voltammetry and amperometry. Stripping voltammetry techniques are suitable for determination of traces of nickel in environmental and biological samples due to its high sensitivity, low cost of instrumentation, portability, simplicity and low detection limit. The sensitivity is increased because of deposition step before recording signal. However, these

techniques are to be carefully controlled to acquire accurate and precise results, it is not suitable for routine analysis due to it is quite sensitive to experimental conditions. Moreover, the problem about selectivity may be happen. For example, the decrease of signal was obtained when the presence of a large excess of Cu(II) and Pb(II) for determination of nickel in the presence of dimethylglyoxime with the formation of the complex adsorbed on the electrode [210]. Unluckily these analytical methods employed large amounts of reagent volume and generated large amounts of toxic chemical waste. Some analytical methods used more time consuming and they also demanded complicated instrument and maintenance. Finally, FIA methods for determination of nickel in various samples have been reported on flow based [191, 194, 201], reversed flow analysis [184] and stop flow analysis [211] with spectrophotometric measurement. Furthermore, FIA with FAAS system has been studied for determination of nickel [203]. FIA with spectrophotometric detection displays some advantages such as instrumental simplicity, low cost of equipment, low running cost, high sensitivity and wide variety of organic reagents for nickel determinations. However, most of existing reagents have poor selectivity and then separation and preconcentration procedures may be essential to improve the determination of nickel. For examples, Vicente et al. [194] described the FIA method for determination of nickel using spectrophotometric detection at 562 nm. This method employed 2-(5-brom-2-pyridylazo)-5-(diethylamino)-phenol (Br-PADAP) as complexing reagent. The C18 mini-column with immobilized 1-(2-thiazolylazo)-2-naphthol is combined to FIA system for suppression of Cu^{2+} interference. The proposed method was sensitive with detection limit of 17 $\mu g \cdot L^{-1}$ and applied to

determination of nickel in standard reference materials. The results showed accuracy and agreement with certified values. Ghasemi et al. [212] developed spectrophotometric method for determination of nickel based on measuring the color complex of Ni(II)-nitroso-R salt at 490 nm. This method is a sensitive, selective and simple. The procedure was successfully applied to determination of nickel in alloy samples. However, a report of flow injection analysis for the determination of Ni(II) in waters using nitroso-R salt as the chromogenic agent has not been yet presented in the literature.

Table 1.3 A brief review of the methods for the determination of nickel

Year	Technique	Sample	LOD (μg L^{-1})	Reference
1995	rFIA-UV-Vis	Waste water	-	[184]
1995	ICP-MS	Human blood	0.04	[185]
1996	HPLC	Mineral ore and phosphate rock residues	2500	[186]
1998	FAAS	Brass (NBS SRM 37e)	87.0	[187]
1998	UV-Vis	Alloys and biological samples	300	[188]
1999	UV-Vis	Aluminium alloy	0.45	[189]
1999	UV-Vis	Silicates and alloys	77	[190]
2000	FIA-UV-Vis	Nickel–copper alloys	-	[191]
2001	FAAS	Reference materials, food	1.1	[192]
2002	AdSV	Tap and mineral water	0.005	[193]
2002	FIA-UV-Vis	Biological materials	17	[194]
2002	ICP-AES	Hair samples	-	[195]
2004	ICP-AES	Natural water	-	[196]
2004	UV-Vis	Waste water samples	10	[197]
2006	ICP-OES	Natural and synthetic water	0.06	[198]
2006	GFAAS	Water samples	0.12	[199]
2006	CL	Synthetic samples	0.33	[200]
2006	FIA-UV-Vis	Plant	40	[201]
2007	ICP-AES	Iron oxide pigment samples	5	[202]
2008	FIA-FAAS	Tobacco samples	0.8	[203]
2008	HPLC	Water	180	[204]
2008	UV-Vis	Medicinal leaves, soil and industrial effluent samples	69	[205]
2008	AdSV	Reference material	0.013	[206]
2010	FAAS	Black tea, rice flour, sesame seeds, tap and river water	0.71	[207]
2010	ICP-OES	Crawfish	-	[208]
2011	ETAAS	Human hair	-	[209]

