APPENDIX A

LIGHT SCATTERING DETECTOR FOR FLOW INJECTION NEPHELOMETRIC DETERMINATION OF SULFATE

Introduction

Light scattering detection has been applied in flow analysis for either determination of size or mass concentration of particles [1]. Multiangle light scattering detectors can be combined with particle separation techniques such as field-flow fractionation (FFF) [2] and size exclusion chromatography (SEC) [3] for size characterization. Mass concentration of particles can be determined by either multiangle or simple uniangle detectors.

Determination of a single analyte by uniangle light scattering detection in flow injection (FI) and sequential injection (SI) analysis has been widely investigated. Either nephelometric (detection of the scattered light at an angle of 90^o to the incident beam) or turbidimetric (detection at 0^o) methods are usually used. Although turbidity can be easily measured using a spectrophotometer, other phenomena (e.g., light absorption or light diffraction) may interfere. Nephelometric measurements are also more sensitive than turbidimetric measurements.

An SI system for determination of chloride based on the precipitation with silver ions has been proposed [4]. Phosphate can be determined by an SI turbidimetric method based on calcium phosphate precipitation [5]. A stopped FI turbidimetric immunoassay using the interaction of concanavalin A (antibody) and yeast mannan (antigen) has been investigated [6]. FI systems based on precipitation reactions have also been widely applied for determination of drugs, such as thiamine [7], promethazine [8], amitriptyline [9], and chlorhexidine [10].

Determination of sulfate using barium chloride as a precipitating agent has previously been applied in both FI [11-14] and SI [15-16] systems. A liquid drop windowless optical cell has been developed for FI turbidimetric or nephelometric determination of sulfate by precipitation with barium ion [17].

In this work, a simple and low cost flow-through light scattering detection system for determination of particle mass concentration was developed. It is based on nephelometric detection, using a laser pointer as a light source and a photodiode IC as a light sensor. The detector was utilized for the flow injection determination of sulfate by precipitation as barium sulfate.

Experimental

Flow Injection Apparatus

The flow injection system used for the determination of sulfate is illustrated in Fig. 1. The system was assembled using a peristaltic pump (FIAlab Instruments, USA), a 6-port injection valve (Upchurch Scientific, USA), a 100 cm mixing coil and a flow-through light scattering cell with a home-built detection system. All connections were made with 0.8 mm i.d. PTFE tubing.

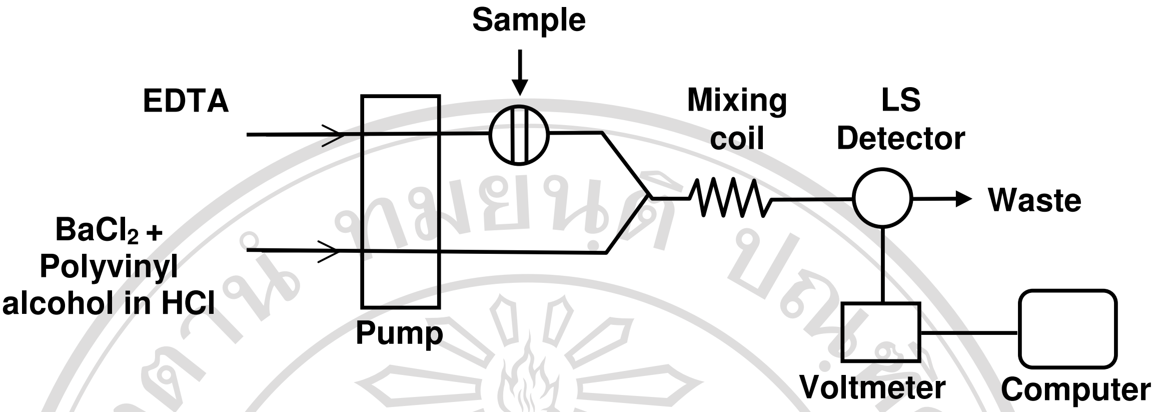


Figure 1 Flow injection nephelometric system for determination of sulfate; Solutions were 0.2%w/v EDTA (or 0.0060M) and 1.2%w/v $BaCl_2$ (or 0.058 M) plus 0.1%w/v PVA in 0.05 M HCl.

