USE OF THE PREDICT-OBSERVE-EXPLAIN TEACHING SEQUENCE FOR FLOW-BASED TECHNIQUE AND NEW DEVELOPMENT OF TWO FLOW SYSTEMS FOR FOOD SAFETY AND QUALITY CONTROL

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ABSTRACT

Flow injection (FI) technique is one of the analytical techniques introduced into the undergraduate curriculum for chemistry students. In order to support students' learning of this topic, a demonstration together with a predict-observe-explain teaching sequence was developed. The developed instructional method was applied to teach flow phenomena occurring in the FI technique. A demonstration tool was assembled by using transparent materials for clear observation of the flow phenomena. Students were engaged to predict, observe the situation, and then encouraged to discuss with peers. This strategy, therefore, promotes students generating their own conceptual knowledge via reconciliation and negotiation between their prior knowledge and new experience.

This dissertation was extended to develop two new methods for food safety and control. A flow system for the rapid screening of formaldehyde contaminated in food was proposed. The system is based on the concept of the hybrid flow analyzer (HFA) with a Hantzsch reaction. An operating procedure was designed for multiple tasking and high sample throughputs. This system was successfully applied to re-hydrated dry squids, vegetables and mushrooms. Another system is a sequential injection (SI) module for simultaneous and real-time monitoring of three key parameters for the beverage industry, i.e., the sucrose content, pigment and dissolved CO_2. The SI module included a vaporization unit where dissolved CO_2 was measured via vaporization of the gas from the liquid phase. This unit was also used to degas the carbonated drink, prior to the measurements of sucrose and pigment within the same system. The method requires no chemicals and is therefore completely friendly to the environment.

KEY WORDS: PREDICT-OBSERVE-EXPLAIN/ FLOW TECHNIQUE/ FORMALDEHYDE/ SUCROSE/ DISSOLVED CO_2

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การใช้รูปแบบการสอนทำนาย-สังเกต-อธิบาย ในการเรียนการสอนเรื่องปรากฏการณ์ที่เกิดขึ้นในระบบวิเคราะห์แบบไหล และการพัฒนาเทคนิควิเคราะห์แบบไหล เพื่อความควบคุมความปลอดภัยและคุณภาพในอาหาร
USE OF THE PREDICT-OBSERVE-EXPLAIN TEACHING SEQUENCE FOR FLOW-BASED TECHNIQUE AND NEW DEVELOPMENT OF TWO FLOW SYSTEMS FOR FOOD SAFETY AND QUALITY CONTROL

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บทคัดย่อ

เทคนิควิเคราะห์แบบไหลเป็นหนึ่งในเทคนิคทางเคมีวิเคราะห์ ที่ถูกบรรจุอยู่ในหลักสูตรระดับปริญญาตรีสำหรับนักศึกษาเคมี เพื่อส่งเสริมการเรียนรู้ในหัวข้อนี้ จึงได้มีการใช้การสาธิตร่วมกับการจัดการเรียนการสอนแบบทำนาย-สังเกต-อธิบาย โดยวิธีที่ได้พัฒนาขึ้นนี้ ได้ถูกนำไปใช้สอนเรื่องปรากฏการณ์ที่เกิดขึ้นในระบบวิเคราะห์แบบไหล ชุดเครื่องมือสาธิตถูกประกอบขึ้น โดยใช้วัสดุโปร่งใส เพื่อให้ผู้เรียนสามารถมองเห็นปรากฏการณ์การไหลได้อย่างชัดเจน ผู้เรียนจะต้องทำนายผลก่อนชมการสาธิต และหลังจากการสาธิต ผู้เรียนจะต้องอภิปรายผลร่วมกับเพื่อน วิธีการเรียนการสอนแบบนี้ จึงถือได้ว่าเป็นการสนับสนุนให้ผู้เรียนสร้างองค์ความรู้ด้วยตนเอง โดยอาศัยการปรองดองและไกล่เกลี่ยความรู้เดิมของผู้เรียนเข้ากับประสบ การณ์การเรียนรู้ใหม่

วิทยานิพนธ์นี้ได้ขยายขอบข่ายงานวิจัย โดยได้มีการพัฒนาระบบวิเคราะห์แบบใหม่สองระบบเพื่อการควบคุมความปลอดภัยและคุณภาพในอาหาร ระบบแรก เป็นระบบวิเคราะห์แบบรู้ผลเร็วเพื่อตรวจสอบสารฟอมัลดีไฮด์ที่ปนเปื้อนในอาหาร ระบบนี้อาศัยการไหลแบบผสมร่วมกับปฏิกิริยาฮันซ์ ระบบถูกออกแบบให้ทำงานได้หลายขั้นตอนในเวลาเดียวกัน รวมทั้งมีจำนวนการวิเคราะห์ตัวอย่างสูง จากผลการทดลองแสดงให้เห็นว่า ระบบนี้ประสบความสำเร็จในการนำไปประยุกต์หาฟอมัลดีไฮด์ในตัวอย่างจำพวกปลาหมึกกรอบ ผัก และเห็ดต่างๆ ส่วนระบบที่สอง เป็นการพัฒนาระบบซีเควนเชียลอินเจคชัน สำหรับติดตามสามองค์ประกอบหลักที่มีอยู่ในน้ำอัดลมในเวลาเดียวกันได้แก่ น้ำตาล, สี และคาร์บอนไดออกไซด์ โดยระบบได้ถูกต่อเข้ากับอุปกรณ์ไอระเหย เพื่อตรวจวัดปริมาณคาร์บอนไดออกไซด์ที่ละลายอยู่ในน้ำอัดลมผ่านหลักการไอระเหย อุปกรณ์ไอระเหยนี้ยังทำหน้าที่ไล่แก๊สที่ละลายอยู่ในน้ำอัดลม ก่อนที่จะเข้าสู่การตรวจวัดปริมาณน้ำตาลและสีต่อไป วิธีการวิเคราะห์ที่ได้พัฒนาขึ้น ไม่มีการใช้สารเคมีเข้ามาเกี่ยวข้อง ดังนั้นจึงถือได้ว่าเป็นระบบที่เป็นมิตรต่อสิ่งแวดล้อม

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LIST OF ABBREVIATIONS

FI	Flow injection
SI	Sequential injection
HFA	Hybrid flow analyzer
POE	Predict-observe-explain
MBL-VP	Membraneless-Vaporization
C^4D	Capacitively coupled contactless conductivity detector
CO_2	Carbon dioxide
RI	Refractive index
NIR	Near infrared
RGB LED	Red/green/blue light emitting diode
LOD	Limit of detection
LOQ	Limit of quantitation
nm	Nanometer
μ	Micro-
min	Minute
rpm	Round per minute
i.d.	Inner diameter
et al.	Et Alli (Latin), and others

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

Flow-based technique is known to be a powerful tool for driving almost all analytical instruments to work at full efficiency. The technique has been mentioned in almost all textbooks concerning basic principles in analytical chemistry. In Thai universities, flow-based technique has been taught in undergraduate classes for at least 15 years. However this technique involves with some abstract concepts, which is difficult to understand. This work thus introduced a new instructional method, which foster student learning in flow topics.

Trends in global food production, processing, distribution and preparation have created an increasing demand for food safety research in order to ensure a safer global food supply. In Thailand, there have been reports of the misuse of formaldehyde in food. This work therefore developed a screening method based on flow format for detection of formaldehyde contamination in food. The method has good sensitivity and high throughputs. The instrument that was developed in this work can be applied for onsite-analysis.

Global consumption demands huge quantities of soft drinks, which results in the growth of soft drink manufacturing. Quality control is important for processing to ensure the homogeneity and value of product. In this work, automatic flow system for monitoring contents, i.e., sugar, pigment and dissolved CO_2 is presented. The system provides simultaneous and real-time detection of three parameters. The system is low-cost comparing to commercial instrument. Therefore, this is an optional method for beverage manufacturing.

CHAPTER I
INTRODUCTION

A goal for undergraduate courses is to provide students with concepts and experience that are vital for their academic. However it is not always successful to achieve the goal. Students often possess knowledge without understanding (Bodner, 1991). This might be due to inadequate active teaching approach to engage students' learning. Thus the key to teaching at the undergraduate level should be an integrated approach, i.e., combination of various teaching methods to ensure the active learning process. In this dissertation, the demonstration together with predict-observe-explain teaching approach was described. The new instructional unit on flow injection was developed for chemistry undergraduate students.

Beyond development of instructional unit for educational purpose, the research work is extended to development of two flow systems for food safety and food control. The reason for implementing flow-based technique in the method developments is that, in author opinion, in-depth understanding in subject is important. The author also learned the flow-based technique through doing research by herself. This fosters the author understanding and skill in flow-based technique, which benefit to teacher and researcher to be.

In this chapter, background of knowledge involving in this dissertation is described. The concept of flow-based technique, predict-observe-explain teaching strategy and trend of food safety and control are provided. Aims of research study are also included in this section.

1.1 Flow-based analysis and its generations

1.1.1 Flow injection

Flow injection (FI) was first described by Ruzicka and Hansen (1975). Its principle is based on injection of a sample zone into a continuously moving carrier stream propelled by a peristaltic pump. During transportation, sample is mixed with a carrier stream. If the carrier stream is a reagent, analyte in the sample solution will react with the carrier stream to form a product. This zone of the product is transported for detection at detector. All sorts of detector such as spectrophotometer, fluorometer and electrochemical detector can be coupled with the FI system. The simplest flow manifold is shown in Figure 1.1. The advantages of this technique are automation, high sample throughput, and small amount of reagent and waste when comparing with batch method.

However FI can consume considerable amount of reagents, if the system is continually operated for long period of time. Also peristaltic pump can not maintain stable flow rate for long operation hours. This resulted in a new generation of FI.

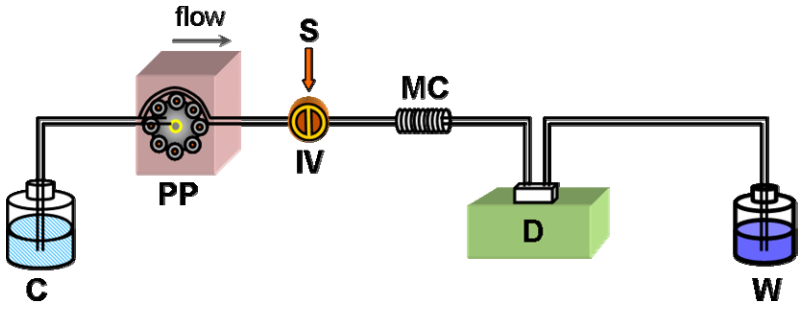


Figure 1.1 An example of simple FI manifold: C, Carrier; S, sample; PP, peristaltic pump; IV, injection valve; MC, mixing coil; D, detector; W, waste

1.1.2 Sequential injection

Sequential injection (SI) is the second generation of FI. SI was introduced by Ruzicka and Marshall (1990). The system typically consists of a syringe pump fitted with a selection valve, which can control multi-flow directions. Liquid sample and reagent are aspirated into the system as small plugs. With this system, water is normally used as the carrier. With this arrangement, the SI system thus has lower

consumption of reagent compared to the FI. Syringe pump also provides constant flow rate and robustness. Nevertheless, some drawbacks emerged. Since plugs of sample and reagent are inserted sequentially, low mixing efficiency with high blank signal is thus obtained. A general configuration of a SI is shown in Figure 1.2.

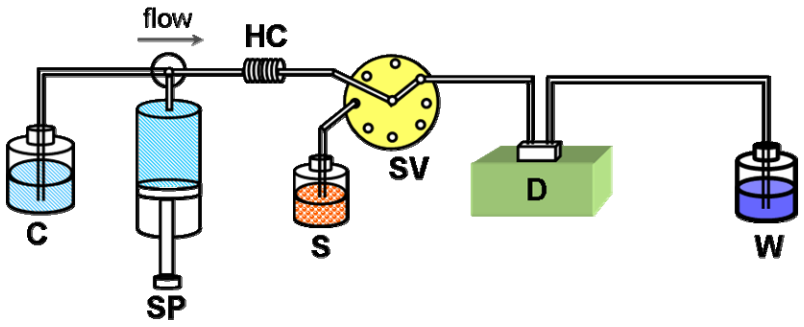


Figure 1.2 A configuration of SI: C, Carrier; S, sample; SP, syringe pump; HC, holding coil; SV, selection valve; D, detector; W, waste

1.1.3 Hybrid flow analyzer

Later a concept of hybrid flow analysis (HFA) was presented by Amornthammarong et al. (2006). The HFA system is a combination between traditional FI and SI schemes. The system compromises the advantages of the two flow-based techniques. A HFA system handles sample and reagents by using two syringe pumps. The operation is carried out in continuous flow mode, good efficiency of mixing and high sensitivity (due to low blank signal) are thus obtained. Alike SI format, robustness and flexibility are overcome. Long-term operation is made possible with the use of syringe pump. Figure 1.3 illustrates a basic HFA system.

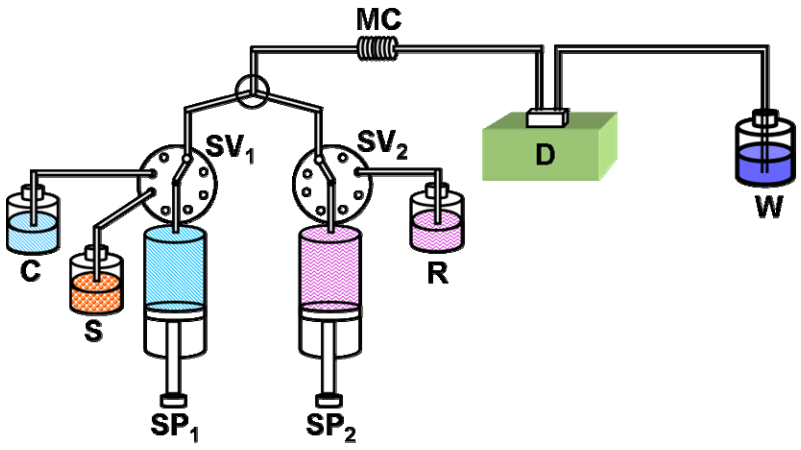


Figure 1.3 A HFA diagram: C, Carrier; S, sample; R, reagent; SP_1 and SP_2, syringe pump; SV_1 and SV_2, selection valve; MC, mixing coil; D, detector; W, waste

1.2 Problems of teaching and learning flow injection

Flow-based analysis can be roughly divided into two categories as macrobore and microbore flow analyses. Macrobore flow analysis is a flow-based system with cylindrical bore ranging from 0.3 to 1.0 mm i.d., such as flow injection, sequential injection, etc. Microbore flow analysis is the concept of a miniaturized system that dimension of a channel is in the range of 1-300 µm (Amatatongchai, 2006).

There are two processes simultaneously occurred in a FI system, namely the physical process of dispersion of sample zone in laminar flow of a carrier stream and the chemical process of the product formation. These processes occurred in FI system are difficult to understand for students who attened just the lecture without any practical experience. Detection is usually carried out before the equilibrium, causing confusion with traditional flask chemistry. It is difficult to visualize the phenomena happening in both macrobore and microbore scales. Thus, if the students do not understand in the fundamental of flow concepts, students would probably have trouble in learning the related topics.

According to personal communication with the class instructor at Mahidol University, teaching FI is not easy. When introducing the technique to inexperienced students, they usually can not perceive what occur in small cylindrical flow channels in FI system. The followings are problems found in learning FI.

1. some flow phenomena in FI [e.g., (a) what is laminar flow and its effect on zone shape (after injection into a continuous flowing stream) in relation to the recorded profile at detector, (b) how zone elongates in the flowing stream, (c) dispersion of an injected zone in laminar flow and (d) dilution due to mixing in laminar flow]
2. how flow in FIA facilitates the chemical reaction of the sample plug and carrier stream

Karlberg, a well-known scientist, stated as appearing in the preface of 'Flow Injection Analysis' 2nd edition written by Ruzicka and Hansen (1988) that "Flow injection analysis should not be explained. It ought to be demonstration". Thus, teaching flow injection would be more effective with demonstration.

1.3 Predict-observe-explain teaching sequence

Although lecture seems to be a common way in teaching at undergraduate level, because it is the most easiest method for a large number of students (Chambers & Blake, 2007), lecture has some drawbacks. Traditional lecture focuses on the direct transmission of knowledge from teacher to students, and thus places students in a passive role (Lujan & DiCarlo, 2006). Nevertheless, there are several ways to make lecture more active and keep students' minds actively engaged. There is a method that can be effectively used with large number of students is a predict-observe-explain or POE (Gunstone & White, 1981; White & Gunstone, 1992). POE strategy was primarily described by Champagne et al. (1980) but in term of DOE or demonstration-observe-explanation. Gunstone and White (1981) later included the prediction term into the strategy. Prediction is important because it reflects students' preconceptions, which students own in scientific concepts and phenomena. POE task begins with posing a situation and asking students for a prediction with good reasons. Then a situation will be demonstrated and students are allowed to observe. Real-time situation is often used in order to encourage student to think about concepts (Kearney, 2004). As Scientific-oriented thinking is important to learning science (Moore, 2008), students are required to explain rationally to the thing they observe, what and why it happens. Unlike other strategies such as concept mapping and interview, results obtained by POE are easy to interpret. Also, The POE strategy can be coupled with

both demonstration and hands-on activities. It has been shown to be a very powerful strategy for science teaching, and at secondary school and university (Parmer, 1995).

1.4 Constructivist view of learning

Constructivism is a theory of learning (Driver, Asoko, Leach, Mortimer, & Scott, 1994; Taber, 2000), which now plays significant to pedagogy in science education. The constructivist view of learning is briefly defined as: learners construct the knowledge by themselves. According to constructivist process, individual learner generates meaning with respect to the received information and adopts this meaning with existing knowledge. If new information conforms to existing knowledge, it is thus fitted in cognitive scheme and saved in a memory. But if it does not match with previous knowledge, cognitive structure is then re-organized (Doğru & Kalender, 2007). Fox (2001) derived constructivist learning process to six short statements: "(1) learning is an active process, (2) knowledge is constructed rather than passively absorbed (3) knowledge is invented not discovered, (4) all knowledge is personal but is through socially constructed, (5) Learning is a process of making sense of the world and (6) effective learning requires meaningful, open-ended and challenging problems for the learner to solve". In order to push student in a way of constructivist learning, teacher must be sure that teaching approaches supply student with active leaning environment and also student perform active role.

The POE teaching strategy is one of the approaches corresponding to theory of constructivism. The strategy helps students to justify the ideas that they bring into the classroom (prediction). When students find some conflictions during doing the activity (the difference between prediction and observation), their initial ideas are thus reshaped. Students are placed in active role, using all senses for receiving or transmitting information (observation and discussion) and then constructing the meaning through out of it (explanation). In this strategy, teacher is not an information resource providing students to absorb knowledge. Teacher is a facilitator who respects on student's role by guiding only useful information and allowing students to freely generate some ideas to predict the scientific phenomena, and allowing students to construct the knowledge by themselves.

1.5 Food safety and quality control

Food safety refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer. Quality refers to all other attributes that influence a product's value such as favor, color and texture. It also includes processing method of food (World Health Organization, 2010). Both food safety and quality are major issues nowadays since they concern in public health sector. National and international legislations have imposed strict regulations on food control in order to protect consumer and to ensure that all foods are safe for human consumption. In order to meet these legislations, either governmental agencies or industries demand new analytical methods for improving their laboratory efficiency (Tóth, Segundo, & Rangel, 2008). The method developments, which are sensitive, accurate, fast and automatic, are thus required for this issue.

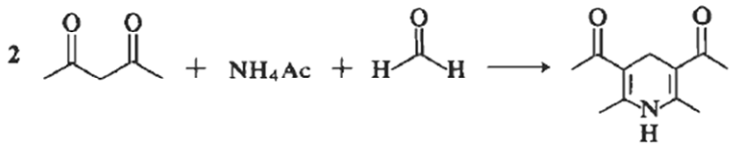
In this work, a new process for formaldehyde analysis in food was developed based on the recent HFA technique. Besides, a flow system based on SI was developed for quality control of soft drink. The followings are useful information concerning the method developments carried out in this work.

1.5.1 Formaldehyde contamination in food

Formaldehyde (HCHO) is a human carcinogen known to increase mortality from nasopharyngeal cancer (Cogliano, et al., 2004). Experiments in Sprague-Dawley rats suggested that long-term consumption of food contaminated with HCHO may cause cancer in humans (Soffritti, et al., 2002). According to the US EPA (1999), the reference dose for chronic exposure (RfD) of HCHO is 0.2 mg kg^{-1} body weight per day. However, enzymatic reduction of trimethylamine oxide in fish could produce HCHO as a product up to 98 mg kg^{-1} (Sotelo, Gallardo, Piñeiro, & Pérez-Martin, 1995). This causes an increase in the amount of HCHO during the storage of frozen fish (Bianchi, Careri, Musci, & Mangia, 2007; Sotelo, et al., 1995). In some countries including Thailand, HCHO is illegally added to foods as preservative (Cui, Fang, Jiang, & Wang, 2007; Ngamchana & Surareungchai, 2004; Wang, Cui, & Fang, 2007). However in our country, the use of HCHO in food is strictly prohibited (Global Complex Co. Ltd. Thailand (GCCThai)). It is therefore necessary to have a method for screening the level of HCHO in food.

1.5.2 Hantzsch reaction for formaldehyde analysis

Hantzsch reaction involves in a cyclisation of diketone compound with formaldehyde in the presence of ammonia. The reaction was reported to be sensitive and specific for formaldehyde. Nash (1953) developed the method relied on the Hantzsch pyridine synthesis, formaldehyde reacts with a reagent consisting of 2,4-pentanedione and concentrated ammonium acetate. The formation of a colored derivative of 3,5-diacetyl-1,4-dihydrolutidine was yielded. The measurement of formaldehyde was carried out at 414 nm. The reaction of Nash method is given in equation 1.1.



..(1.1)

In FI method, the Hantzsch reaction has been widely explored for analyses of air (Eom, Li, Li, & Dasgupta, 2008; Li, Dasgupta, & Luke, 2005; Sakai, Nagasawa, & Nishikawa, 1996; Sakai, Tanaka, Teshima, Yasuda, & Ura, 2002), breath (Ueda, Teshima, & Sakai, 2008), beverage (de Oliveira, Sousa, & de Andrade, 2007; Tsuchiya, et al., 1994) and food (Zhao & Zhang, 2009). Different diketone compounds, such as 2,4-pentanedione (de Oliveira, et al., 2007; Eom, et al., 2008; Li, et al., 2005), 1,3-cyclohexadione (Sakai, et al., 1996; Zhao & Zhang, 2009), 5,5-dimethylcyclohexane-1,3-dione (Sakai, et al., 2002; Ueda, et al., 2008), were employed. Amongst these, 2,4-pentanedione is recommended as the best reagent due to its reactivity at lower temperature and low sensitivity to interferences (Li, et al., 2005). However it is also suggested that the diketone compound and ammonium acetate should be prepared separately rather than as a single mixed reagent. The mixed reagent gradually exhibits the green fluorescence of 3,5-diacetyl-1,4-dihydrolutidine at room temperature (Li, et al., 2005).

1.5.3 Carbonated soft drink manufacturing

The worldwide beverage trade has grown continuously in every year due to increasing demand. It is estimated that for the years 1988-1994 to 1999-2004, the percentage of sugar-sweetened beverage drinker increased from 58 % to 63% in the US (Bleich, Wang, Wang, & Gortmaker, 2009). A research report for developing Asian countries, such as China, India, Vietnam and Thailand, showed that drinking of carbonated drinks has also become popular resulting in an expansion of soft drink manufacturing (Ismail, Tanzer, & Dingle, 1997). Sweetener, flavor, color and carbonating gas (CO_2) are added to beverage in order to make the product more attractive and pleasing to consumers. In the process of quality control, these ingredients must be closely monitored, both on-line and off-line (in the laboratory) to ensure the homogeneity of the production.

1.5.4 Schlieren effect and its application

The deflection of light, as it passes through a medium due to the temperature or the concentration gradient (hence the RI gradient), is called the schlieren effect (Dias, Borges, Zagatto, & Worsfold, 2006; Fang, 1993; Zagatto, Arruda, Jacintho, & Mattos, 1990). The existence of the schlieren effect in FI was first pointed out by Krug et al. (1977).

In FI system with spectrophotometric detection, the schlieren effect can cause serious errors. Several approaches have been used to compensate this effect such as increasing the system's dispersion, injection with matrix matching technique (McKelvie, Peat, Matthews, & Worsfold, 1997), employment of dual wavelength detection (Zagatto, et al., 1990), utilization of a so-called multi-reflective flow cell (Ellis, Lyddy-Meaney, Worsfold, & McKelvie, 2003) or the recent 'total internal reflection photometric detection cell' (Ellis, Gentle, Grace, & McKelvie, 2009).

Nevertheless, the schlieren effect also has a positive side. In 1978, Betteridge et al. made use of this phenomenon for determining the RI of some solutions with an LED flow-cell. Injection of a non-absorbing bolus of liquid into a flowing stream of another liquid leads to formation of RI gradient or regarded as series of lenses. These liquid lenses either focus the light onto detector or divert the light away from detector, depending upon the order of the refractive indices of the liquids

and the flow direction. Consequently, the spectrophotometric signal decreases or increases, respectively (Betteridge, Dagless, Fields, & Graves, 1978). The simulation of schlieren effect on light deflection at a light detection flow through cell is depicted in Figure 1.5.

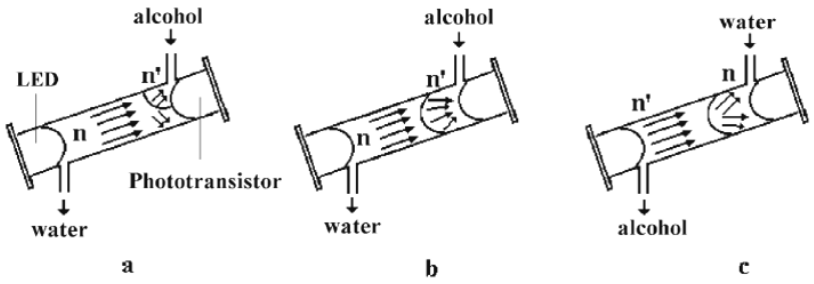


Figure 1.4 Simulation of light direction when alcohol sample zone penetrates into the optical path of the flow cell and relatively changes in the refractive zones (n and n' are RI of water and alcohol respectively) (dos Santos, De Araújo, & Barbosa, 2002). (a) The alcohol sample zone is just entering the flow cell, diverting light away from detector (phototransistor), (b) light is focused on detector when passing from water carrier to alcohol interface and (c) light is off again when changing the medium from alcohol to water.