1.4.3 Zinc [213, 214, 215, 216, 217]

Zinc (Figure 1.12) is one of the important elements in the earth's crust. It is one of the transition elements of the periodic table with the chemical symbol Zn and an atomic number 30, the density is 7.14 $g \cdot cm^{-3}$, melting point and boiling point of 419.53 and 907 ^{o}C respectively, electrical resistivity and thermal conductivity of 59.0 $n\Omega \cdot m$ and 116 $W \cdot m^{-1} \cdot K^{-1}$ respectively. It is a silver-grey solid that is almost insoluble in water. This metal has the most common of oxidation state +2. It is found in natural with in a wide variety of mineral (sphalerite, smithsonite and zincite). It is the important commercial minerals.

Figure 1.12 Zinc [213]

The property of zinc is similar to the character of the first-row transition metals nickel and copper. There are many compounds of zinc that is dominated by the +2 oxidation state. For examples, ZnO is a white powder which is closely insoluble in neutral aqueous solutions, but it can dissolve in strong acid and base. $ZnCl_2$ is low melting points, strong covalent bond and highly soluble in water.

There are various applications of zinc and its compounds. First is used as an anti-corrosion reagent. It is employed for the coating of iron or steel to against corrosion process. Second is applied as an anode material for fabrication of batteries such as lithium batteries. Moreover, it plays as the cases and anodes in zinc–carbon batteries. Third is the composition of brass as alloy that is stronger than copper for corrosion resistance and alloy is beneficial for hardware and water valves. In addition, ZnO is widely employed as a catalyst in the production of rubber, zinc sulfide (ZnS) is utilized in luminescent colors such as on the hands of clocks and many zinc salts are applied in wood preservation, photographic paper, ceramics, textiles, fertilizers and as dietary supplements.

Zinc in natural water is found in various forms such as hydrated ions, metal-inorganic complexes and metal-organic complexes. The spent alkaline and zinc–carbon batteries and no disposal represent an increasing many metal including zinc released to environment. The waste batteries cause a severe problem because of their toxicity and no decomposition in the environment. One of the results is that rivers are depositing zinc-polluted slush on their banks. Concentrations of zinc in surface water and groundwater normally do not above 0.01 and 0.01-0.04 $mg \cdot L^{-1}$ [215], respectively, levels in tap water can be much higher as an effect of the leaching of zinc from pipes and fittings [216]. In food, meat and sea food contain high levels of zinc usually 10–50 $mg \cdot kg^{-1}$ of wet mass, while grains, vegetables and fruit are low in zinc typically less than 5 $mg \cdot kg^{-1}$.

Zinc is an essential human nutrient for good health. It is a cofactor of enzyme and found in all tissues, bone and muscle. It is generally expected that zinc is non-

toxic due to the strong homeostatic regulation of procedures controlling the absorption and secretion of this element. Zinc in the human body contains about 2–3 g. The quantity of zinc for the recommended dietary allowance for men and women are 11 $mg \cdot day^{-1}$ and 8 $mg \cdot day^{-1}$ respectively. Moreover, zinc intake is suggested as 2–3 $mg \cdot day^{-1}$ for infants and 5–9 $mg \cdot day^{-1}$ for children because of their lower mean of body weights. Zinc-deficient human can occur when they intake insufficient zinc to allow body to grow normally such as infancy, adolescence, pregnancy and lactation. The symptoms of severe zinc deficiency in humans are losing hair, diarrhea, skin lacerations, delayed sexual maturation, impaired growth in infants, weakened immune system, poor healing of wounds, loss of appetite leading to anorexia, white spots, bands, or lines on fingernails, mild anemia, loss of taste, smell and vision impairments [218]. In contrast, the upper level of zinc intake for human lead to the acute health effects due to usually the result of short-term and high dose exposure. The symptoms and signs of zinc excess are lethargy, focal neuronal deficits, respiratory disorder after inhalation of zinc smoke, metal fume fever, nausea-vomiting, epigastic pain, diarrhea, elevated risk of prostate cancer, copper deficiency and sequelae and altered lymphocyte function [214].

