

CHAPTER 2

EXPERIMENT

2.1 Chemicals and reagent

All chemicals and reagent used in this study are listed as following in Table 2.1.

Table 2.1 List of chemicals and reagent

Chemicals/Reagent	Assay, %	Molecular Formula	Company	Country
Potassium chloride	99.5	KCl	Merck	Germany
Sodium bicarbonate	≥99.0	NaHCO ₃	Merck	Germany
Ammonium chloride	99.8	NH ₄ Cl	Scharlab	Spain
Sodium sulfite	98.0	Na ₂ SO ₃	Carlo Erba	France
Sodium sulfide	60	Na ₂ S	Loba Chemie	India
Sulfuric acid	98	H ₂ SO ₄	QRëC	Thailand
Phosphoric acid	85	H ₃ PO ₄	RCI	Thailand
Potassium hydrogen phthalate	≥99.95	KC ₈ H ₅ O ₄	Sigma-Aldrich	USA
Sodium hydroxide	≥97.0	NaOH	Sigma-Aldrich	USA
Hydrochloric acid	37	HCl	RCI	Thailand
Bromocresol green	-	C ₂₁ H ₁₄ Br ₄ O ₅ S	Merck	Germany
Phenolphthalein	-	C ₂₀ H ₁₄ O ₄	Merck	Germany
Potassium permanganate	99.0	KMnO ₄	Ajax	New Zealand
ortho-Phthalaldehyde	≥97.0	C ₈ H ₆ O ₂	Sigma-Aldrich	USA
di-Sodium hydrogen phosphate dihydrate	99.5	Na ₂ HPO ₄ •2H ₂ O	Scharlab	Spain
Sodium salicylate	≥99.0	C ₆ H ₄ (OH)COONa	Sigma-Aldrich	USA
Sodium nitroprusside	98.0	Na ₂ [Fe(CN) ₅ NO] •2H ₂ O	BDH	UK
Sodium hypochlorite	5	NaOCl	RANKEM	India

2.2 Apparatus and components

All apparatus and components used in this study are listed as following in Table 2.2.

Table 2.2 List of apparatus and components

Apparatus/Components	Model	Company	Country
Analog to digital converter unit	Emant300	Emant Pte	Singapore
Conductometer	712	Metrohm	Switzerland
Peristaltic pump	400 series	Watson-Marlow	UK
UV-Vis spectrophotometer	Evolution201	Thermo Fisher Sci.	New Zealand
Spectrofluorophotometer	RF-5301PC	Shimadzu	Japan

2.3 Preparation of standard solution and reagents

All chemical reagents used in this study are analytical-reagent grade. Deionized (DI) water was used for preparing all reagent solution. All glassware was rinsed with DI water before use.

➤ Potassium chloride stock standard solution

The potassium chloride stock standard solution of 50.00 mmol L⁻¹ was prepared by dissolving 0.3746 g of potassium chloride salt in DI water and adjusting to 100.00 mL in volumetric flask.

➤ Sodium bicarbonate stock standard solution

The sodium bicarbonate stock standard solution of 50.00 mmol L⁻¹ was freshly prepared by dissolving 0.4222 g of sodium bicarbonate salt in DI water and adjusting to 100.00 mL in volumetric flask.

➤ Phosphoric acid solution

0.1 mol L⁻¹ phosphoric acid solution was prepared by adding 3.40 mL of 85% phosphoric acid into 500 mL DI water in breaker and transferring to polyethylene bottle.

➤ Sulfuric acid absorbing solution

0.5 mol L⁻¹ sulfuric acid solution was prepared by adding 2.70 mL of 98% sulfuric acid in 100 mL of DI water. The absorbing solution of 5 mmol L⁻¹ sulfuric acid solution

was prepared by diluting 10.00 mL of 0.5 mol L⁻¹ sulfuric acid solution with DI water and adjusting to 1.0 L.

➤ Phosphate buffer pH 11.0

0.1 mol L⁻¹ phosphate buffer was prepared by dissolving 17.89 g of di-Sodium hydrogen phosphate dihydrate salt in 900 mL of DI water, adjusting its pH to be 11.0 with 2 mol L⁻¹ NaOH and adjusting a final volume to 1 L.

➤ Ammonium stock standard solution

The ammonium stock standard solution of 50.00 mmol L⁻¹ was freshly prepared by dissolving 0.1340 g of ammonium chloride salt in 5 mmol L⁻¹ sulfuric absorbing solution and adjusting to 50.00 mL in volumetric flask.