In the late 1980s, Pawliszyn exploited the schlieren effect by building some concentration gradient detectors for liquid chromatography (Pawliszyn, 1986a) and for capillary electrophoresis (Pawliszyn, 1988) that were based on laser beam deflection by the schlieren effect. Besides, the same author mentioned some results of the application of the laser-based detector in flow injection analysis for sucrose measurement (Pawliszyn, 1986b, 1988). So far there has been no reported application in the beverage industry. In addition, the concept of schlieren effect was also adopted in quantitative flow methods for verification of adulteration in alcoholic beverages (da Costa, et al., 2004), determination of alcoholic grade (dos Santos, et al., 2002), determination of glycol (Wijk & Karlberg, 1994) and measurement of wine density (Mataix & Luque de Castro, 2001).

1.6 Aims of the study and research questions

This study aimed at developing a demonstration set of simulated FI system to promote student learning through visualization. A tool was constructed in magnify-scale with transparency materials to enable students to observe the flow phenomena by naked eyes. The demonstration together with POE teaching sequence was applied into a lecture classroom for teaching FI, a topic for chemistry undergraduate. The developed instructional method was expected to challenge students with real-world phenomena and to foster students learning in constructivist environment. Group discussion was also organized in order to provide student experience with collaborative learning. The method supposed to be completed by a certain time of classroom lecture.

The study focused on supporting students' learning of flow phenomena by means of POE teaching sequence. The research questions for this work thus are:

1. Does the lecture demonstration together with POE teaching sequence support students' learning of flow phenomena in FI system?
2. What are the students' attitudes toward the newly developed POE-based instructional unit?

The aim of study was extended to develop a flow system suitable for rapid screening of formaldehyde contaminated in food. The system is based on the concept of a robust HFA format with Hantzsch reaction for detection of formaldehyde. An operating procedure was developed for multiple tasking. The capability in multiple processing is supposed to a significant increase in sample throughput. A thermostated heating device was also built for heating of the reagent solution of 2,4-pentanedione and ammonium acetate, prior to reaction with formaldehyde. The heating device was supposed to be effective in terms of uniform heat distribution and stability over extended operation period, leading to significant improvement of analytical sensitivity.

A practical technique suitable for on-line measurement and process control of sugar content in the beverage industry would be a flow analysis technique based on light deflection due to a refractive index (RI) gradient. The arrangement can be easily

set up by employing a simple U-shape flow-cell with a LED light source for achieving instantaneous measurement. Therefore this study also developed a new SI method and a module for simultaneous and real-time monitoring of three key parameters for the beverage industry, i.e., the sugar content, pigment and dissolved CO_2.

Detection of the light deflection at the liquid interface (the schlieren effect) of sucrose and water was utilized for sucrose content measurement. A portable near infrared spectrometer was chosen as the light source to ensure that all the ingredients and dyes in soft drinks will not interfere by contributing light absorption. The module also has a section where dissolved CO_2 is measured via vaporization of the gas from the liquid phase. The module includes a vaporization unit that is also used to degas the carbonated drink, prior the measurements of sucrose and pigment within the same system. The method requires no chemicals and is completely friendly to the environment. This method thus expected to be an option system for quality control in beverage manufacturing.

CHAPTER II
LITERATURE REVIEW

This chapter summarizes the literatures related to the content or issue of this thesis. It begins with a description of a predict-observe-explain as a teaching strategy for science classroom. Previous attempts in teaching flow injection technique are also reviewed. This chapter includes methods for determination of formaldehyde in food and beverage such as chromatographic, spectrometric and flow techniques. In addition available methods for quality control of some contents, i.e., sucrose, pigment and dissolved CO_2 in carbonated soft drink manufacturing are presented.

2.1 Previous application of the predict-observe-explain strategy for teaching science

Liew and Treagust (1995) used the POE procedure as a practical lesson to teach grade-11 students in the topic of heat and expansion of liquids. At the same time, POE was used to investigate students' prior knowledge. The results indicated that students held different background in the concept of heat and expansion of liquids, and this more or less influenced to their observation and interpretation of new encounter. Thus response of students on POE is useful information to assist teacher insight what students think, and teacher is able to plan the lesson and strategy appropriately. Chairam et al. (2009) developed a chemical kinetics experiment that incorporated the use of POE sequence. This work was a case study involving first-year undergraduate students at a Thai university. Following the activities, students had to identify the problem, design an experiment and predict the results including offered explanations on POE tasks. The finding showed that POE sequence along with small group and whole class negotiations, successfully encouraged students to use critical and logical thinking as well as consideration of alternative explanations. After finishing the activities, students were able to explain the changes of the rate of a

chemical reaction, and were able to develop a better conceptual understanding of chemical kinetics.

Since multimedia has now an impact on educational section, the developments of multimedia within a POE framework also have been already reported (Kearney, 2004; Tao & Gunstone, 1999). Tao and Gunstone (1999) developed a set of computer simulation programs carrying out POE tasks. The tasks provided cognitive conflicts that fostered students' conceptual change. Students worked in pairs on the topic of force and motion. Although the finding showed that students probably achieved conceptual change without any peer conflict, collaborative work helped students get though the tasks and develop shared understanding. Kearney (2004) used video of real-life events supported POE strategy to engage a meaningful learning. Students worked collaboratively on computers. POE tasks motivated students' thinking and conversation, since the program was designed not to allow students to watch the video until they have completed the prediction and reason. The study showed a successful contribution of technology media based POE strategy for teaching and learning in science classroom.

In most of the literatures, POE teachings were developed and tried out at secondary and tertiary level. Palmer (1995) however had tried out this method at primary level. It was found that children were excited with POE activity. Children liked responding their ideas by verbalization.

The concept of POE was extended to be other teaching strategies. Thorley and Woods (1997) and Dial et al. (2009) were interested in the reasons of students' prediction. Those authors thus applied a model-based POE, called PEOE (predict-explain-observe-explain) in their studies. They mentioned that the explanation student gave for prediction was vital for identifying students' prior-understanding and belief. Tien et al. (1999), Mattox and Reisner (2006) and Tien et al. (2007) employed the model-observe-reflect-explain (MORE) framework as an instructional tool. This model was a refinement and expansion of POE format. Under MORE framework, students brought their initial understanding to the class (model), conducted experiments to test their ideas (observe), considered the implications of their observations, and used these to refine their initial ideas (reflect and explain). Sokoloff and Thornton (1997) developed an interactive lecture demonstration (ILD) as a model

for teaching physics. Although ILD was constructed separately, its procedure was similar to the formerly reported POE. Alike benefits getting from POE format, ILD method could enhance students understanding.

2.2 Previous attempts in teaching flow injection

Hansen and Ruzicka (1979) primarily introduced flow injection (FI) into instrumental laboratory for undergraduate chemistry students. The experiments were separated into three parts. The first one was determination of chloride. The second experiment was determination of phosphate, and the third one was an acid-base titration. The experiment successfully fulfilled students' experience in flow injection technique. Nóbrega et al. (1991) developed a quick lab exercise that introduces students to fundamentals of the FI technique. A reaction, in which chemical equilibrium is not attained, was chosen in order to demonstrate the role of kinetics. Students developed understanding of kinetics and applied the concept to compromise the decisions concerning sensitivity, selectivity and designing of flow system. Nóbrega and Rocha (1997) introduced FI apparatus to evaluate the effect of ionic strength on the rate of reduction of hexacyanoferrate (III) by ascorbic acid. The experiment illustrated to students that a flow system is not only an instrumental tool to obtain analytical data, but it is also a powerful process to obtain physical chemistry data. Wolfe et al. (1998) described a simple FI system for performing protein assays. This experiment showed an application of flow method to biology and biotechnology. Real-world samples such as foods and saliva were employed for motivation of students' attention. Sartini et al. (2000) mentioned that undergraduate classes on classical gravimetric analysis are usually tedious. This is due to its long procedure and time consuming. Sartini et al. therefore developed the gravimetric-based flow method for presenting modern analytical approach. This resulted in a more interesting lesson, which stimulated the students' curiosity. A final year project on flow technique was also presented by Economou et al. (2004). The project required multidisciplinary, i.e. chemical instrument, graphical programming and analytical chemistry, the project is therefore suitable as a teamwork exercise. In this project students had to integrated knowledge to design, construct and apply the flow apparatus for quantitative purpose. Determination of nitrite in meat (Penteado, Angnes, Masini, & Oliveira, 2005) and

hypochlorite in bleaching products (Ramos, Prieto, Gomes Cavalheiro, & Schmitt Cavalheiro, 2005) are proposed. These experiments initiated students to recognize some important flow parameters and system operation. A gradient flow-injection titration was introduced for determination of acidity constants (Conceição & Minas da Piedade, 2006). The experiment demonstrated the implementation of automated and rigorous titration by flow format. Recently, an open-ended experiment of flow analysis was established with the goal of enhancing student motivation (Petrozzi, 2009). The situation was set as a fictitious industrial problem concerned with off-flavors from phenol in fermented beverages. The students started with batch method and finally a flow system was set up. The results showed that students were more motivated when working in an interactive and realistic way. The students realized that as in a real-life situation, the experimental results should not be considered only in terms of their analytical aspects, but also as a means of gathering chemical information in a simple, rapid, and economical manner.

Demonstration sets for teaching flow-based technique using overhead projector also developed (Grudpan & Thanasarn, 1993; McKelvie, Cardwell, & Cattrall, 1990). McKelvie et al. constructed a microconduit flow system using a 35-mm slide projector. The slide was etched to make narrow manifold channel (0.5 mm i.d.). Grudpan and Thanasarn used polyethylene tubing with 1.3 mm id. for a demo assembly. Both demonstrations were performed by the instructors to illustrate the flow principles in classroom lectures.

Although there are many FI experiments proposed for laboratory, those experiments only assist students to learn the performance of FI instrument and its application, as well as to train students' science process skills. On the other hand, an actual instrument which typically composes of small tubing size (~ 0.3-1.0 mm of diameter), acts like a 'black box' itself. Students have difficulty in visualizing the flow phenomena inside those small tubing.

2.3 Methods for determination of formaldehyde in food

2.3.1 Chromatographic method

Liquid chromatography coupled to mass spectrometric detection (LC-MS) was used for the analysis of formaldehyde in shitake mushroom (Mason, Sykes,

Panton, & Rippon, 2004). The method was based on the derivatization with β-diketones. As other aldehydes would form derivatives with different molecular structures, this reaction provided a derivative that was specific for formaldehyde, which could be characterized by MS detection. A high-performance liquid chromatography (HPLC) method was also developed for determination of formaldehyde in squid and squid products (Li, Zhu, & Ye, 2007). Based on steam distillation and 2,4-dinitrophenylhydrazine derivatization, formaldehyde was analyzed by HPLC using ODS-C18 column with UV detector at 355 nm.

A modified-headspace gas chromatographic method (GC), based on polycondensation with formaldehyde, was developed for derivatization of formaldehyde in foods (Tashkov, 1996). The results could be obtained within three minutes by this method. The method was therefore proposed for the processing in manufacturing. The formaldehyde content in different fish species was evaluated using a solid phase microextraction (SPME) with GC-MS method (Bianchi, Careri, Musci, & Mangia, 2007). Although the method was capable to detect formaldehyde at trace levels, the extraction was carried out at high temperature with long period of time.

Method developments, based on chromatography, generally provide low limits of detection. However, the formerly developed techniques required expensive instruments and skilled technician, with no portability.

2.3.2 Electrochemical method and sensor

Pulse amperometric detection (PAD) was developed for determination of formaldehyde in rinsed water of vegetable and fruit and iced-melt from seafood (Ngamchana & Surareungchai, 2004). This method was proposed for monitoring the misuse of formaldehyde, which contaminated in food at milli-molar level. An application of the electronic nose (E-nose) for spoiling and formaldehyde-containing detection in octopus was presented (Zhang, et al., 2009). However this device could be used for only qualitative assessment with need of complex statistics to interpret the results.

Detection and analysis of formaldehyde, based on electrochemical or sensing detection has good potential in miniaturization to make the system portable.

Nevertheless, the stability and robustness for on-site analysis may not be attained from this kind of detection.

2.3.3 Spectrometric method

Measurement of formaldehyde can be done spectrometrically. A test for the qualitative detection of formaldehyde in food as recommended by the Association of Official Analytical Chemist (1990) is the chromotropic acid test. A quantitative spectrometric method based on chromotropic acid is also available (Mason, et al., 2004). However the method is inconvenient since it involves heating of concentrated acid mixture in boiling water bath. A kinetic spectrometric method using the catalytic effect of formaldehyde on the oxidation of rhodamine B by potassium bromate in sulfuric acid was presented for food (Cui, Fang, Jiang, & Wang, 2007). This method also requires heating in boiling water for 6 min.

Amongst the spectrometric methods, the most practical and effective technique for detection of the formaldehyde in food is the method reported by Nash (1953). The reaction is also known as the Hantzsch reaction. This Hantzsch reaction was used to measure the formaldehyde accumulated in fish during cold storage at different temperatures (Sotelo, Gallardo, Piñeiro, & Pérez-Martin, 1995), and was also adopted for a test kit with a reference color card for estimating the level of formaldehyde in food (Wang, Cui, & Fang, 2007).

2.3.4 Flow injection method

In FI method, the Hantzsch reaction has been explored by some researchers for analyses of food and beverage. The flow method involving on-line condensation of formaldehyde with 2,4-panetanedione and ammonia, followed by fluorescence detection of the product 3,5-diacetyl-1,4-dihydrolutidine, was reported (de Oliveira, Sousa, & de Andrade, 2007; Tsuchiya, et al., 1994). The procedure was successfully applied to analyze in either alcoholic or non-alcoholic beverage samples. The results showed that this method does not suffer from the blank responses and interferences by contamination from the other aldehydes. The FI fluorometric method based on Hantzsch reaction was re-employed but with different diketone compound (Zhao & Zhang, 2009). The 1,3-cyclohexadione was used instead of 2,4-panetanedione, in

order to enhance the fluorescent intensity of product. However this reaction requires higher temperature condition, 95 °C. This procedure was applicable to bean curds, beer and juice samples and the method gave a sample throughput of 18 samples per hour.

Although fluorometry offers good sensitivity, this method requires sample preparation to filtrate micro-particulates in food extract. (Zhao & Zhang, 2009).

2.4 Quality control of major contents in soft drink

2.4.1 Sucrose measurement

Refractometry is the conventional method for measuring sucrose (Bhuyan, 2007), and typically, sugar content in water is expressed as Brix or degree Brix (°Brix). For example, a solution that has 10 Brix contains 10 grams of sugar and 90 grams of water (Shachman, 2005). In addition to the refractrometric method, there are some other methods reported for determination of sucrose in beverages.

Fourier transform near infrared (FT-NIR) spectrometry with multivariate analysis was developed for determination of individual sugars in fruit juices (Rodriguez-Saona, Fry, McLaughlin, & Calvey, 2001). Sucrose, glucose and fructose were used as a model of sugar mixtures. The FT-NIR allowed 3 min of analysis time. The method was therefore claimed that it is appropriate for quality control in beverage manufacturing. Sucrose content was also determined by dispersive Raman spectroscopy (Silveira Jr, et al., 2009). A Raman spectrometer was employed using 830 nm laser as light source and an imaging spectrograph coupled to a CCD camera. With multivariate analysis, the results showed small error for quantitation in lemon-type soft drinks. A liquid density sensor using acoustic waves was developed for analysis of sucrose (Turton, Bhattacharyya, & Wood, 2006). A wide-range measurement (0-50% sucrose by weight) with high accuracy was an advantage of this sensor. Enzymatic sensors with flow system were proposed for automated measurement of sucrose in juices (Guemas, Boujtita, & El Murr, 2000) and milk (Maestre, Katakis, Narváez, & Domínguez, 2005). However, it may be difficult to apply this kind of sensors to food and beverage industries. The enzymatic sensor had drawback in terms of life-time and stability of enzyme.

2.4.2 Dissolved carbon dioxide measurement

Dissolved CO_2 found in a typical can of carbonated soft drink is in range of 3-10 g CO_2/L. A total pressure inside a can at temperature of 20 °C is approximately 3 bars (kg/cm^2).

Manufacturing of carbonated soft drinks requires efficient monitoring of CO_2 concentration in the liquid during the carbonation process. The traditional technique for measuring dissolved CO_2 is an indirect technique based on Henry's law. Total pressure and temperature are used to calculate the concentration of CO_2 (Cash, Broski, & Slier, 2008; Sachon, 2007). This technique is likely to provide inaccurate result, as total pressure is used in the calculation but not the partial pressure of CO_2. Therefore, some other techniques for direct measurements based on infrared spectroscopy have been introduced (Cash, et al., 2008; Wilks QC Inc.). The infrared absorption detector produces quick response times and the results are reliably quantitative. However the detector is usually bulky and expensive. A membrane gas-diffusion FI system was successfully applied for off-line analysis of beer and soft drinks with spectrometric detection using a pH indicator (Ljunggren & Karlberg, 1995). Commercial instruments are also available for monitoring CO_2 in beverage industry (Rosemount analytical, 1998; Sachon, 2007).

2.4.3 Pigment measurement

Food dye additives are commonly added into beverages to improve their appearance and texture, since the visual aspect is an important factor for the selection of products by consumers. For quantitative purpose, complicate instrument such as chromatography with spectrometric detection was required for identifying and determination of dye quantities (Alves, Brum, Branco de Andrade, & Pereira Netto, 2008). However for routine monitoring, in which the same formulation is carried out, semi-quantitative measurement is sufficient for monitoring pigment level from batch to batch of production. A portable photometer, fitted with multi-wavelength light emitting diode (LED) (Gros, 2007) is thus the most common and inexpensive apparatus used. Absorbance reading is measured and directly interpreted to pigment content.

CHAPTER III
METHODOLOGY

This chapter provides the methods for research work which comprises of three sections: development of flow demonstration, method for analysis of formaldehyde and method for monitoring some contents in soft drink. The first part describes construction of the demonstration set, classroom activity and how to collect and interpret data. The second and third parts show flow configurations developed for determinations of formaldehyde and measurements of contents in soft drink, respectively. Chemicals, sample preparation and operation for both analytical systems are also given.

3.1 Experimental design for teaching flow phenomena in flow injection (FI) system

3.1.1 Chemicals and materials used for the demonstration

Chemicals

For showing the flow phenomena, no chemical was involved in order to avoid confusion between physical and chemical processes. Therefore water carrier and a solution of aqueous food dye were employed as the carrier and the injected plug, respectively. Concentration of dye must be intense enough for the observation using naked eyes, thus red dye solution at 1.0 absorbance was used for the demonstration. For showing the chemical process, 0.1 M potassium thiocyanate (KSCN) and 0.005 M iron chloride ($FeCl_3$) were exploited as the carrier and the injected liquid sample, respectively. Both thiocyanate and iron solutions were prepared in 0.1 M hydrochloric acid (HCl). The chemical preparation of reagents is shown in Table 3.1

Table 3.1 Preparation of chemical solutions used for demonstration of the chemical process occurred in FI system

Reagent	Preparation
0.1 M HCl	Slowly add 500 ml of distilled water into 4.2 ml of 37% conc. HCl (LabScan, Ireland).
acidic 0.1 M KSCN	Dissolve 2.43 g of KSCN (Ajax, Australia) in 250 ml of 0.1 M HCl
acidic 0.005 M $FeCl_3$	Prepare 0.1 M of iron stock solution by dissolving 0.81 g of $FeCl_3$ (Merck, Germany) in 50 ml of 0.1 M HCl. Pipette 5 ml of the stock into a volumetric flask and then make the volume up to 100.0 with 0.1 M HCl.

Materials and construction

A portable demonstration set consisted of transparent plexiglass sheet (30x30x0.2 cm), aquarium apparatus like air tubes (0.4 cm i.d.) and simple air valves. These aquarium pieces were arranged and fixed onto the plexiglass base to make a see-through set. A pump was used for introducing liquid flow. The demonstration set is shown in Figure 3.1. During demonstration a sheet of pexiglass was put on a visualizer for the live-show to the whole class.

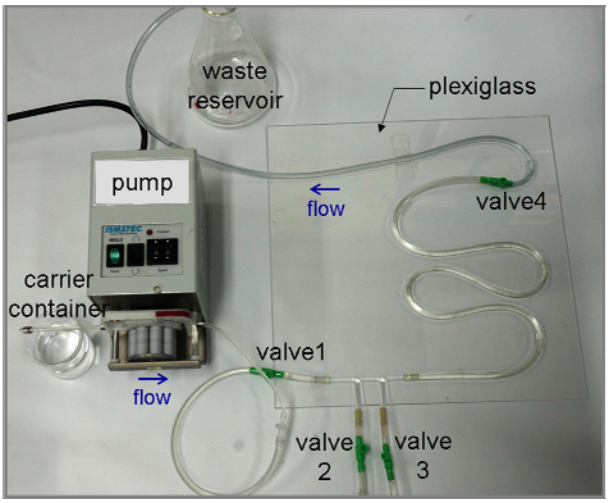


Figure 3.1 The demonstration set used for teaching the flow phenomena in FI system

3.1.2 Demonstration procedure

As shown in Figure 3.1, carrier solution was propelled from carrier container to the waste reservoir by turning on the pump and only valves 1 and 4 are opened. Introduction of sample was carried out by stopping the pump, closing valves 1 and 4 as well as opening valves 2 and 3. And then sample solution was injected to come in at valve 2 and flow out at valve 3. When valves 2 and 3 were switched off while valves 1 and 4 as well as a pump were switched on again, a plug of sample held at running channel is suddenly transferred by carrier to the waste.

Students observed the change of sample zone after it was injected into the system and during it was moving along the channel.

3.1.3 Participants

Participants of this study were 43 senior chemistry students from Mahidol University, Thailand. They were 30 females and 13 males with age of 21-23 years old. These students enrolled in a course on SCCH 412 Special Topics in Analytical Chemistry. The students were taught with the developed POE by a volunteer chemistry faculty who had teaching experience at undergraduate level for more than ten years.

3.1.4 Classroom design

The demonstration was divided into two parts: (1) illustration of physical phenomena occurs upon flowing of liquid in a small tubular channel, (2) demonstration of how flow system facilitates the chemical reaction. In each demonstration, three steps of POE, i.e., prediction, observation and explanation were used before, during and after the demonstration, respectively. The details of activities which lasted 90 minutes are shown in Table 1. Over the time teacher acted as facilitator to give necessary information and encourage students to shape up their ideas.

Table 3.2 Classroom activities based on POE sequence for teaching FI topic

Step of POE	Activity	Period (min)
***Part I** : demonstration of phenomena in FI system*		
Prediction	Students are engaged to independently foresee what would happen in FI system when a plug of dye is transferred from one place to another in continuous flowing stream of a water, and explain why such phenomena happened.	10
Observation	Teacher performs the demonstration, while students observed and recorded what they did actually see.	10
Explanation	Groups of three students discussed with peers. They are asked to amend or add their explanation, reflected and re-shaped the understanding.	15
***Part II** : demonstration of chemical reaction proceeded in FI system*		
Prediction	Individual student makes an assumption with reasons on how chemical process occurs during transportation of chemical solutions.	10
Observation	Students focus on the demonstration (by teacher), and write down the observation.	10
Explanation	Students share and articulate ideas with peers, leading to consensual meaning conclusion.	15
Groups of students present their conclusion to class. Whole-class knowledge is constructed through discussion and negotiation.		20
	Total	**90**

3.1.5 Data collection

POE written document

During the classroom activity, students had to complete 2 written documents that were developed based on the POE format (White & Gunstone, 1992). All of these documents were checked for practicability by trialing with the students of previous academic year. Details of the developed POE documents are shown in Appendix A.

Questionnaire

Perception of students on their classroom experience was collected by using a 26-item questionnaire. The questionnaire comprised of four main aspects such as perception to the demonstration (5 items), POE approach (5 items), collaborative work (9 items) and role of teacher (7 items). The Likert scale was set as five degrees; strongly agree, agree, neutral, disagree and strongly disagree (Oppenheim, 1992). One open-ended statement asking for suggestion was provided at the end of questionnaire. The developed questionnaire is shown in Appendix B.

Interview

The students were randomly selected for the interview by using a guide-format interview (Patton, 1990). A 10-min face-to-face interview was conducted for each student two days after the class was completed. Individual interview was recorded and transcribed.

3.1.6 Data analysis

Students' understanding was evaluated by examination the prediction, observation and explanation, which students gave on their POE documents.

The data from questionnaire was analyzed to obtain a qualitative result. The Likert-score was set as following; strongly disagree = 1, disagree = 2, neutral = 3, agree = 4 and strongly agree = 5. A mean score with standard deviation in each item was calculated. The value of agreement was categorized based on the interval (Doğru & Kalender, 2007) as the followings;

1.00-1.80 = strongly disagree
1.81-2.60 = disagree
2.61-3.40 = neutral
3.41-4.20 = agree
4.21-5.00 = strongly agree

The data from interview provided supplementary information. This information was used to ascertain the results gained from the POE documents and the questionnaires.

3.2 Experimental design for analysis of formaldehyde

3.2.1 Chemicals and sample preparation for analysis of formaldehyde

Chemicals

All reagents were of analytical grade. Deionized-distilled water used throughout this experiment, was obtained from an EASYpure II system (Branstead, USA).

A stock solution of formaldehyde standard (0.1 M) was prepared by diluting 0.8 ml of 37.5% HCHO (Ajax, Australia) to 100 ml with water. The exact concentration was obtained by standardization using the iodometric method (Jeffery, Bassett, Mendham, & Denney, 1989). This stock was kept refrigerated at 4 °C. Formaldehyde calibrators (10-100 μM) were prepared daily by appropriate dilution with water from the stock.

Ammonium acetate solution (2 M) was prepared by dissolving 38.5 g of ammonium acetate (Ajax, Australia) in 200 ml of water, followed by adjusting the pH in the range of 5.6-6.2, using glacial acetic acid (J. T Baker, USA). Water was added to this solution to 250 ml.

