

Review

Recent Electrochemical and Optical Sensors in Flow-Based Analysis

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Abstract: Some recent analytical sensors based on electrochemical and optical detection coupled with different flow techniques have been chosen in this overview. A brief description of fundamental concepts and applications of each flow technique, such as flow injection analysis (FIA), sequential injection analysis (SIA), all injection analysis (AIA), batch injection analysis (BIA), multicommutated FIA (MCFIA), multisyringe FIA (MSFIA), and multipumped FIA (MPFIA) were reviewed.

Keywords: Flow injection analysis (FIA), FI, SIA, AIA, BIA, MCFIA, MSFIA, MPFIA, electrochemical detection and optical detection

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1. Introduction

Chemical flow-based analysis was first introduced in the middle of 20th century [1]. This technique has been developed from an attempt to collect the fractions eluted in the chromatographic separation process in an industrial plant. At the end of 1950s, such a concept was outlined in a practical way and become a sample delivery system within a fluid, known as segmented flow analysis (SFA) [2]. Using air segments within the flowing stream in the SFA technique increases the homogeneity between the sample and reagent. Many laboratories rapidly accepted this approach. But the two main drawbacks are the debubble processing of the fluid before entering the detector and the high reagent and sample consumption due to the continuous flow.

A new technique, flow injection analysis (FIA) [3], was introduced by Ruzicka in 1975. It became one of the most popular techniques for analytical applications within a short period of time because of its simplicity and ease of use. An injection valve (6-port valve with a small-size sample loop) is used to minimize the sample consumption. No air segments are required in this approach, thus, it offers more simplicity and robustness. Easy implementation together with the high sample throughput is the main advantages, whereas the high reagent consumption rate is unsolved. The FIA was originally designed for an automated system. It has been used as a general solution for handling or sample introduction systems. A current literature survey reveals that this technique can also be used in many branches of analytical research such as a routine methodology in chemical monitoring processes, chemical and bioanalytical sensors, and clinical applications including food and drug industries. Moreover, numerous variant techniques based on FIA, such as sequential injection (SIA), batch injection (BIA) [4-7], multicommuted flow injection analysis (MCFIA), all injection analysis (AIA) [8], multisyringe flow injection analysis (MSFIA), and multipumping flow injection analysis (MPFIA), have been developed over the past few decades. However, FIA is still the most widely used technique because no complicated instruments or computers are required. In SIA [9], sample and reagent segments are sequentially introduced in a holding coil by using selection valve before the sample and reagent are impelled by piston pump through the mixing coil toward the detector. This process was controlled by a microprocessor or software. The great advantage of this technique is an important saving in the samples and reagents, whereas a low analysis rate and complicated software, including an expensive instrument setup are the main drawbacks.

Another approach, BIA, is conceptually similar to FIA in that the injected sample zone is transported toward the detector. In this technique, a small liquid sample is injected toward the nearby flat sensor surface resulting in a transient signal similar to those in the FIA readout. Although this technique is considered a “non-flow injection-based technique” from the fact that no transport of either sample or reagents takes place within the tubing, it has been demonstrated to be a very attractive approach for automation analysis. In MCFIA, the sample injector used in FIA is replaced by three-way

solenoid commutation valves, which are controlled by computer. The injected volume can also be adjusted by controlling the commutation timing via software. Since the sample and reagents are injected into the flow system only when necessary, the consumption rate and waste generation can be minimized. Peristaltic pumps are still the most common liquid-propelling drives in MCFIA. The flexible pump tubing needs to be replaced periodically. This factor is one of the main problems of this technique. The pump tubing lifetime can be eliminated in MSFIA. This technique is based on multiple syringes which are used as liquid-propelling devices. MSFIA takes advantage of manifold operation and high sample throughput of FIA together with the robustness and versatility of SIA. But the main drawback of the technique is that the liquid carrier needs to be refilled periodically. In MPFIA, a series of individual solenoid micro-pumps are used instead of a peristaltic pump. Each micro-pump is independently responsible for the introduction of carrier, sample, reagents, and eluent. In comparison with MSFIA, MPFIA is more practical by the fact that a sample injector or holding coil is not required. Moreover, the periodic refilling procedure of the liquid carrier can be eliminated. Some interested cost-effective flow-based analysis have been reviewed by Grudpan [10]. The schematic diagrams of FIA and variant configurations are shown in figure 1.