➤ ortho-Phthalaldehyde (OPA) reagent

The ortho-Phthalaldehyde (OPA) reagent was prepared by dissolving 0.268 g of *o*-Phthalaldehyde in 20 mL of 0.1 mol L⁻¹ phosphate buffer in 65°C water bath and 0.0761 g of sodium sulfite was dissolved in 180 mL of 0.1 mol L⁻¹ phosphate buffer and finally mixing both solutions together.

➤ Hot spring water sample preparation for DIC determination

In this study, the hot spring water samples were collected from the different locations in Chiang Mai province. The samples were collected in polyethylene bottle and kept in a refrigerator at 4°C. The KMnO₄ was added to the sample for removing its interference (sulfide/sulfite). The sample was filtered with nylon syringe filter (0.22 μm pore size) before injecting sample into the GD-FI system.

2.4 Experimental setup

2.4.1 Fabrication of the direct current conductivity detector

The conductivity cell was fabricated from two stainless steel tubes (0.4 mm ID, 0.6 mm OD) with 1 cm-length. Both stainless steel tubes were used as conductivity electrodes and let the electrode gap approximate 1 mm. Such stainless steel tubes were inserted and fitted in 3 pieces of Teflon tube (0.5 mm ID, / 1.6 mm OD). The 0.8 mm copper wire was coiled on stainless steel tubes as electrode connector to the conductivity

detector circuit. All components were placed on a glass slide and sealed with an epoxy glue as shown in Figure 2.1. The copper wire were then connected to the conductivity detector. This conductivity cell has been used for investigating the analytical performance of the proposed detector in flow system. Two duplicate conductivity cells were fabricated and setup as dual cell detectors connected in series in FI system for comparison the analytical signal of the proposed detector with the commercial pulse conductivity detector (712 conductometer, Metrohm).