Light Scattering Detector

A cross-section of the cell is shown in Fig. 2. A perspex plastic block was drilled in order to insert a straight glass tube (2 mm i.d.) with o-ring sealing. A light sensor (described below) was mounted at right angles to the tube axis as shown. A glass microscope cover slide, attached to the block using epoxy glue, acted as a window and sealed the flow-through cell. The block was painted black to minimize stray light. Finally, the flow cell, the light source and the detector were placed inside a black box.

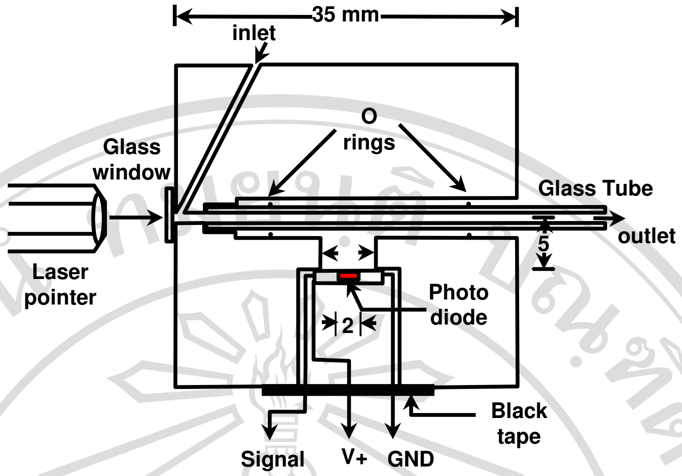


Figure 2 Schematic diagram of flow through light scattering cell

Procedure for Sulfate Analysis

A standard or sample (100 µL) was injected into a stream of 0.2% w/v EDTA pumped at 1.5 ml min^{-1} using a peristaltic pump (see Fig. 1), which then passed through a mixing coil to merge with a stream of 1.2% w/v $BaCl_2$ and 0.1% w/v polyvinyl alcohol (PVA) in 0.05 M HCl before entering the flow-through cell. Light scattering caused by the barium sulfate precipitate was monitored by the detector and recorded on the computer. An FIAgram (a plot of output voltage linearly proportional to the light scattering intensity vs time) was obtained for each injection. Data for a series of standard sulfate solutions were plotted (peak height versus sulfate concentration) and the resulting calibration graph was used in the determination of the concentration of sulfate in the unknown water samples.

Reagents

Deionized water (Milli Q, Millipore) was used throughout. All reagents were of analytical grade, unless otherwise stated. Sodium sulfate (Merck) was used to prepare a standard sulfate stock solution of 1000 mg SO_4^{2-} l^{-1}. Solutions of 1.2 %w/v $BaCl_2$ and 0.1 %w/v polyvinyl alcohol (PVA) in 0.05 M HCl were prepared from the chemicals from Merck. Ethylenediamminetetraacetic acid disodium salt (Na_2EDTA, Merck) was used to prepare 0.2 %w/v EDTA. A Polystyrene latex bead suspension (particle diameter 0.144 µm) was obtained from Seradyn (IN, USA).

Results and Discussion

Testing of FI-LSD Instrument

A series of 0.144 µm polystyrene (PS) latex suspensions with concentrations in the range 2-10 mg l^{-1} were injected into the FI system and allowed to flow-through the LS detector. The maximum difference between replicates of the resulting FIAgrams (detector voltage versus time) was 10%. The pooled standard deviation between replicates was 1 mV or 2% at the mid-range of the data. There was little baseline drift during the 5 min of the experiment. The resultant calibration line is plotted in Figure 3. The data demonstrate the linearity of the detection system. The uncertainty in the slope of the line (the standard error as determined by a least squares fitting procedure), was ±1.