1.4.4 Determination of zinc

Many biological and environmental samples contain low concentrations of zinc, it is easy to interfere in various samples. Thus, it is necessary to develop the new sensitive and selective methods for determination of zinc. There are several analytical techniques available for determination of zinc in various sample matrices such as GFAAS [219, 238], ICP-AES [220, 221], FIA [222, 229, 231, 233, 239, 240],

ICP-MS [223], DPASV [224], LC-MS [225], ETAAS [226, 241], AdSV [227], HPLC [230], SIA [232], FAAS [234, 236], ICP-OES [235, 237], and UV-Vis [238] as shown in Table 1.4. Selection of the most suitable method depends on various parameters; the aim of the analysis, the nature of the analyte, the concentration of the zinc, the presence of interferences in the sample matrix, the accuracy and the precision required and the available of methodology and instrument. A brief overview for zinc quantity is shown in Table 1.4. FAAS and ICP-OES are not appropriate for the determination of zinc at trace levels in well water, tap water and river water due to the insufficient of sensitivity. Therefore, preconcentration step prior to analysis is necessary. Most of the sensitive and selective techniques current have been presented such as ICP–AES, ICP–MS and GFAAS are also expensive to be employed in routine analysis, whereas a disadvantage of ETAAS is a single element analysis, high background absorption and interference effects of constituents in sample matrix [226]. In addition, the main drawback in the simultaneous analysis of copper and zinc by electroanalytical methods such as ASV is the creation of intermetallic compound between copper and zinc that is oxidized from mercury at the same potential [242, 243, 244]. Moreover, other analytical methods include FIA and SIA have been widely employed to determine very low levels of zinc in various samples. These methods exhibited very high sensitivity, low detection limits, good precision and selectivity at trace concentrations of zinc. However, they must consume a large volume of chemical when compare with LOC systems. In summary, the previously reported analytical methods for Zn determination (Table 1.4) offered sensitive, selective and high sample throughput (with the exception of FIA and SIA), but they produced large quantity of

toxic chemical waste with very expensive analytical instrument, some of them desired more consuming time and also complex system operation and maintenance.

Table 1.4 A brief review of the methods for the determination of zinc

Year	Technique	Sample	LOD ($\mu g \cdot L^{-1}$)	Reference
1995	GFAAS	Sea water	0.024	[219]
1998	ICP-AES	Copper-base alloys	-	[220]
2000	ICP-AES	Human teeth	-	[221]
2000	FIA-DPASV	Water	14.7	[222]
2001	ICP-MS	Plant	-	[223]
2002	DPASV	Wines and spirits	-	[224]
2002	LC-MS	Urine	50	[225]
2004	ETAAS	Botanic and biological	0.03	[226]
2004	AdSV	Water	0.06	[227]
2004	GFAAS	Delta waters	-	[228]
2004	FIA-UV-Vis	Plant materials	200	[229]
2005	HPLC	Tobacco	0.240	[230]
2006	FIA-UV-Vis	Vitamin	60	[231]
2007	SIA-UV-Vis	Pharmaceutical	20	[232]
2007	FIA-ETAAS	Human saliva	0.35	[233]
2008	FAAS	Mineral water	0.8	[234]
2009	ICP-OES	Water and biological	0.8	[235]
2010	FAAS	Lubricating oil	-	[236]
2010	ICP-OES	Tap water	0.08	[237]
2010	UV-Vis	Metalloproteins	6.3	[238]
2011	FIA-UV-Vis	Pharmaceuticals and galvanizing	50	[239]
2011	FIA-UV-Vis	Natural waters	2	[240]
2012	ETAAS	Food stuffs	-	[241]

1.5 Nitroso-R salt [212]

Van Klooster was first presented disodium 1-nitroso-2-napthol-3,6-disulfonate (Nitroso-R salt) in 1921 for the determination of cobalt [245] and then it is employed with several reactions for the determination of cobalt [246, 247], iron [248], copper