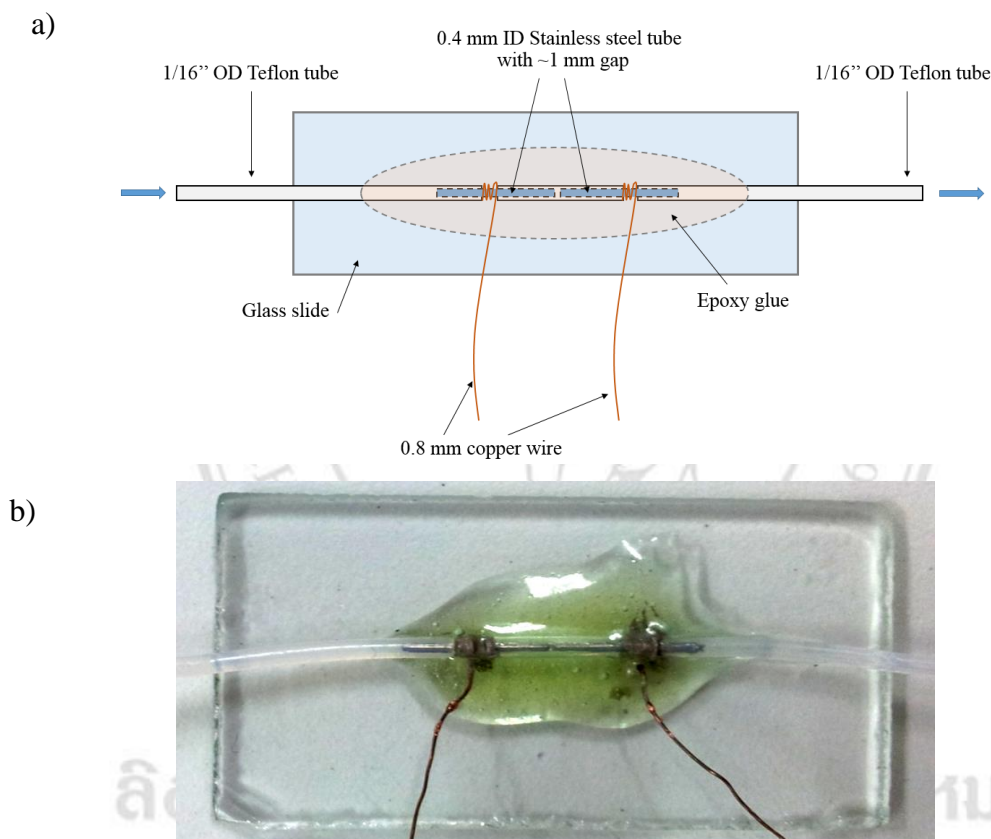


Figure 2.1 Schematic diagram (a) and photograph (b) of conductivity cell

The DC conductivity circuits were fabricated in two formats. The first format, simple voltage regulator IC 7805 or 7815 to provide +5 V and +15 V, respectively, as a constant voltage to conductivity cell. The second format, the wheatstone bridge circuit and cooperated with operational amplifier circuit (WB-OA), which was suggested by Qi et al [54], as shown in Figure 2.2c. The response signal of DC conductivity detector was recorded to a computer through 22-bit A/D card (Emant-300, Emant pte, Singapore) with 200-ms sampling rate with in-house data acquisition software. The detector can be operated with external power supplied from either $\pm 12\text{V}$ DC desktop power supply or

two of 9V-battery. The components were combined into the metal box as illustrated in Figure 2.3.

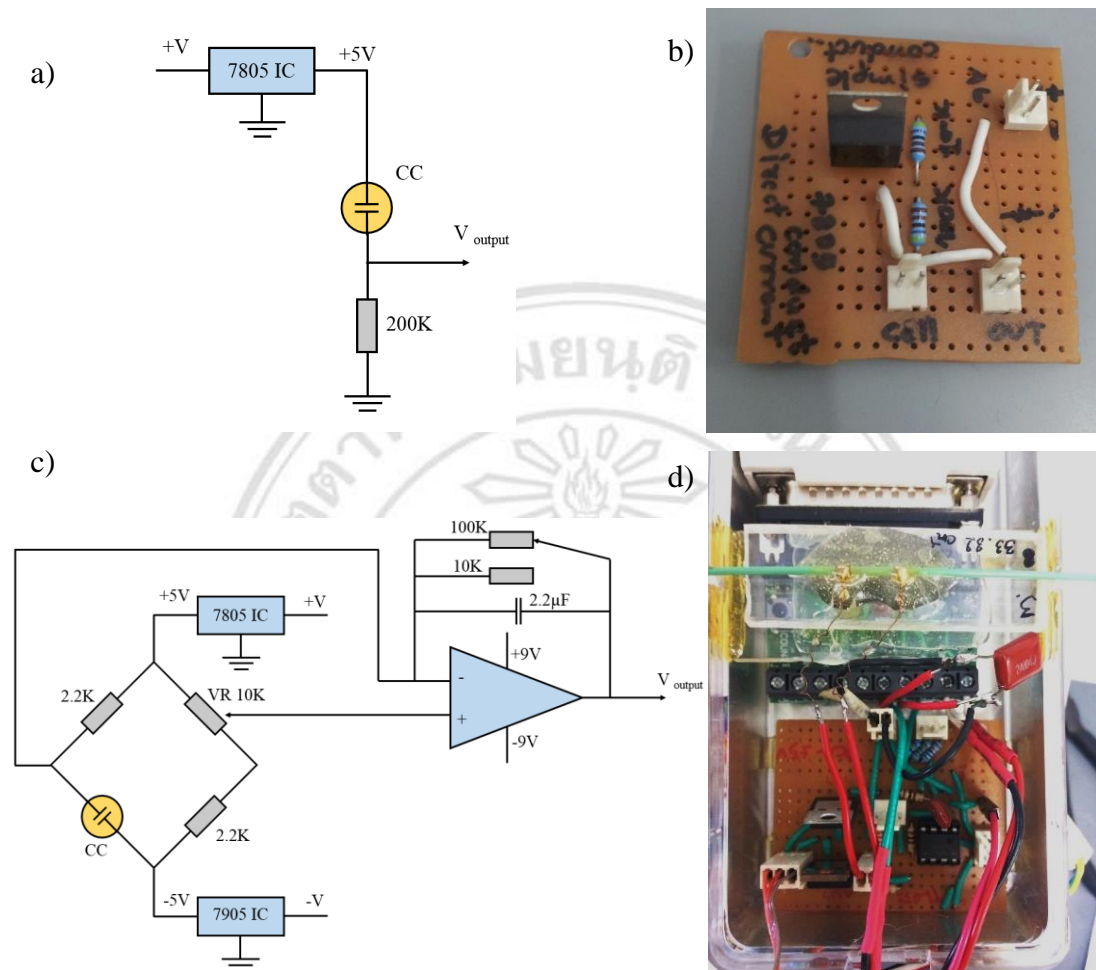


Figure 2.2 The DC conductivity circuits: a) circuitry diagram of using 78xx IC; b) a photographical circuit using 78xx IC; c) circuitry diagram using WB-OA; CC, conductivity cell and d) a photographical circuit using WB-OA

