CHAPTER 1

INTRODUCTION

1.1 Ions in natural water

Natural water is an important resource for the human life and environment, in terms of being an aquatic habitat and has been used for human and animal consumption [1]. Some free ions in the natural water such as ammonium, nitrate, carbonate, phosphate, sulfide, sulfite etc., affect to the environmental quality. Most of ammonia comes from not only the respiration process of fish and the aquatic animals but also waste degradation by bacteria such as food particles and industrial waste [2]. The high concentration of ammonia is toxic to aquatic animals [3] as a result, dissolved ammonium in natural water can be a deterioration indicator of water [4]. Moreover, dissolved inorganic carbon (DIC) or bicarbonate/carbonate ion is a one component of the carbon cycle and relate to the atmospheric CO_2 [5]. The CO_2 involves in the metabolism of all organisms and plays an important role in the occurrence of photosynthesis and pH of natural water [6]. Most of increasing atmospheric CO_2 through human activity leads to climate change that impact to ecosystems and human [7]. In addition, sulfide is usually found in hot springs or rotten water. The amount of sulfide in the water indicates the amount dissolved oxygen and acidic condition of water. Sulfide in acidic solution can be converted to be hydrogen sulfide gas, which is toxic to the respiratory system of human [8]. Therefore, the determination of these dissolved ions in natural water is very important. The analytical techniques for ions determination are required from environmentalist to monitor and indicate the quality of the environment. Moreover, the development of higher accuracy, precision, less analysis time, and low cost analytical technique is required.

In this study, some convertible gas species such as HCO_3^-, NH_4^+ have been selected to be model ions. Such ions can be converted to a gaseous analyte in an appropriate condition and can be liberated out from interference matrices in sample solution such as HCO_3^- can be converted to CO_2 in an acidic solution and ammonium ion

can be converted to be ammonia gas in a basic solution.

1.1.1 Dissolved inorganic carbon (DIC)

DIC is one of the major chemical species dissolved in geothermal water and other natural water, with concentrations in a range of micromolar as HCO_3^- [9]. DIC is the important parameter which the environmental scientist needs to monitor. Moreover, the Concentration of DIC in natural water relates with the amount of carbon dioxide flux in an atmosphere. The carbon dioxide is one of greenhouse gas which affect the global warming crisis and lead to the other environmental problems such as the increasing number of ocean storm and the sea level rising [10].

The dissolved inorganic carbon (DIC) or total inorganic carbon (TIC) is the sum of concentrations of three inorganic carbon species, carbonic acid/carbon dioxide, bicarbonate ion and carbonate ion, in an aqueous solution [11]. The relation among these inorganic carbon species is shown as in Equation 1.1. The concentrations of various DIC species depend on the pH of solution as illustrated in Bjerrum plot [12]. The Bjerrum plot is a graph of the various species concentration of polyprotic acid in solution such a H_2CO_3 acid. Carbon dioxide, bicarbonate, carbonate are dominant species in lower pH solution ($pH \leq 4$), middle pH solution (pH ~7), higher pH solution ($pH \geq 11$) respectively as shown in Figure 1.1.

$$[DIC] = [H_2CO_3/CO_2] + [HCO_3^-] + [CO_3^{2-}] \quad [1.1]$$

[DIC]	Dissolved inorganic carbon of total inorganic carbon
$[H_2CO_3/CO_2]$	The sum of carbon dioxide and carbonic acid concentration
$[HCO_3^-]$	The bicarbonate concentration
$[CO_3^{2-}]$	The carbonate concentration