Acetyl acetone stock solution (1 M) was prepared by diluting 5.0 ml of 95 % acetyl acetone (Carlo Erba, Australia) to 50 ml with water. Acetyl acetone working reagent (0.04 M) was obtained by diluting 10 ml of 1 M acetyl acetone to 250 ml with water.

Pararosaniline solution (5.2 mM in 2.4 M HCl) was prepared by dissolving 0.16 g of pararosaniline (Fluka, USA) in 20 ml of conc. HCl (LabScan, Ireland). This mixture was gradually transferred into 20 ml of water. The solution was then made up to 100 ml with water.

Sodium sulfite solution (8 mM) was daily prepared by dissolving 0.10 g of anhydrous sodium sulfite (Ajax, Australia) in water sufficient to total 100 ml.

Sample preparation

The extraction of HCHO from food sample was adopted from the method of Wang et al. (2007). A food sample was cut into small pieces (4 to 5 mm), accurately weighed (12.5 g) and placed into a 50-ml centrifuge tube containing 25.00 ml water, which was extraction solvent. Sample tubes were capped and shaken at 240 rpm for 15 min (IKA Labortechnik, Germany) to extract the HCHO. The sample tubes were then loaded onto the rack of the autosampler (PerkinElmer AS90, USA).

3.2.2 Hybrid flow analyzer and multiple processing

Hybrid flow analyzer

The HFA system consisted of two commercial syringe pumps, equipped with an eight-port selection valve (Kloehn Versa Pump 6, USA). 5-mL zero dead volume syringes (Kloehn, USA) were employed for the pumps. Swinnex filter holder, 13 mm diameter (Millipore, USA) fitted with Whatman paper no. 1 (Whatman, England) was used for online filtration of sample. A manifold was assembled with 0.75 mm i.d. PTFE tubing. A small water bath (Isotemp 205, USA) was used to heat up reaction coil to accelerate the Hantzsch reaction. A spectrometer (Jenway 6405, UK), fitted with a quartz flow cell with 5-cm light path, was used for monitoring the absorbance at 412 nm. The control of the syringe pumps and the data acquisition were made using in-house software written using LabVIEW 8.0^{TM}. The HFA configuration is shown in Figure 3.2.

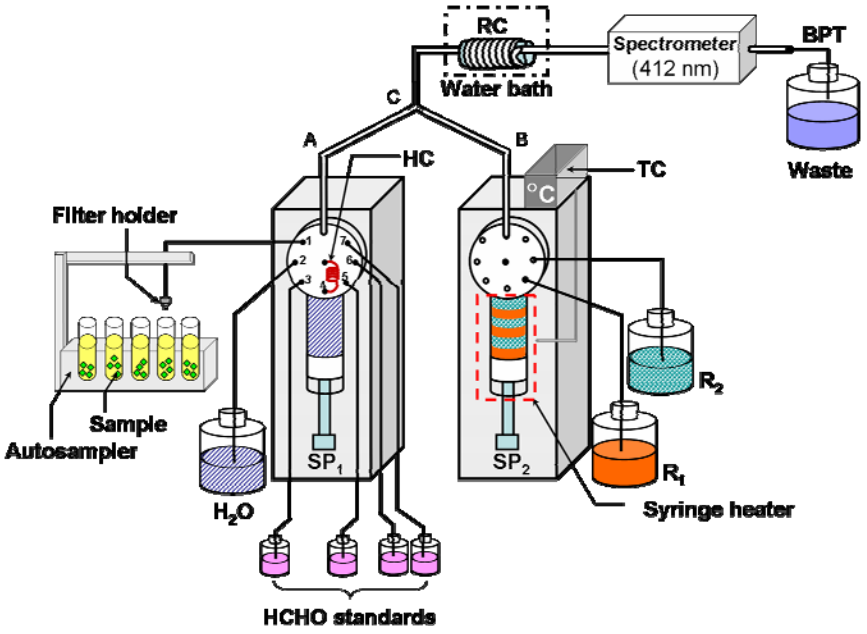


Figure 3.2 The HFA system developed for detection of formaldehyde in food: SP_1 and SP_2, syringe pumps; HC, holding coil (1 ml); TC, temperature controller set at 70 °C; RC, reaction coil (0.8 mm i.d. x 150 cm length); BPT, back pressure tube (0.5 mm i.d. x 15 cm length); R_1, 0.04 M 2,4-pentanedione; R_2, 2.0 M ammonium acetate.

In general, the HFA configuration for determination of HCHO was similar to the system presented for ammonia (Amornthammarong, Jakmunee, Li, & Dasgupta, 2006). As shown in Figure 3.2, the flow outlets from syringe pumps were connected at point C. The first pump (SP_1) was used for pumping a water carrier together with a sample into the system. The eight-port selection valve on SP_1, was slightly modified to fit a holding coil (HC) on top. The HC was used for holding a sample extract or HCHO standard zone. The second pump (SP_2) was used for introducing the reagents, 2,4-pentanedione and ammonium acetate, into the flow system. The syringe heater was constructed on pump SP_2. Heater was used to pre-heat these two reagents prior to introducing into the system for reaction with HCHO.

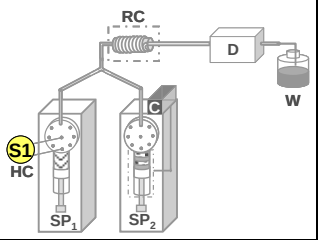
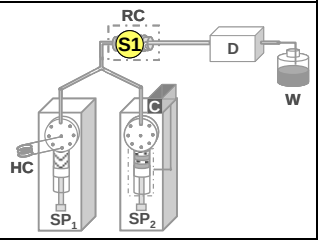
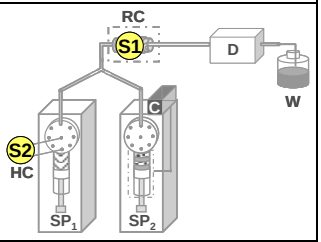
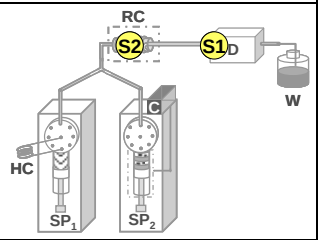
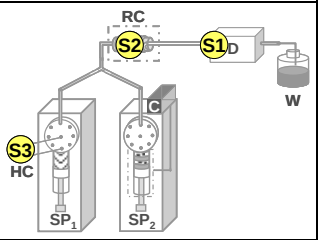
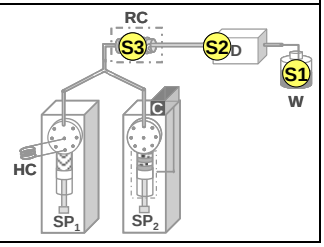
For the system in Figure 3.2, filtration of the sample was done on-line via a filter holder fitted at the end of the sampling probe. Small debris of sample extracts was then filtered out to prevent clogging of the flow system. A supernatant (350 µl) was introduced into the flow system by drawing from the autosampler (via port 1) into

the holding coil. Similarly to the introduction of sample, the HCHO standards were individually loaded into the HC coil (via ports 3, 5, 6 and 7).

Operation of multiple processing

Briefly, from Figure 3.2, the SP_2 was programmed to alternately introduce 2,4-pentanedione (R_1) and ammonium acetate (R_2) into the syringe allowing the two reagents to pre-mix and pre-heat. The SP_1 was programmed for aspiration of water carrier, following by aspiration of the aqueous extract. The analyte (from SP_1) and the mixed reagents (from SP_2) were transferred for merging at the confluence point C and then stopped in the reaction coil (RC). Degree of the cyclisation reaction of HCHO was enhanced. A yellow product of 3,5-diacetyl-1,4-dihydrolutidine (DDL) was formed. The product was moved further to detector in next cycle of operation. The detail of operation is summarized in Table 3.3. This procedure was designed for capable of three samples at the same time, which made analysis time faster.

Table 3.3 The procedure for multiple processing of the HFA system in Figure 3.2 for determination of HCHO in food

Step	Motion of pump	Duration (s)	Position of analytical zone
1 (load)	850 µl of carrier and 350 µl of sample 1 (S1) are drawn from the sample tube into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into the SP_2 syringe until total volume reaches 1,200 µl	30	
2 (inject)	SP_1 and SP_2 push all solutions from the two syringes to mix and stop at RC	56	
3 (load)	850 µl of carrier and 350 µl of sample 2 (S2) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1,200 µl	30	
4 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	
5 (load)	850 µl of carrier and 350 µl of sample 3 (S3) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1,200 µl	30	
6 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	

According to the procedure in Table 3.3, as the first sample (S1) is being heated in the reaction coil RC in step 3, the next sample (S2) is introduced into the holding coil, HC. During this time, pre-mixing and pre-heating of the R_1 and R_2 is being processed inside the heated syringe SP_2. Following this process, in step 4, S2 and the mixed reagents are then pushed by both syringe pumps into the reaction coil. When the syringe pistons are moved upward, the head of the S1-zone is therefore pushed further and stopped by at detector. In step 5, the third sample (S3) is now introduced into the coil, HC. Again, pre-mixing and pre-heating of R_1 and R_2 occur inside the SP_2 heated syringe. In step 6, S3 and the mixed reagents are pushed by SP_1 and SP_2, simultaneously, to replace S2 in the reaction coil. In step 6, the entire zone of S1 is now driven through the detection cell to waste, whereas S2 is just entering flow cell of the detector.

3.2.3 Syringe heater

In Figure 3.2, the dashed line drawn on SP_2 indicated approximate region of the syringe heater. The heater was assembled in order to pre-heat the mixed reagents. The schematic drawing of syringe heater is depicted in Figure 3.3.

The syringe heater was built using an electrical heating tape. The first layer was a sheet of aluminum foil, which was wrapped around the 5-mL glass syringe. On top of the foil, a temperature sensor was placed such that it was touching the foil layer for measurement of the temperature. The second layer was another layer of aluminum foil. The third layer was a layer in heating tape. This electrical heating tape was placed to surround the whole area of the foil to supply heat to the syringe. Another foil sheet was again wrapped around this syringe as the fourth layer. A microcontroller (TC in Figure 3.2) was employed for control the temperature.

In order to protect heat loss and to ensure that the heat was distributed evenly, the heating system was covered with sand bag. A rectangular sand bag was made from a piece of cotton cloth. The sand bag was simply sealed by sewing up all open ends. This bag was wrapped around the syringe to make the fifth layer (Figure 3.3). The sand bags were made to the right size to fit exactly the cavity of the PVC case, to achieve minimum heat loss. Finally, the whole syringe was covered up with 2 pieces of cylindrical PVC (Φ = 3.4 cm, used in general plumbing).

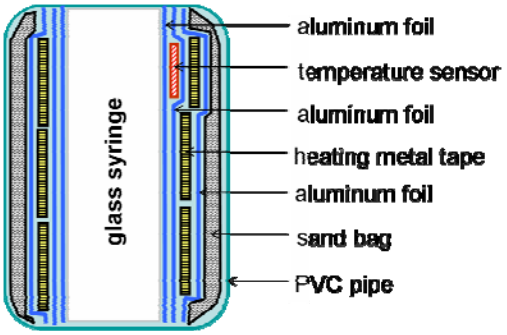


Figure 3.3 A schematic drawing (side view) representing construction of the syringe heater built on SP_2.

3.2.4 Pararosaniline (PRA) method for validation

A conventional method known as the pararosaniline method (Miksch, et al., 1981) was employed to validate the developed HFA method. The sample extract (1.00 ml) was transferred into a 5-ml volumetric flask containing 500 µl of 5.2 mM pararosaniline in 2.4 M HCl. To this mixture, 500 µl of 8 mM sodium sulfite was added. The mixture was made up to the mark with water and allowed to stand for 60 min before reading its absorbance at 570 nm. For this PRA method, a calibration graph from 5 to 50 µM was prepared.

3.3 Experimental design for measurement of sucrose, dissolved carbon dioxide and pigment in soft drink

3.3.1 Chemicals used for the monitoring of contents in soft drink

All solutions in this experinment were prepared in ultrapure water (Branstead EASYpure II, USA). Sucrose standard used in this work was commercial food grade (Mitr Phol, Thailand). A 60 Brix stock sucrose solution was prepared by dissolving exactly 60.00 g of solid sucrose in approximately 40.00 g of water with heating on a hot plate until the entire solid has been dissolved. After cooling to room temperature, water was added to this solution to give exactly 100.0 g. Working standards were obtained by appropriate dilution. The stock solution was kept refrigerated at 4 °C.

Sodium hydrogen carbonate ($NaHCO_3$) was prepared as a standard solution for CO_2 analysis. A stock of $NaHCO_3$ (1 M) was obtained by dissolving 8.4 g of $NaHCO_3$ (Merck, Germany) with 100 ml of water. Working standards for calibrator (0.075-0.15 M) were prepared daily by dilution with water from the stock.

Acid solution of 3 M hydrochloric acid (HCl) was prepared by transferring 25 ml of conc. HCl (LabScan, Ireland) into 50 ml of water. The final volume was made up to 100 ml with water.

3.3.2 Soft drink sample

Soft drink samples were purchased from Thai supermarkets. These samples were selected with different brands and colors to verify broad range of application of the developed SI method. Any pretreatment does not require.

3.3.3 Degassing unit (membraneless-vaporization unit)

A membraneless-vaporization (MBL-VP) unit (Sereenonchai, et al., 2007) was incorporated into the sequential injection (SI) module for on-line measurement of dissolved CO_2. The unit also serves as an on-line degassing in order to remove the CO_2 in sample prior the detections of sucrose and pigment. The unit was thus so-called a 'degassing unit'. This degassing unit allows for selective detection of CO_2 via gas-liquid separation by vaporization.

A schematic diagram of the degassing unit is shown in Figure 3.4. The design of this unit was identical to the MBL-VP unit used for analysis of carbonate in natural water (Sereenonchai, 2010). Water acceptor stream was flowed into acceptor channel while soft drink sample was drawn into donor chamber (see Figure 3.4(a)). Vaporization of CO_2 was accelerated by stirring with magnetic bar (400 rpm). The dissolved CO_2 in sample diffused via headspace and re-trapped into water acceptor. Re-dissolved CO_2 in water was then further transferred to detection.

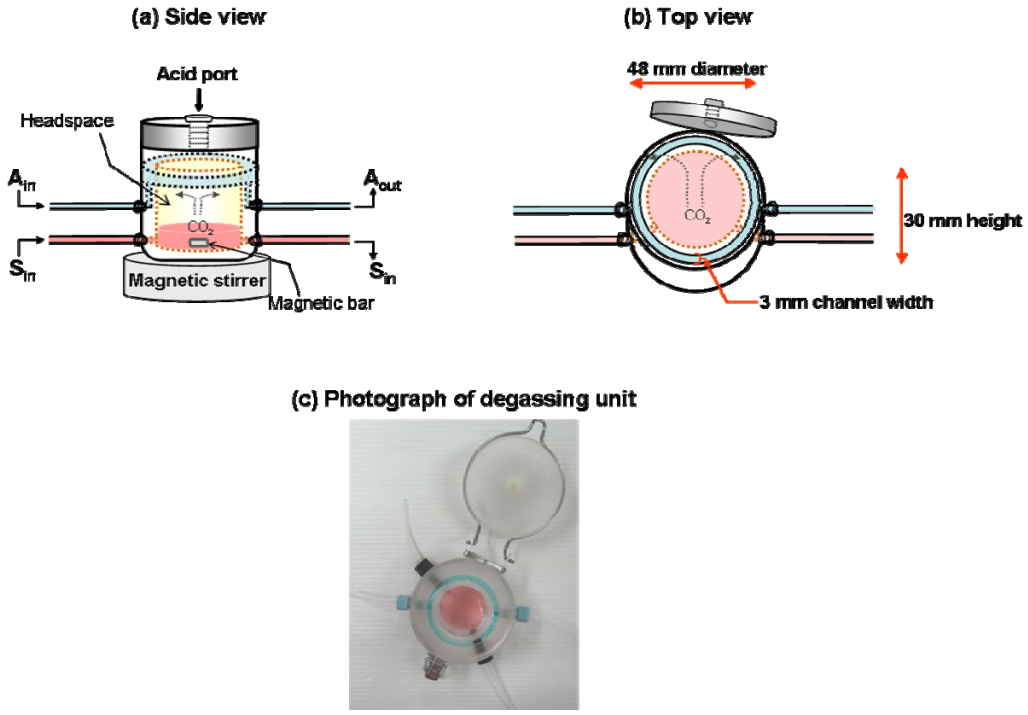


Figure 3.4 A schematic diagram of degassing unit: (a) side view, (b) top view, and (c) the photograph of the unit taken from top view.

For making CO_2 calibrator, $NaHCO_3$ standards were drawn into donor chamber. HCl solution was injected via acid port. Gaseous CO_2 was produced relying on the reaction shown in equation 3.1.

$$NaHCO_{3(aq)} + HCl_{(aq)} \rightarrow H_2O_{(aq)} + NaCl_{(aq)} + CO_{2(g)} \quad ..(3.1)$$

3.3.4 Capacitively coupled contactless conductivity detector (C^4D)

A C^4D (Kubáň & Hauser, 2008) was employed for detection of dissolved CO_2 in acceptor solution. The detection is based on changes in conductivity of acceptor solution, which is related to the amount of absorbed CO_2. In this work, axial tubular electrodes for the C^4D flow cell were used (da Silva & do Lago, 1998).

The flow cell of C^4D was made from PEEK tube (1 mm i.d. and 1.6 mm o.d.). The total length of the tubing was approximately 150 mm. The length of the two electrodes were 10 mm each and were made by painting the PEEK tubing with silver paint varnish (da Silva & do Lago, 1998), and with a gap of (0.2 ± 0.05 mm)

separating the electrodes. An AC signal (30 V, 100 kHz) was fed to the first electrode by using a function generator (Bangkok High Lab Co., LTD., Thailand). The AC current arisen at the second electrode was amplified, rectified and converted to voltage. The output voltage was later digitized before recording using software written on LabVIEW 8.0^{TM}. A configuration of C^{4}D flow cell is shown in Figure 3.5.

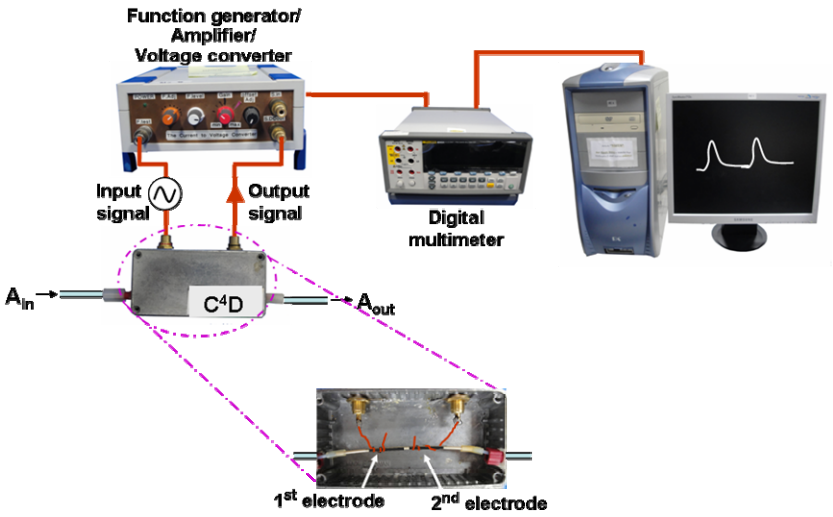


Figure 3.5 A configuration of C^{4}D flow cell; A_{in} and A_{out}, is flow direction of acceptor solution in and out the C^{4}D cell respectively. The enlargement shows arrangement of two electrodes inside the shielding box (5cm width x 10 cm length).

3.3.5 Sequential injection module

As shown in Figure 3.6, the SI module was assembled from two syringe pumps (SP_1 and SP_2) each equipped with eight-port selection valve (SV_1 and SV_2) (Kloehn Versa Pump 6, USA). The 10-ml zero dead volume syringes were fitted on the pumps. A 1 ml holding coil (HC) was connected to SP_2. Section A in Figure 3.6 was arranged for sucrose measurement. A portable photometer (Bangkok High Lab Co., LTD., Thailand) was equipped with a near infrared (NIR) light emitting diode (LED) (L890-06AU, Epitex, Japan). This light source emitted radiation in 890±40 nm. The selection valve SV_2 was also furnished with another portable photometer (Bangkok High Lab Co., LTD., Thailand), equipped with a Red-Green-Blue (RGB) LED (SMT-RGB, Epitex, Japan), which emits blue (470 ± 25 nm), green (525 ± 40

nm) and red (640 ± 20 nm) lights. This second detector was used in the measurement of pigment content in the soft drink (Section B, in Figure 3.6). Quartz flow cells, 10 mm light path and 80 µl internal volume (Hellma, Germany), were used in these photometers. The software for recording the signals of the photometers was written using Visual Basic.

The SI module was also assembled with a degassing unit for removing CO_2 from the sample. This manifold was denoted as section C in Figure 3.6. The unit also had a separate channel to flow in water as acceptor stream (section D, Figure 3.6), in order to trap CO_2 vapor for detection by a C^4D. The acceptor flow was continuously maintained by using a peristaltic pump (Ismatec827, Switzerland).

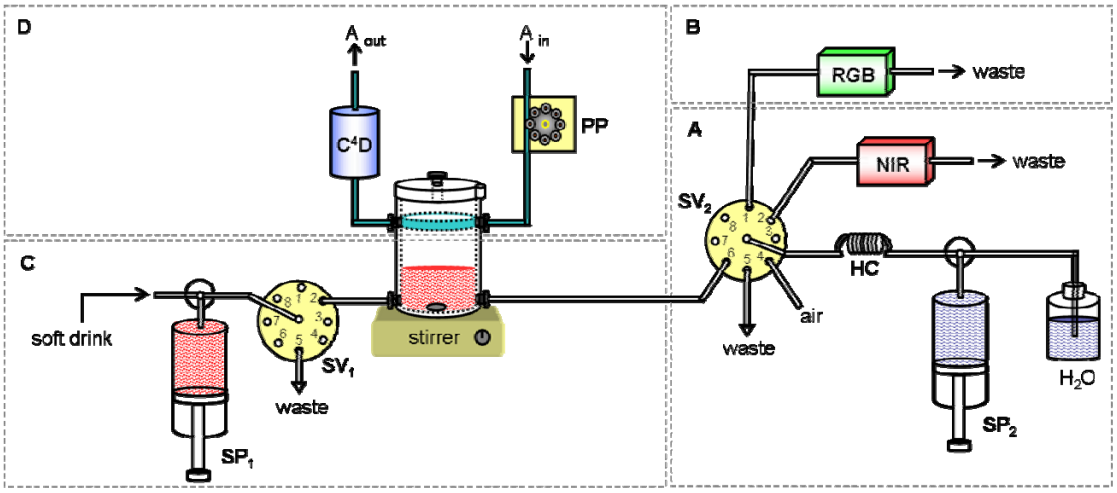


Fig. 3.6 The SI module for on-line measurement of soft drink; for sucrose content (section A) and pigment (section B). The module includes a degassing unit (section C) with detection for CO_2 content (section D). SP_1 and SP_2; syringe pumps, SV_1 and SV_2; selection valves, NIR; portable NIR-photometer, RGB; portable LED-photometer, C^4D; capacitively coupled contactless conductivity detector, PP; peristaltic pump, A_{in} and A_{out}; acceptor stream (water) flowing in and out.

3.3.6 System operation

At the start of the analysis cycle, carbonated drink sample was transferred into the degassing unit (section C in Figure 3.6) by a syringe pump SP_1, while water stream was filled in acceptor channel by using a peristaltic pump. The sample was

degassed via agitation, some of the gaseous CO_2 dissolved in the acceptor stream. When water acceptor absorbed with CO_2 was flowed to C^4D (section D in Figure 3.6), the signal then increased. Meanwhile, degassed sample was drawn into a section A for consecutive measurements of sucrose and pigment contents, respectively.

In section A of Figure 3.6, a water carrier was drawn into a syringe pump SP_2. A 300 μl of degassed sample was subsequently drawn into a holding coil (HC). A selection valve SV_2 was then switched to connect a NIR spectrometer via port 2. A plug of sample was finally propelled to the NIR spectrometer. Due to the difference of RI between water carrier and sample, the schlieren signal is thus obtained. The schlieren signal corresponds to amount of sucrose in soft drink.

For measurement of pigment, a 3000 μl of air was aspirated into the SP_2. Afterward 800 μl of degassed sample from the unit was introduced into a holding coil (HC). An air-sandwiched segment sample (Skeggs, 1966) was then pushed to a RGB detector via port 1 of SV_2 for measurement of intensity of pigment (section B). The entire steps of operation are given in Table 3.4.

Table 3.4 Operational step for the SI module shown in Figure 3.6 for analysis of sucrose, pigment and dissolved CO_2 content in carbonated soft drink

Step	Pumps			Connection on SV_1	Connection on SV_2	Flow rate (mL min^{-1})	Motion	Period (s)	Type of analysis
	SP_1	SP_2	PP						
1	on	off	on	SP_1-SV_1-P_2	-	12.5 (SP_1) 3.5 (PP)	3500 µl of carbonated sample is aspirated into the degassing unit. The acceptor line is being filled with water.	15	
2	off	off	off			-	Sample solution is stirred to remove CO_2. CO_2 gas partially dissolves into water acceptor.	60	CO_2
3	off	off	on			3.5	The zone of CO_2 is transferred to C^4D.	20	
4	off	on	off		P_6-SV_2-SP_2	12.5	6000 µl of water carrier is introduced into SP_2, subsequently 300 µl of degassed sample is drawn from unit to the holding coil.	30	Brix
5	off	on	off		SP_2-SV_2-P_2	2.0	All solutions were pushed to NIR spectrometer	180	
6	off	on	off		P_4-SV_2-SP_2 P_6-SV_2-SP_2	12.5	3000 µl of air carrier is aspirated into SP_2. 800 µl of degassed sample is pulled into holding coil.	15	
7	off	on	off		SP_2-SV_2-P_1	4.0	Sample plug is transferred to RGB detector.	60	Pigment
8	on	off	off	P_2-SV_1-P_5		12.5	The rest of sample in degassing unit is flushed out to waste	10	
							Total /cycle	**390**	

SP: syringe pump
PP: peristaltic pump
P: port
SV: selection valve

3.3.6 Refractometric method for validation

The results of sucrose analysis by the SI system were validated with the refractometry (Ramasami, et al., 2004). A measurement was carried out by using the Abbe refractometer (Bausch & Lomb, USA). The room temperature was controlled at 25 °C during the measurement. Few drops of degassed sample were put on prism assembly of the refractometer. The lamp was turned on. The lamp position was adjusted in order to sharpen the borderline between the light and the dark regions (look through eyepiece). Finally, the index of refraction was read out. For this refractometric method, a calibration graph from 10 to 60 Brix was prepared.