In the next two sections, we will briefly review flow-based electrochemical detection and flow-based optical detection.

2. Flow-based electrochemical detection

Normally, a flow system with electrochemical detection is carried out based on a single-line configuration. This is because no reagent is required to develop the color of the sample. However, many systems, such as SIA or MSFIA, use a manifold in order to increase versatility. One of the most important parts in flow system is a flow cell, which is a place to hold the electrodes. An electrochemical reaction takes place inside the flow cell, and then the electrochemical data such as current or potential will be recorded. Many configurations of a commercial flow cell, such as wall-jet, thin-layer, and flow-through cell can be applied to this system [11]. The cost of an instrument can be reduced by using a homemade-version [12].

2.1. Amperometric detection

In this technique, a constant potential is applied at the working electrode, then the oxidation or reduction reaction of an analyte takes place. A three-electrode system is required for conventional amperometric detection. Metal working electrodes such as Au, Ag, and Pt can be used in this technique. The current responses are directly proportion to the concentration of the analyte. A major drawback of amperometry is the lack of reproducibility due to some adsorption of by-products and/or impurities on the electrode surface (electrode fouling). To obtain reproducible results during analysis, the electrode surface needs to be cleaned frequently by either mechanical polishing or an electrochemical cleaning procedure. FIA with amperometric detectors has been widely used in pharmaceutical applications including food and drink analysis. The main advantages are the low consumption of reagents and samples, better repeatability, high sample throughput, reduction of the risk of contamination during the analysis step, and the relatively low-cost of the instrumentation.