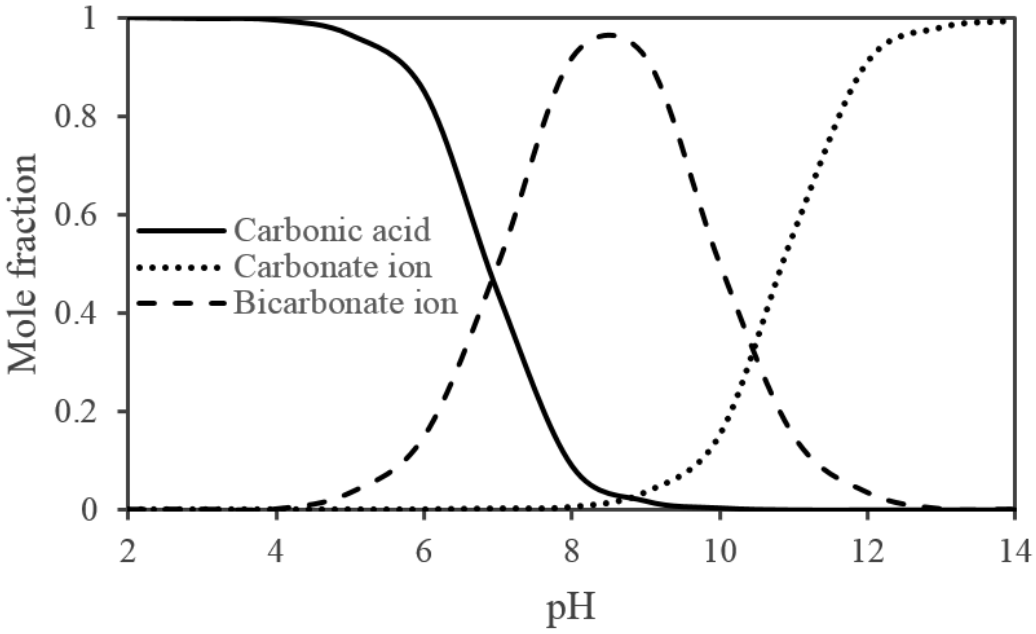

Figure 1.1 Bjerrum diagram shows a mole fraction of different DIC species vs the pH

For DIC determination, the total alkalinity analysis with titration method have been referred to use as the standard method [13]. This titration method is based on the acidification reaction of sample by adding an acidic standard solution into a sample and indicating the equivalence point as stoichiometry. Moreover, many analytical flow methods were developed with using sample acidification to convert the DIC to CO_2. The gas from analytical ion in sample liberates from the sample matrix and diffuses across the hydrophobic membrane to the acceptor stream where it is detected by several detection methods as explained in section 1.3. This diffused process was occurred in the gas diffusion unit.

1.1.2 Ammonium ion

Ammonia (NH_3) consists of two primary forms in water, ammonia form and ammonium ion (NH_4^+). The protonation of ammonia would form ammonium ion as explained in chemical equation (in Equation 1.2) [14]. The conversion of ammonia species depend on the pH of solution. The lower pH solution provides a dominant form of ammonium and the higher pH solution provides a dominant form of ammonia gas as illustrated in Figure 1.2. The ammonia was reported as one of the toxic species to an aquatic animals. Its effects include damage to the gills and resulting in a poor gas exchange, ion regulation and blood pH regulation [15]. In addition, there is also the study

and report about ammonia toxicity to the zooplankton community and freshwater amphipods [16].

$$NH_{3(aq)} + H^{+}_{(aq)} \rightarrow NH^{+}_{4(aq)} \qquad [1.2]$$

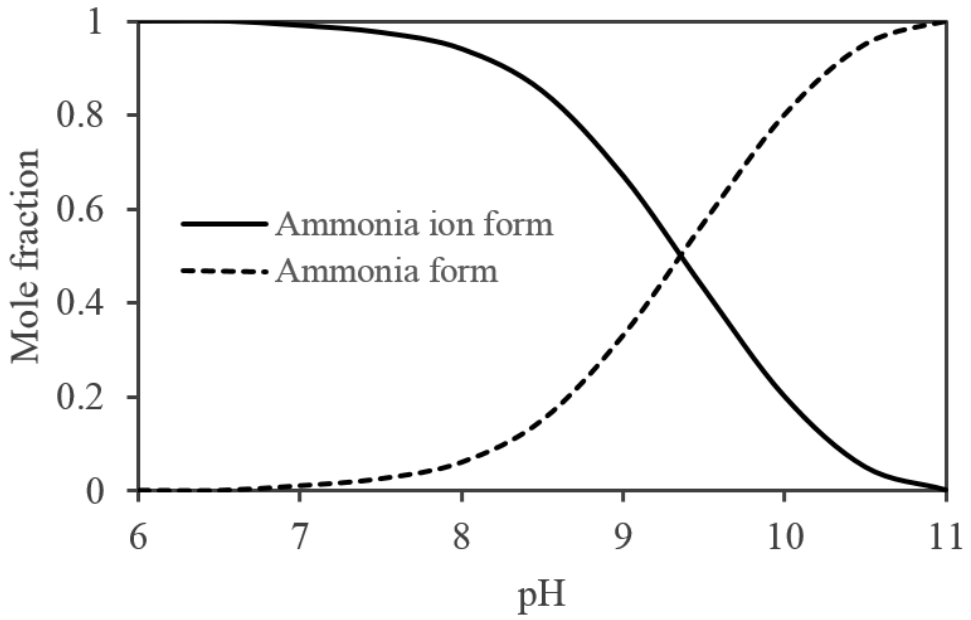

Figure 1.2 The mole fraction of ammonia and ammonium as a function of pH

In addition, the risk of indoor ammonia gas has been reported; the ammonia gas can damaged a historical picture and murals in museums or churches. There are many sources of the produced NH_3 such as human bodies, plant and cleansing agent. The concentration of NH_3 in ambient air is the important parameter to monitor continuously for protecting a human health and some historical working [17]. Moreover, the human bodies typically produce NH_3 from metabolism of urea and protein, and then release it through the urine, exhale breath and skin. The kidney disease patient produced NH_3 in exhale breath with higher amount than normal people. Therefore, NH_3 in exhale breath is an interesting biomarker to monitor and diagnose the kidney disease.