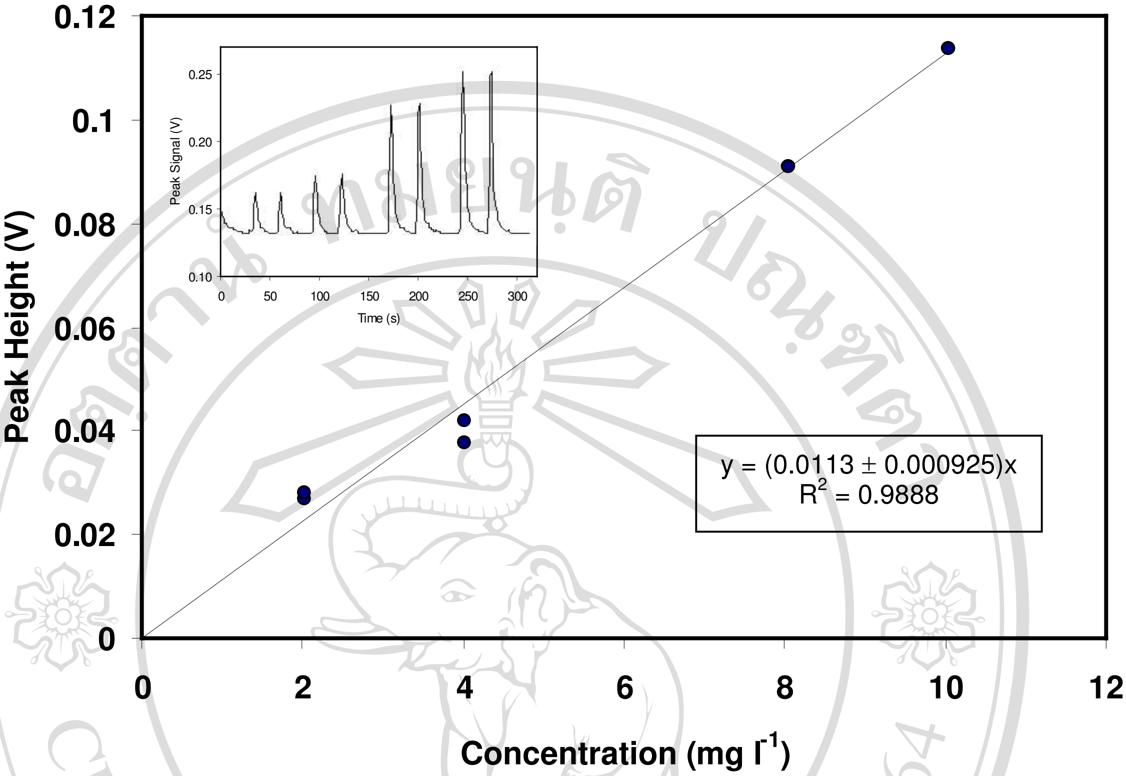


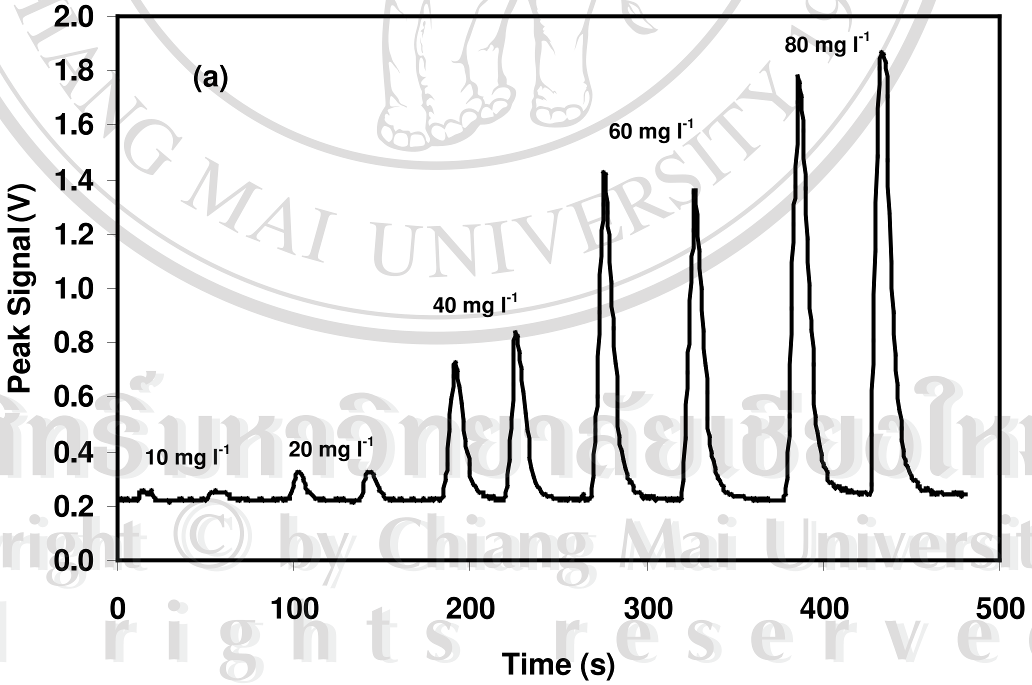
Figure 3 Calibration plot of detector voltage versus polystyrene (monodisperse 0.144 μm beads) concentration fitted to a straight line through the origin.

Flow Injection Nephelometric Determination of Sulfate

Using the FI manifold in Fig. 1, the effects of various measurement conditions, concentrations of reagents (EDTA, $BaCl_2$, polyvinyl alcohol), flow rates, injection volume and mixing coil length were studied. From this information the run conditions selected were: Na_2EDTA concentration 0.2% (w/v) or 0.0060 M, $BaCl_2$ concentration 1.2% (w/v) or 0.058 M, PVA concentration 0.1% (w/v), sample injection volume 100 μl, mixing coil length 100 cm and flow rate for each line 1.5 ml min^{-1}.

The carrier stream of alkaline EDTA acts as a wash solution as well as binds some metal ions in the sample [14]. Sulfate reacts with the excess barium ions to form a precipitate before reaching the light scattering cell. The polyvinyl alcohol would help to stabilize the precipitate by forming a protective layer on the particles.

The FIAgrams obtained for a series of sulfate injections in the concentration range 10-80 mg l^{-1} are shown in Figure 4a. No baseline drift was observed for at least 50 injections. The reproducibility of the peak heights was generally not as good as for the PS latex sample with the maximum variation between replicates being 17% and the pooled standard deviation (f = 5) over all the samples being 40 mV or 10% at mid-range. This may be due to the variation in the particle size of the precipitate formed, which will affect the scattering of light [14].



