Simultaneous Injection Effective Mixing Flow Analysis (SIEMA): Its Development and Application

Nuanlaor Ratanawimarnwong^{1,2}, Norio Teshima³, Tadao Sakai^{3,*}, Duangjai Nacapricha^{1,4} and Shoji Motomizu⁵

¹Flow Innovation-Research for Science and Technology Laboratories (FIRST Labs).

²Department of Chemistry, Faculty of Science, Srinakharinwirot University, Sukhumvit 23 Road, Bangkok 10110, Thailand

³Department of Applied Chemistry, Aichi Institute of Technology, 1247 Yachigusa, Yakusa-cho, Toyota 470-0392, Japan ⁴Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

⁵Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan

Abstract

The development and application of a new simultaneous injection- effective mixing flow analysis (SIEMA) is described. The manifold is based on one syringe pump with a two-way solenoid valve, a set of holding coils and three-way solenoid valves, two multiport connectors, a mixing coil and a detector. The system is compact and is controlled by a laptop computer. Some analytical features of SIEMA are compared with other flow-based systems in terms of mixing efficiency, reagent consumption and sample throughput. Applications of the SIEMA system are demonstrated for environmental and clinical analysis.

Keywords Flow-based method, simultaneous injection effective mixing flow analysis, SIEMA

1. Introduction of flow-based techniques

The high demand for chemical analyses in clinical, agricultural, pharmaceutical and industrial laboratories has led to a tremendous number of research papers published on development of automation of analysis. Automation provides precision, low cost per assay and reliability of operation. The first practical automated continuous flow method was introduced by Skeggs in 1957 [1]. The system was commercialized by Technicon under the trade name Autoanalyzers [2]. The instrument has been widely applied in clinical and environmental monitoring laboratories. A characteristic of the system is that the sample stream is segmented by air in order to avoid contamination between the samples. At predetermined points, reagents are added at fixed flow rates. Then reaction stream flows to the detector with removal of the air segment before the liquid zone enters the detection cell for precise measurements.

Flow injection analysis (FIA or FI), first introduced in 1975 by Ruzicka and Hansen [3], offers automation and rapid analysis suitable for dealing with large numbers of samples. The concept of FI is based on injection of the sample into a rapidly flowing carrier stream without segmentation by air. Thus the sample becomes a part of a continuously moving stream. The sample zone is mixed with another stream of reagent at a fixed flow rate for chemical reaction to take place. The processed stream finally flows through the cell of a detector where the signal is continuously recorded. FIA technique is now recognized as a standard tool in quantitative analysis. However it has some weakness with regards to the consumption of large amounts of reagents and carrier solutions due to the continuous monitoring process. In 1990, Ruzicka and Marshell introduced sequential injection analysis (SIA or SI), a fully automatic system operated through a computer [4]. In SI, small volume of sample and reagent(s) are sequentially aspirated into a holding coil using a syringe pump with a selection valve. The

stacked layers of sample and reagent(s) in the holding coil are then propelled in the opposite direction of flow to a detector. The volume of carrier and reagents are much less compare to the FIA technique. In addition the SIA system is more robust and versatile by the use of syringe pump instead of the peristaltic pump. However, SIA has limitations due to the low mixing efficiency of the stacked zones and lower sample throughput.

Cerda et al. proposed the 'multi-syringe' flow analyzer or 'MSFIA' in 1999 [5]. The system employed automated syringe pumps for robustness and versatility. The basic system of MSFIA comprises four syringes that are connected in one block to the same stepping motor. А three-way solenoid valve is fitted at the head of each syringe for selection of the flow direction. These valves allow injection of precise volume at the required time. Thus like SI, MSFIA uses less volume of reagents than FIA with comparable sensitivity [6]. Multicommutation flow analysis (MCFA), introduced by Reis et al.[7] is a continuous flow strategy in which handling of sample and reagent is carried out by computerized control of three-way solenoid valves and a peristaltic pump. In the arrangement of the MCFA, the peristaltic pump is placed at the end of the flow line after detection cell. Recent development of MCFA has been reviewed by Feres et al.[8].