The colorimetric based with indophenol blue method or Berthelot's method was referred as conventional method for NH_4^+ determination in water [18]. This method is based on the reaction of ammonium and hypochlorite in the basic pH medium to form monochloroamine, which reacts to the phenolic compound to produce the indophenol blue complex. Sodium nitroprusside is used as a catalyst to enhance rate of such chemical

reaction as well as heating to 60°C [19]. In addition, the fluorescence-based detection method has been widely used for nanomolar level NH_4^+ determination. The method is based on the reaction of ammonium and ortho-phthalaldehyde (OPA) in alkaline medium solution and the presence of sulfite as a strong reducing agent [20]. The fluorescence based ammonium determination is a highly sensitive and selective method to detect NH_4^+. Many previous studies for NH_4^+ determination by using the colorimetry, fluorescence and others have been reviewed [19]. In this work, a brief summarized of some flow based analytical techniques for NH_3/NH_4^+ determination is shown in Table 1.2.

1.2 Flow injection analysis (FIA)

Flow analysis technique provides not only high throughput continuous analysis but also automation chemical analysis without using volumetric glassware requirement [21]. Flow analysis technique has been widely developed in various liquid manipulation formats such as segmented flow analysis (SFA), flow injection analysis (FIA), sequential injection analysis (SIA), multi-commutated flow injection analysis (MCFIA), multi-syringe flow injection analysis (MSFIA) and multi-pumping flow system (MPFS). The typical flow analysis technique was illustrated in Figure 1.3. FIA is the first generation of flow-based technique. The principle of measurement is based on the injection of a certain volume of liquid sample into continuous moving carrier stream of the reagent. The injected sample zone disperses and reacts with the reagent stream in a tube and forms the detectable product. The detector continuously records the change of physical or chemical signal, which obtained from the change of the injected analyte. The typical peak signal (peak height, peak width and peak area) is proportional to the concentration of analyte. Some factors, such as a flow rate and manifold geometry, which affect to the degree of mixing and dispersion are controlled to obtain the similar experimental condition.