[249, 250, 251] and nickel [252]. The structure formula of nitroso-R salt is shown in Figure 1.13. It is chelating bidentate ligands. There are various donor atoms in the molecules of this reagent, that are a nitrogen atom as nitroso or oxime groups and an oxygen atom as hydroxy or oxy groups, which make it potential to coordinate a given metal ion. This reagent forms soluble complexes with certain metal ions such as Fe^{3+}, Ni^{2+}, Co^{2+} and Cu^{2+}. The sensitivity and the selectivity of the reagent depend on the pH of the solution and the character of the complexing agent. For Nickel(II)-nitroso-R salt complex has been reported about optical and colorimetric characteristics [253]. The ratio of Ni(II) : nitroso-R salt of 1:2 are required for complete color complex, but there are no literature data on FIA-UV-Visible of the Ni(II)-nitroso R salt complexes.

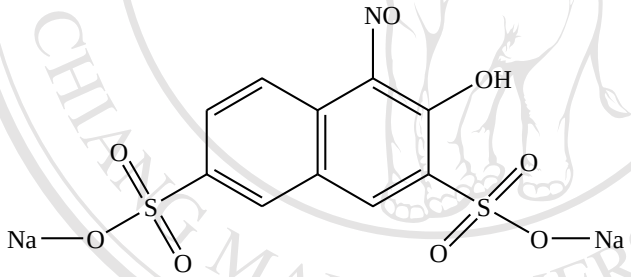


Figure 1.13 The structure formula of Nitroso-R salt

1.6 Xylenol orange [254]

IUPAC name of xylenol orange is 3,3-Bis[N,N bis(carboxymethyl)aminomethyl]-o-cresolsulfonephthalein tetrasodium salt with the molar mass of 672.67 g mol^{-1} and soluble in water. It appears yellow color at low concentration and red color at high concentration in water. Commonly, it is used for metal titrations for indicate endpoint. Moreover, xylenol orange form complexes with different metal ions like Al(III) and Fe(III) [255], Ni(II) and Fe(II) [256], Zn(II) and

Fe(III) [257]. In this case, spectrophotometric determination of zinc has been reported [258]. This method is based on the detection of the absorbance of the ternary complex formed by the reaction between Zn(II) and xylenol orange in the presence of cetylpyridinium chloride in the ratio 1:1:2 respectively. The proposed method was a facile, rapid and sensitive and successfully applied to determination of Zn in biological and pharmaceutical samples. However, this method employed a large amount of reagent leading to a large volume of chemical waste.

1.7 Reasons for creating this work

Nowadays, there are various analytical techniques that are sensitive, reproducible, simple, cost effectiveness, flexible and rapid methods have been reported for metals determination. However, some instruments for metals analysis are very expensive such as ICP-AES, ICP-MS. In addition, most of analytical methods generated a large amount of chemical wastes, resulting in contaminate to environment, difficult to elimination and harmfulness to human health. In some situation, the chemicals are employed more dangerous than analyte. As a consequence, the work of some analytical chemists has been focused on the development of methodologies which are less harmful to humans and to the environment. Therefore, this work was developed flow injection system to microflow analysis system or LOC due to LOC use very small amounts of chemicals and samples, rapid and high sensitivity. However, laser machines such as CO_2 laser, Excimer laser are very expensive, for that reason, part of the research was to develop a mini-CNC machine modified with a low cost diode laser for fabrication of a PMMA

chip and then the PMMA chip was applied to microflow analysis system for determination of some metals. On the other hand, the selected reaction for LOC using micro-spectrophotometer in visible region should be the molar absorptivity more than 10000. Due to the small amount of reagent and analyte in the tiny detection cell that cause decreased sensitivity of analysis. Therefore, the reaction between metal and reagent to use in FIA may not be applied in LOC.

1.8 Research Objectives

The objectives of this research described herein are:

(i) To design and construct a low-cost flow injection analysis with spectrophotometric detection system for nickel determination in water samples

(ii) To modify the mini-CNC machine using diode laser for fabrication of a PMMA chip and application to microflow analysis system

(iii) To design and fabricate PMMA chip using CO_2 laser and application to microflow analysis system with spectrophotometric detection for zinc determination in water samples