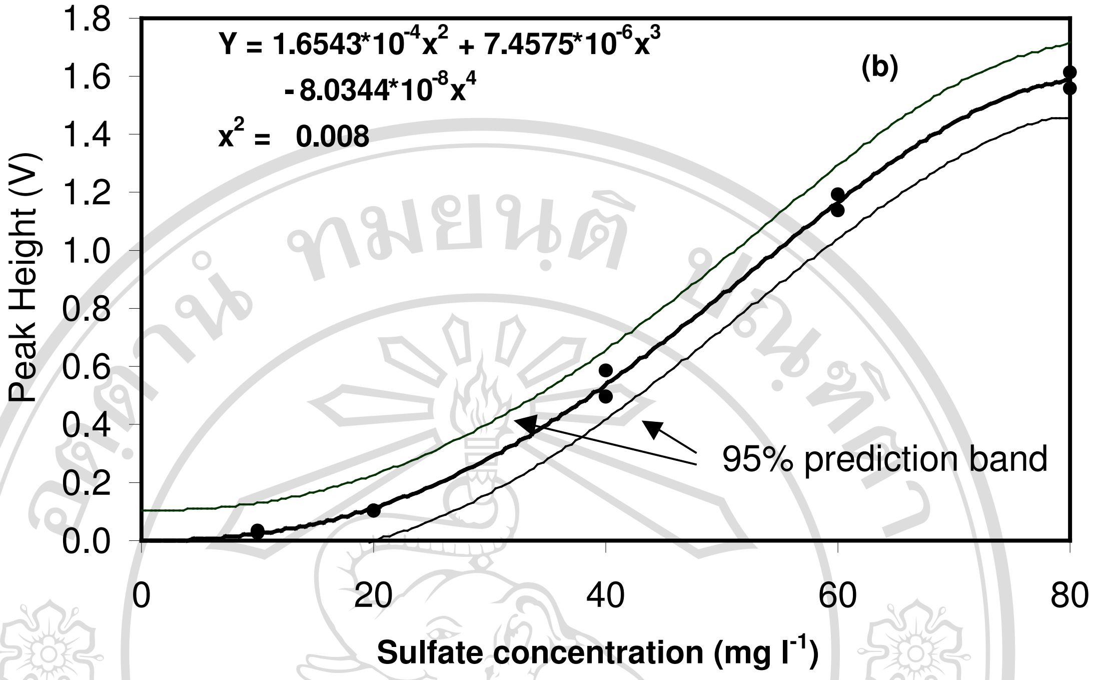


Figure 4 (a) FIAgrams obtained with the FIA-LS system shown in Fig. 1 using 100 µl injections of sulfate of various concentrations. (b) Calibration plot of detector voltage versus sulfate concentration fitted to a truncated quartic equation as explained in the text. X^2 = sum of squares of deviations of the points from the line. 95% prediction band such that 95% of the measured points are expected to lie within the band.

The data are plotted in Fig. 4b and are clearly nonlinear. In the absence of any theoretical model for describing the non-linearity, several functions (exponential, sigmoid, polynomial) were evaluated (using the software package *Igor Pro* [19]) for use as an interpolation function. The simplest function that adequately represented the data was the truncated quartic

$$y = k_2 x^2 + k_3 x^3 + k_4 x^4 \qquad (1)$$

The fitted curve is shown in Figure 5b together with the prediction bands drawn such that 95% of measured points are expected to lie within the band.

The FIA-LS system was then utilized to analyse sulfate in some natural water samples. The reproducibility is similar to that of the sulfate standards: the maximum difference between replicates was 0.1V or 17% and the pooled standard deviation over all the samples was 0.05V (f = 7).

Using the quartic calibration curve obtained with the sulfate standards the concentration of sulfate in each natural water was estimated. The results are given in Table 1 together with the 95% confidence limits determined as follows.

$$95\% \text{ confidence limit} = \frac{\text{0.5 the width of the 95\% prediction band}}{(\text{number of replicates})^{1/2}} \quad (2)$$

Table 1 Sulfate contents of natural water samples using the FIA-LS system.

Sample	Peak height (V)			$[SO_4^{2-}]$ mg l^{-1}	
	(i)	(ii)	Mean	Mean	95% confidence
A	0.378	0.362	0.370	30.3	±3
B	0.614	0.511	0.5625	41.8	±3
C	1.005	1.14	1.0725	58.7	±3
D	0.138	0.124	0.131	19.1	±6
E	0.549	0.491	0.52	34.8	±3
F	0.189	0.189	0.189	21.1	±5
G	0.104	0.102	0.103	18.7	±9

Conclusions

A simple low cost flow-through light scattering detector was developed for use in FI systems where precipitates are generated and the resulting turbidity is used as the basis for chemical analysis. The detector employed a readily available laser pointer as a light source and photodiode IC as a light sensor. The detector was tested using polystyrene latex beads and found to be quite sensitive and reliable.