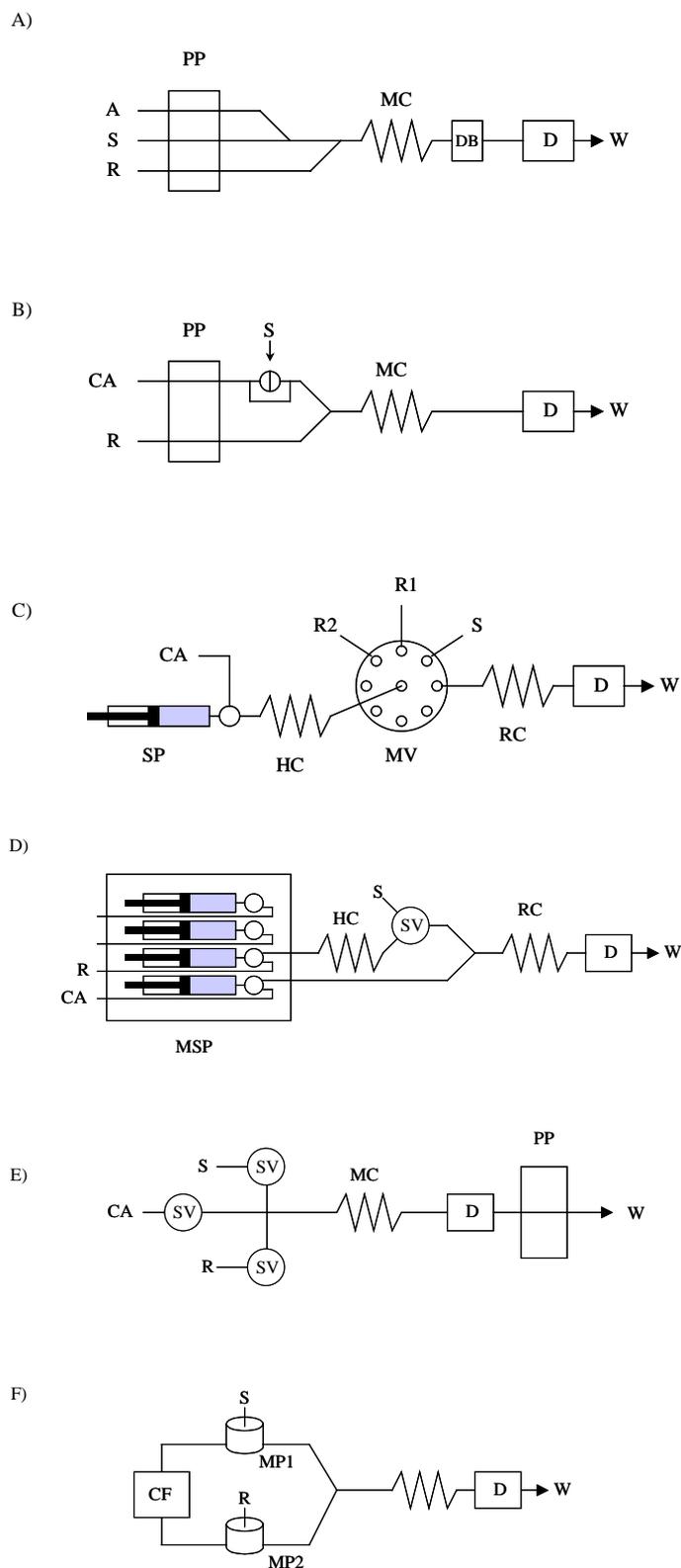


Figure 1. Schematic diagram of flow system: A) Segmented flow analysis (SFA), B) Flow injection analysis (FIA), C) Sequential injection analysis (SIA), D) Multisyringe flow analysis (MSFIA), E) Multipumped flow injection analysis (MPFIA), and F) Multicommutated flow injection analysis (MCFIA): PP = peristaltic pump; A = Air; S = Sample; R = Reagent; MC = Mixing Coil; DB = Debubbler; D = Detector; W = Waste; CA = Carrier; SP = Syringe Pump; HC = Holding Coil; MV = Multi-Ports Valve; RC = Reaction Coil; MSP = Multisyringe Pump; SV = Solenoid Valve; CF = Control Interface; MP = Micro Pump.

As mentioned above, the main disadvantage of amperometry is a deposition of the by product or solution impurities on the electrode surface. Another technique, pulsed amperometric detection (PAD), has been introduced to overcome that kind of problem. PAD offers the possibility of cleaning and reactivating the electrode surface effectively after the measuring cycle without mechanical polishing. In the simplest implementation of PAD, the potential of the working electrode is stepped between the potentials for detection, E_{det} ; cleaning, E_{oxd} ; and reactivation, E_{red} . All three steps of PAD require the following: (a) the oxidation of analyte during the detection step; (b) the oxidative desorption of adsorbed detection products or solution impurities during the cleaning step; and (c) the cathodically dissolution of inert oxide product during reactivation step. Pulsed amperometric detection has been used for the sensitive detection of numerous compounds such as tetracycline [13] and chlortetracycline [14].

Another interesting system is a gas-diffusion flow injection (GD-FI) which can be used for the determination of trace levels of Hg (II) in aqueous samples [15]. The analytical procedure was carried out by injection of Hg(II) samples and standards into an acid stream which was subsequently merged with a reagent stream of SnCl_2 to reduce Hg(II) to metallic mercury. The mercury vapor diffused across a Teflon membrane into an acceptor stream, and then it was amperometrically detected at a gold electrode. This system was successfully applied for the determination of mercury in freshwater samples.

Combination of the FIA system and immobilized cell reactor can be used for dopamine determination. PC 12 cells were immobilized on to the lumen of fused silica microbore tubing. At the optimized conditions, the cells could be attached to the poly-L-lysine layer in approximately 2 h, after which they could be maintained inside of the tubing for a period of up to 5 days. A release of dopamine by Ca^{2+} -induced can be amperometrically detected at a Nafion[®] modified platinum ultramicro electrode [16]. Non-metallic electrodes such as carbon paste, glassy carbon, and boron-doped diamond (BDD) electrodes can also be used as well. Use of a non-metal electrode, especially the BDD electrode, can minimize the fouling effect on the electrode surface. BDD electrode has been proved to be an excellent working electrode in many analytical applications. An extremely low background current over a wide range of potentials offers a lower detection limit. Amperometric detection of acetaminophen [17], *D*-penicillamine [18], homocysteine [19], captopril [20], and tiopronin [21] in pharmaceutical samples using BDD electrodes has been reported. Chailapakul et al. [22] also reported the determination of iodide in nuclear emergency tablets using BDD electrodes. A schematic diagram of flow injection manifold with amperometric detection is shown in figure 2. Moreover, this electrode can also be used to study the electrochemical behaviors of 3,6-dihydroxyphenanthrene in both non-aqueous and aqueous media [23]. The FIA manifold with a relocated detector for renewing the working electrode surface has been reported for the amperometric determination of diltiazem and nimesulide in pharmaceutical formulations [24]. A flow system was constructed with two independent channels based on the concept of multi-site detector locations. An electrochemical cell can be relocated between the two flow channels. Each measurement that was carried out in one of the channels could alternate with the regeneration of the glassy carbon surface in the other channel through the passage of an adequate conditioning solution.