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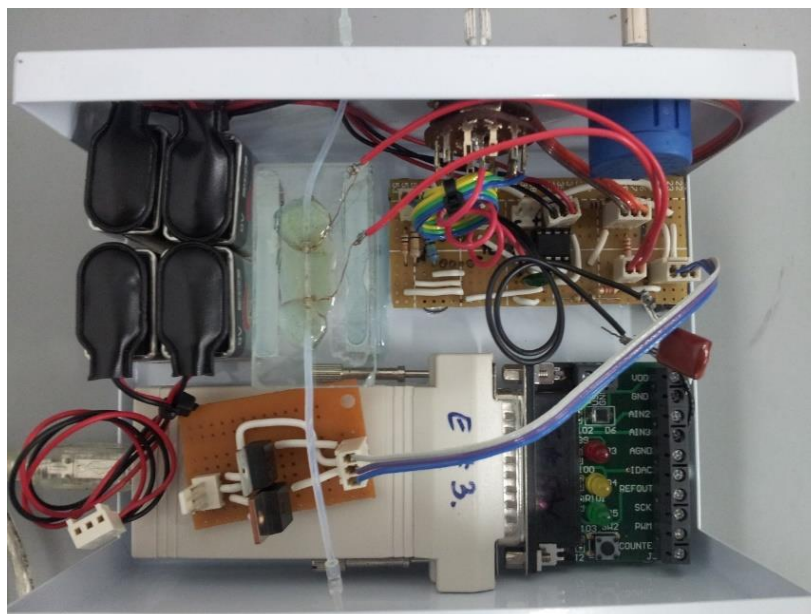


Figure 2.3 Photograph of DC conductivity detector using 9V-battery to supply the WB-OA based DC conductivity detector

2.4.2 Fabrication of the LED-Photodiode based fluorescence detector

The fluorescence cell was fabricated by drilling 1.6 mm thru-hole-cross connector (0.5 mm thru hole ID). The 3-cm-length optical fiber (1.6 mm OD) was cut and polished with scrubbing material. The optical fibers were inserted to the hole of the cross connector with 90° direction and locked the position with nut and ferrule. The other holes of cross connector were inserted with PEEK tube as inlet/ outlet of solution as illustrated in Figure 2.4. The UV-LED (365 nm emission) was used as the light source of fluorescence detector and placed to attach the end of the optical fiber. The photodiode (OPT301) was placed to collect the fluorescence light from optical fiber and was locked into the position with the acrylic block holder. The dimensional design of the acrylic block is shown in Figure A1 in Appendix. The UV filter sheet was cut and placed in front of the photodiode.

The circuit of fluorescence detector was fabricated according to the previous study [17]. The photodiode circuit was supplied from $\pm 12\text{V}$ DC desktop power supply. The response signal of photodiode was amplified by the operational amplifier (TL082) circuit as shown in Figure 2.5. The output signal was recorded to a computer through the 22-bit ADC card (Emant300 or Emant380 with Bluetooth DAQ) with 200ms sampling rate with in-house data acquisition software. The box of fluorescence detector was coated with

acrylic black color paint for protecting stray light. All components were placed in the black box as illustrated in Figure 2.6.