In order to demonstrate the applicability of the LS detector it was incorporated into an FI system, which was, designed to measure sulfate concentrations using an approach based on the standard turbidimetric method of barium sulfate precipitation. Reasonable results were obtained considering the known limitations of this method, which result from variations in the particle size of the precipitate. This method has considerable promise due to the very reproducible mixing conditions that can be obtained in FI systems. Improvements in the accuracy of the analysis should be possible by optimizing the precipitation conditions to obtain more precise control of the particle size.

References

1. S. Ross and I.D. Morrison, **Colloidal Systems and Interfaces**, John Wiley, New York, 1988.
2. B. Wittgren and K-G. Wahlund, *Carbohyd. Polym.*, **43** (2000) 63.
3. H.H. Stuting and I.S. Krull, *Anal. Chem.*, **62** (1990) 2107.
4. R.B.R. Mesquita, S.M.V. Fernandes and A.O.S.S. Rangel, *J. Environ. Monitor*, **4** (2002) 458.

5. B.M. Simonet, F. Grases and J. G. March, *Fresen. J. Anal. Chem.*, **369** (2001) 96.
6. P.J. Worsfold and A.Hughes, *Analyst*, **109** (1984) 339.
7. C.O. Costa-Neto, A.V. Pereira, C. Aniceto and O. Fatibello-Filho, *Talanta*, **48** (1999) 659.
8. J.M. Calatayud, S.N. Sarrion, A.S. Sampedro and C.G. Benito, *J. Microchem.*, **45** (1992) 129.
9. J.M. Calatayud and C.M. Pastor, *Anal. Lett.*, **23** (1990) 1371.
10. J.M. Calatayud, P.C. Falco and A.S. Sampedro, *Analyst*, **112** (1987) 87.
11. I.P.A. Morais, A.O.S.S. Rangel and M.R.S. Souto, *J. AOAC Int.*, **84** (2001) 59.
12. V. Kuban, *Fresen. J. Anal. Chem.*, **346** (1993) 873.
13. F.J. Krug, E.A.G. Zagatto, B.F. Reis, O. Bahia Filho, A.O. Jacintho and S.S. Joergensen, *Anal. Chim. Acta*, **145** (1983) 179.
14. S. Baban, D. Beetlestone, D. Betteridge and P. Sweet, *Anal. Chim. Acta*, **114** (1980) 319.
15. R.A.S. Lapa, J.L.F.C. Lima and I.V.O.S. Pinto, *Analusis*, **28** (2000) 295.
16. J.F. van Staden and R.E. Taljaard, *Anal. Chim. Acta*, **331** (1996) 271.
17. H. Liu and P.K. Dasgupta, *Anal. Chim. Acta*, **326** (1996) 13.
18. J. G. Graeme, **Photodiode Amplifiers**, McGraw Hill, London, 1995.
19. Igor Pro, Wavemetrics, Inc. Oregon USA

APPENDIX B

DEVELOPMENT OF LIQUID LIQUID DYNAMIC SURFACE TENSION DETECTOR

A new development in instrument design was attempted from the previous liquid-air analyzer to a novel flow-based liquid-liquid analyzer for a dynamic surface tension detection and study of active species at the liquid-liquid interface. By applying Young-Laplace equation, a similar drop pressure measurement technique and sensor calibration method, a novel DSTD for liquid-liquid interface system was developed in this work. Due to that the similar principle was used in both interface types and it could also be used for study at liquid-liquid interface. A home-made flow cell which included sensor tip, in which a drop will be formed at the end of the tip, pneumatic detachment part, water reservoir to hold the bulk phase and the reference pressure measurement part were constructed to be a flow cell detector. Thus the detector was coupled with the continuous flow technique for demonstrating performance. For demonstrating the ability of this detector, two different chemicals (Cholesterol and Brij®30) were employed for studying the interfacial property at the hexane/water interface. In this work hexane was used as a mobile phase and water was used as a bulk phase for study the hexane water interface property.