Itabashi et al. proposed 'all injection analysis' system or 'AIA', in 2001 [9]. In a contrast to the other flow systems, AIA is manually operated. All solutions are injected into a reaction coil via six-port valve that are connected together as one big loop. Circulation of solutions inside the loop is carried out using a double plunger micro-pump to promote mixing of the solutions before detection. In 2007, an alternative stopped-flow technique, called 'stopped-in-loop flow analysis' or 'SILFA' was introduced by Teshima et al [10]. With the SILFA system, both sample and reagents are propelled and mixed on-line in the This stream of mixed solution is then loaded into a system. six-port valve. When the six-port valve is switched, the mixture is trapped in a loop. In this position, only carrier is flowing through the detection cell. Finally the six-port valve is

^{*}Corresponding author. E-mail address: tadsakai@aitech.ac.jp

switched back to the original position and the carrier now propels the reaction mixture to the detector. Both AIA and SILFA have relatively low reagent consumption and are suitable for relatively slow chemical reaction.

Recently, another flow injection strategy named 'cross injection analysis' or 'CIA' was introduced by Nacapricha et al. in 2013 [11]. The basic system of CIA comprises two peristaltic pumps and a flow platform made from a rectangular acrylic block, with crossing cylindrical channels drilled out along the x- and y- axis. The main (or x-axis) channel is for the carrier stream whereas three (or more) channels in the y-axis are for sample and reagent streams. Both ends of these later channels are connected with tubing to the same set of rollers of one peristaltic pump. Thus sample and reagents solutions simultaneously fill the y-axis channels. At the same time, carrier stream flows through a x-axis channel by the second pump to propel solutions at the crossing zone to a detector. This cross-flow results in prior mixing in the platform, leading to high sensitivity. CIA system uses no valves and is a cost effective technique.

2. Concept of simultaneous injection-effective mixing flow analysis (SIEMA)

In 2010, another flow injection called 'simultaneous injection-effective mixing flow analysis' or 'SIEMA' was introduced [12]. The SIEMA system is based on simultaneous aspiration of small plugs of sample and reagent with mixing at one confluence point via multiple ports connector to attain efficient turbulent mixing. Chemical reaction is allowed to take place inside a mixing coil before flowing to a detector. The basic system consists of a bidirectional syringe pump, three (or more) three-way solenoid valves and a two-way solenoid valve all connected together via PTFE tube and four-way cross connector (Fig.1).



Fig.1 Schematic diagram of typical three-channel SIEMA system. AC, auxiliary coil; $4C_1$ and $4C_2$, 4-way cross connector; HC_1 , HC_2 and HC_3 , holding coils; $3V_1$, $3V_2$, $3V_3$ and $3V_4$, 3-way solenoid valves; MC, mixing coil.

Selected volume of sample and reagent solutions are aspirated into separate holding coils through individual solenoid valves before propelling (or injecting) simultaneously toward the detector through mixing coil. All tubing from $4C_1$ (Fig.1) to waste are flushed with the carrier stream to prevent contamination from the preceding sample. The SIEMA system has distinguishing performance as follow:

- Rapid analysis compared to SI; as all solutions are simultaneously aspirated and dispensed.
- Efficient mixing comparable to FI by employing multiple connection ports to facilitate radial mass transfer.
- Robustness similar to SI; as the syringe pump is used. Eliminating imprecision from the flexible tubes of a peristaltic pump.
- Low reagent consumption compared to FI; as only micro-liter of reagents is used.
- Simple flow manifolds compare to FI without use of multi-channel pumps or complicated flow schemes.