CHAPTER IV
RESULTS

The data obtained from the experiments are presented in this chapter. These data are analyzed and categorized into three main parts. Students' understanding and perception on the flow demonstration together with POE strategy are evaluated in the first part. Results from the method development for analysis of formaldehyde are explained in the second part. And the third part showed the results from a new method for monitoring contents in soft drink.

4.1 Development of the predict-observe-explain (POE) strategy for teaching chemistry at undergraduate level

4.1.1 Students' understanding in flow phenomena

In author opinion, the POE document is equivalent to a pretest and a posttest by itself. In the prediction phase, each student justifies their prediction with reasons based on their existing knowledge. This not only can be used for finding students' prior concepts, but also helps teacher to plan the teaching in action. Thus result obtained from prediction is comparable to pretest outcome. Observation is an essential stage of POE strategy. This process requires all senses for collecting data and use of the scientific method for transferring the encounter into knowledge (Oğuz-Ünver & Yürümezoğlu, 2009). A social constructivist activity involves in constructing consensual meaning through discussions and negotiations by learners (Kearney, 2004). In the explanation phase, small group discussions were arranged. Students had opportunity to construct their own meaning via peer process. Group discussion also provides students to practice the discourse of science. The information which students provided on their observation and explanation of POE task is thus comparable to the post-learning outcome.

The first demonstration of flow phenomena was to inject an aqueous dye solution into a flowing stream of water carrier. Figure 4.1 is an example of the first POE document. An example of student's preconception is shown in Figure 4.1(a). It shows that student could picture the extending and fading of sample zone when it travels along carrier stream. However the student did not realize the laminar flow effect which makes a zone shape like bullet. Also heterogeneity of zone has not been considered yet. After observation, all flow phenomena could be detected by student (Figure 4.1(b)). Group discussion was encouraged by teacher. Some vital information and scientific terms such as convection, diffusion, linear velocity and laminar flow profile were introduced. Thus students could keep on conversation and gave description with meaningful words.

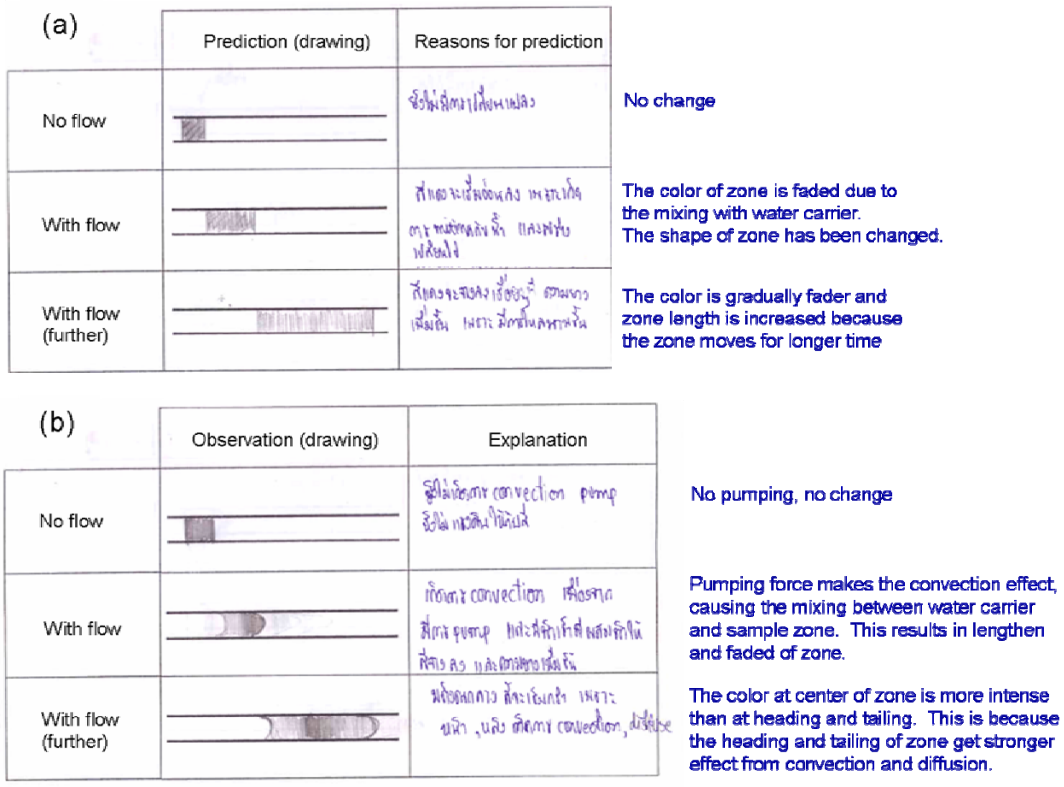


Figure 4.1 An example of the first POE document obtained from a student, (a) prediction with reason and (b) Observation with explanation

The overall students' responses both before and after the demonstration, are shown in Figure 4.2. In the prediction stage, more than 90% of the students recognized the change of sample zone in terms of elongation and dilution. Half of the students mentioned the laminar flow pattern. Less than 12% of the students mentioned the heterogeneity of zone. After observation students up to 95% perceived all flow phenomena and drew the shape of sample zone correctly. The laminar shape was raised up to discuss. Students attempted to explain the phenomena, with guidance from teacher. It was discussed that liquid flowing in cylindrical tubing, has some velocities with different values at various positions. The core of zone has the highest linear velocity. The velocity gradually decreases until at the contact of the tubing wall, the liquid there has the lowest velocity due to retardation with friction against tubing wall. This causes a bullet shape and elongation of the dye zone when liquid is flowing. Molecular diffusion, which was driven by such a concentration gradient together with the convection occurred by mechanical flow agitation, were also discussed. Both kinds of dispersion were described in either axial or radial direction. Students explained the dilution and heterogeneity of the zone based on the dispersion effect.

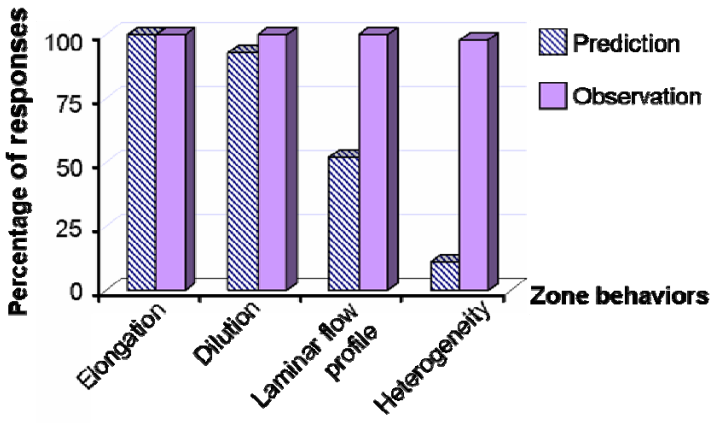


Figure 4.2 Student responses to a situation of what phenomena happen when a plug of dye travels along stream of water carrier. Students' prediction and observation were compared.

In the second demonstration, reaction of ferric ion (Fe^{3+}) and thiocyanate (SCN^-) was employed for presenting how FI system facilitates the degree of a chemical reaction. The teacher introduced the reaction to students so that students could detect when the reaction occurs. When the two reagents are mixed, some red-colored products were generated. The chemical reaction is shown in equation 4.1.

$$\underset{\text{colorless}}{Fe^{3+}_{(aq)}} + \underset{\text{colorless}}{SCN^-_{(aq)}} \rightarrow \underset{\text{red}}{Fe(SCN)_x^{(3-x)}_{(aq)}} \qquad ..(4.1)$$

Note: x = 1 to 6

POE task was run again to probe student's idea. It was found that up to 80% of students can prefigure physical characteristic of sample zone when it moved due to laminar flow inside small channel. At this moment students have not yet imagined how much impact of flow convection can proceed the mixing of the two colorless reagents, causing red product. The demonstration started by injecting a plug of ferric solution into the running channel filled up with thiocyanate reagent carrier. Under no flow condition, reaction took place only at the interface of two solutions due to dynamic diffusion. About 30% of students predicted this situation correctly, while 52 % of them guessed that reaction had not started yet and the rests guessed that reaction went through the zone immediately after injection. Figure 4.3 illustrates three examples of drawings and descriptions that students reflect their predictions.

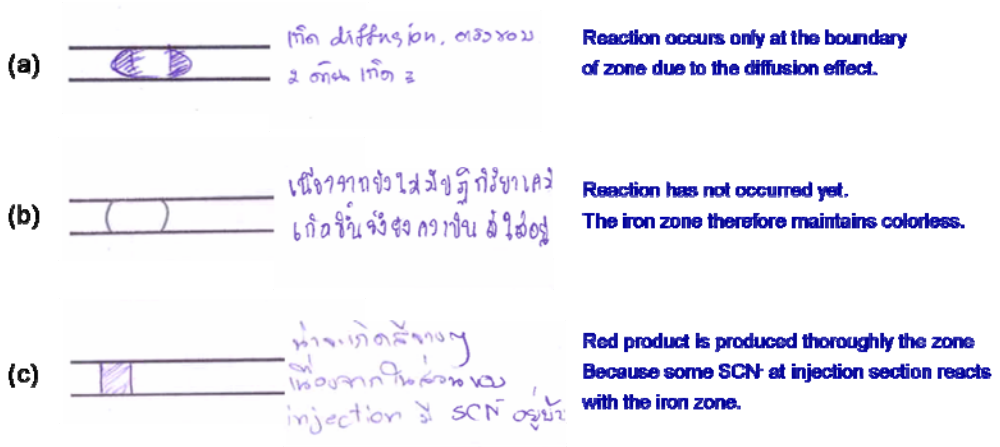


Figure 4.3 Student predictions on the second POE task of when ferric solution was injected into a thiocyanate stream with no flow. Students predicted (a) reaction occurred at boundary of sample zone, (b) no reaction occurred after injection and (c) reaction occurred immediately after injection.

The red-colored product of the iron complexes was generated thoroughly in the zone in few seconds with flowing condition. This shows that the chemical reaction was easily produced via flow convection. Students observed the circumstance and then recorded and discussed about what they saw. The example of observation that a student drew down on the second POE is given in Figure 4.4

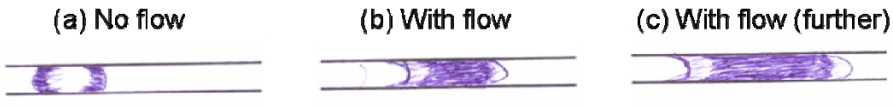


Figure 4.4 A student's observation on the second POE task. The drawing represents (a) reaction occurred at boundary with no flow, (b) and (c), reaction was produced through the zone with flow. The physical phenomena were also observed and recorded.

To ensure complete understanding of students without missing any concept, oral presentation was carried out prior to ending the class. Groups of three students presented their consensual conclusion to class and then class discussion and

negotiation was initiated simultaneously. Teacher contributed profound knowledge to the class for an in-depth understanding of the students. At the end of class discourse, students understood the flow phenomena including their effects which influenced to behaviors of liquid bolus. The students perceived that flow not only facilitates chemical reaction, but also transports chemical solutions to detection automatically. Students thus realized that the flow method makes chemical analysis faster than batch method and also reduces manual work of chemists.

4.1.2 Student perception to the demonstration

Students provided their reflections about the demonstration after finishing the activities. They strongly agreed that the demonstration helped them to visualize what phenomena could occur and how chemical reaction could be facilitated to a certain degree under laminar flow in a FI system. The demonstration supported them understand the flow phenomena, including the concept of FI technique. Students also strongly agreed that the demonstration made a class interesting. This also enhanced their learning in FI topic. The students' perception to demonstration presents in Table 4.1

Table 4.1 Students' response on items relating to the perception of students to the demonstration

Items	Cat.	Mean	SD
▪ The demonstration helped student understanding flow phenomena occurred in FI system	D	4.67	0.56
▪ The demonstration helped student visualize flow phenomena occurred in FI system	D	4.65	0.57
▪ The demonstration helped student understanding a concept of FI technique	D	4.51	0.59
▪ The demonstration was interesting	I	4.44	0.66
▪ The demonstration supported student to learn FI technique	D	4.40	0.53

D = Roles of the demonstration

I = Effect of demonstration on students interest

4.1.3 Student perception to the POE approach

According to the results in Table 4.2, the students agreed that teacher clearly explained how to conduct POE documents before started activity. They therefore understood how to use these documents. The students reflected that POE was a tool to make them think critically. They used scientific knowledge to explain what they observed. Also, they attempted to correlate their prediction and observation. The students felt that the POE was a valuable material for their learning. In addition students enjoyed learning through the POE way.

Table 4.2 Students' response on items relating to the perception of students to the learning with POE

Items	Cat.	Mean	SD
▪ Teacher explained the use of POE document clearly	C	4.40	0.75
▪ POE supported student to think rationally	E	4.19	0.58
▪ Student made a comparison between the prediction and observation.	C	4.19	0.45
▪ Student explained the observed phenomena based on scientific knowledge	C	4.09	0.52
▪ POE was a useful material for student's learning	E	4.01	0.62
▪ Student understood what to do with POE	U	3.86	0.66
▪ Student enjoyed the use of POE	I	3.70	0.82

C = Classroom interaction U = Effect of POE on students understanding
E = Efficiency of POE I = Effect of POE on students interest

4.1.4 Participation in a group discussion

An expected outcome of group learning is that students should be able to communicate and argue with peer in order to justify and defend their ideas. Group learning provides co-construction of understanding among peer. Another reason for implementing group discussion in the activity is to provide team work learning, which is the one of scientist's manners.

From questionnaire, the students mentioned that group discussion allowed them to share and learn from each other. They learned to work as a group. The students made a discussion based on scientific information. The conceptual understanding was developed through collaborative discussion. Finally, they had opportunity to contribute the consensual conclusion to the whole class for discussion. The students' reflection to group discussion is summarized in Table 4.3

Table 4.3 Students' response on items relating to the participation in a group discussion

Items	Cat.	Mean	SD
▪ Student used scientific knowledge to support a discussion	P	4.14	0.55
▪ Student developed understanding in flow concept.	P	4.09	0.67
▪ Student learned ideas from peer	P	4.09	0.56
▪ Student shared own ideas with peer	P	4.02	0.66
▪ Student learned how to work in a team	P	3.88	0.81
▪ Students shared a consensual conclusion to a whole class discussion.	P	3.79	0.74
▪ Student had opportunity to ask questions	P	4.35	0.64

P = Students participation

4.1.5 Role of teacher in the classroom

In an environment of constructivist learning, teacher acts as a facilitator who provides important and relevant information as well as leads discussion to a classroom. This is to foster students to learn and construct their own meaning knowledge.

The students strongly agreed that teacher played an important role to encourage discussions. They also felt that teacher was very friendly. In the views of students, teacher had posing questions as well as asked students for opinions. Students had a chance to ask questions and the teacher paid attention to answer their questions. The rating students gave to teacher shows in Table 4.4.

Table 4.4 Students' response on items relating to the teacher performance in the classroom

Items	Cat.	Mean	SD
▪ The teacher was friendly	T	4.63	0.48
▪ The teacher encouraged student to discuss in a group	R	4.55	0.54
▪ The teacher is interested to answer student's questions	T	4.47	0.62
▪ The teacher asked student's opinions	R	4.44	0.54
▪ The teacher posed questions to arouse student thinking	R	4.33	0.46

R = Roles of the teacher T = Teacher expression

4.2 Multiple processing hybrid flow system for analysis of formaldehyde contamination in food

Besides the development of the POE strategy for teaching flow injection in a classroom, the author also carried out two types of method development for chemical analyses based on use of the flow-based format. The followings are results of development of a multiple processing HFA system for monitoring formaldehyde contamination in food.

4.2.1 Efficiency of the syringe heater

From literatures, it is known that heating can improve the sensitivity of the Hantzsch reaction (Sritharathikhun, Oshima, & Motomizu, 2005; Zhao & Zhang, 2009). In the building of a HFA for formaldehyde analysis, an electrical heating jacket for the syringe SP_2 was therefore constructed for the system. This heating jacket surrounded the glass syringe SP_2 for pre-heating the mixture of reagents stored in the syringe.

In order to test the effectiveness of the heating device, the flow system in Figure 3.2 was used with and without operating the heating device. Calibration graphs

of formaldehyde standard solutions from 10 to 100 μM were obtained by operating the flow system as shown in Table 4.5.

Table 4.5 The initial procedure employed for operating the flow system in Figure 3.2 during the preliminary studies and system optimization for formaldehyde analysis

Step	Motion of pump	Duration (s)
1 (load)	3100 μl of carrier and 500 μl of sample are respectively drawn from the sample tube into the syringe by SP_1. 100 μl of reagents (R_1/R_2) are alternately drawn from reservoirs into the syringe of SP_2 until total volume reaches 3600 μl.	150
2 (inject)	SP_1 and SP_2 push all solutions from the two syringes through to the detector.	300
	Total	**450**

R_1: 0.04 M 2,4-panetanedione, R_2: 2.0 M ammonium acetate

As expected, the sensitivity of the system using the heated jacket was increased by a factor of 2. The calibration equation obtained for 70 °C was $\Delta A = (8.67\pm0.53)x10^{-3}$ [HCHO, μM] + $(2.08\pm2.96)x10^{-3}$. The calibration obtained at 25 °C was $\Delta A = (4.55\pm0.10)x10^{-3}$ [HCHO, μM] + $(0.22\pm0.55)x10^{-3}$. In Figure 4.5, the highly reproducible signals (%RSD = 0.7) obtained over extended operating period indicated that the heating jacket was efficient. Another indicator of poor heating efficiency is baseline shift. In this design, the baseline is very stable (Figure 4.5). A constant baseline over 36 h of operation could be accomplished. This electrical heating jacket was thus employed for all further experiments and was operated at 70 °C.

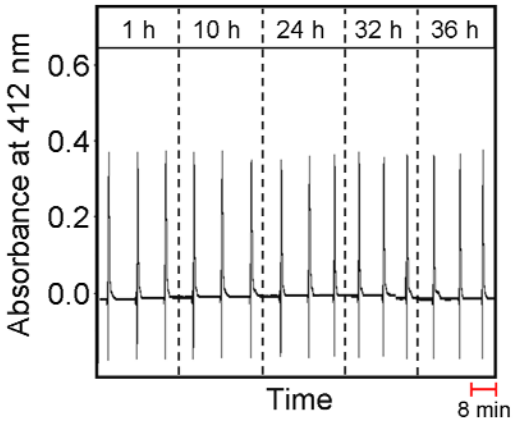


Figure 4.5 Reproducible signals of 40 μM HCHO illustrating the efficiency of the homebuilt syringe heater (the operating procedure in Table 4.5 was used).

4.2.2 Design of a procedure for multiple processing

Initially, a normal procedure listed in Table 4.5 was used to operate the HFA system for analysis of formaldehyde. Although this procedure seems to be practical, the operation based on this procedure is carried out sample by sample. The analysis time for a sample is 7.5 min, which includes the time taken for sample introduction, premixing and pre-heating of the reagents (R_1 and R_2) inside the SP_2 syringe and finally for detection of the signal at spectrometer. Introduction of the next sample does not start until the cycle of the previous sample is completed. The sample throughput of this procedure is therefore very low (8 samples h^{-1}).

In order to improve the sample throughput, a new procedure was then designed to operate the same flow system. Table 3.3 shows the new procedure, which can offer a reasonably high sample throughput up to 51 samples h^{-1}. With this new operating procedure, the flow system in Figure 3.2 is capable of doing multiple processing of 3 samples at a time.

According to step 4 in Table 3.3, as a result of the push by the pumps, the head of the S1 zone is moved forward and then stopped at a detector. A part of the signal of S1 therefore appeared on a recorder. In step 5, the third sample (S3) is introduced into the coil (HC) while S2 was heated in the reaction coil (RC). The S1 zone stays at the same place as in step 4, at the detector. The signal level of S1 thus stays constantly during the system processing in step 5. In step 6, the S3 is pushed to replace S2 at the RC. With this moment of step 6, the entire zone of S1 is finally

driven through the detection cell resulting in achievement of the complete signal of S1 (shown in Figure 4.6a), whereas S2 is just entering at the detector. After achieving the complete profile of S1, the system then handles 3 samples at a time, by repeating step 1 to step 6 in Table 3.3. At this second repetition, the profile of S2 will appear in step 1 and finish at the end of step 2 (Figure 4.5b). The profile of S3 will appear in step 3 and step 4 (Figure 4.5c), and so on.

Since the developed system has multiple processing, the signal profiles obtained from this system (Figure 4.6) are therefore different to what normally seen with general flow systems. In Figure 4.6c, the measurement of the height of a signal obtained from 100 μM HCHO (ΔA =0.994) is illustrated. The zero absorbance reading of the detector was set with water. The calculation is from the zero absorbance of baseline reading to the top of the signal.

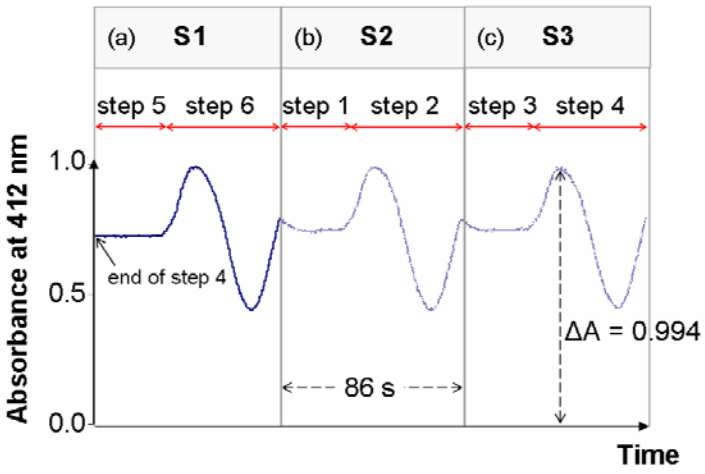


Figure 4.6 Signal profiles obtained from consecutive introduction of the liquid standard of HCHO (100 μM), representing the ability of the flow system in multiple processing of three samples (S1, S2 and S3) all at the same time, under the operating procedure in Table 3.3.

4.2.3 System optimization

Effect of flow rate

Effect of flow rates for lines AC and BC of the system (Figure 3.2) were examined. To simplify the system optimization, flow rates of the two lines were set equally. Since the increase in the flow rate would reduce reaction time, it was observed that the signal decreased as the flow rate was increased from 0.1 to 0.8 ml min^{-1} (Figure 4.7). As a compromise between the signal size and speed of analysis, 0.6 ml min^{-1} was chosen as the optimum flow rate.

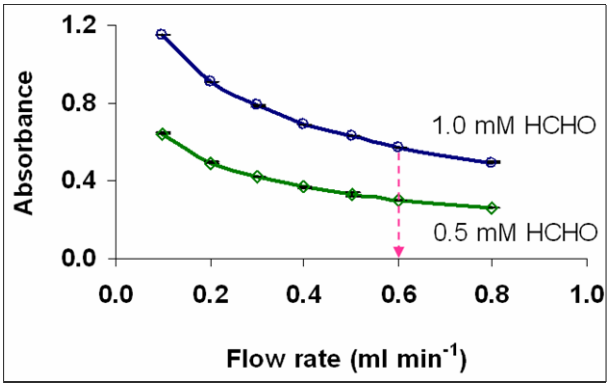


Figure 4.7 Effect of flow rate on absorbance signal. Condition: 200 µl of injection volume, 0.04 M 2,4-panetanedione and 2.0 M ammonium acetate. Two standards of HCHO were tested to ascertain the results.

Effect of Injection volume

Various sample volumes ranging from 50 to 1,200 µl were investigated. The signal increased with increasing in the volume of sample, before reaching a plateau when greater than 500 µl (Figure 4.8). In this work, we selected 350 µl as the sample volume instead of 500 µl to reduce the time to process the sample.

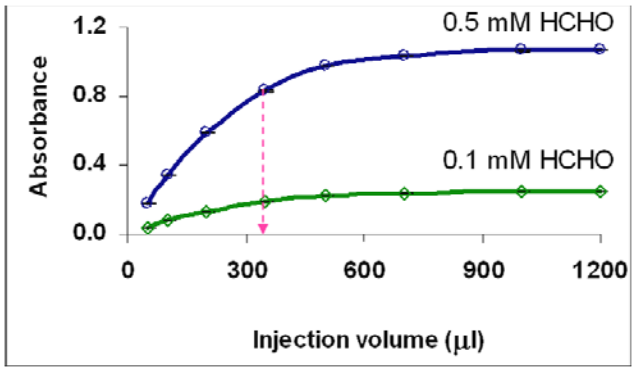


Figure 4.8 Effect of injection volume on absorbance signal. Condition: 0.6 ml min^{-1} of flow rate, 0.04 M 2,4-panetanedione and 2.0 M ammonium acetate. Two standards of HCHO were tested to ascertain the results.

Effect of concentration of reagents

The effect of concentrations of 2,4-pentanedione was studied. The signal increased drastically from 0.02 to 0.03 M, and stayed more or less constant from 0.03 to 0.05 M. Therefore, 0.04 M of 2,4-pentanedione was chosen (Figure 4.9a). Ammonium acetate concentrations of 1 to 4 M were also investigated and it was found to have insignificant effect on the signal at concentrations above 2 M. For this work, 2 M of ammonium acetate was chosen (Figure 4.9b).