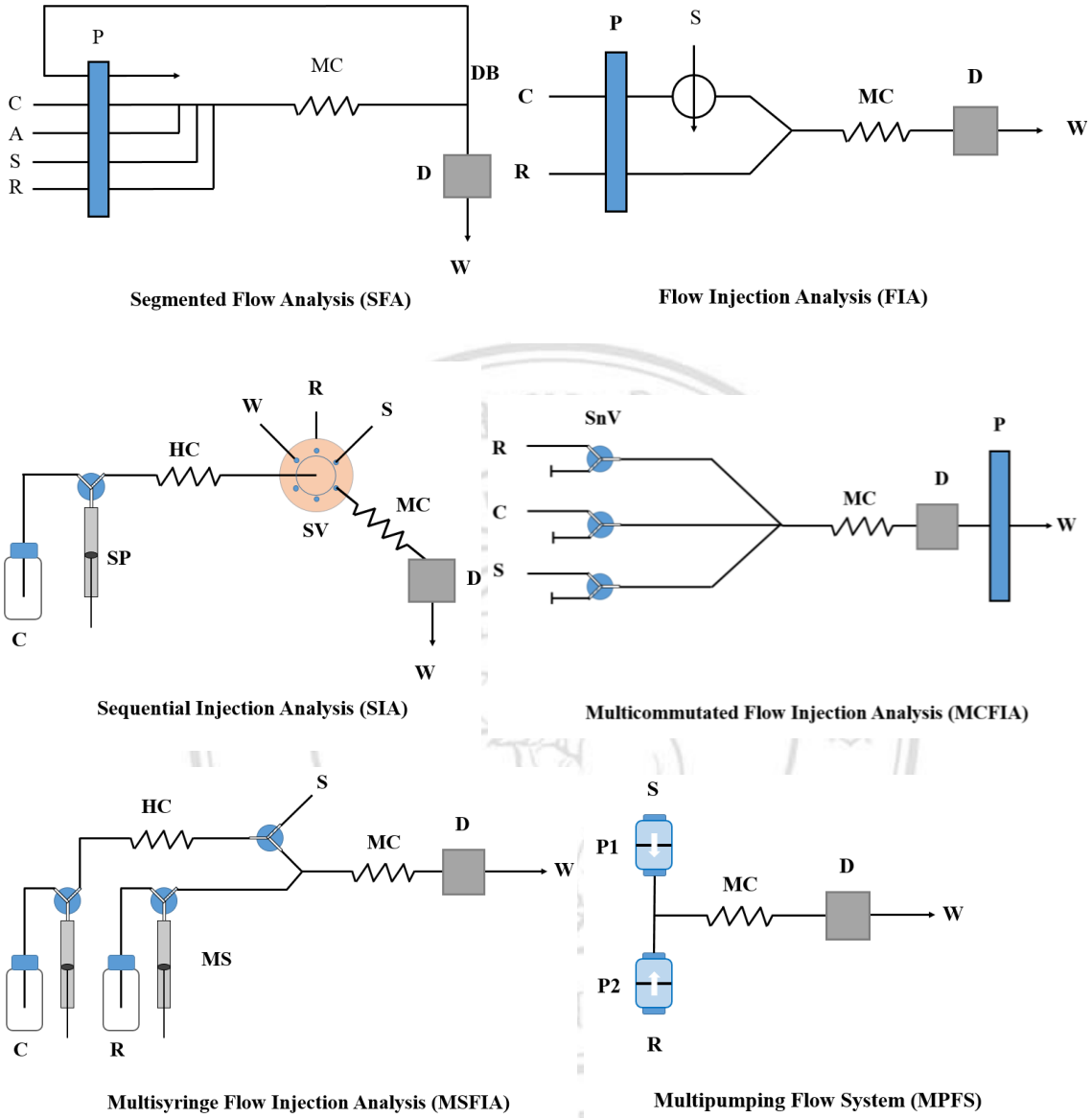

Figure 1.3 Typical flow technique: C, Carrier; R, Reagent; A, Air; S, Sample; D, Detector, W, Waste; MC, Mixing coil; P, Peristaltic pump; DB, Debubbler; SP, Syringe pump; HC, Holding coil; SV, Selection valve; SnV, Solenoid valve; MS, Multi-syringe pump; P1, P2, Solenoid micro pump [19]

The simple FIA consist of the main components such as solution propelling unit (typically used the peristaltic pump or the gravity-driven propulsion system), standard/ sample injection unit (2-positions-6-ports injection valve), mixing/reactor unit, detection unit and signal recording unit as shown the schematic diagram in Figure 1.3. Although FIA is fast analysis technique, it is not fully automation device. Many research groups have been developed the other flow formats such as SIA to enhance a degree of

automation [22]. However, SIA technique requires the more expensive component such as syringe pump with the computerized control unit. Recently, the automation of SIA and FIA has been developed to be the portable measuring device which based on the cost effective open source platform [23]. The device can be controlled and monitored through the computer and applied to be the standalone device. Moreover, flow based analytical technique encounters the problem of interference of the complicated metrics in the real sample such as the gas diffusion unit. The gas diffusion system has been developed to couple with flow injection analysis (FIA) to improve the selectivity of analytical system by converting analyte to gaseous phase and liberating out from interferences of the sample [6, 19].

1.3 Gas diffusion flow injection analysis (GD-FI)

The gas diffusion unit (GDU) consists of two solution channels; donor channel and acceptor channel. Both channels are separated by using the hydrophobic membrane as illustrated in Figure 1.4. The gas diffusion flow injection (GD-FI) system is based on the injection of sample to the continuous flow of reagent solution in the donor channel and convert to be the gaseous phase. The gaseous analyte diffuses through the hydrophobic membrane and dissolve into the acceptor solution in acceptor channel. An appropriate detector detects the change of diffused zone due to gas in the acceptor stream. The GD-FI system has been applied for the determination of some convertible gas species such as carbonate/bicarbonate, ammonium/ammonia, sulfide/hydrogen sulfide, sulfite/sulfur dioxide and cyanate/hydrogen cyanate.

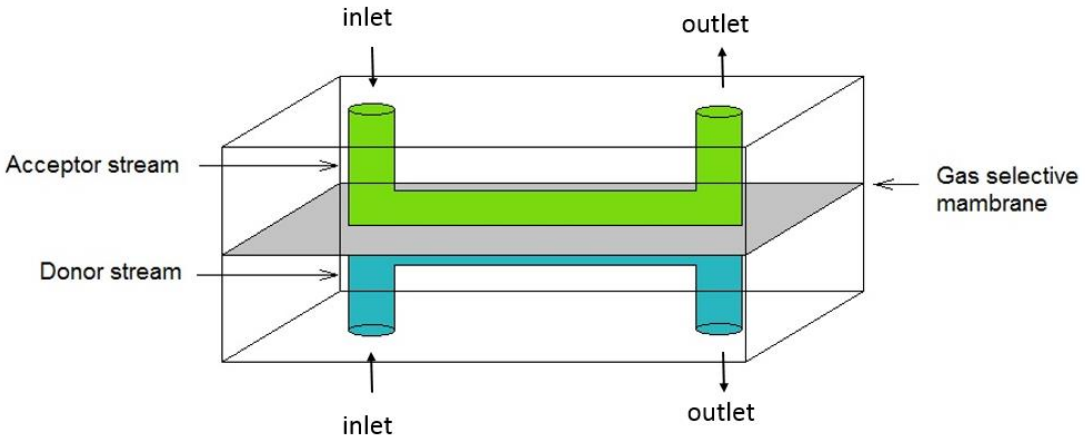

Figure 1.4 Simple diagram of gas diffusion unit (GDU)