3. Application of SIEMA

Motomizu reported the initial study of SIEMA in 2006 [13]. The first publication concerning SIEMA system was in 2010 for the determination of palladium [12] using a three-channel SIEMA system with spectrophotometric detection palladium on the reaction between based and 2-(5-bromo-2-pyridilazo)-5-[N-n-propyl-N-(3-sulfopropyl)-amino]aniline. Comparison of analytical performance between SIEMA and FIA system was also discussed. It was found that sensitivity and repeatability of SIEMA system was comparable to FIA system. However, the reagent consumption was approximately 3-times less. The emphasis was on effective mixing with minimal reagent consumption. Application to other spectrophotometric determination was carried out for residual chlorine in tap water [14] using the same three-channel SIEMA system, based on reaction of chlorine with 4,4'-bis-dimethylaminobenzophenone in the mixture solution of Triton X-100, 2-methoxyethanol and formate buffer, to form blue compound. Accuracy of the method was successfully validation with the official N.N-diethyl-p-phenylenediamine (DPD) method [15]. Sample throughputs of 44 h⁻¹ and of 87 h⁻¹ were attained for analysis of palladium and residual chlorine, respectively.

Clinical analysis usually involves large numbers of samples, and a flow based technique is suitable in terms of simplicity, rapid analysis and automation. Use of SIEMA technique for the analysis of bilirubin in urine has also presented [16]. The color development was based on the reaction of free bilirubin with diazotized sulfanilic acid in the present of *n*-octyl- β -D-thioglucoside (OTG). The system was applied to 10 healthy adults and the results obtained by the proposed method were found to be in good agreement with those obtained by the batch-wise diazo method [17]. The sample throughput of this method was 45 h⁻¹. The method is sensitive and applicable as a liver function test in the clinical laboratory.

A four-channel SIEMA system has also been assembled for determination of urinary albumin [18]. Albumin reacts with tetrabromophenol blue anion (TBPB) in Triton X-100 micelle to from a blue ion complex that can be detected at 625 nm. TBPB, Triton X-100, acetate buffer and albumin in sample (or standard solution) were aspirated into four individual holding coils before pushing simultaneously to the detector flow cell. This work presented modified operation of SIEMA system to have additional washing step for removal of TBPB-albumin complex on the wall of hydrophobic PTFE tubing by aspiration of Triton X and buffer solution prior to the step of simultaneous aspiration of all solutions. When the washing step was included, baseline drift was minimized and sharp signal peaks with constant baseline were obtained. In addition, the assay of protein in urine using this method was in good agreement with Bradford's method [19]. Therefore, the SIEMA system was proposed as an alternative method for routine diabetic screening.

In 2013, SIEMA system was developed for successive determination of urobilinogen and bilirubin in human urine [20]. A four-channel SIEMA system was employed. Fig.2 presents schematic diagram of the proposed SIEMA system. For urobilinogen assay, the measurement was based on reaction between urobilinogen the and p-diethylaminobenzalehyde (p-DEABA) in hydrochloric acid. For analysis of bilirubin, the reaction with diazotized sulfanilic acid in OTG was used. Absorbance detection at 560 nm was employed for both measurements. The operational sequence started with bilirubin determination using two channels (two holding coil) for separate aspiration of a bilirubin solution and a mixed reagent followed by pushing these two aliquot to merge subsequent analysis. Subsequently, for urobilinogen determination was carried out in the same manner as for bilirubin but using the other two channels of the system. The method was successfully applied to 20 urine samples taken from subjects with different ages. The speed of analysis was 30 h⁻¹ for dual analysis.



Fig.2 Schematic diagram of typical three-channel SIEMA system for successive determination of bilirubin and urobilinogen. AC, auxiliary coil; $5C_1$ and $5C_2$, 5-way cross connector; HC_1 , HC_2 , HC_3 and HC_4 , holding coils; $3V_1$, $3V_2$, $3V_3$, $3V_4$ and $3V_5$, 3-way solenoid valves; MC, mixing coil.

Conclusion

In this article, the new flow technique, SIEMA, was reviewed. The system was developed to have the advantages of FIA, SIA and multicommutation in flow-based analysis. Basically, it involves the adoption of an automated syringe pump to aspirate sample and reagent solutions into individual holding coils before simultaneous injection of these zones to mix in a holding coil before flowing to a detector. By using multichannel connector, effective mixing of all zones was accomplished. Applications of the SIEMA system have been successfully demonstrated to environmental analysis and clinical analysis. The SIEMA system provided features of efficient mixing of reagents, low reagent consumption and rapid analysis.

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