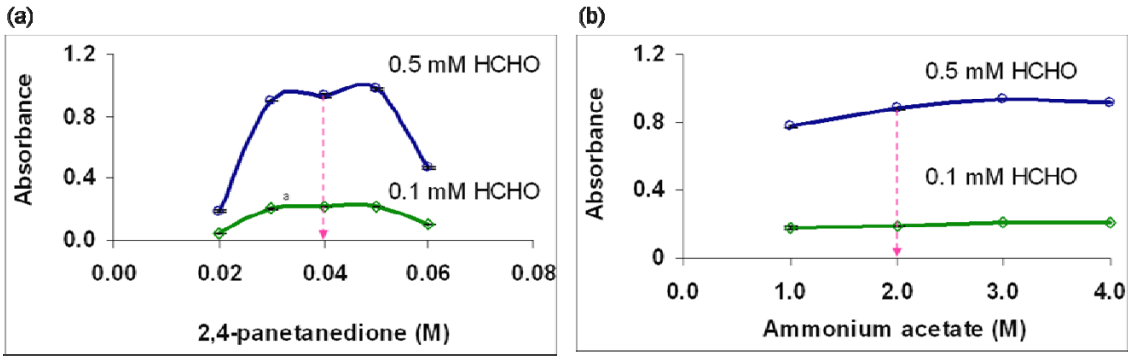


Figure 4.9 Effect of reagents concentration on absorbance signal. (a) Condition: 0.6 ml min^{-1} of flow rate, 500 µl of injection volume and 3.0 M ammonium acetate. (b) Condition: 0.6 ml min^{-1} of flow rate, 500 µl of injection volume and 0.04 M 2,4-panetanedione. Two standards of HCHO were tested to ascertain the results for each reagent.

Effect of temperature

In order to utilize the Hantzsch reaction more proficiently, heat is often involved to accelerate the reaction rate. The reaction coil (RC in Figure 3.2) was immersed in a thermostatic bath. As expected, the signal was improved as the temperatures at RC were increased from 25°C to 80°C (Figure 4.10). However, we observed interfering effect from air bubbles occurs from time to time when temperature was set at higher than 50°C. By the addition of a back pressure tube (BPT) in Figure 3.2, temperatures of up to 70°C were able to be used without interference from bubbles during the day. In this work, the bath temperature at 70°C was thus selected.

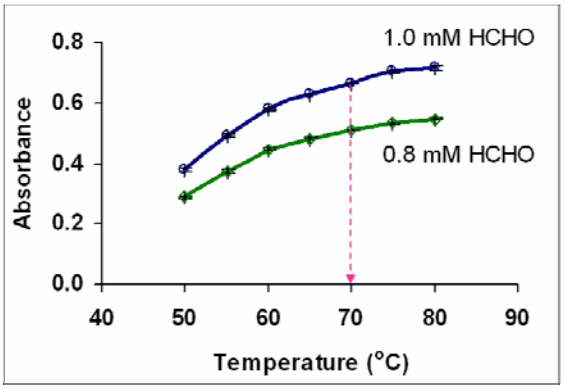


Figure 4.10 Influence of temperature on absorbance signal. Condition: 0.6 ml min^{-1} of flow rate, 200 µl of injection volume, 0.04 M 2,4-panetanedione and 2.0 M ammonium acetate. Two standards of HCHO were tested to ascertain the results.

For the HFA system in Figure 3.2, heating systems were finally employed at two places. As described in the section 3.2.3, a syringe heater was constructed at SP_2 syringe for warming up the reagents before reaction. Another heating system was also employed at the reaction coil (RC). The temperature was set equally at 70°C for residual experiments.

4.2.4 Analytical performance of hybrid flow system

Under the multiple procedure listed in Table 3.3 and the optimum conditions, the plot between peak height (ΔA) and concentration of HCHO over the range of 10 to 100 μM was linear (ΔA = (9.85±1.34)x10^{-3} [HCHO, μM] – (7.35±1.20)x10^{-3}, r^2 = 0.999). The signal profiles with calibration are illustrated in Figure 4.11. Ten replicate injections of 40 μM HCHO were carried out, from which the system's precision of 0.9% (RSD) was obtained. A value of 1 μM was shown experimentally to be the limit of detection or LOD (3SD of blank signal, n = 7) of a sample extract. This leads to the LOD of 0.06 mg kg^{-1} for solid sample. For HFA method, the limit of quantitation or LOQ (5SD of blank signal) was found to be 0.1 mg kg^{-1}. The LOQ of the method is sufficient and meets the acceptable levels in vegetable (63 mg kg^{-1}) (Food and Environmental Hygiene Department (FEHD), 2002) and sea food (10 mg kg^{-1} for crustaceans and 60 mg $kg^{-1}$for *Gadidae* fish) (Bianchi, Careri, Musci, & Mangia, 2007).

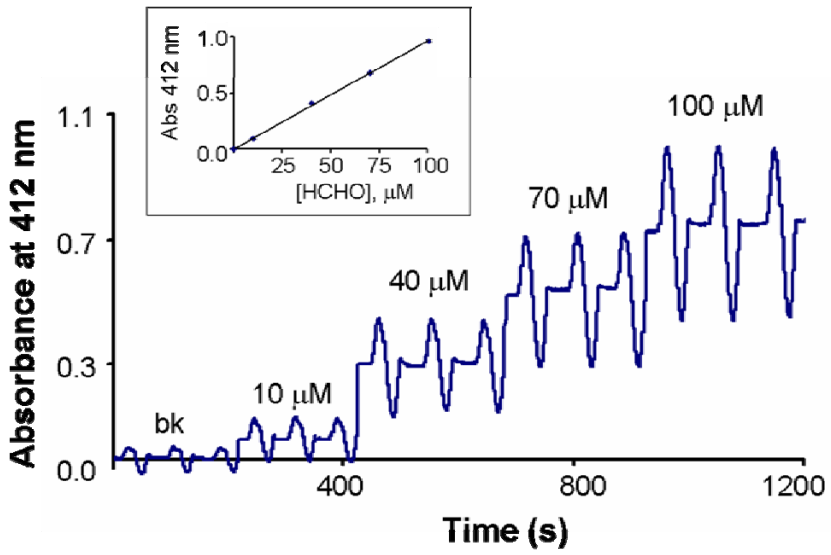


Figure 4.11 Signal profiles obtained from the multiple processing hybrid flow system developed for determination of HCHO (the values are concentrations of HCHO standards). The calibration curve is shown as the inset figure.

4.2.5 Application to food samples

The HFA method was applied to local food samples that were high in risk of contamination with HCHO. The first one selected to analyze was re-hydrated dry squid, which HCHO was sometimes found in a distinctive amount to preserve the freshness after re-hydration (Office of Food and Drug Administration Thailand, 2002). Some vegetable leaves were also known to sometimes sprayed with HCHO to make the leaves look fresh and appealing. For this work, yard long beans, cabbages and kales were chosen as representative vegetable sample. Other three kinds of mushroom such as shitake, Jew's ear and straw mushroom were also tested. These selected samples are commonly used in Thai dishes.

The total 18 samples of vegetables and mushrooms were analyzed by using the developed HFA method. The results indicated fortunate news that the collected samples were clean.

Analysis carried out by the HFA method for re-hydrated dry squid (Table 4.6) showed that there were two samples (Sq1 and Sq2) found to contain high level of HCHO (from 11.81$\pm$ 0.02 to 12.37 $\pm$ 0.03 mg kg^{-1}). For Sq3 and Sq4, the levels were more or less comparable with the normal levels found in cuttlefish (2.91 to 3.27 mg kg^{-1}) (Bianchi, et al., 2007). The levels of HCHO in Sq5 and Sq6 were considered low, however lying at detectable levels of the developed method.

Table 4.6 The results of formaldehyde analysis in re-hydrated dry squid obtained from current HFA method and from the conventional paraosaniline (PRA) method

Sample of re-hydrated dry squid	[HCHO, mg kg^{-1}], n = 3	
	Our method	PRA method
Sq1	12.37 $\pm$ 0.02	12.67 $\pm$ 0.03
Sq2	11.81 $\pm$ 0.03	10.51 $\pm$ 0.05
Sq3	4.51 $\pm$ 0.04	4.24 $\pm$ 0.00
Sq4	4.10 $\pm$ 0.10	4.30 $\pm$ 0.00
Sq5	0.26 $\pm$ 0.01	0.16 $\pm$ 0.00
Sq6	0.31 $\pm$ 0.02	0.24 $\pm$ 0.00

Beside analysis by using the developed HFA method, the samples were also analyzed for HCHO by using the conventional PRA method (Miksch, et al., 1981). The results given by the two methods in Table 4.6 were adopted for statistical comparison using the paired *t*-test (Miller & Miller, 1993). There was no significant difference ($t_{observe}$ = 1.188, $t_{critical}$ = 2.776, P = 0.05) in the sets of results obtained from the developed method and from the conventional method. This consistency between the results from the two methods clearly demonstrated that the newly developed flow method is suitable for analysis of HCHO contamination in food samples.

Recovery studies were carried out using another set of squid samples (n = 8) containing low level of formaldehyde from 0.26 to 0.88 mg kg^{-1}. A 20 μM of standard HCHO was spiked in all sample solutions. Analytical recoveries from 93 to 112 % were obtained from these squid samples. The recoveries carried out on vegetables' and mushrooms' extracts were from 92 to 113 % (18 samples). These reasonable recoveries indicate that components in the sample matrices did not interfere in the current HFA method.

The method was applied for analysis of frozen fishes. The interference of alpha-amino acid on the analysis of formaldehyde content was investigated. Recoveries found in fish samples were very poor in range of 60-80%. These results correspond with those obtained from Lu et al. (2005). They found that the consumed quantity of formaldehyde is correlative with the protein concentration in fish. Therefore, the determination of formaldehyde content in fish is not actual amount, but just a residue after its reaction with the alpha-amino acids or free amino groups on the protein surface.

4.3 Sequential injection module for monitoring of sucrose, carbon dioxide and pigment in soft drink

This section presents results from the second piece of work based on use of the knowledge in flow analysis. In this work, the second generation of FI called SI technique was employed in the construction of a system capable of on-line monitoring of three parameters often required for beverage industry.

4.3.1 Measurement of sucrose

4.3.1.1 Selection of light source

In this work, measurement of sucrose was carried out using schlieren effect. Theoretically, any wavelength not absorbed by the sample should be suitable for detection of the schlieren effect. For a colorless aqueous sample containing sucrose, any visible wavelength would be appropriate. For colored samples such as those in popular carbonated drinks, e.g., orange, strawberry, mixed fruit and cola drinks, the wavelength must be carefully chosen to ensure that only the RI effect is detected. Results from the absorption spectrums obtained from carbonated drinks (Figure 4.12), suggested that if a NIR light source is used, sucrose content can be detected without prior decoloration of the samples. In this work, a near infrared LED (890 ± 40 nm) was fitted into the photometer used for sucrose analysis.

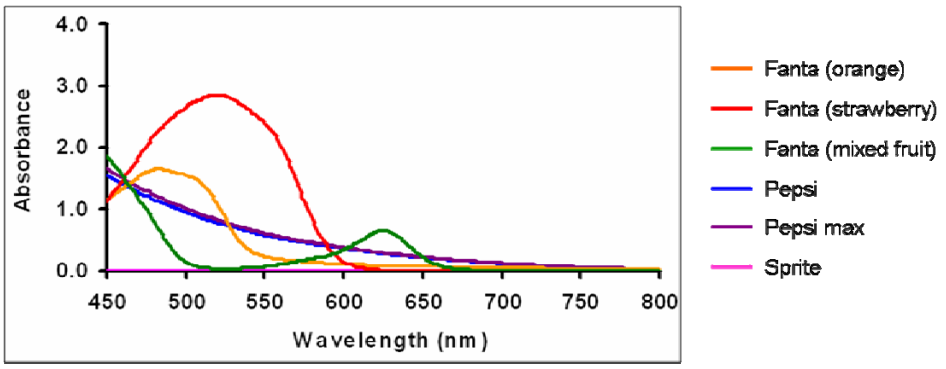


Figure 4.12 Absorption spectrums of some carbonated drinks

4.3.1.2 Schileren profile and calibration

Preliminary, a system based on a SI technique was designed (Figure 4.13) and tested for sucrose content measurement. Figure 4.13a depicts the contour of sample zone with parabolic profile as the sucrose sample is propelled towards the holding coil. However, when the selection valve is switched to connect 'HC-SV-NIR', part of the zone was chopped off leading to a flat boundary at the left end (Figure 4.13b). As the laminar flow was introduced towards detector, this left end becomes the leading parabolic interface, which is responsible for the schlieren signal in this system.

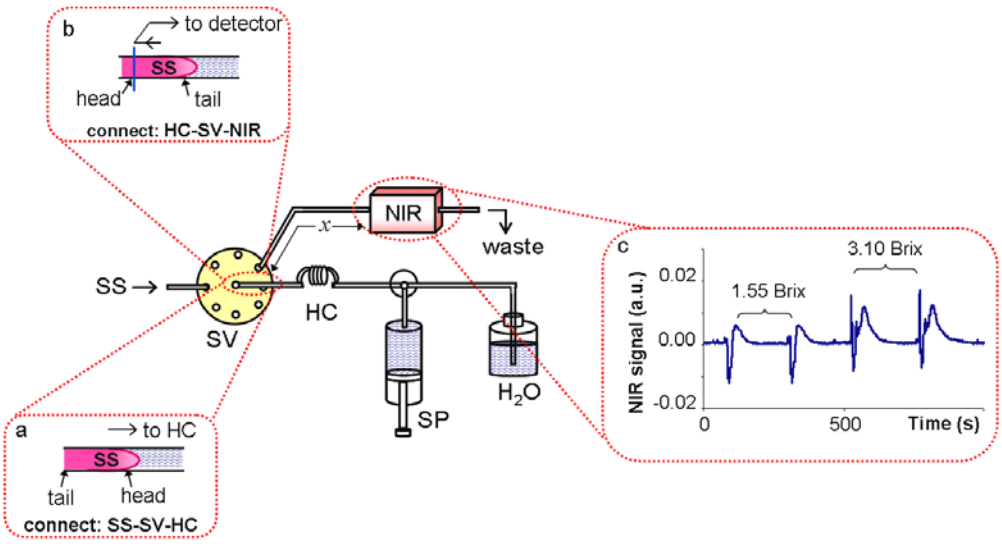


Figure 4.13 Design and set up of the SI system for sucrose content measurement with (a) the contour of sample zone being aspired into the holding coil (HC, 127 cm), (b) the contour of final zone of sample prior being pushed toward the NIR detector and (c) signal profiles of sucrose solutions (SS) at 1.55 and 3.10 Brix (300 µl injection). SV: selection valve, SP: syringe pump

The results in Figure 4.13c indicated that the schlieren profiles of a dilute sucrose solution (1.50 Brix) began with a sharp negative first peak, following by a sudden positive second peak. This pattern of diluted sucrose is similar to the pattern of the schlieren profiles reported for sodium chloride (McKelvie, Peat, Matthews, & Worsfold, 1997) and for glycol (Wijk & Karlberg, 1994). However, the first peak became less negative as the concentration of sucrose was increased to 3.10 Brix (Figure 4.13c). This negative first peak disappeared with injection of sucrose beyond 7.75 Brix, but was replaced by a positive signal. Finally a two-positive peaks signal (Figure 4.14) was obtained for an injection of sucrose solutions from 7.75 to 46.50 Brix.

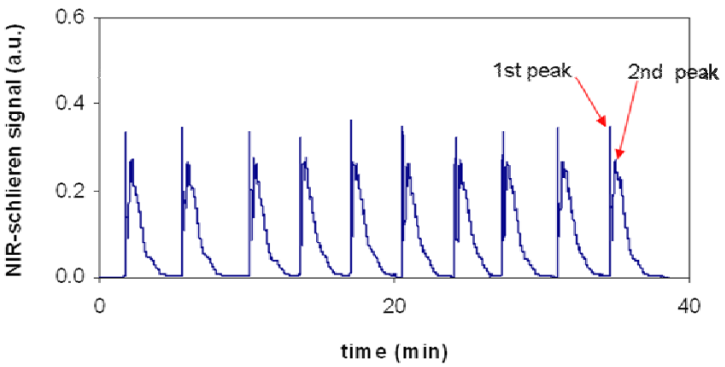


Figure 4.14 The doublet-positive peaks profile of the schlieren signal for a sucrose solution of 15.50 Brix (ten replicates). SI system in Figure 4.13 was employed with 300 µl of standard volume and flow rate 2 ml min^{-1}.

This pattern of two-positive peaks signal, observed for high sugar concentrations in Figure 4.14, are similar to the profiles as reported for sucrose solution (Dias, Borges, Zagatto, & Worsfold, 2006) as well as for sucrose, glycol and ethanol (Zagatto, Arruda, Jacintho, & Mattos, 1990). The schlieren pattern may change accordingly to the concentration and the viscosity of the solution, as observed here. Moreover, various schlieren profiles can also occur depending upon optical arrangement of instrument and the flow path.

4.3.1.3 Optimization study

Since no chemical reaction was involved, the SI system for measurement of sucrose content was optimized by investigating only physical parameters such as sample volume, flow rate, and tubing size. The optimization was carried out using the stand alone SIA system in Figure 4.13.

a. Effect of sample volume on the profile

Figure 4.15a to Figure 4.15d show the profiles obtained from aspirating a standard sucrose at different volumes. Doublet signals were observed for volumes 170 µl (Figure 4.15a) and 300 µl (Figure 4.15b), and the signals were well separated into two peaks for larger volumes such as 3,000 µl (Figure 4.15c) and 6,000 µl (Figure 4.15d). For the results in Figure 4.15c and Figure 4.15d, the first peak was

the result from the schlieren effect at the head of the zone, and the second peak was the result of the same phenomenon at the tail of the same bolus (Grudpan & Jakmunee, 2008). The distance between the gap of the first and the second peaks is related to the sample volume.

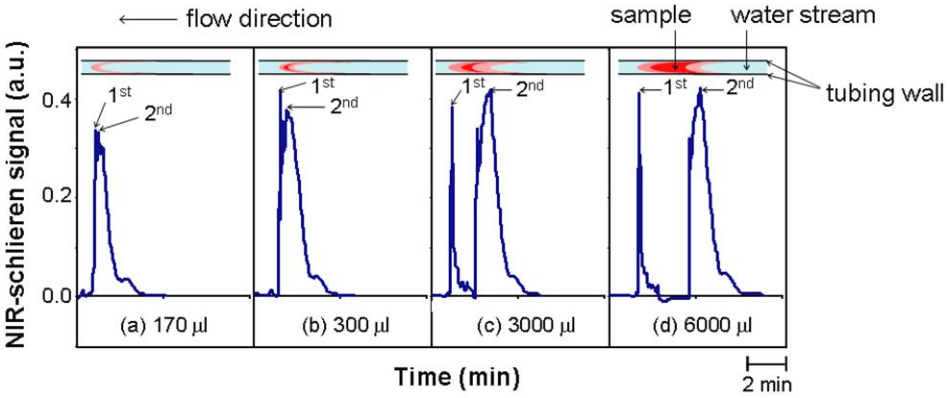


Figure 4.15 Effect of sample volume on the profiles of the schlieren signal for a sucrose solution of 15.50 Brix. The shape of sample zone was simulated comparing to the schlieren profile.

At low volumes such as 170 µl and 300 µl, the dispersion of the sucrose zone exists throughout the sample zone. However, for longer zone, such as 3,000 µl and 6,000 µl, there is only dispersion at the 'head' and the 'tail'. Theoretically, a non-dispersed zone for 3,000 µl sample would have a zone length of 382 cm inside a 1 mm i.d. tube. As the flow path (denoted as 'x' in Figure 4.13) was comparatively short (20 cm), the middle region of the zone of sucrose would have the 'dispersion coefficient (D)' (Ruzicka & Hansen, 1988) equal to 1, or which means no dispersion. Therefore, when this middle region of the zone traveled through the 1 cm light path, no schlieren effect is observed. This behavior is the basis of the so-called "large-injection" approach, in which only the central zone, remote from the schlieren peaks, is used for quantification (Yamane, 1995). This explains the two separate peaks which obtained from injection of the volumes 3,000 and 6,000 µl, in Figure 4.15c and Figure 4.15d, respectively.

Herein, the first peak is obtained when the heading of sucrose sample moves through an optical path of flow cell. The concentration gradient of water carrier and sucrose sample causes a differential signal. Without the differentiation of concentration, absorbance signal thus downs to zero. On the other hand, the second peak is obtained from the tailing interface between sucrose solution and water carrier.

In this work, the signal height obtained from the first peak was used for making the calibration. In order to measure purely the schlieren effect from the leading interface, well separate profiles like the ones in Figure 4.15c and Figure 4.15d seem to be ideal. However these profiles were attained at the condition in which extremely large volumes of sample were used. The analysis time would have been unnecessary long if these volumes were adopted. However it was tested and found that 300 μl was also usable. Although the first peak was partially separated from the second peak for this volume, it could still identify and measure only the height of the first signal. For further experiments, a volume of 300 μl was employed for the sucrose analysis.

b. Flow rate

The effect of flow rate was investigated over the range of 1 to 4 ml min^{-1}. Although higher flow rates provided greater sample throughput, this was at the expense of precision. The %RSDs of repeated injections (n = 10) of a 31.00 Brix sucrose solution were 3.1, 3.6 and 6.0, at 1, 2 and 4 ml min^{-1}, respectively. The sensitivity of 1 ml min^{-1} was the lowest. The observed sensitivities were comparable for 2 and 4 ml min^{-1} flow rates. In order to achieve fast analysis with good precision, a flow rate of 2 min ml^{-1} was therefore chosen.

c. Tubing size

It is known that tube diameter can affect the dispersion of the sample zone. In a measurement based on the schlieren effect, minimal carrier-sample mixing is preferable in order to maintain the integrity of the interfacial boundary between the zones of different RI, and thus the intensity of the schlieren signal. In this SI system (Figure 4.13), the diameter of the tube connecting the selection valve and the detector (*x*) would have the most influence on dispersion and thus the size of

schlieren signal, and this was investigated using 20 cm tubes of 1.0, 1.6 and 3.2 mm i.d. The sensitivities of the 1.0 and 1.6 mm i.d. tubes were not significantly different. However, the dispersion was very large for the 3.2 mm i.d. resulting in very low signals as outcome, and thus the 1.0 mm i.d. tube was used in the final module configuration.

For the same reasons, the shortest possible length of tubing (20 cm) between the selection valve and the detector was used.

4.3.1.4 Analytical features of sucrose monitoring

At the optimum condition (sample volume: 300 μl; carrier flow rate: 2 ml min^{-1}; tubing i.d.: 1 mm and length = 20 cm), absorbance profiles were obtained as shown in Figure 4.16, and used to prepare a calibration graph of the first peak and Brix value. The inset figure of Fig. 3a illustrates a satisfactorily linear calibration from 3.10 to 46.50 Brix ((peak height, a.u.) = (0.026±0.001)[sucrose, Brix] – (0.097±0.018), r^2 = 0.998). The system provided reasonable recoveries ranging from 95.8 to 105% with good precision (%RSD = 3.2, n = 10). Precision diminished at sucrose concentrations of greater than 46.50 Brix, because of the effect of increased viscosity. The system tolerates temperature change within 3-4 °C.

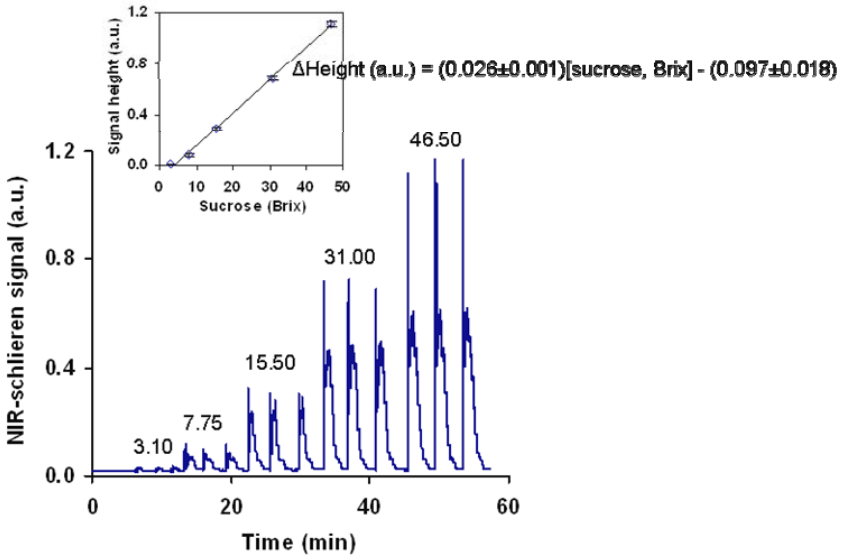


Figure 4.16 Signal profiles obtained from the SI system in Figure 4.13 developed for determination of sucrose (the values are concentrations of sucrose standards in Brix). Calibration curve is shown in an inset figure.

4.3.2 Measurement of pigment

After the optimum condition for measurement of sucrose content was attained, the main module (Figure 4.13) for sucrose content was modified (Figure 3.6) to include sections for pigment measurement (section B) and another for monitoring of dissolved CO_2 (section D).

In this work, an incorporation of a small part to the SI core system (section B, Fig. 3.6) was presented for simple detection of pigment content in production of soft drink. A portable photometer, with an RGB-LED, was attached to port 1 of SV_2. With the feature of the RGB-LED, the applicability of this photometer covers all the absorption wavelength of most types of soft drink.

According to Table 3.4, the air-segmented technique (Skeggs, 1966) was adopted in the SI module for monitoring absorbance of the beverage. The system was designed to feed the liquid sample to the flow cell with no dilution. To avoid sample dispersion, air was used to sandwich the liquid sample during the delivery to detector. After the degassing process in section C (Figure 3.6), the selection valve SV_2 connects port 4 (air)-SV_2-HC. Air (3,000 μl) was drawn into the holding coil. SV_2 then

connects ports 6 (sample)-SV_2-HC for aspiration of 800 µl of soft drink into the holding coil. SV_2 was finally switched to port 1 (to connect 'HC-SV2-RGB'), and the air-sandwiched segment sample was then pushed from the holding coil to the detector. Figure 4.17 shows examples of the obtained profiles. This section for on-line measurement of the pigment offers a robotic feature with high standard of precision with %RSD of 0.3 to 1.7 (n = 15). This indicated that the carryover was not a problem in this system, even though it was operated in the segmented flow mode.