GDU has been used to couple with FIA as GD-FI system. The GD-FI system has been used for DIC determination. The DIC sample is injected and mixed in acidic solution as a carrier/donor solution stream, and converts bicarbonate/carbonate ion to the carbon dioxide gas. The produced CO_2 gas diffuses through the gas selective membrane into an acceptor solution stream and is detected by the suitable detector. Several detectors were coupled with the GD-FI system and used for DIC determination. The selective detectors with GD-FI system for DIC determination were summarized in Table 1.1. Oshima et al. [24] developed a new colorimetric change system with 4-(2', 4'-dinitrophenylazo)-1-naphthol-5-sulfonic acid (DNN5S) as reagent in an acceptor solution measure change of absorbance of the solution at 450 nm. Cresol red was also used as an acceptor stream and detected absorbance at 410 nm, this approach was developed by Sanada et al. [25]. The system with bromothymol blue was also used by Pencharee et al. [26], which measured absorbance at 615 nm. Monser et al. [27] used a tungsten oxide electrode and a silver/silver chloride electrode as a potentiometric detector for an acceptor stream which contained sodium acetate buffer. Yao and Su [28] used bulk acoustic wave detector on the basis of sensitive response for change of the solution conductance and using Tris buffer and KCl as an acceptor solution for DIC determination. As well as, conductivity detectors including contact [5-7, 29] / contactless [22, 30] formats, have been used a coupling with GD-FI system for DIC determination. Perez-Ponce et al. [31-32] used Fourier transform infrared spectroscopy (FTRI) to detect carbon dioxide directly without acceptor stream solution.

Table 1.1 The brief review of detection methods using GD-FI system for DIC determination

Method	**Type of sample**	**Donor solution**	**Acceptor solution**	**Working range, mM**	**LOD, mM**	**Sampling rate, h^{-1}**	**Ref.**
Absorption spectrometry	Natural water	H_2SO_4	Cresol red	Up to 2.87	0.02	20	[25]
	Water	H_2SO_4	DNN5S	0.001-1	0.001	20	[24]
	Seawater	H_2SO_4	BTB[b]	0.2-10	0.42	90	[26]
FTIR[a]	Water	HNO_3	-	Up to 16	240	15	[31]
	Seawater	HNO_3	-	Up to 5	75	30	[32]
Bulk acoustic wave	Natural/waste water	H_2SO_4	Tris and KCl	0.05-10	0.01	45	[28]
Potentiometry	Water	HCl	Sodium acetate	0.08-1.7	3	30	[27]
	Vines	-	-	1-11		50	[33]
Conductometry	Natural water	-	-	0.05-5	0.01	-	[6]
	Water	-	-	0.05-0.5	0.003	45	[7]
	Natural water	-	MilliQ	0.01-0.1	0.003	15	[29]
	Seawater and water	H_2SO_4	NaOH	0.08-9	0.05	15	[5]
CCD[c]	Water	-	-	0.01-0.1	0.006	15	[30]
	Seawater	H_2SO_4	MilliQ/NaOH	0.2-2	0.08	90	[26]

[a]FTIR, Fourier transform infrared spectroscopy

[b]BTB, Bromothymol blue

[c]CCD, Contactless conductivity detector

In addition, the GD-FI has been used for NH_4^+ determination by using many detection method as briefly summarized in Table 1.2, Cerda et al. [34] used conductometric detection for NH_4^+ determination in waste water with GD-FI system by using sodium hydroxide as carrier stream in donor channel of GDU. Ammonium ion was converted to ammonia, and then diffused through the gas membrane into deionized water or boric acid as acceptor stream. Moreover, absorption spectrophotometry was used for NH_4^+determination by using acid-base indicator as the acceptor stream such as bromothymol blue solution (BTB) [35], phenol red [36], and potentiometric pH sensor [37]. These approaches have been achieved in micromolar range of sensitivity for NH_4^+determination. Moreover, GD-FI also enhances sensitivity by improving in term of

GD configuration and surface area. However, fluorometric detection method is a high sensitivity with NH_4^+ [19]. The fluorometric method involves the reaction of ammonium with ortho-phthalaldehyde (OPA) in basic solution in the presence of a strong reducing agent such as 2-mercaptoethanol in GD-FI system [38, 51]. But this reducing agent has interfered from volatile amines. Therefore, Genfa and Dasgupta [20] has been used sodium sulfite as a reducing agent, to reduce the sensitivity of amino acids, thus it provides the selective method for NH_4^+ determination. An intensely fluorescent product is formed and allows the fluorometric assay to reach the nanomolar range. Watson et al. [39] combined the advantages of GD-FI with the OPA-sulfite approach for improving sensitivity and selectivity for NH_4^+ determination in seawater. Since the reaction of ammonium with OPA and sulfite is more selective to ammonium ion than to primary amines, the GDU may be unnecessary. There are several reports for NH_4^+ determination involve flow-based methods using the OPA-sulfite reaction as fluorometric method without GDU such as shipboard FIA [40], SIA [41] and autonomous batch analyzer (ABA) [42]. In the other hand, the same reaction (NH_3-OPA-sulfite) can determine amount of sulfide in fresh water and seawater [43].