A carbon paste electrode can also be used as an amperometric detector in FIA for dipyrone determination in pharmaceutical formulations [25]. This system offers a fast, simple, and precise

method that is suitable for dipyrone analysis and quality control of pharmaceutical samples. The carbon paste electrode was found to be very stable over several hours without requiring any chemical or mechanical surface renovation. On-line interfacing of a rotating bioreactor and continuous-flow/stopped-flow/continuous-flow processing for the determination of lactate in milk samples has been developed. The high sensitivity can be attained using a bienzymatic system and redox polymer [Os(bpy)₂CIPyCH₂NHpoly(allylamine)] (Os-PAA) as a mediator. This system allows analyzing undiluted milk samples with a good stability and reproducibility with a sample throughput of 30 samples per hour [26]. Amperometric detection of carbohydrates and galactose using single-wall carbon-nanotube (CNT)-modified glassy-carbon electrodes has been reported. The amperometric response is extremely stable, with operational stability of more than 2 hours. The flow-injection response is characterized with a detection limit of 10 μ M and a relative standard deviation of 3.45% (n=20) [27]. A stopped-flow technique with amperometric detection of chlorate has been developed. The reaction of chlorate with excess potassium iodide and hydrochloric acid, forms iodine/triiodide that is further reduced at the glassy carbon electrode. Sensitivity can be improved by increasing the reaction time and stopping the flow while the injected zone is in a mixing coil. This can be done without increasing dispersion of the product zone. This system was successfully applied to the determination of chlorate in soil samples [28].

FIA has also been used in immunosensor applications. An amperometric immunosensor based on graphite paste has been reported for the assay of azidothymidine (AZT) [29]. The potential used for the AZT assay was 435 mV vs. Ag/AgCl electrode. The advantage of using graphite paste is that a surface regeneration for a new assay can be simply done by polishing. This immunosensor was successfully used as a detector in a sequential injection analysis system, and gave reliable results for assays of AZT purity in raw materials and AZT contents in pharmaceutical formulations. Determination of β -D-glucose in different commercial medical. The experimental results are in good agreement with those reported by the manufacturer. The reproducibility of the system in terms of its standard deviation was 2.9% with a 95% confidence level with a lifetime of over 100 days.

Four types of Barbera wines from different origins in northern Italy were characterized using an electronic tongue based on amperometric detection in a flow injection system. A commercial electronic nose has also been used. All data from both E-nose and E-tongue were compared and elaborated on together with those of chemical analysis and color evaluation. All data were treated by multivariate data processing based on principal component analysis (PCA), linear discriminant analysis (LDA), and classification and regression trees (CART) analysis [31]. A sequential injection analysis/amperometric biosensor for the enantioselective analysis of the S-enantiomer of enalapril, ramipril, and pentopril has also been reported [32]. The biosensor used can be prepared by immobilization of L-amino acid oxidase in carbon paste. By using the proper enzymatic reactions to prepare the biosensors, the same system can be used for the determination of L- and D-carnitine [33] or L- and D-methotrexate [34] with a sample throughput of 34 samples per hour.

The determination of tramadol hydrochloride using a flow injection analysis system with amperometric detection has been developed [35]. Tramadol could be determined in concentrations between 9 and 50 μ M with a detection limit of 1.7 μ M at 90 samples per hour. These methods were successfully applied to the determination of tramadol in pharmaceutical dosage forms, without any

pre-treatment of the samples. Recovery trials were performed to assess the accuracy of the results; the values were between 97 and 102%.