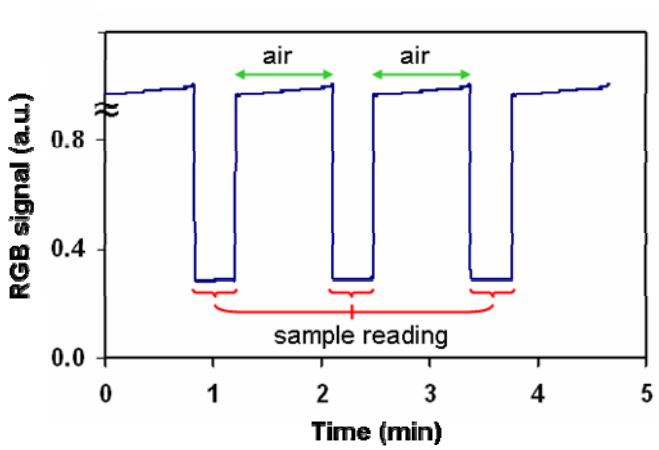


Figure 4.17 Signal profiles obtained from the SI module in Figure 3.6 for pigment, condition: triplicate injection of a cola drink with LED set at 'RED' emission

4.3.3 On-line degassing and measurement of carbon dioxide

If samples are directly aspirated into the SI system (section A and section B in Figure 3.6) without prior degassing, dissolved CO_2 strongly interferes in the determination of sucrose and pigment content by creating lots of bubbles. Thus, it is necessary to include a mechanical apparatus, which can be installed on-line with the SI system for degassing the CO_2. In this work, the system was furnished with a degassing unit that was constructed similarly to the MBL-VP unit (Sereenonchai, 2010).

4.3.3.1 Recommended condition for C^4D

From literature, an excitation frequency applied to the input electrode, normally affects to C^4D signal (Hong, et al., 2005). In this work, a dependence of C^4D signal upon frequency was investigated by using a single line manifold. De-ionized water carrier was continuously flowed in the C^4D cell and was set as a baseline. Then an individual standard solution of KCl in range of 25-750 µM was inserted into the carrier line. The calibration of signal heights and concentrations of KCl were plotted. Then the sensitivity (or slope) at different frequency values were compared (Figure 4.18).

It was observed that sensitivity was higher when the excitation frequency increased until reaching the plateau at frequency in range of 75-150 kHz. The sensitivity then slightly dropped down when the frequency was raised up to 200 kHz. Thus 100 kHz of input frequency was adopted for further experiment for CO_2 analysis.

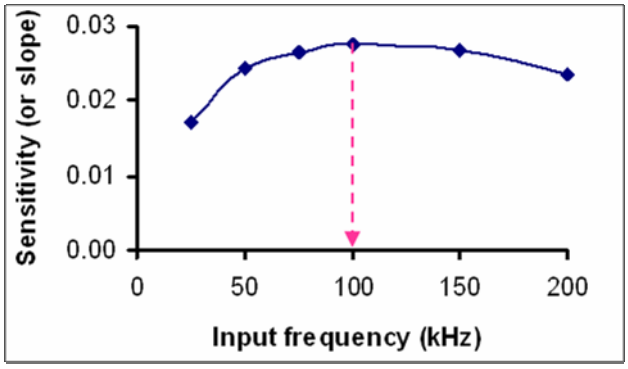


Figure 4.18 Effect of input frequency on sensitivity by using a function generator (Bangkok High Lab Co., LTD) with constant input voltage at 20 V

4.3.3.2 Analytical features of dissolved carbon dioxide monitoring

For the analysis of dissolved CO_2, a satisfactorily linear plot of C^4D signal versus concentration of CO_2 was obtained (Figure 4.19). An example of calibration equation is (peak height, V_{dc}) = (1.11±0.02)[CO_2, g CO_2 L^{-1}]-(2.35±0.09). The linear regression was close to unity (r^2 = 0.999). The linear range covers the

concentration of CO_2 in the soft drinks. The system gave a reasonable precision with %RSD ranging from 2.9 to 6.3 (n = 15).

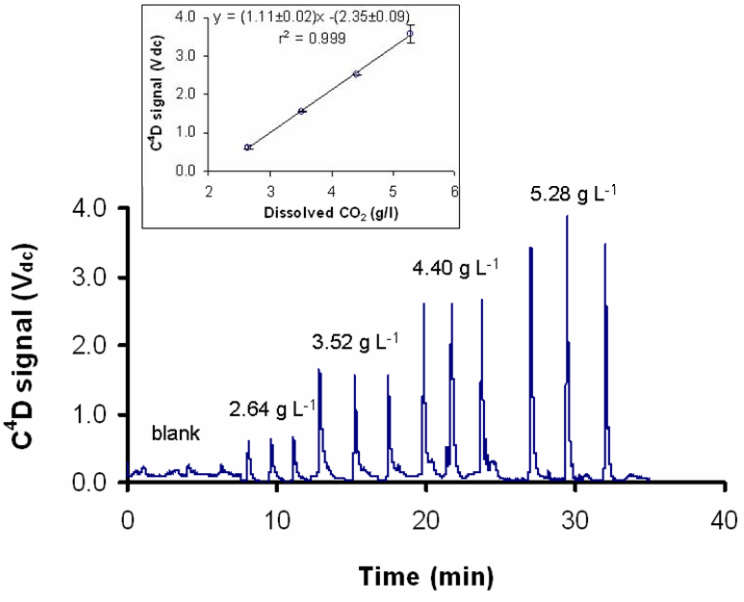


Figure 4.19 Signal profiles and calibration graphs (inset) obtained from the SI module for CO_2 with triplicate analyses of standard $NaHCO_3$ equivalent to the dissolved CO_2.

4.3.4 Application to soft drink

The SI module in Figure 3.6 was applied and tested in the analysis of some soft drink samples. The results (Table 4.7) from the developed method for sucrose content measurement agreed significantly well with the refractometric method at 95% confidence (t_{stat} = 0.975, t_{crit} = 3.182). The system was also tested for measuring the pigment of soft drinks. All the readings from the RGB photometer lied in appropriate range of detection signal. For our tested samples, only two wavelength regions were needed (red and blue light). It was found that the level of dissolved CO_2 of all the carbonated drinks is comparable at 3.2 ± 0.2 g CO_2 L^{-1}.

Table 4.7 Analysis of carbonated soft drinks for sucrose, pigment and dissolved CO_2 using SI module.

Carbonated drink	Sucrose content (Brix)		Pigment	Dissolved CO_2
	SI method	Refractometry	(Absorbance)	(g CO_2 L^{-1})
1. Pepsi	5.3 ± 0.2	6.8 ± 0.1	0.305 ± 0.001[b]	3.1 ± 0.1
2. Pepsi max	n.d.[a]	0.85 ± 0.0	0.290 ± 0.002[b]	3.1 ± 0.1
3. Fanta strawberry	12.5 ± 0.2	11.1 ± 0.1	1.130 ± 0.008[c]	3.2 ± 0.2
4. Fanta orange	11.7 ± 0.2	10.5 ± 0.1	0.178 ± 0.013 [b]	3.3 ± 0.2
5. Fanta mixed fruit	11.9 ± 0.3	11.2 ± 0.1	0.432 ± 0.004 [b]	3.4 ± 0.1

[a]n.d.: non-detectable

[b]with the RGB-LED set at 640 ± 20 nm

[c]with the RGB-LED set at 470 ± 25 nm

CHAPTER V
DISCUSSION

The results from previous chapter are discussed here in this chapter. First, the findings from the use of POE strategy are revealed and compared with other works. Secondly, the superiors of the developed method 'multiple processing hybrid flow system' is described. Results from the application of formaldehyde analyses in food samples are also discussed in the term of safety to consumer. Finally, the developed SI module was offered to monitoring contents in non-carbonated drinks. The versatility of this module in beverage manufacturing is discussed.

5.1 The findings of the use of predict-observe-explain strategy for teaching flow injection technique

5.1.1 The finding on students' learning

The results obtained from the prediction of the first POE (Figure 4.1) showed that the students could correctly predict some behaviors of sample zone such as dilution and elongation. However they did not give a clear description why the zone behaves like that in laminar flow. Deficiency in explaining a prediction often happens because students may not be able to define appropriate words for their description. Students often said, they know but can not explain. Similar trouble was also found in other studies (Gunstone & White, 1981; M. Kearney & Treagust, 2000). Gunstone and White (1981) found that students failed to explain because they sometime used their intuition rather than rational assessment. Kearney and Treagust (2000) found that limitation of science vocabulary restricted the extent of students' elucidation. However, after the discussion, students could give reasonable reasons based on scientific knowledge for their observation. They used proper words to explain the flow phenomena.

The students revealed via the interview that they learned a topic of gas and fluid since their first-year in physics class. This makes sense that why most of them (>90%) can prefigure some flow features such as elongation and dilution at the beginning of the task (prediction in Figure 4.1). This information also suggests that teaching the FI technique by using of the POE is suitable because students already have some experience in liquid phenomena. White and Gunstone (1992) suggested that POE is a strategy well-apply with events that students already have some ideas. If students have no clue, they may not possibly give any relevant concepts for their explanation. And this causes worthlessness to learning.

In the second POE, it is overwhelming to find that 80% of students could apply the flow phenomena, which was obtained from first POE (previous situation) to the second POE (another situation). This means students can apply previous experience with new encounter. It is in accordance with the prior study (Liew & Treagust, 1998) showing that students derived their reasons from the previous experience. Liew and Treagust (1998) found that earlier POE task, i.e., solubility of salt in water, influenced to students' view on a subsequent task of salt in oil which made students critically considered the solubility phenomena.

In group discussion, students learned and shared ideas with peers, compiled the ideas and then reconciled or negotiated with existing knowledge prior to construction of their own conceptual knowledge. The finding showed that collaborative learning helped students to develop understanding. This learning reflects the process of constructivist learning. Tao and Gunstone (1999) concluded that the collaborative learning, in which students shared knowledge and understanding, was an aspect of social constructivist learning. According to students' responses, in this work, classroom is thus considered as running under social constructivist environment.

A weak point found in this work is that students sometime did not write their descriptions clearly. This circumstance reflects a problem in science communication. Deficiency in science communication makes the difficulty to interpret what students already know or do not know. However the trouble could be solved by doing a parallel oral communication during running the activity, in order to perceive reliable students' responses.

5.1.2 The finding on instructional method and teacher role

Students gave positive perspectives to the demonstration tool. The findings clearly showed that the demonstration was interesting to the students. Learning through visualization helped them understand the flow concept. The results are similar to those obtained by McKelvie et al. (1990) and Grudpan and Thanasarn (1993) who also developed the visualized tools, illustration of the flow processes and factors involved in FI technique. Although those works did not show data how much demonstration improved students in term of learning, the authors mentioned that practical demonstration could convinced students' interest. Students gained appreciation to observation of flow processes in action.

In this work, students enjoyed and perceived that POE is a valuable instructional method supported their learning. It is consistent with a case of Chairam et al. (2009), who developed chemical kinetics experiment based POE tasks. Chairam et al. also found that POE tasks enhanced their students learning.

Students stated that they looked over the prediction and observation, in order to compare the similarities or differences between two sections. This process was described by Liew and Treagust (Liew & Treagust, 1995; Liew & Treagust, 1998). When students saw the difference between their prediction and observation, they then paid attention to reconstruct their view, developing conceptual understanding.

Although most of students enjoyed the POE tasks, four of them seemed not to appreciate with this approach. One of those students expressed as shown in the following excerpt:

"*I was bored to write down what I predicted or explained. The demonstration was interesting but it would be better if teacher performed the show and then described to students. I preferred to take a note from her talk*."

It is possible to have a case of students who feel uncomfortable with new learning format. Some students might be familiar with a traditional lecture where they normally just follow explicit information provided by teacher. However active

methods are believed to be more effective rather than traditional teaching (Kvam, 2000; Lujan & DiCarlo, 2006). Therefore the POE is still considered as an impressive and effective way for teaching science. In this work, the teacher had important role to encourage discussion and collaboration of learning.

In summary, this work shows the success of the use of demonstration together with POE teaching sequence for FI topic at undergraduate level. The POE method works well under a certain time of classroom lecture, and also keep classroom active. The findings of this study including previous works (Chairam, et al., 2009; Gunstone & White, 1981; M. Kearney, 2004; Liew & Treagust, 1995; Parmer, 1995) imply that the POE should be an effective strategy for teaching in science classroom at all levels.

5.2 The advantages and assessment of the multiple processing hybrid flow system for analysis of formaldehyde in food

The results obtained from the development of a multiple processing HFA system for monitoring formaldehyde contamination in food are discussed in aspects of its performance and safety to consumers.

5.2.1 Performance of the multiple processing HFA system

The HFA system combines features of flow injection (FI) and sequential injection (SI) techniques. The system thus gathers both advantages of FI and SI techniques, which are low blank signal and high baseline stability together with flexibility and robustness of the system.

When the HFA was initially constructed, it was observed that each analytical cycle required a rather long period of time to fill in the carrier and reagents into the syringes (the minimum possible time was 2.5 min). The awaiting time for transferring the sample to pass through the reaction and to reach the detector was even longer (the minimum possible time was 5 min). This resulted in a very slow sample throughput. Therefore a new operating procedure was developed for multiple tasking. This enables three samples to be processed at the same time. During the signal recording of the first signal, the reaction mixture of the second sample is heated to

increase the colored product. During this time, the third sample is introduced into the system and the reagents are being mixed and are ready to react with the third sample in the next push of the pumps. The capability in multiple processing resulted in a significant increase in sample throughput to 51 samples h^{-1}. This throughput is much faster than most flow methods previously reported for food and beverage (Bechmann, 1996; Li, Ma, Lu, & Tao, 2008; Tsuchiya, et al., 1994; Zhao & Zhang, 2009).

Due to it's specificity towards formaldehyde, the Hantzsch reaction was adopted in this research. A heating device was built to heat up the reagents of 2,4-pentanedione, and ammonium acetate, prior to react with formaldehyde. The employment of this heating device significantly improved the sensitivity of the spectrometric detection.

For food and beverage analysis, the spectrometric detection is more practical than the use of fluorometric detection. Although, in principle fluorometry offers a supreme sensitive detection, this kind of detection is easily upset by the effect of light scattering from micro-particulates in food extract. Therefore with fluorometric detection, analysis of food extracts must be carefully filtered through special micropore membrane, following by further dilution if necessary (de Oliveira, Sousa, & de Andrade, 2007; Tsuchiya, et al., 1994; Zhao & Zhang, 2009). Hence measurement with molecular absorption might be a better option, if the limit of detection meets the requirements. In this work, the HFA system is connected to an autosampler, where on-line filtration can be simply performed by a millipore filter attached at the end of the sampling probe.

Under optimized conditions, a linear calibration is in range of 10 to 100 μM. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.06 and 0.10 mg kg^{-1}, respectively. These values of LOD and LOQ suggest that the method has sufficient sensitivity to detect HCHO in food. The system is robust and suitable for heavy duty use such as the screening of food export, for formaldehyde as required by the regulation.

5.2.2 Formaldehyde analysis and assessment

HCHO has serious toxicology properties causing cancer in animals, and it may also cause cancer in humans (Cogliano, et al., 2004; Soffritti, et al., 2002). It is therefore necessary to pay more attention to the contamination level as well as risk in oral administration.

Results from Table 4.6 shows that there were detectable amounts of HCHO in the aqueous extracts of all re-hydrated dry squids. The amounts found in Sq1 and Sq2 have outstandingly high HCHO levels, this may be coming from misuse of HCHO as food preservatives. However, there is not enough information to conclude that the measurable levels found in the rest of the squid samples is natural or illicit.

The reference dose for chronic exposure (RfD) of HCHO is 0.2 mg kg^{-1} body weight per day(US Environment Protection Agency (EPA), 1999). Therefore, the level of HCHO intake would be only 3 mg HCHO by eating 250 g of the re-hydrated dry squid, which is far from the RfD value for a person who weigh 60 kg (calculated RfD = 12 mg HCHO per day). This means customer would not be in a health risk from eating these Sq1 and Sq2 samples.

Although Thailand has not set a maximum limit for the level of HCHO in food, preparation to face new regulations set by other countries for the export of food should be aware. For the control of formaldehyde level in food, the developed HFA method here can be a useful tool for screening formaldehyde misuse.

5.3 Versatility of sequential injection module to quality control in beverage manufacturing

A merit of the developed SI module for monitoring contents in carbonated drink is discussed. An extension of the module to non-carbonated drink is also included in this section.

5.3.1 Extension of the SI module for non-carbonated drink

Although the SI module in Figure 3.6 was developed for the manufacturers of carbonated soft drinks, the system can be easily modified to fit manufacturers producing other types of drinks. For non-carbonated drinks, the manifold in section C

(for degassing) and section D (for CO_2 analysis) in Figure 3.6 can be omitted. The applicability of the method in measuring sugar contents in 10 samples of non-carbonated drinks was tested. These included green tea, black teas (2 samples), chrysanthemum tea, amino drink, energy drink, apple juice, syrup of palm-sugar, syrup of red sala-cider, and syrup of green cream soda. The results of sugar contents obtained by the schlieren method for these samples agreed statistically well with the results obtained by a refractometric method (Ramasami, et al., 2004) (t_{stat} = 0.509, t_{crit} = 2.306).

5.3.2 Merits of SI module

A completely reagent-free module based on SI is presented for process analysis in the manufacturing of carbonated soft drinks. The module offers a rapid and simultaneous analysis of contents of total sugar, pigment and dissolved CO_2, within 6.5 min. In this work, SI was utilized to achieve automatic liquid handling of the sample. The light sources in the two photometers were deliberately selected based on the priority of being small in size, robust and minimum cost. Since the samples contain only one type of sugars that is sucrose, only a simple LED detector emitting a non-absorbing wavelength by the sample is sufficient. The detection of the schlieren effect could be simply achieved by employing commercial 10-mm pathlength flow cell with the NIR photometer. However for samples with complexity and requirement in identification of sugar contents, a more sophisticated type of detection such as the FTIR spectrophotometer with a suitable chemometric evaluation should be employed (Lethanh & Lendl, 2000; Schindler, et al., 1998).

Here the schlieren effect was utilized for monitoring sugar contents in carbonated soft drinks. Two other parameters such as pigment and dissolved CO_2 can be concurrently determined by this system in real time. The SI system is totally environmentally friendly since the detection systems do not rely on use of any chemical. Therefore the system is attractive to industrial users. As well as the application in process analysis, the system is also suitable in the laboratory for quality control purposes. If the system in Figure 3.6 is used as an off-line system, a reasonable throughput of 9 samples h^{-1} would be obtained with complete results of the 3 parameters. Nevertheless if the system is detached and used separately, faster

throughput for single analysis of 17 (sugar), 42 (pigment) and 37 (CO_2) samples h^{-1} could be achieved.

With the feature of precise liquid handling of the syringe pumps, frequent re-calibration is not necessary for this method. Syringe pumps have facilitated system's robustness and constant flow rate. The system is therefore appropriate for long-term use or for analyzing large numbers of beverage samples in process line.

CHAPTER VI
CONCLUSION

At the end of research work, a new instructional method based on POE approach for teaching FI technique at undergraduate level was achieved. The developed POE method promoted students' understanding in the concept of flow phenomena by learning through visualization. The students had positive attitude toward the new teaching strategy, which express a meaningful of learning.

Two new flow systems were also developed for food safety and control. The first system is suitable for rapid screening of formaldehyde contaminated in food. An operating procedure was developed for multiple tasking and high sample throughputs. Another flow system is for quality control in soft drink manufacturing. Three major parameters, i.e., sucrose, pigment and dissolved CO_2 can be measured simultaneously. The system does not require any chemical, which makes the system very attractive and friendly to the environment.

All these years of study, the author has a strong experience in teaching and learning as well as carrying out research on flow-based techniques. This will support the author to be a good teacher and researcher in the future. Two international publications of original research paper of flow-based analysis were achieved. At least two more publications are expected in the near future.

Recommendations:

For next study, it would be better to try out the developed POE method with students in next academic years, in order to ascertain a reliability of the method. Perception of teacher to the developed method is important because teacher may not continue using the method if they do not impress with. Therefore this factor also should be investigated.

The instructional unit based on POE strategy should be effectively applied for teaching other chemistry topics, especially for teaching the topics involving

abstract concepts such as an instrumentation course. Fundamental principles of chemical instruments are often hidden behind the instrument cases, students often only learn how to control those cases with specific software. Thus teaching through visualization with active POE approach may help students to understand ‘how the instrument works’ and also engage students’ mind of learning.

Two method developments for analysis of formaldehyde in food and of major contents in soft drink can be adapted for educational purpose. With this in mind, author thus plan to developed those experiments for teaching and learning in aspect of real-life situation.

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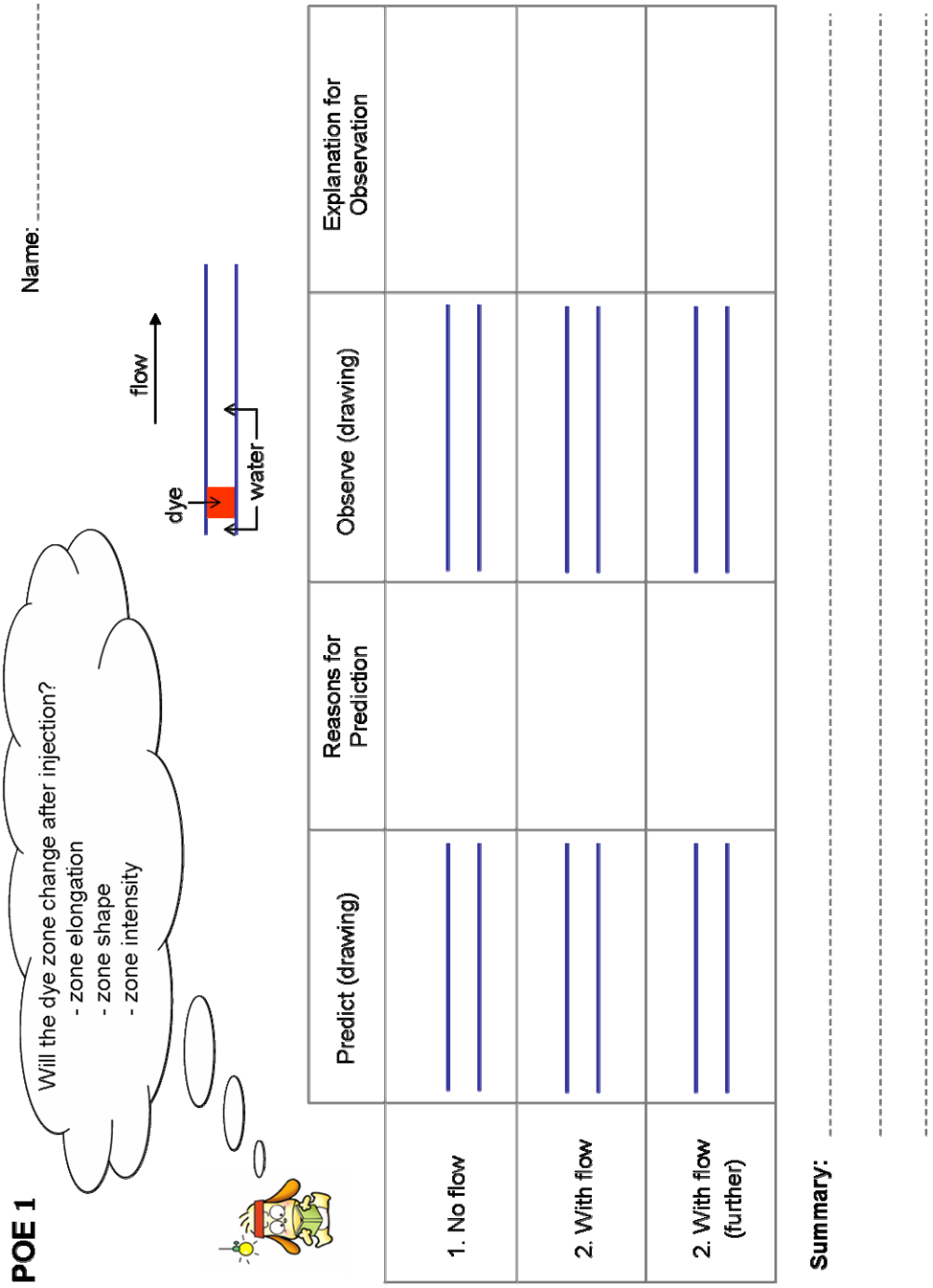
APPENDICES

APPENDIX A

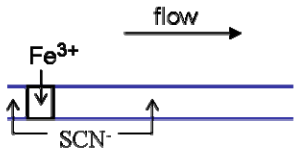

Name:
POE 1
Will the dye zone change after injection?
- zone elongation
- zone shape
- zone intensity
flow
dye
water
Predict (drawing)
Reasons for Prediction
Observe (drawing)
Explanation for Observation
1. No flow
2. With flow
2. With flow (further)
Summary:

POE 2

Name: .....

What would happen to the zone of Fe^{3+} after injection into the stream of SCN^-?

$Fe^{3+} + SCN^- \rightarrow Fe(SCN^-)_x^{3-x}$ (red cpx.)



	Predict (drawing)	Reasons for Prediction	Observe (drawing)	Explanation for Observation
1. No flow				
2. With flow				
2. With flow (further)				

Summary: .....

.....

.....

APPENDIX B

What are the students' attitudes toward the POE-based instructional unit?

Student Identification Number : ……..
Gender : ……..
Age : …….....

Directions for students

These questionnaires contain statements about the experience you found in the demonstration with POE teaching approach, including interactions you had with teacher and other students in learning flow-based technique, a part of special topics in analytical chemistry SCCH 412.

Please indicate your honest views by circling what you think actually happened in the class. For each statement, circle only one number corresponding to your answer. There are no 'right' or 'wrong' answers. Your opinion is what is wanted.

Draw a circle around:

1 if you **Strongly Disagree**
2 if you **Disagree**
3 if the you are **Neutral**
4 if you **Agree**
5 if you **Strongly Agree**

Suppose that you felt the statement: 'The teacher asked for my opinion.' is not always true, you would circle 1. However, if your teacher always asks your opinion, you would circle 5, or you can circle the number 2, 3, and 4 if one of these seems like a more accurate answer.

Section A	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
The teacher asks for my opinion.	(1)	2	3	4	5

If you change your mind about an answer, cross it out and circle a new number.

Section A	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
The teacher asks for my opinion.	⊗	2	3	4	(5)

Please give your opinion for all statements.