In addition, the membraneless based GDU was used for determination of some samples because it provided a lower cost and greater mass transfer than the membrane based GDU which easily to apply with the solid sample. For example, the ethanol determination in an alcoholic drinking sample which the potassium dichromate in acidic solution was used as an acceptor solution. The ethanol vapor can diffuse in the sample chamber without the membrane and dissolving in the dichromate solution to produce chromium (III), which is detected by spectrophotometric method [44]. Sereenonchai et al. also used the membraneless GDU for calcium determination in a calcium supplement as a solid sample. The cresol red solution was used as an acceptor solution. The hydrochloric acid was dropped to the solid sample to produce a carbon dioxide gas. The gas was diffused through the headspace and dissolved in the acceptor solution. The color of indicator solution was detected by spectrophotometry [45]. Recently, the generated ammonia gas was dissolved in the liquid drop manipulation with the membraneless GDU for NH_4^+ determination in the natural water. NaOH solution was added to a sample to generate an ammonia gas and purged to flow over the acidic absorber drop. The decreasing of conductivity was detected and evaluated [46].

Table 1.2 The brief review of detection methods using GD-FI system for NH_4^+ determination

Method	**Type of sample**	**Donor solution**	**Acceptor solution**	**Working range, µM**	**LOD, µM**	**Sampling rate, h^{-1}**	**Ref.**
Absorption spectrometer (acid-base indicator)	Canal water	NaOH	BTB	Up to 100	1	100	[35]
	Waste water	NaOH	BTB	111-3333	55.6	-	[34]
	Estuarine water	NaOH	BTB	Up to 214	0.64	135	[47]
	Estuarine water , water		BTB	Up to 222	3	28	[48]
	Estuarine water	NaOH	BTB	2.8-55.6	1	20	[14]
	Ammonia excretion	NaOH	Phenol red	Up to 10	0.05	60	[36]
(Indophenol based method)	Waste water	NaOH	HCl	55.6-3333	55.6	-	[34]
Potentiometry	Seawater	NaOH	BTB	1-50	0.2	30	[37]
Conductometry	Waste water	NaOH	HCl	55.6-3333	1.7	-	[34]
	Estuarine water	NaOH	HCl	Up to 252	<0.2	8	[49]
Fluorometry	water	NaOH/ citrate	OPA / 2-mercapto-ethanol	Up to 2	0.001	30	[38]
	Seawater	NaOH/ citrate	OPA / 2-mercapto-ethanol	-	0.001	18	[50]
	Seawater	NaOH	OPA / sulfite	Up to 4	0.007	30	[39]

BTB, Bromothymol blue

OPA, ortho-Phthalaldehyde

1.4 Conductivity detector

The conductometric detection is based on the measuring of electrical conductance of the ionic solution caused by mobility of ions toward respective metallic electrodes [51]. The conductance is an inverter of resistance and relates with a potential according to the Ohm's Law as shown in Equation 1.3.

$$L = \frac{1}{R} = \frac{I}{E} \qquad [1.3]$$

Where, L: Conductance, Siemens (S) or (Ω^{-1})

R: Resistance, ohm (Ω)

I: Current, ampere (A)

E: Potential, volt (V)

The conductance cell for measuring conductivity in a solution comprises of two inert electrodes, which is generally platinum as shown in Figure 1.5. Many designs of the conductance cell was fabricated and used for measuring the conductivity because the conductance directly varied with the surface area of electrode (A) and inversed with an electrodes distance (d). The call constant (θ) is a ratio of electrode distance (d) and their surface area. A conductance value of varied cell dimension is improved by using the specific conductance or conductivity (K), which relates with a conductance (L) and a cell constant (θ) as shown in Equation 1.4. Therefore, the conductivity (K) does not depend on the conductance cell and measurement device.

$$K = L \cdot \theta = L \frac{d}{A} \qquad [1.4]$$

Where, K: Specific conductance/Conductivity, (S cm^{-1})

L: Conductance, (S)