The use of microfluidic cells for micro-flow analysis has been reported [36]. These cells can be designed and assembled by thermal transfer of laser printed masks and CD-Rs. Microprocessor-controlled electronic pipettors can be used as liquid propulsion devices instead of conventional oversized peristaltic pumps. This system can be operated in three different modes similar to FIA, SIA, and direct injection analysis (DIA). Various analytes such as promethazine, dipyrone, and chlorpromazine in pharmaceutical formulations can be determined at low picomole quantities with an excellent linearity and high repeatability.

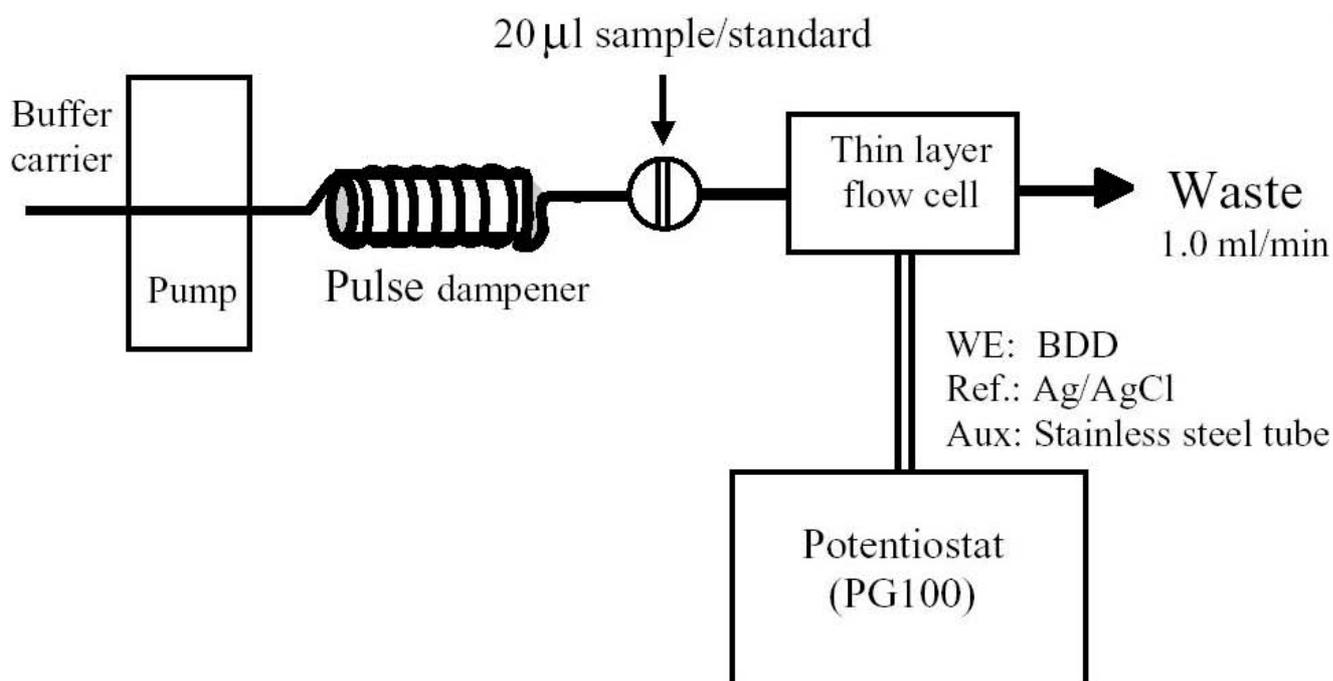


Figure 2 The FI manifold with amperometric detection using BDD as the working (WE) electrode. Optimal condition: potential, 1.0 V; carrier, 60mM phosphate buffer at pH 5.