Section A	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1. The teacher asks questions that motivate me to think.	1	2	3	4	5
2. I have opportunity to ask my questions when I have doubt.	1	2	3	4	5
3. The teacher is interested to answer my questions.	1	2	3	4	5
4. The teacher asks for my opinion.	1	2	3	4	5
5. Teacher encourage group discussions	1	2	3	4	5
6. The teacher is friendly to me.	1	2	3	4	5
7. The teacher explains the use of Predict-Observe-Explain (POE) document clearly.	1	2	3	4	5
Section B	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
8. I attempt to explain flow phenomena I observed.	1	2	3	4	5
9. I use scientific knowledge to explain flow phenomena I observed.	1	2	3	4	5
10. I share ideas with peers in my group.	1	2	3	4	5
11. I learn ideas from peers in my group.	1	2	3	4	5
12. I use scientific knowledge to support discussion in a group.	1	2	3	4	5
13. I connect a conclusion of my group to scientific knowledge.	1	2	3	4	5
14. Group discussion helps me to develop understanding in flow concept.	1	2	3	4	5
15. I learn to work in a team	1	2	3	4	5
16. I share a consensual conclusion to a whole class discussion.	1	2	3	4	5

Section C	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
17. I understand how to use POE document.	1	2	3	4	5
18. I enjoyed the use of POE.	1	2	3	4	5
19. I feel that the POE is a useful tool to my learning.	1	2	3	4	5
20. I attempt to make comparisons between my prediction and my observation.	1	2	3	4	5
21. I feel that the POE supports me to think rationally.	1	2	3	4	5
Section D	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
22. The demonstration is interesting to me	1	2	3	4	5
23. The demonstration helps me to visualize flow phenomena happened in flow injection (FI) system	1	2	3	4	5
24. The demonstration helps me to understand the flow phenomena in FI system	1	2	3	4	5
25. The demonstration helps me to understand the concept of FI technique.	1	2	3	4	5
26. I feel that the demonstration support my learning in FI technique.	1	2	3	4	5

APPENDIX C

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Notes

A Multiple Processing Hybrid Flow System for Analysis of Formaldehyde Contamination in Food

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This work proposes a flow system suitable for the rapid screening of formaldehyde contaminated in food. The system is based on the concept of a flow analyzer with a Hantzsch reaction. An operating procedure was developed for multiple tasking and high sample throughput. This resulted in a significant sample throughput of 51 samples h^{-1}. Under the optimized conditions, linear calibration from 10 to 100 µM was obtained. The system gave a limit of detection and a limit of quantitation of 0.06 and 0.10 mg kg^{-1}, respectively. The system was successfully applied to re-hydrated dry squids, vegetables and mushrooms.

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Introduction

Formaldehyde (HCHO) is a human carcinogen known to increase mortality from nasopharyngeal cancer.[1] According to the US EPA,[2] the reference dose for chronic exposure (RfD) of HCHO is 0.2 mg kg^{-1} body weight per day. However, the enzymatic reduction of trimethylamine oxide in fish could produce HCHO as a product at levels of up to 98 mg kg^{-1}, causing an increase in the amount of HCHO during the storage of frozen fish.[3,4] In some countries, HCHO is illegally added to foods as a preservative.[5-8] It is therefore necessary to have a method for the detection and monitoring of the level of HCHO in food.

Conventional colorimetric methods[7,9,10] for the qualitative and quantitative analyses of HCHO in food are laborious. A pulsed amperometric method[6] and an "electronic nose"[11] have been reported as alternatives to the conventional methods for the detection of HCHO abuse in seafood. A review of methods for the determination of HCHO in the diet has recently been published.[12] In our view, the most practical and effective technique for detection of the HCHO in food is a method reported by Nash.[13] This method is based on a reaction between HCHO and a diketone in the presence of ammonium acetate. The reaction is also known as the Hantzsch reaction.

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In flow injection analysis (FIA), the Hantzsch reaction has been applied to the analysis of air,[14-17] breath,[18] beverage[19,20] and food.[21] Different diketones, such as 2,4-pentanedione,[16,17,20] 1,3-cyclohexadione[14,21] and 5,5-dimethylcyclohexane-1,3-dione[15,18] have been employed. Amongst these, 2,4-pentanedione is recommended as the best reagent due to its reactivity at lower temperature and low sensitivity to interferences.[16] In a recent flow system, 2,4-pentanedione and ammonium acetate solutions were arranged to mix together in one of the syringes prior to a reaction with HCHO.[17] This flow system for the analysis of HCHO in air is based on the concept of a hybrid flow analyzer.[22] The system is more robust than the conventional FI system due to using syringe pumps.

In this work, a flow system based on hybrid flow[22] was developed for the analysis of HCHO in food. The procedure is different from one presented earlier by Eom *et al*. for monitoring HCHO in air.[17] A new operating procedure has been designed that allows for the processing of 3 samples at one time to achieve a considerably improvement in the sample throughput.

Experimental

Reagents and chemicals

All chemicals used were of analytical reagent grade. Deionized-distilled water used throughout the work was obtained from an EASYpure II system (Branstead, USA).

Ammonium acetate solution (2.0 M) was prepared by

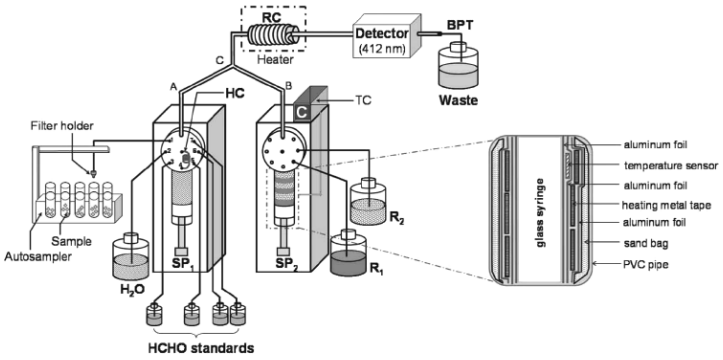


Fig. 1 Flow system developed for the detection of formaldehyde contamination in food: SP_1 and SP_2, syringe pumps providing a flow rate of 0.6 ml min^{-1}; HC, holding coil (1 ml); TC, temperature controller; RC, reaction coil (0.8 mm i.d. × 150 cm length); BPT, back pressure tube (0.5 mm i.d. × 15 cm length); R_1, 0.04 M 2,4-pentanedione; R_2, 2.0 M ammonium acetate. The temperature of syringe SP_2 and the reaction coil RC were always set at the same value.

dissolving 38.5 g of ammonium acetate (Ajax, Australia) in 200 ml of water, followed by adjusting the pH to the range of 5.6 to 6.2 using glacial acetic acid (J. T. Baker, USA), and then diluted to 250 ml with water. A 2,4-pentanedione stock solution (1.0 M) was prepared by diluting 5.0 ml of 95% 2,4-pentanedione (Carlo Erba, Australia) to 50 ml with water. A standardized[23] stock solution of HCHO (0.1 M) was prepared by diluting 0.8 ml of 37.5% HCHO (Ajax, Australia) to 100 ml with water, and was stored in a refrigerator. HCHO calibrators were prepared daily by appropriate dilution with water from the stock reagent.

Sample preparation

A food sample was cut into small pieces (4 to 5 mm), accurately weighed (12.5 g) and placed into a 50-ml centrifuge tube containing 25.00 ml of water, the extraction solvent. The extraction of HCHO from food sample was adopted from a method of Wang *et al.*[5] Sample tubes were capped and shaken at 240 rpm for 15 min (IKA Labortechnik, Germany) to extract the HCHO. The sample tubes were then loaded onto the rack of an autosampler, as shown in Fig. 1.

The flow system

The flow system in Fig. 1 consisted of two syringe pumps (Kloehn V6 Pump, USA), each equipped with an eight-port selection valve (Kloehn 17620, USA). Two glass syringes with a 5-ml zero dead volume (Kloehn, USA) were fitted to the pumps. A spectrometer (Jenway Model 6300, UK), with a 5-cm light-path quartz flow cell was used for monitoring absorbance at 412 nm. The flow system was coupled to an autosampler (PerkinElmer AS90, USA). The control of the syringe pumps and data acquisition were carried out using in-house software written using LabVIEW 8.0.™

A sample extract (350 µl) was introduced into the flow system by first drawing the supernatant from the autosampler (*via* port 1) into the holding coil (HC in Fig. 1), and then driving the sample extract to merge with the mixed reagents at point C. Similarly to the introduction of a sample, the HCHO standards were individually loaded into the HC coil (*via* ports 3, 5, 6 and 7).

For the system shown in Fig. 1, filtration of the sample was done on-line *via* a filter holder (Millipore-Swinnex, USA) fitted at the end of a sampling probe and a glass fiber filter (13 mm diameter) placed inside this holder. Small debris of sample extracts was filtered out to prevent clogging of the flow system.

Syringe heater

It is known that heating can improve the sensitivity of the Hantzsch reaction,[21,24] so an electrical heating jacket was constructed for the glass syringe SP_2. This syringe SP_2 pre-heated a mixture of reagents R_1 and R_2 stored in the syringe. The inset of Fig. 1 shows the assembly of the heating apparatus. A temperature controller (TC in Fig. 1) was used to control the temperature. The dashed line around syringe SP_2 indicates the region of the thermostated heating jacket.

Results and Discussion

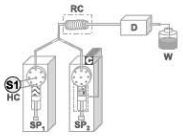
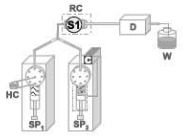
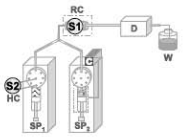
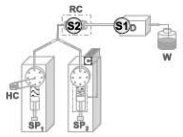
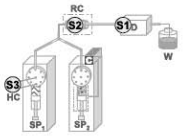
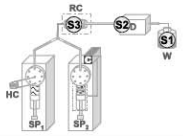
Efficiency of the electrical heating jacket

In order to test the effectiveness of the heating device, the flow system in Fig. 1 was used with and without operating the heating device. As expected, the sensitivity of the system using the heated jacket increased by a factor of 2. The calibration equation obtained at 70°C was $\Delta A = (8.67 \pm 0.53) \times 10^{-3}$[HCHO, µM] + $(2.08 \pm 2.96) \times 10^{-3}$. The calibration obtained at 25°C was only $\Delta A = (4.55 \pm 0.10) \times 10^{-3}$[HCHO, µM] + $(0.22 \pm 0.55) \times 10^{-3}$. The highly reproducible signals (%RSD = 0.7) obtained over an extended operating period of 36 h indicated that the heating jacket was efficient. Therefore, this electrical heating jacket was employed for all further experiments, operating at 70°C.

Design of a procedure for multiple processing

With the normal procedure, the analysis time for one sample is 7.5 min, which includes the time for sample introduction, premixing and pre-heating of the reagents (R_1 and R_2) in the SP_2 syringe, and for detection of the signal at the spectrometer. The introduction of a following sample does not start until the cycle

Table 1 Procedure selected for multiple processing of the flow system in Fig. 1 for the determination of HCHO in food

Step	Motion of pump	Duration/s	Position of analytical zone
1 (load)	850 µl of carrier and 350 µl of sample 1 (S1) are drawn from the sample tube into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into the SP_2 syringe until total volume reaches 1200 µl	30	
2 (inject)	SP_1 and SP_2 push all solutions from the two syringes to mix and stop at RC	56	
3 (load)	850 µl of carrier and 350 µl of sample 2 (S2) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1200 µl	30	
4 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	
5 (load)	850 µl of carrier and 350 µl of sample 3 (S3) are drawn into SP_1 100 µl of reagents (R_1/R_2) are alternately drawn into SP_2 until total volume reaches 1200 µl	30	
6 (inject)	SP_1 and SP_2 push all solutions from the syringes to mix and stop at RC	56	

R_1, 0.04 M 2,4-pentanedione; R_2, 2.0 M ammonium acetate. From step 6 onwards, the flow system handles 3 samples at a time, leading to the throughput of 51 samples h^{-1}.

of the previous sample has been completed. The sample throughput for this normal procedure is therefore low (8 samples h^{-1}). In order to improve the sample throughput, we designed a new operating procedure for the flow system. Table 1 gives this new procedure, which now has a considerably higher sample throughput.

With this new procedure (Table 1), as the first sample (S1) is being heated in the reaction coil RC in step 3, the next sample (S2) is introduced into the holding coil, HC. During this time, pre-mixing and pre-heating of reagents R_1 and R_2 is being processed inside the heated syringe SP_2. Following this step, S2 and the heated reagents are pushed by both syringe pumps into the reaction coil. When the syringe pistons are moved upward, the head of the S1-zone is therefore pushed further into the detector. At the end of step 4, we see the first part of the signal of S1 (Fig. 2a). In step 5, S1 is stationary, and therefore the signal level of S1 stays constant. In step 5, the third sample (S3) is now introduced into the coil, HC. Again, the pre-mixing and pre-heating of R_1 and R_2 occur inside the SP_2 heated syringe. In step 6, S3 and the mixed reagents are pushed by SP_1 and SP_2, to replace S2 in the reaction coil. In step 6, the entire zone of S_1 is now driven through the detection cell to waste giving the complete signal of S1, as shown in Fig. 2a, whereas sample S2 is just entering the flow cell of the detector. After the complete profiling of S1, the system then handles 3 samples at a time, by repeating steps 1 to 6. In this second cycle, the profile of S2 appears in step 1, and finishes at the end of step 2 (Fig. 2b). The profile of S3 appears in steps 3 and 4 (Fig. 2c), and so on.

System optimization

The effect of the flow rates for lines AC and BC of the system (Fig. 1) were examined. It was observed that the signal

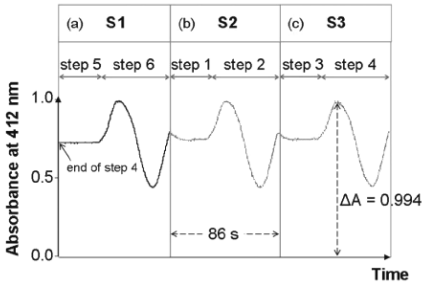


Fig. 2 Signal profiles obtained from the consecutive introduction of the liquid standard of HCHO (100 µM), representing the ability of the flow system in multiple processing of three samples (S1, S2 and S3) all at the same time, under the operating procedure given in Table 1. The baseline is set at zero absorbance.

decreased as the flow rate was increased from 0.1 to 0.8 ml min^{-1}. As a compromise between the signal amplitude and the speed of analysis, 0.6 ml min^{-1} was chosen.

Various sample volumes ranging from 50 – 1200 µl were investigated. The signal increased with increasing sample volume, reaching a plateau for a volume greater than 500 µl. In this work, we selected 350 µl as the sample volume instead of 500 µl to reduce the processing time of a sample.

The effect of various concentrations of 2,4-pentanedione was also studied. The signal increased sharply from 0.02 to 0.03 M, but stayed more or less constant between 0.03 and 0.05 M. Therefore, 0.04 M of 2,4-pentanedione was chosen. Ammonium acetate concentrations from 1 to 4 M were investigated. It was found to have only a little effect on the signal at concentrations above 2 M. For this work, 2 M of ammonium acetate was chosen.

Analytical features

Using the procedure given in Table 1 and the optimum conditions, the plot between the peak height (ΔA) and the concentration of HCHO, over the range of 10 to 100 µM, was linear ($\Delta A = (9.85 \pm 1.34) \times 10^{-3}$[HCHO, µM] $- (7.35 \pm 1.20) \times 10^{-3}$, $r^2 = 0.999$). Ten replicate injections of 40 µM HCHO were carried out to obtain the system's precision of 0.9% (RSD). A concentration of 1 µM was shown experimentally to be the limit of detection, LOD (3SD of blank signal, $n = 7$), of a sample extract. This translates to a LOD of 6.0×10^{-2} mg kg^{-1} for a solid sample. For our method, the limit of quantitation, LOQ (5SD of blank signal), was found to be 1.0×10^{-1} mg kg^{-1}. The LOQ of our method meets the acceptable residue levels in vegetable (63 mg kg^{-1})[25] and seafood (10 mg kg^{-1} for crustaceans and 60 mg kg^{-1} for *Gadidae* fish).[4]

This developed flow system has a special feature of multiple processing of samples and reagents. With this capability of handling multiple tasks at the same time, the flow system has a high sample throughput of up to 51 food samples h^{-1}.

The signal profiles obtained from our system (Fig. 2) are different from what is normally seen with general flow systems. In Fig. 2c, we show the measurement of a signal obtained from 100 µM HCHO ($\Delta A = 0.994$). The zero absorbance reading of the detector was set with water.

Table 2 Results of formaldehyde analysis in re-hydrated dry squid obtained from our flow method and from the PRA method

Sample of re-hydrated dry squid	HCHO/mg kg^{-1} ($n = 3$)	
	Our method	PRA method[26]
Sq1	12.37 ± 0.02	12.67 ± 0.03
Sq2	11.81 ± 0.03	10.51 ± 0.05
Sq3	4.51 ± 0.04	4.24 ± 0.00
Sq4	4.10 ± 0.10	4.30 ± 0.00
Sq5	0.26 ± 0.01	0.16 ± 0.00
Sq6	0.31 ± 0.02	0.24 ± 0.00

Applications in food samples

The current method has been applied to local food samples. A total of 18 vegetable samples were analyzed. Samples were selected from cabbages, string beans, kales, together with three kinds of mushroom, shitake, Jew's ear and straw mushroom commonly used in Thai and Chinese cooking. The results showed that the samples were not contaminated with HCHO.

Analyses carried out by our method for re-hydrated dry squid (Table 2) showed that two samples (Sq1 and Sq2) contained high levels of HCHO. However, there is insufficient information to conclude whether the levels found in these two samples are natural levels, or from an exogenous source. For Sq3 and Sq4, the levels were more or less comparable with the normal levels found in cuttlefish (2.91 to 3.27 mg kg^{-1}).[4] The levels of HCHO in Sq5 and Sq6 were at the detectable limit of the developed method.

The conventional PRA method[26] was also employed for validation purpose. The results obtained by the two methods (Table 2) were compared using the Paired *t*-test.[27] There was no significant difference ($t_{observe} = 1.188$, $t_{critical} = 2.776$, $P = 0.05$) in the sets of results obtained from the current method and from the conventional method. This equality between the two methods demonstrates that the newly developed flow method is suitable for the analysis of HCHO contamination in food samples.

Recovery studies were carried out using another set of squid samples ($n = 8$) containing low levels of HCHO, from 0.26 to 0.88 mg kg^{-1}. Analytical recoveries from 93 to 112% were obtained from these squid samples. Recoveries carried out on vegetables' and mushrooms' extracts varied from 92 to 113% (18 samples). These good recovery values indicate that sample matrices did not interfere with our method.

Conclusions

We developed a new operating procedure for multiple tasking of a hybrid flow system for the determination of HCHO. The system allows much faster throughput than previous systems presented for food samples.[19,21,28,29] The employment of the heating device significantly improved the sensitivity of the spectrometric detection by the Hantzsch reaction. The method has sufficient sensitivity to detect HCHO in food, especially in re-hydrated dry squid. Although Thailand has not yet set a maximum limit for the level of HCHO in food, our flow system will be a useful tool for screening the misuse of HCHO.

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APPENDIX D

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A reagent-free SIA module for monitoring of sugar, color and dissolved CO_2 content in soft drinks

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ABSTRACT

This work presents a new sequential injection analysis (SIA) method and a module for simultaneous and real-time monitoring of three key parameters for the beverage industry, i.e., the sugar content (measured in Brix), color and dissolved CO_2. Detection of the light reflection at the liquid interface (the schlieren effect) of sucrose and water was utilized for sucrose content measurement. A near infrared LED (890 ± 40 nm) was chosen as the light source to ensure that all the ingredients and dyes in soft drinks will not interfere by contributing light absorption. A linear calibration was obtained for sucrose over a wide concentration range (3.1–46.5 Brix). The same module can be used to monitor the color of the soft drink as well as the dissolved CO_2 during production. For measuring the color, the sample is segmented between air plugs to avoid dispersion. An RGB-LED was chosen as the light source in order to make this module applicable to a wide range of colored samples. The module also has a section where dissolved CO_2 is measured via vaporization of the gas from the liquid phase. Dissolved CO_2, in a flowing acceptor stream of water resulting in the change of the acceptor conductivity, is detected using an in-house capacitively coupled contactless conductivity detector (C^4D). The module includes a vaporization unit that is also used to degas the carbonated drink, prior the measurements of sucrose and color within the same system. The method requires no chemicals and is therefore completely friendly to the environment.

1. Introduction

The worldwide beverage trade has grown continuously in every year due to increasing demand. It is estimated that for the years 1988–1994 to 1999–2004, the percentage of sugar-sweetened beverage drinker increased from 58% to 63% in the US [1]. A research report for developing Asian countries, such as China, India, Vietnam and Thailand, showed that drinking of carbonated drinks has also become popular resulting in an expansion of soft drink manufacturing [2]. Sweetener, flavor, color and carbonating gas (CO_2) are added to beverage in order to make the product more attractive and pleasing to consumers. In the process of quality control, these ingredients must be closely monitored, both on-line and off-line (in the laboratory) to ensure the homogeneity of the production.

Sucrose is the major sugar used as sweetener in soft drink. Refractometry is the conventional method for measuring sucrose [3], and typically, sugar content in water is expressed as Brix or degree Brix (Brix). For example, a solution that has 10 Brix contains 10 g of sugar and 90 g of water [4]. In addition to the refractometric method, there are some other methods reported for determination of sucrose in beverages, including Fourier transform infrared (FTIR) spectrometry with multivariate analysis [5], mass spectrometry [6], acoustic wave [7] and enzymatic sensors [8–10].

In our opinion, a practical technique suitable for on-line measurement and process control of sugar content in the beverage industry would be a flow analysis technique based on light deflection due to a refractive index (RI) gradient. The deflection of light, as it passes through a medium due to the temperature or the concentration gradient (hence the RI gradient), is called the schlieren effect [11–13]. The existence of the schlieren effect in flow injection analysis (FIA), was first pointed out by Krug et al. in 1977 [14].

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In a FIA system with spectrophotometric detection, the schlieren effect can cause serious errors. Several approaches have been used to compensate this lenses effect such as increasing the system's dispersion, injection with matrix matching technique [15], employment of a dual wavelength detection [13], utilization of a so-called multi-reflective flow-cell [16] or the recent 'total internal reflection photometric detection cell' [17].

Nevertheless, the schlieren effect also has a positive side. In 1978, Betteridge et al. [18] made use of this phenomenon for determining the RI of some solutions with an LED flow-cell. Injection of a non-absorbing bolus of liquid into a flowing stream of another liquid leads to formation of RI gradient or regarded as series of lenses. These liquid lenses either focus the light onto detector or divert the light away from detector, depending upon the order of the refractive indices of the liquids and the flow direction [18]. Consequently, the spectrophotometric or colorimetric signal decreases or increases, respectively. In the late 1980s, Pawliszyn exploited the schlieren effect by building some concentration gradient detectors for liquid chromatography [19] and for capillary electrophoresis [20] that were based on laser beam deflection by the schlieren effect. Besides, the same author mentioned some results of the application of the laser-based detector in flow injection analysis for sucrose measurement [20,21]. In addition, the concept of schlieren effect was also adopted in quantitative flow methods for verification of adulteration in alcoholic beverages [22], determination of alcoholic grade [23], determination of glycol [24] and measurement of wine density [25].

In this work, we present a newly complete module suitable for on-line monitoring of soft drinks during production. The system is capable of analyzing for sucrose content (Brix), color and dissolved CO_2 of the soft drink. Sucrose analysis was developed based on the concept of Sequential Injection Analysis (SIA) [26], with manipulation of the schlieren effect. Although some early literature states that the schlieren effect can be utilized for the determination of sucrose by flow injection [20,21], there has been no reported application in the beverage industry. The laser incident flow-cell reported in the late 1980 s was specifically designed for detection in narrow bore techniques, i.e., chromatography and capillary electrophoresis [19–21]. For on-line measurement, a larger scale of flow-through detector with a simpler optical arrangement is preferable. Herein, we employed a simple U-shape flow-cell with a LED light source for achieving instantaneous measurement of sucrose. The LED source emits light in the near infrared (NIR) region, which is beneficial because most of the ingredients do not have significant optical absorption in this region. This light source is therefore universal and covers the entire range of soft drinks in the market.

Dissolved CO_2 is also a key parameter in carbonated beverage industry. The conventional technique for measuring dissolved CO_2 is an indirect technique based on Henry's law. Total pressure and temperature are used to calculate the concentration of CO_2 [27–29]. This technique is likely to provide inaccurate result, as total pressure is used in the calculation but not the partial pressure of CO_2. Therefore, some other techniques for direct measurements based on infrared spectroscopy have been introduced [27,29]. Some membrane-based techniques have also been employed. A membrane gas-diffusion FIA system was presented for off-line analysis of beer and soft drinks with spectrometric detection using a pH indicator [30]. A commercial in-line system with a selective membrane sensor is also available [31].

Here we incorporated a membraneless vaporization (MBL-VP) unit [32] into the SIA module for on-line measurement of dissolved CO_2. The unit also serves as an on-line degassing unit for removing the CO_2 in the sample prior the detections of sucrose and color. This degassing unit allows for selective detection of CO_2 via gas-liquid separation by vaporization. The detection of CO_2 in the acceptor stream was carried out based on use of the capacitively coupled contactless conductivity detection (C^4D) [33]. In addition to the feature of sucrose content analysis and dissolved CO_2 contents, color of the soft drink is often required in the process control of carbonated drink manufacture. The color can be determined concurrently using this SIA module furnished with a portable photometer, fitted with multi-wavelength light emitting diode (LED) as light source [34]. The module offers entirely environmentally friendly analysis, since none of the measurements involves the use of toxic chemicals.

2. Experimental

2.1. Chemicals and samples

Sucrose standard used in this work was commercial food grade (Mitr Phol, Thailand). All solutions were prepared in ultrapure water (Branstead EASYpure II, USA). A 60 Brix stock sucrose solution was prepared by dissolving exactly 60.00 g of solid sucrose in approximately 40.00 g of water with heating on a hot plate until the entire solid has dissolved. After cooling to room temperature, water was added to this solution to give exactly 100.0 g. Working standards were obtained by appropriate dilution. The stock solution was kept refrigerated at 4 °C.

Soft drink samples were purchased from Thai supermarkets. These samples were selected with different brands and colors to verify broad range of application of the developed method.