A schematic diagram of the DSTD flow cell for the liquid-liquid interfacial analyzer and the system of liquid-liquid dynamic surface tension detector is shown in Fig.B1 and B2. The flow cell was machined from polyacrylic acid, since it is transparent and easy to drill.

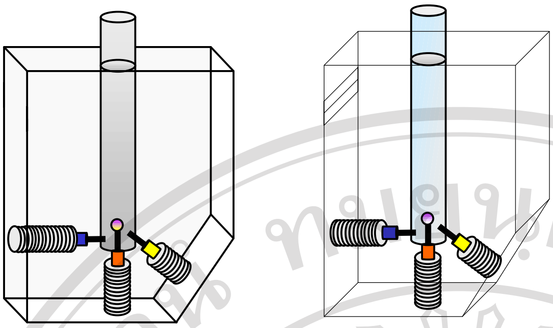


Figure B1 The developed flow cell for liquid-liquid dynamic surface tension detector

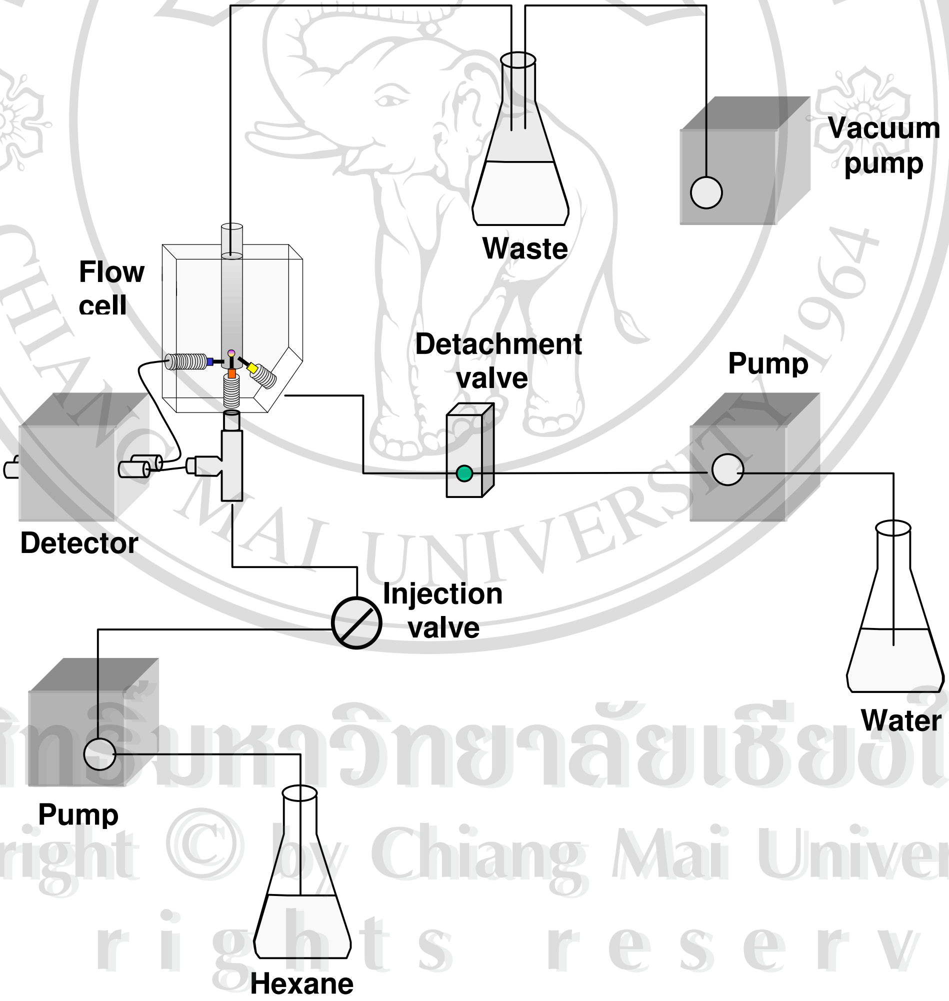


Figure B2 The system of liquid-liquid dynamic surface tension detector

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List of Publications:

International Journals

1. K. Grudpan, P. Ampan, Y. Udnan, S. Jaysavati, S. Lapanantnoppakhun, J. Jakmunee, G.D. Christian and J. Ruzicka, “Stopped-flow Injection

Simultaneous Determination of Phosphate and Silicate Using Molybdenum Blue", Talanta, 2002.