θ: Cell constant, (cm^{-1})

d: Electrode distance

A: Electrode surface area

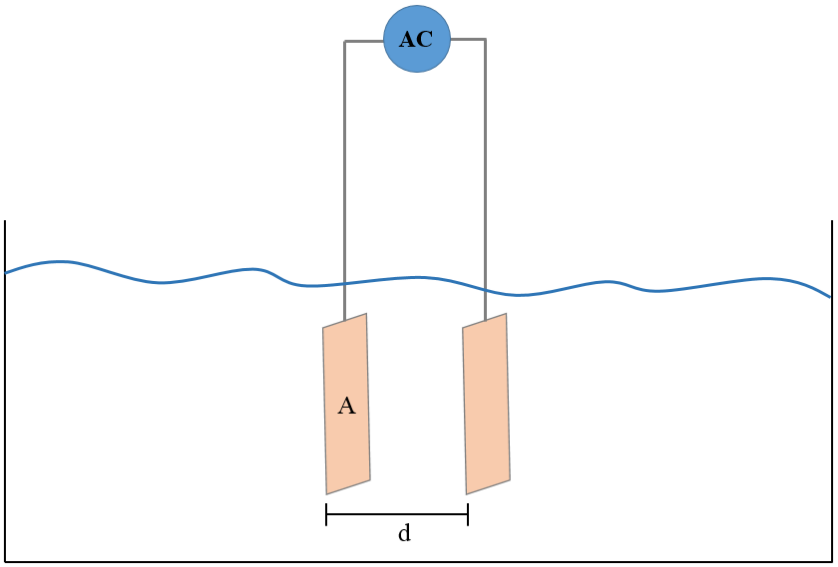

Figure 1.5 Conductance cell for measuring a conductivity in solution

The conductivity value (K) depends on the type of ion, concentration of ion, size of ion, viscosity of solution, temperature, solvation and the dimension of the electrodes. The brief equation of conductivity was shown in Equation 1.5.

$$K = F \sum_i |z_i| u_i C_i \qquad [1.5]$$

Where, F: Faraday constant (96485 C mol^{-1})

Z: Charge of ion

U: Mobility of ion

C: Concentration of ion

Total conductivity of the solution is directly proportional to the sum of ion concentration. The conductometric technique has been widely applied for ion determination in water sample although this technique is not a selective method. The disadvantage have been improved by using the GDU [6-7]. The commercial conductometer usually uses the pulse generator to apply the voltage to a conductivity cell but some studies used the pulseless circuitry to apply the direct current to a conductivity cell for chemical analysis applications [52, 54]. The DC conductivity detector could be an alternative detection system since the simple pulseless circuitry and this would be good for the budget limit laboratory. The principle of DC conductivity detector or pulseless conductivity detector is mentioned in section 3.1.1.

1.5 Fluorescence detector

The fluorometry is highly sensitive technique and is widely used in biochemical, clinical and analytical chemistry. The principle of fluorometry is based on the measurement of fluorescence intensity of the fluorescent molecules. After molecules absorb the excited radiation, the electrons in molecules are excited to higher energy states as demonstrated in Figure 1.6. Various processes lead to relaxation of the excited molecules which involves vibrational relaxation, internal conversion, intersystem crossing and emitted radiation as a fluorescence and phosphorescence. The fluorescence and phosphorescence are usually referred as photoluminescence due to similarity of photon absorption. Fluorescence differs from phosphorescence in term of the electronic energy transition. Fluorescence does not change in electron spin which result in the relaxation of electrons in excited state occur through vibrational relaxation and internal conversion process, which the lifetime of fluorescence is short (10^{-10}-10^{-5} seconds). However, phosphorescence changes the excited electron spin and produces external conversion which is a longer process (10^{-4}-10 seconds) as demonstrated in Figure 1.6. Fluorescence and phosphorescence occur at longer wavelength than the excitation radiation [53].