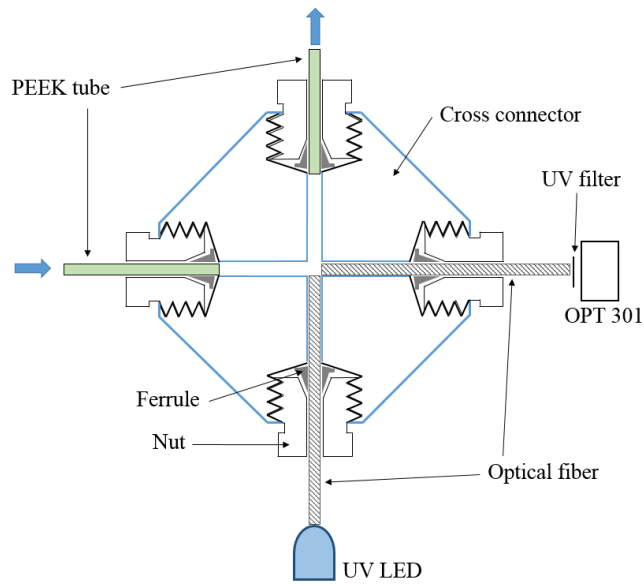


Figure 2.4 Schematic diagram of fluorescence cell

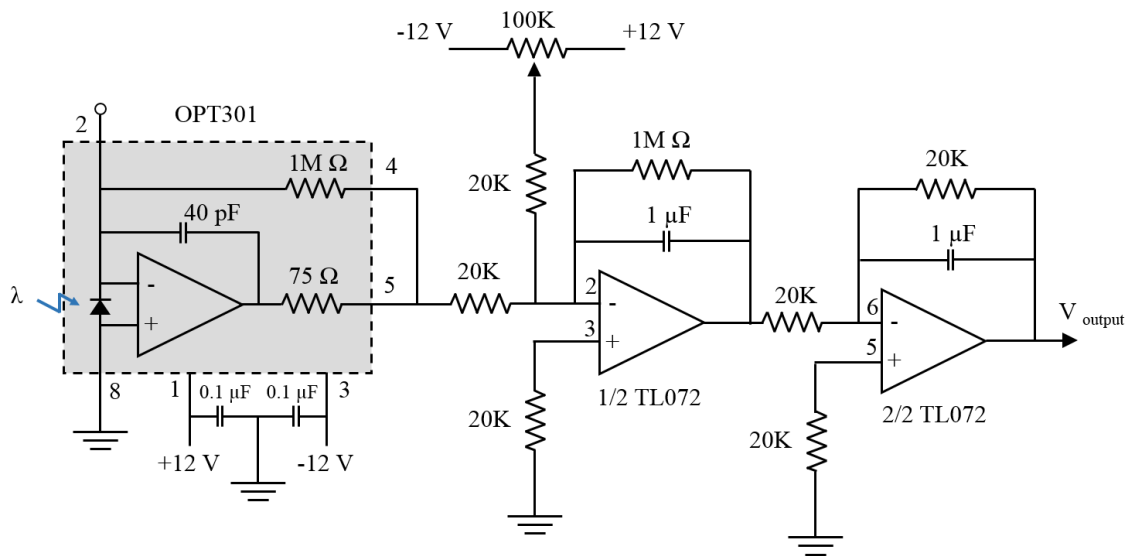


Figure 2.5 The Circuit of integrated photodiode (OPT301 model) and operational amplifier