2.2. The SIA module

As shown in Fig. 1, the SIA module was assembled from two syringe pumps (SP1 and SP2) each equipped with eight-port selection valve (SV1 and SV2) (Kloehn Versa Pump 6, USA). The 10-ml zero dead volume syringes were fitted on the pumps. A 1 ml holding coil (HC) was connected to SP2. Section A in Fig. 1 was arranged for sucrose measurement. A portable photometer (Bangkok High Lab Co., LTD., Thailand) was equipped with a near infrared (NIR) LED (L890-06AU, Epitex, Japan). This light source emitted radiation in 890 ± 40 nm. The selection valve SV2 was furnished with another portable photometer (Bangkok High Lab Co., LTD., Thailand), equipped with a Red-Green-Blue (RGB) LED (SMT-RGB, Epitex, Japan), which emits blue (470 ± 25 nm), green (525 ± 40 nm) and red (640 ± 20 nm) lights. This second detector was used in the measurement of color content in the soft drink (Section B, in Fig. 1). Quartz flow cells, with 10 mm light path and 80 μL internal volume (178.010-QS Hellma, Germany) were used in these photometers. This regular type of flow cell was selected as reported in the literature [12] for examination of the factors affecting the schlieren effect in flow analysis. The software for recording the signals of the photometers was written using Visual Basic.

The SIA module was also assembled with a manifold for degassing the sample. This manifold was denoted as section C in Fig. 1. A modified MBL-VP unit [32] was adopted for degassing carbonated soft drink via stirring with magnetic bar (400 rpm) to accelerate vaporization of CO_2. This MBL-VP unit also had a separate channel to flow in water as acceptor stream (section D, Fig. 1) to trap CO_2 vapor for detection by an in-house C^4D: input voltage at 30 V and 100 kHz. The output voltage was rectified and amplified before recording using software written on LabVIEW 8.0™. The acceptor flow was continuously maintained by using a peristaltic pump (ISM827 Ismatec, Switzerland). The system in Fig. 1 was operated as given in Table 1. The results of sucrose analysis by our method were validated with a method employing a refractometer (Bausch & Lomb, USA).

The flow cell of C^4D in section D (Fig. 1) was made from PEEK tube (1 mm i.d. and 1.6 mm o.d.). The total length of the tubing

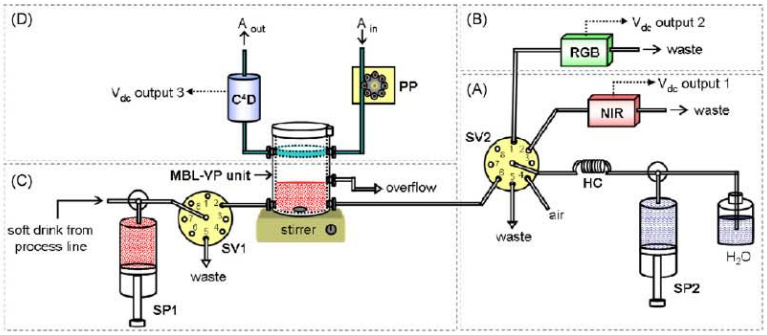

Fig. 1. Diagram of complete module based on sequential injection analysis for on-line measurement in soft drink industry; for sucrose content (section A), color (section B). The module includes a degasser unit (section C) with dissolved CO_2 analyzer (section D). SP1 and SP2; syringe pumps, SV1 and SV2; selection valves, HC; holding coil 127 cm, NIR; portable NIR photometer, RGB; portable LED-photometer, C^4D; capacitively coupled contactless conductivity detector, PP: peristaltic pump, A_{in} and A_{out}: acceptor stream (water) flowing in and out, MBL-VP unit: membraneless vaporization unit [27], V_{dc}: output in dc voltage.

was approximately 150 mm. The length of the two electrodes were 10 mm each and were made by painting the PEEK tubing with silver paint varnish [35], and with a gap of (0.2 ± 0.05 mm) separating the electrodes. An AC signal was fed to one of the electrodes from a function generator (Bangkok High Lab Co., LTD., Thailand). The AC current from the second electrode was amplified, rectified, digitized and recorded as reported by da Silva and do Lago [35], but using an in-house detector unit coupled to a personal computer.

2.3. System operation

According to Table 1, the system in Fig. 1 enables the drawing of sample from process line into the first section for degassing (section C). While the sample is being degassed in the MBL-VP unit, we can detect the CO_2 content in the second section using C^4D (section D). C^4D detects the re-dissolved CO_2 in a water-acceptor stream flowing through the unit in a separate channel to the flow channel of the sample. CO_2 content could be determined using the external-calibration method.

After 1 min, the degassed sample is then aspirated from the MBL-VP unit into section A and section B, in which there is a SIA system furnished with two photometers. In our work, SIA was utilized to achieve automatic liquid handling of the sample. Zones of the degassed sample are alternately fed into the NIR (section A) and RGB (section B) photometers for detections of sucrose and color contents, respectively.

3. Results and discussion

3.1. Sucrose content analysis

3.1.1. SIA design and the contour of liquid interface

Initially, a system based on a SIA technique was designed (Fig. 2) and tested for sucrose content measurement. Fig. 2a depicts the contour of sample zone with parabolic profile as the sucrose sample is propelled towards the holding coil. However, when the selection valve is switched to connect 'HC-SV-NIR', part of the zone was chopped off leading to a flat boundary at the left end (Fig. 2b).

Table 1
Operational step for the SIA module shown in Fig. 1 for analysis of sucrose, color and dissolved CO_2 contents in carbonated soft drink.

Step	Pumps			SV1	SV2	Flow rate (mL min^{-1})	Motion	Period (s)	Type of analysis
	SP1	SP2	PP						
1	On	Off	On	SP1-SV1-P2	–	12.5 (SP1)	3500 μL of sample from process line is aspirated into the MBL-VP unit.	15	
						3.5 (PP)	The acceptor line is being filled with water.		CO_2
2	Off	Off	Off	–	–	–	Sample solution is stirred to remove CO_2. CO_2 gas partially dissolves in water-acceptor.	60	
3	Off	Off	On	–	–	3.5	The zone of CO_2 is transferred to C^4D.	20	
4	Off	On	Off	–	P6-SV2-SP2	12.5	6000 μL of water carrier is introduced into SP2. 300 μL of degassed sample is subsequently drawn from MBL-VP unit to the holding coil.	30	Brix
5	Off	On	Off	–	SP2-SV2-P3	2.0	All solutions were pushed to NIR photometer.	180	
6	Off	On	Off	–	P4-SV2-SP2	12.5	3000 μL of air is aspirated into SP2. 800 μL of degassed sample is aspirated into holding coil.	15	
					P6-SV2-SP2				Color
7	Off	On	Off	–	SP2-SV2-P1	4.0	Sample plug is transferred to RGB photometer.	60	
8	On	Off	Off	P2-SV1-P5	–	12.5	The rest of sample in MBL-VP unit is flushed out to waste.	10	
Total/cycle								390	

SP: syringe pump, PP: peristaltic pump, P: port, SV: selection valve. *Note*: when changing from one sample to another, the first readings of all the parameters should be ignored. The readings from second injections onwards are usable (to ensure the complete flushing of the system with new sample).

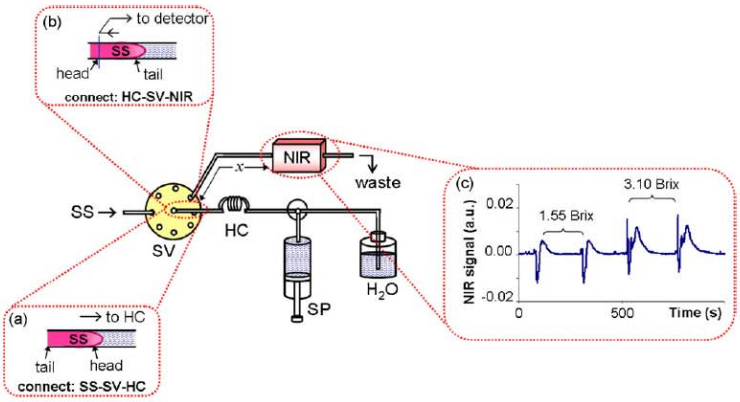

Fig. 2. Design and set up of the SIA system for sucrose content measurement with (a) the contour of sample zone being aspired into the holding coil (HC, 127 cm), (b) the contour of final zone of sample prior being pushed toward the NIR detector and (c) signal profiles of sucrose solutions (SS) at 1.55 and 3.10 Brix (300 μL injection). SV: selection valve, SP: syringe pump.

As we introduce the laminar flow towards detector, this left end becomes the leading parabolic interface, which is responsible for the schlieren signal in our system.

3.1.2. Light source

Theoretically, any wavelength not absorbed by the sample should be suitable for detection of the schlieren effect. For a colorless aqueous sample containing sucrose, any visible wavelength would be appropriate. For colored samples such as those in popular carbonated drinks (e.g., orange, strawberry, mixed fruit and cola drinks), the wavelength must be carefully chosen to ensure that only the RI effect is detected. Results from the absorption spectrum obtained from target samples, including all sorts of carbonated drinks, as well as natural drinks such as juice and tea drinks, suggested that if a NIR light source is used, sucrose content can be detected without prior decoloration of the samples. In this work, a near infrared LED (890 ± 40 nm) was fitted directly into the photometer used with the SIA system for sucrose analysis in Fig. 2.

3.1.3. Signal profile

We used the SIA set up in Fig. 2 for carrying out injections of sucrose solutions (1.50–46.50 Brix) and compared with the results obtained from a single-line FIA manifold (not shown). Fig. 2c and Fig. 3a depict some of the signal profiles obtained from the SIA experiment. We observed that the FIA results are similar (r^2 = 0.998).

The results in Fig. 2c indicated that the schlieren profiles of a dilute sucrose solution (1.50 Brix) began with a sharp negative first peak, following by a sudden positive second peak. This pattern of diluted sucrose is similar to the pattern of the schlieren profiles reported for sodium chloride [15] and for glycol [24]. However, the first peak became less negative as we increased the concentration of sucrose to 3.10 Brix (Fig. 2c). This negative first peak disappeared with injection of sucrose beyond 7.75 Brix (Fig. 3a), but was replaced by a positive signal. Finally we obtained a two-positive peaks signal for an injection of sucrose solutions from 7.75 to 46.50 Brix.

This pattern of two-positive peaks signal, observed for high sugar concentrations in Fig. 3a, are similar to the profiles as reported for sucrose solution [12] as well as for sucrose, glycol and ethanol [13]. We think the schlieren pattern may change accordingly to the

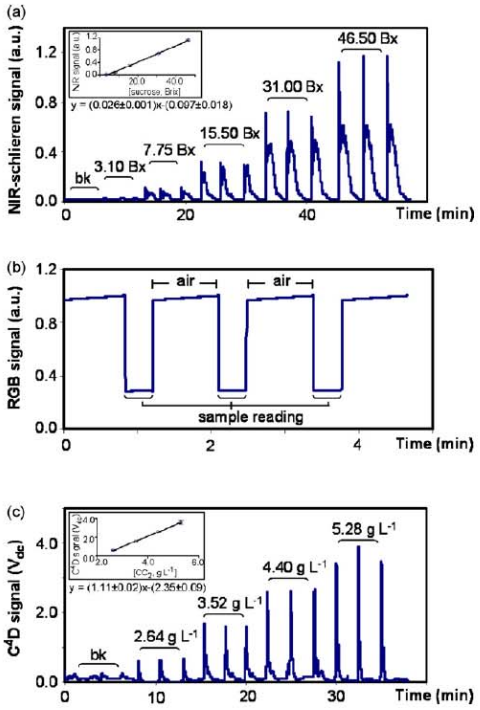

Fig. 3. Signal profiles and calibration graphs obtained from the reagent-free SIA module for (a) sucrose with triplicate injections of a series of sucrose standards, (b) color with triplicate injection of a cola drink: LED set at 'RED' emission and (c) CO_2 with triplicate analyses of standard $NaHCO_3$ equivalent to the dissolved CO_2.

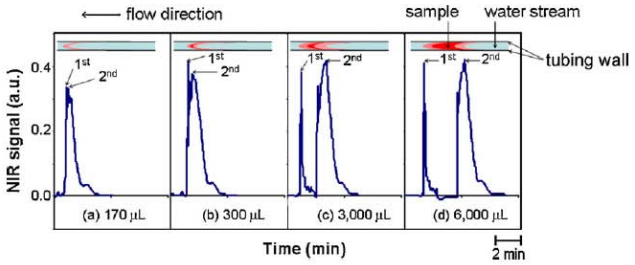

Fig. 4. Effect of sample volume on the profiles of the schlieren signal for a sucrose solution of 15.50 Brix.

concentration and the viscosity of the solution, as observed here. We think that various schlieren profiles can also occur depending upon optical arrangement of instrument and the flow path.

3.1.4. Optimization study

Since no chemical reaction was involved, the SIA system for measurement of sucrose content was optimized by investigating only physical parameters such as sample volume, flow rate, and tubing size. The optimization was carried out using the stand alone SIA system in Fig. 2.

3.1.4.1. Effect of sample volume on the profile. Fig. 4a–d shows the profiles obtained from aspirating a standard sucrose at different volumes. We observed doublet signals for volumes 170 μL (Fig. 4a) and 300 μL (Fig. 4b), and the signals were well separated into two peaks for larger volumes such as 3000 μL (Fig. 4c) and 6000 μL (Fig. 4d). For the results in Fig. 4c and d, the first peak was the result from the schlieren effect at the head of the zone, and the second peak was the result of the same phenomenon at the tail of the same bolus [36]. The distance between the gap of the first and the second peaks is related to the sample volume. At low volumes such as 170 and 300 μL, the dispersion of the sucrose zone exists throughout the sample zone. However, for longer zone, such as 3000 and 6000 μL, there is only dispersion at the 'head' and the 'tail'. Theoretically, a non-dispersed zone for 3000 μL sample would have a zone length of 382 cm inside a 1 mm i.d. tube. As the flow path (denoted as 'x' in Fig. 2) was comparatively short (20 cm), the middle region of the zone of sucrose would have the 'dispersion coefficient' or D equal to 1 and thus when this middle region of the zone traveled through the 1 cm light path, no schlieren effect is observed. This behavior is the basis of the so-called "large-injection" approach, in which only the central zone, remote from the schlieren peaks, is used for quantification [37]. This explains the two separate peaks which obtained from injection of the volumes 3000 and 6000 μL, in Fig. 4c and d, respectively.

In this work, the signal height obtained from the first peak was used for making the calibration. In order to measure purely the schlieren effect from the leading interface, well separate profiles like the ones in Fig. 4c and d seem to be ideal. However these profiles were attained at the condition in which extremely large volumes of sample were used. The analysis time would have been unnecessary long if these volumes were adopted. However we have tested and found that 300 μL was also usable. Although the first peak was partially separated from the second peak for this volume, we could still identify and measure only the height of the first signal. For further experiments, a volume of 300 μL was employed for the sugar analysis.

3.1.4.2. Flow rate. The effect of flow rate was investigated over the range of 1–4 mL min^{-1}. Although higher flow rates provided greater sample throughput, this was at the expense of precision. The %RSDs of repeated injections (n = 10) of a 31.00 Brix sucrose solution were 3.1, 3.6 and 6.0, at 1, 2 and 4 mL min^{-1}, respectively. The sensitivity of 1 mL min^{-1} was the lowest. The observed sensitivities were comparable for 2 and 4 mL min^{-1} flow rates. In order to achieve fast analysis with good precision, a flow rate of 2 min mL^{-1} was therefore chosen.

3.1.4.3. Tubing size. It is known that tube diameter can affect the dispersion of the sample zone. In a measurement based on the schlieren effect, minimal carrier-sample mixing is preferable in order to maintain the integrity of the interfacial boundary between the zones of different RI, and thus the intensity of the schlieren signal. In this SIA system (Fig. 2), the diameter of the tube connecting the selection valve and the detector (x) would have most influence on dispersion and thus the size of schlieren signal, and this was investigated using 20 cm tubes of 1.0, 1.6 and 3.2 mm i.d. The sensitivities of the 1.0 and 1.6 mm i.d. tubes were not significantly different. However, the dispersion was very large for the 3.2 mm i.d. resulting in very low signals as outcome, and thus the 1.0 mm i.d. PTFE tube with 1.6 mm o.d. was used in the final module configuration.

For the same reasons, the shortest possible length of tubing (20 cm) between the selection valve and the detector was used.

3.1.5. Analytical features

At the optimum condition (sample volume: 300 μL; carrier flow rate: 2 mL min^{-1}; tubing i.d.: 1 mm and x = 20 cm), absorbance profiles were obtained as shown in Fig. 3a, and used to prepare a calibration graph of the first peak and Brix value. The inset figure of Fig. 3a illustrates a satisfactorily linear calibration from 3.10 to 46.50 Brix ((peak height, a.u.) = (0.026 ± 0.001)[sucrose, Brix] − (0.097 ± 0.018): r^2 = 0.998). The system provided reasonable recoveries ranging from 95.8 to 105% with good precision (%RSD = 3.2, n = 10). The limit of detection (LOD, 3S/N) and limit of quantitation (LOQ, 10S/N) of 2.79 and 2.88 Brix were obtained for sugar, respectively. Precision diminished at sucrose concentrations of greater than 46.50 Brix, because of the effect of increased viscosity. The system tolerates temperature change within 3–4 °C.

3.2. Extension of the module for monitoring of color and dissolved CO_2

After the optimum condition for measurement of sucrose content was attained, the main module (Fig. 2) for sucrose content was modified (Fig. 1) to include sections for color measurement (section B) and another for monitoring of dissolved CO_2 (section D).

Table 2
Analysis of carbonated soft drinks for sucrose, color and dissolved CO_2 using SIA module.

Carbonated drink	Sucrose content (Brix)		Color (absorbance)	Dissolved CO_2 ($g\,CO_2\,L^{-1}$)
	Our method	Refractometry		
1. Regular cola	5.3 ± 0.2	6.8 ± 0.1	0.305 ± 0.001[a]	3.1 ± 0.1
2. Sugar-free cola	n.d.[b]	0.85 ± 0.0	0.290 ± 0.002[a]	3.1 ± 0.1
3. Strawberry	12.5 ± 0.2	11.1 ± 0.1	1.130 ± 0.008[c]	3.2 ± 0.2
4. Orange	11.7 ± 0.2	10.5 ± 0.1	0.178 ± 0.013[a]	3.3 ± 0.2
5. Mixed fruit	11.9 ± 0.3	11.2 ± 0.1	0.432 ± 0.004[a]	3.4 ± 0.1

[a] With the RGB-LED set at 640 ± 20 nm.
[b] n.d.: non-detectable.
[c] With the RGB-LED set at 470 ± 25 nm.

3.2.1. Quality control of the color

Color appearance of soft drink is a necessary feature in the quality of carbonated drinks. In this work, we present an incorporation of a small part to the SIA core system (section B, Fig. 1) for simple detection of color content in production of soft drink. A portable photometer, with an RGB-LED, was attached to port 1 of SV2. With the feature of the RGB-LED, the applicability of this photometer covers all the absorption wavelength of most types of soft drink.

According to Table 1, we adopted the air-segmented technique [38] in our SIA module for monitoring absorbance of the beverage. The system was designed to feed the liquid sample to the flow cell with no dilution. To avoid sample dispersion, air was used to sandwich the liquid sample during the delivery to detector. After the degassing process in section C (Fig. 1), the selection valve SV2 (Fig. 1) connects port 4 (air)-SV2-HC. Air (3000 μL) was drawn into the holding coil. SV2 then connects ports 6 (SS)-SV2-HC for aspiration of 800 μL of soft drink into the holding coil. SV2 was finally switched to port 1 (to connect 'HC-SV2-RGB'), and the air-sandwiched segment sample was then pushed from the holding coil to the detector. Fig. 3b shows examples of the obtained profiles. This section for on-line measurement of the color offers a robotic feature with high standard of precision %RSD = 0.3–1.7 (n = 15). This indicated that the carryover was not a problem in this system, even though it is operated in the segmented flow mode.

3.2.2. On-line degassing unit and monitoring of CO_2

If samples are directly aspirated into the SIA system (section A and section B in Fig. 1) without prior degassing, dissolved CO_2 strongly interferes in the determination of sucrose content and color by creating lots of bubbles. Thus, it is necessary to include a mechanical apparatus, which can be installed on-line with the SIA system for degassing the CO_2. In this work, the system was furnished with a degassing unit that was constructed similarly to the MBL-VP unit, formerly developed for analysis of calcium supplement [32].

Originally, the MBL-VP unit was designed for generating a gaseous form of the analyte species inside its chamber. The unit has a groove under the lid for the flow of acceptor solution from the outside through to detector. With this arrangement, the compartment of acceptor and the gas–donor mixture are separated by air space inside the unit. Vaporization of the gas from the donor up to the top of the unit can be accelerated by introducing magnetic stirring. Basically, this unit offers a sample preparation via gas–liquid separation.

Degassing of carbonated drink could also be achieved by fast stirring of the sample, but this work, the MBL-VP unit was adopted for degassing the soft drink. The efficiency of the degassing of CO_2 (step 2 in Table 1) is sufficient. While the sample was being degassed, CO_2 re-dissolved in the acceptor stream could also be detected. For this work, the former MBL-VP unit was slightly modified (sections C and D in Fig. 1) by the addition of three ports. Two ports at the bottom were for the sample, while the port situated slightly above the port at the bottom right was for the overflow.

At the start of the analysis cycle, carbonated drink was drawn from the process line into the unit, equipped with a continuous stirring magnet. While sample was being degassed via agitation, some of the gaseous CO_2 dissolved in the acceptor stream, resulting in an increased signal from the C^4D (Fig. 3c). Meanwhile, 1100 μL of degassed sample was drawn into the holding coil for consecutive measurements of sucrose and color contents, respectively.

For the analysis of dissolved CO_2, a satisfactorily linear plot of C^4D signal versus concentration of CO_2 was obtained (inset, Fig. 3c). An example of calibration equation is (peak height, V_{dc}) = (1.11 ± 0.02)[CO_2, $g\,CO_2\,L^{-1}$] − (2.35 ± 0.09). The linear regression was close to unity (r^2 = 0.999). The linear range covers the concentration of CO_2 in the soft drinks. The system gave a reasonable precision with %RSD ranging from 2.9 to 6.3 (n = 15). We determined the LOD and the LOQ for CO_2 using repetitive injections of a standard solution of 2.64 $g\,CO_2\,L^{-1}$ The LOD (3SD) and LOQ (10SD) of 2.23 and 2.53 $g\,CO_2\,L^{-1}$ were obtained, respectively.

3.3. Applications

The SIA module in Fig. 1 was applied and tested in the analysis of some can soft drink samples. The results (Table 2) from the proposed method for sucrose content measurement agreed significantly well with the refractometric method at 95% confidence (t_{stat} = 0.975, t_{crit} = 3.182). We also tested the system for measuring the color of soft drinks. All the readings from the RGB photometer lied in appropriate range of detection signal. For our tested samples, we needed only two wavelength regions (red and blue light). We found that the level of dissolved CO_2 of all the carbonated drinks is comparable at 3.2 ± 0.2 $g\,CO_2\,L^{-1}$. Since the analysis had to be carried out after opening the cans, it is therefore expected that some of the CO_2 would have escaped during the transfer of samples. Thus, this average level of CO_2 found in the samples will be less than the actual level before sampling [30].

3.4. Versatility of the system

Although the SIA module in Fig. 1 was developed for the manufacturers of carbonated soft drinks, the system can be easily modified to fit manufacturers producing other types of drinks. For non-carbonated drinks, the manifold in section C (for degassing) and section D (for CO_2 analysis) can be omitted. We have tested the applicability of the method in measuring sugar contents in 10 samples of non-carbonated drinks, including green tea, black teas (2 samples), chrysanthemum tea, amino drink, energy drink, apple juice, syrup of palm-sugar, syrup of red sala-cider, and syrup of green cream soda. The results of sugar contents obtained by our schlieren method for these samples agreed statistically well with the results obtained by a refractometric method (t_{stat} = 0.509, t_{crit} = 2.306) [39].

As well as the application in process analysis, the system is also suitable in the laboratory for quality control purposes. If the system in Fig. 1 is used as an off-line system, a reasonable through-

put of 9 samples h^{-1} would be obtained with complete results of the 3 parameters. Nevertheless if the system is detached and used separately, faster throughput for single analysis of 17 samples h^{-1} (sugar), 42 samples h^{-1} (color) and 37 samples h^{-1} (CO_2) could be achieved.

4. Conclusions

We present a completely reagent-free module based on SIA for process analysis in the manufacturing of carbonated soft drinks. The module offers a rapid and simultaneous analysis of contents of total sugar, color and dissolved CO_2, within 6.5 min. In our work, SIA was utilized to achieve automatic liquid handling of the sample. The light sources in the two photometers were deliberately selected based on the priority of being small in size, robust and minimum cost. Since our samples contain only one type of sugars that is sucrose, only a simple LED detector emitting a non-absorbing wavelength by the sample is sufficient. The detection of the schlieren effect could be simply achieved by employing commercial 10-mm pathlength flow-cell with the NIR photometer. However for samples with complexity and requirement in identification of sugar contents, a more sophisticated type of detection such as the FTIR spectrophotometer with a suitable chemometric evaluation has been employed for SIA [40–42].

Here we have utilized the schlieren effect for monitoring sugar contents in soft drinks. Two other parameters such as color and dissolved CO_2 can be concurrently determined by this system in real-time. Our system is totally environmentally friendly since the detection systems do not rely on use of any chemical. Therefore the system is attractive to industrial users. Either dual-measurement of sugar and color contents, or the full-option measurement of the three parameters, may be used as required. The module can also be used as an off-line system in laboratory for the quality control of the finished products.

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AWARD

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PUBLICATIONS

1. "A multiple processing hybrid flow system for detection of formaldehyde contamination in food", **S. Teerasong**, N. Amornthammarong, K. Grudpan, N. Teshima, T Sakai, D. Nacapricha and N. Ratanawimarnwong, *Anaytical Science* 26 (2010) 629-633.

2. "A reagent-free SIA module for monitoring of sugar, color and dissolved CO_2 contents in the manufacture of soft drinks", **S. Teerasong**, S. Chan-Eam, K. Sereenonchai, N. Amornthammarong, N. Ratanawimarnwong and D. Nacapricha, *Analytica Chimica Acta* 668 (2010) 47-53.

3. "Development of a predict-observe-explain strategy for teaching chemistry at undergraduate level", **S. Teerasong**, P. Ruenwongsa and D. Nacapricha, Submitted to *International Journal of Learning*.

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