2. J. Jakmunee, Y. Udnan, R. Morrison, R. Beckett, I. Mckinnon and K. Grudpan, "Light Scattering Detector for Flow Injection Nephelometric Determination of Sulphate", to be submitted.
3. Y. Udnan, J. Jakmunee, I.D. McKelvie, M. Grace and K. Grudpan "Flow injection amperometry for phosphate determination in high salinity water and on-line preconcentration for low level phosphate water", Analytical and Bioanalytical Chemistry, to be submitted.
4. Y. Udnan, J. Jakmunee, I.D. McKelvie, M. Grace and K. Grudpan "Online sulfide removal for phosphate determination", Limnology and Oceanography; method, to be submitted.

International Conferences

1. Y. Udnan, S. Jaysavati J. Jakmunee, J. Ruzicka and K. Grudpan "A Simple Semi-automatic stopped Flow Injection Analyzer" International Congress on Analytical Science (ICAS), Tokyo, Japan, 2001.
2. Y. Udnan, P. Ampan, S. Lapanantnoppakhun, S. Jaysavati, J. Jakmunee, G.D. Christian, J. Ruzicka and K. Grudpan, "Stopped-flow Injection Simultaneous Determination of Phosphate and Silicate" 1st International Conference on Flow Injection Analysis, Chiang Mai, 2001.
3. J. Jakmunee, Y. Udnan, R. Morrison, R. Beckett, I.D. McKelvie, I. Mckinnon and K. Grudpan, "Flow Injection Light Scattering Detector for

Determination of Sulphate" 9th International Conference on Flow Analysis, Geelong, 2003.

National Conferences

1. Y. Udnan, J. Jakmunee, R. Morrison, R. Beckett, I.D. McKelvie, I. Mckinnon and K. Grudpan, "Flow Injection Light Scattering Detector for Determination of Sulphate" 27th Congress on Science and Technology of Thailand, Songkla, 2001.
2. Y. Udnan, J. Jakmunee, S. Jaysavati, and K. Grudpan, "Determination of Phosphate Using Stopped-Flow Injection Analysis" The first PERCH Annual Scientific Conference (PERCH Conference I), Chonburi, 2002 (Poster presentation).
3. Y. Udnan, J. Jakmunee, S. Jaysavati, and K. Grudpan, "Simultaneous Determination of Phosphate and Silicate in Water Samples by Stopped-flow Injection Analysis" The second PERCH Annual Scientific Conference (PERCH Conference II), Chonburi, 2003.

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Cost effective analytical instrumentation and/or procedures for both iron Fe (II) and Fe(III) and phosphate are needed for the situation in Thailand.

This research work concerns development of analytical techniques for the determination of Fe(II), Fe(III) and phosphate. Acetyl salicylic acid obtained from antipyretic powder is proposed as a new color reagent for FIA for Fe(III). Its application to pharmaceutical preparations is demonstrated. Capillary electrophoresis and voltammetric determination of Fe(II) and Fe(III) for speciation was investigated. A new procedure employing a flow injection system with amperometric determination for phosphate is proposed. An in-valve column for on-line preconcentration and single standard calibration were incorporated. A novel on-line sulfide removal system using potassium permanganate was introduced to the conventional spectrophotometric FIA for phosphate using molybdenum blue. A new procedure for simultaneous determination of phosphate and silicate by stopped-FIA was obtained. A light scattering detector for FI nephelometric determination, and development of a liquid-liquid dynamic surface tension detector, were studied.

Those developed procedures are suitable for the situation not only in Thailand but also elsewhere.