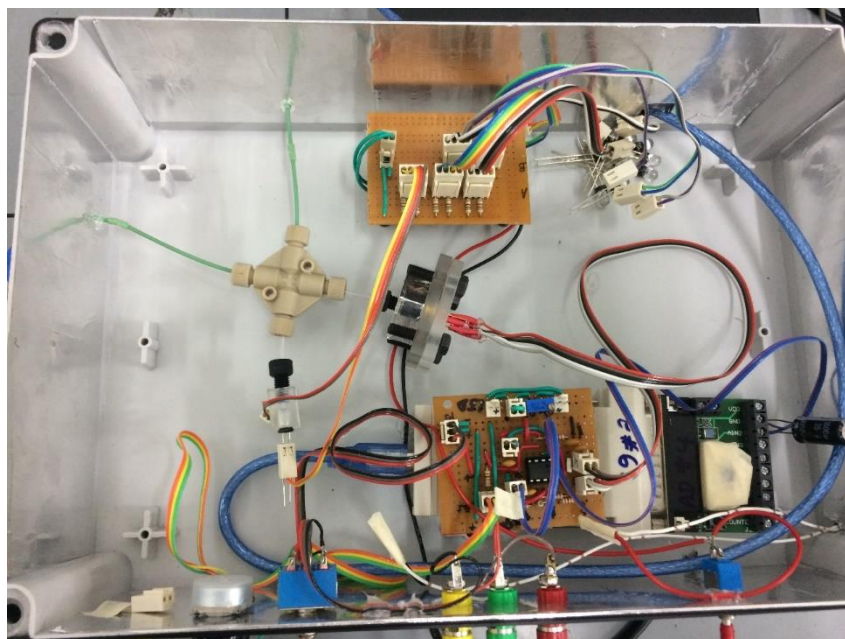


Figure 2.6 Photograph of the LED-photodiode based fluorescence detector

Both fabricated detectors were applied to use in the flow system. The simple single line FI system was used to investigate the characteristic signal and the analytical performance of both detectors. In some experiment, the flow format was selected to use with the appropriate application. The GD-FI system was used with the DC conductivity detector for DIC determination. In addition, the single line FI system was used with the simple fluorescence detector for ammonium determination. The Teflon tube (0.50 mm ID/1.6 mm OD, IDEX health & science) was used for all flow experimental setup.

2.4.3 FI system with the DC conductivity detector

The DC conductivity detector was used in two flow formats. First, single line FI for evaluating the analytical performance of DC conductivity detector and the GD-FI system for gas convertible determination application.

➤ Single line FI with the DC conductivity detector

The single line FI system was setup to investigate the analytical performance of DC conductivity detector. The DC conductivity detector was placed afterward the 6-ports-2-position injection valve in the flow system. The propelling system was used by hanging a bottle on the stand as the gravity-driven propulsion system. The solution flow rate was controlled by adjusting a height of bottle. The KCl standard solution was injected

with 40 μL injection volume into the DI water stream via the 6-port-2-position injection valve.

➤ The GD-FI system with the DC conductivity detector

The GDU consists of two acrylic blocks and Teflon tape membrane (bought from the local equipment shop). The acrylic block was designed with dimension 5 x 16 x 2 cm (wide, long, and tall respectively) and gouging an acrylic surface deep 1 mm following the Figure 2.7a. Two acrylic blocks were closely spliced and locking with 2 inches long nuts. The Teflon tape was function as porous hydrophobic membrane placed between both acrylic boxes as illustrated in Figure 2.7b. The length of membrane is approximately 30 cm. A hydrophobic surface of a Teflon tape membrane separates the aqueous solutions between both sides and permits only the produced gaseous analyte to pass from a donor stream to an acceptor stream. The GDU was used in the flow system for enhancing the selectivity of analysis.