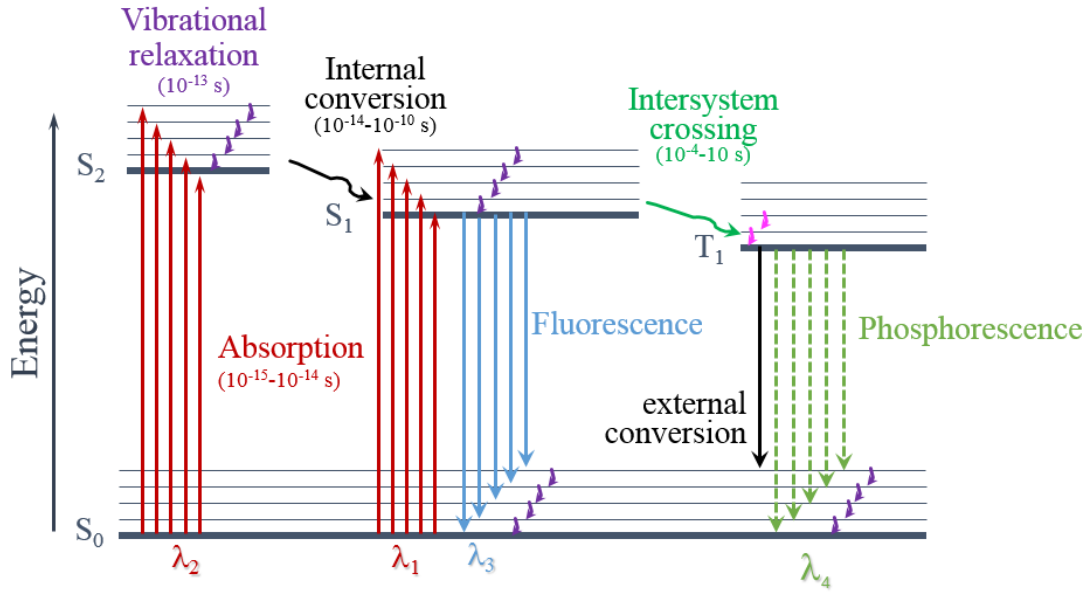

Figure 1.6 The energy level diagram for photoluminescent molecule; S_0, Ground electronic singlet state; S_1, S_2, First and second excited electronic singlet state; T_1, First excited electronic triplet state

The relationship of fluorescence intensity and concentration can be readily derived from Beer's law that the fluorescence intensity F is given in Equation 1.6.

$$F = \phi P_0 (1-10^{-abc}) \qquad [1.6]$$

Where ϕ is the quantum yield, which is the efficiency of conversion of absorbed radiation to fluorescent radiation. The other terms in the equation are the same as Beer' law (P_0 is the intensity of incident radiation, *a* is absorptivity, *b* is light path of the solution and *c* is concentration of the substance). The equation shows that if the product *abc* is large, the term 10^{-abc} becomes negligible compared to 1, and F becomes constant. In the other hand, if *abc* is small (≤ 0.01), it can be shown in Equation 1.7.

$$F = 2.303 \phi P_0 abc \qquad [1.7]$$

Hence, for low concentration, the fluorescence intensity is directly proportional to the concentration. Also, it is proportional to the intensity of incident radiation.

The equation generally holds for concentration up to a few part per million, depending on substance. At high concentration, the fluorescence intensity may decrease with increasing concentration because the first part of solution in the path will absorb more of the radiation. But in dilute solutions, the absorbed radiation is distributed equally through the entire depth of the solution. So the equation can hold only when most of the radiation goes through the solution with 92% transmittance [53]. In addition, the fluorescence technique provides a much wider dynamic range of concentration. Some factors affect to the fluorescence intensity such as the existing of some fluorescence quenching substance, pH, temperature, viscosity of the solution and self-absorption effect.

The simple fluorometer comprises of three basic components, i) light source (typically is tungsten-halogen lamp, mercury arc lamps, xenon arc lamps, lasers, LED), ii) a sensitive light sensor (photomultiplier tube, photodiode), and iii) sample holder. The light sensor was designed to place 90° direction with the light source and as a result, the radial emitted fluorescence was assumed to be detected without the excitation light. The sample is located at the intersection of the two beam paths. The light filter is placed in front of the light sensor for removing the incident light from the light source and the scattered light from sample as shown in Figure 1.7. Several studies have been fabricated

the cost-effective fluorometer with using UV-LED as the light source and the integrated photodiode circuit as a light sensor because these components were a low cost, low power consumption, highly sensitive and compact devices. This in-house fabricated fluorometer would serve to develop the portable measuring system in Thailand.

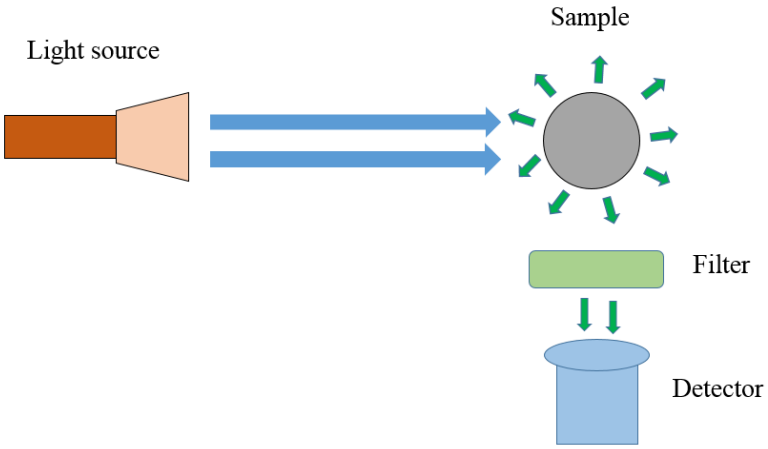

Figure 1.7 Schematic diagram of the simple fluorometer

1.6 Research aims

The objectives of this research are as follows:

1. To develop the DC conductivity detector to couple with GD-FI for DIC determination in some natural water samples.
2. To develop the LED-photodiode based fluorescence detector to couple with flow injection system for NH_4^+ determination in some environmental samples.