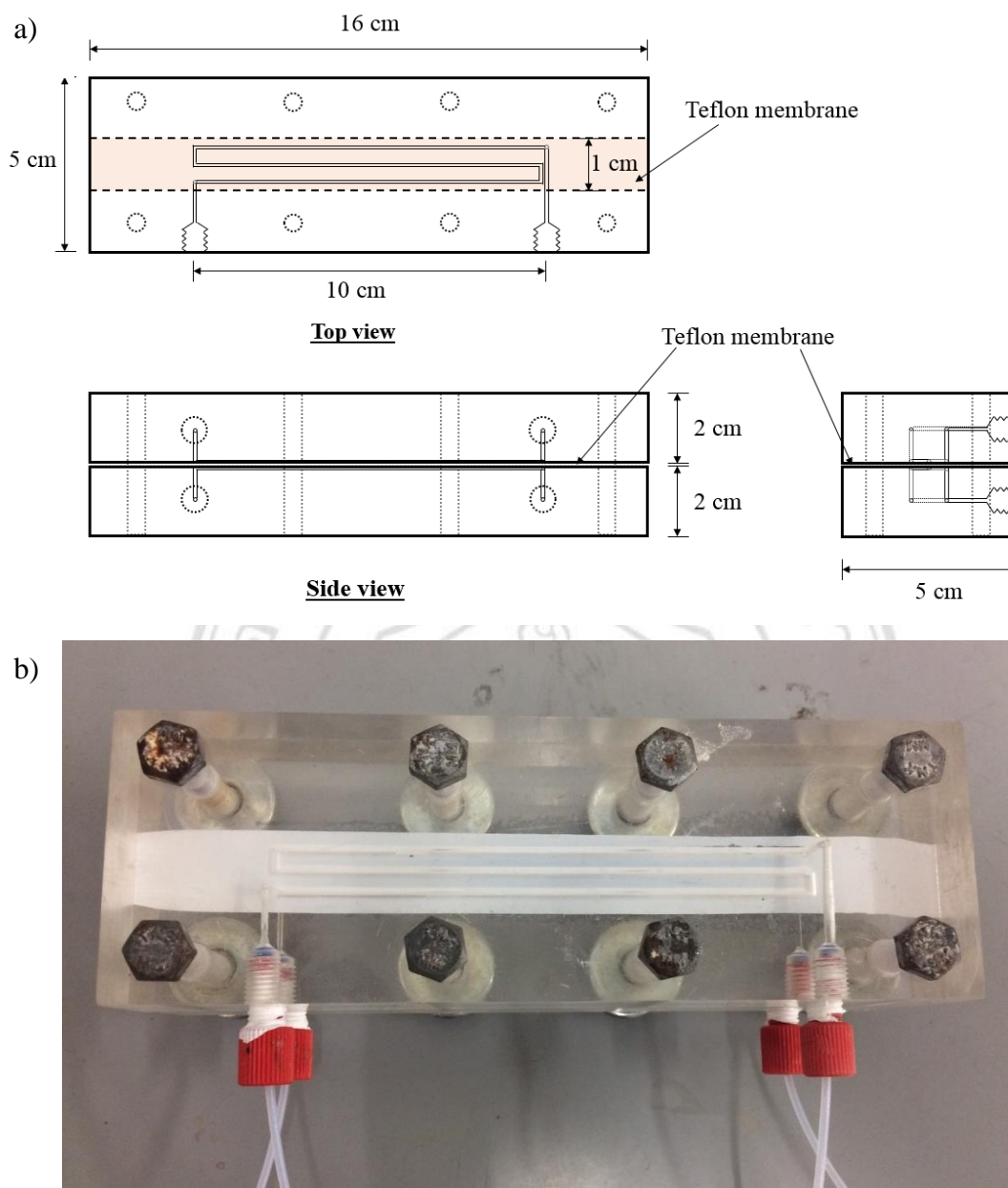


Figure 2.7 The dimensional diagram (a) and photograph (b) of gas diffusion unit (GDU)

The schematic diagram of flow analysis system for DIC determination is shown in Figure 2.8. The flow analysis system was used with the GDU to separate a gaseous analyte from a matrix of sample. The dimensional detail of GDU was illustrate in appendix. Two of 100 mL Duran bottles were filled with 0.1 mol L^{-1} of phosphoric acid solution as a donor stream line and DI water as an acceptor stream line. Both solutions was propelled by hanging the bottles on the stand to perform the gravity-driven propulsion system. The flow rate of solutions were controlled by adjusting the relative bottles height. Moreover, the other propelling system used in this study was the

pressurized propulsion system. The dry nitrogen gas was used to provide a constant pressure to the bottles and adjusting the pressure regulators to control the flow rate of both solutions. The solution flow rate was estimated by measuring solution volume per minute flow. For the donor line, the 6-ports-2-position injection valve was used for injecting the sample or standard solution into the flow system. A 8.0 cm of Teflon tube (0.75 mm ID/1.6 mm OD) equaling 40.2 μL was used as the injection loop and 20 cm mixing coil length. For the acceptor line, the DC conductivity detector was placed after the GDU. The waste solutions from both terminal lines were stored together in a polyethylene bottle. The 50 mmol L^{-1} of HCO_3^- stock standard solution was standardized by using titration method with standard hydrochloric acid and finally estimate the concentration of HCO_3^- . The HCO_3^- standard solution series of 1.0-10.0 mmol L^{-1} was prepared by diluting the HCO_3^- stock standard solution with DI water and containing in polyethylene bottle. After that, the DC conductivity detector was operated and continuously to record the signal. The standard HCO_3^- solution series and hot spring water samples were injected to the GD-FI system with 3 duplicates injections per sample.

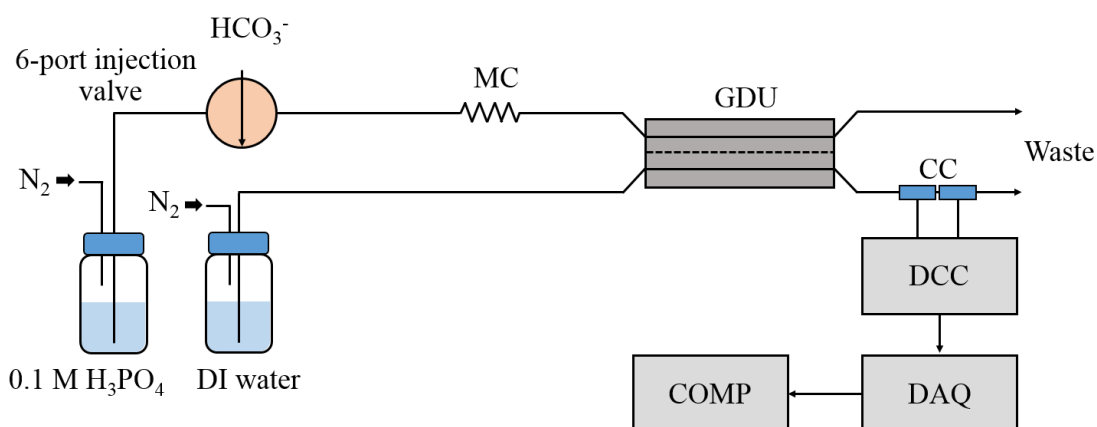


Figure 2.8 The manifold of gas diffusion flow injection (GD-FI) system for DIC determination: MC, mixing coil; GDU, gas diffusion unit; CC, conductivity cell; DCC, direct current conductivity circuit; DAQ, data acquisition unit; COMP, computer

2.4.4 The single-FI system with the FL detector for NH_4^+ determination

The schematic diagram of flow analysis system for NH_4^+ determination is shown in Figure 2.9. The OPA reagent was filled in 250 mL Duran bottle. The peristaltic pump was used as the propelling system with 0.5 mL min^{-1} solution flow rate. The 6-ports-2-

position injection valve was used for injecting the sample or NH_4^+ standard solution into the flow system. A 15.0 cm-length of Teflon tube (0.75 mm ID/1.6 mm OD) was used as the injection loop to provide 66.3 μL injection sample. The 200 cm length of Teflon tube was used as a mixing coil and immersed in 65°C water bath. The 50 mmol L^{-1} of NH_4^+ stock standard solution was standardized to calculate the exact concentration by using indophenol blue method. The NH_4^+ standard solution series of 0.5-10.0 $\mu\text{mol L}^{-1}$ was diluted with 5 mmol L^{-1} of sulfuric acid solution and note that this solution was also used as an absorbing solution for ambient air sampling experiment. After the fluorescence detector was operated and continuously recording the signal, the NH_4^+ standard solution series and air sample solutions were injected to the FI system with 3 duplicates injection.

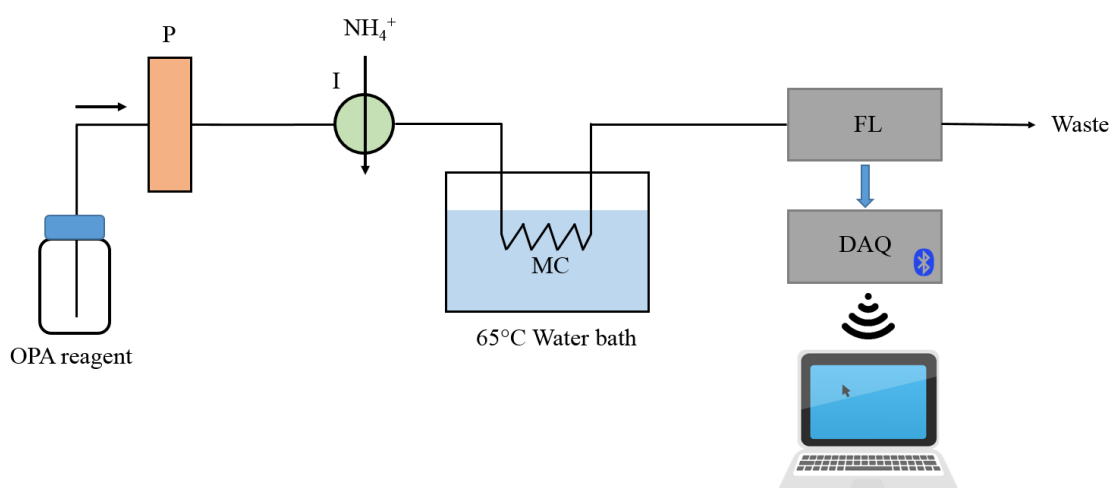


Figure 2.9 The FI-manifold for NH_4^+ determination; P, peristaltic pump; I, 6-ports-2-position injection valve; MC, mixing coil; FL, LED-photodiode based fluorescence detector; DAQ, data acquisition unit (Bluetooth)

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