2.2. Potentiometric detection

Potentiometric methods require a working electrode, a reference electrode, and a device for measuring the potentials. Potentiometry is the most widely used analytical technique because of its simplicity, versatility, and low cost. It is used to detect analytes in titration, biological fluids, etc. The flow injection analysis of hydrogen peroxide using a redox electrode and an Fe(II)-Fe(III) potential buffer solution has been developed [37]. The analysis of high concentrations of hydrogen peroxide up to ~10 M was conducted successfully with a relative standard deviation of 0.7%.

A differential potentiometric method for the determination of lysine based on a flow injection (FI) system has been reported. The lysine biosensor was prepared by chemically immobilized lysine oxidase on a nylon membrane and attached to an ammonium electrode. A circular ammonium electrode was used as a reference. By using differential potentiometry, the possible interferences of

endogenous ammonium can be partly corrected. The sensitivity can be improved from 20 to 40 mV per decade by using stopped-flow technique [38].

A highly sensitive method for the determination of manganese(II) using FIA with a flow-through type redox electrode detector has been developed [39]. This system utilized a redox reaction between manganese (II) and hexacyanoferrate(III) in near neutral media, and the signal can be obtained potentiometrically. Linearity was found over the concentration range from 10^{-4} to 10^{-7} M with a detection limit of 1×10^{-7} M at a throughput of 20 samples per hour. This method was successfully applied to the determination of manganese (II) in soil samples.

Polymer membrane type ion-selective electrodes for a potentiometric determination of phenylpropanolamine hydrochloride have been constructed. A membrane of the first electrode was modified with the phenylpropanolamine-tetraphenylborate (PPA-TPB) ion-pair. Another electrode was modified with the phenylpropanolamine-phosphotungstate (PPA-PT) ion-associate. When applied in the FIA system, both sensors showed fast, stable, and Nernstian slopes over the concentration ranges of 10^{-5} to 10^{-2} M and 10^{-5} to 10^{-2} M in the case of PPA-TPB and PPA-PT, respectively. The advantages of the modified electrodes are its rapid response, simple operation, and low cost [40].

SIA with a potentiometric detection system has been widely used as an automation system for determination of both inorganic cations and anions, such as chloride, iodide, and nickel [41, 42]. Another two SIA system, Lab-at-Valve (SIA-LAV) [43] and Lab-on-Valve (SIA-LOV) [44], for the determination of inorganic ions using ion-selective electrodes have been reported. The LOV concept was introduced in 2000 by Ruzicka [45]. Sample-processing channels with a multipurpose flow cell were integrated on top of a selection valve. This concept can be used to perform wet chemistry at microlitre levels. A simpler designed, LAV, was introduced later [46]. In this new approach, the integrated device (sample-processing channel and detection unit) was simply attached to one port of the selection valve. LAV has more simplicity than LOV in terms of construction. The main advantages in terms of analytical performance should be the same.

Apart from ion-selective electrodes, other electrodes such as carbon paste electrodes can also be used for potentiometric detection. This electrode material can be easily modified with many chemicals to obtain the desired properties or improve the selectivity. The modified carbon paste electrode for the potentiometric determination of the silver content of silver sulphadiazine (burning cream) in both static mode and flow injection analysis has been demonstrated by Ibrahim [47]. This electrode was modified with silver ethylmercurithiosalicylate (silver thimerosal) and also used as an indicator electrode in the potentiometric titration of thiopental and thimerosal with AgNO_3 . Another modified carbon paste electrode which is selective to piribedil (PD) has been reported from the same author [48]. This electrode was prepared by using piribedil phosphomolybdate as an ion-exchanger. The electrode exhibits good selectivity for PD with respect to a large number of inorganic cations, organic cations, sugars, and amino acids. Ibrahim also reported the use of plastic membrane electrodes for the determination of dicyclomine hydrochloride (DcCl) [49] and mebeverine hydrochloride (MvCl) [50]. In case of dicyclomine, the membranes of these electrodes consist of dicyclominium-silicotungstate (Dc-ST), silicomolybdate (Dc-SM), phosphotungstate (Dc-PT), phosphomolybdate (Dc-PM), or tetraphenylborate (Dc-TPB) ion-associations dispersed in PVC matrix with dibutyl phthalate plasticizer. The potentiometric determinations of dicyclominium ions in pharmaceutical preparations, serum, urine, and milk were performed in both batch and flow injection (FI) systems. Mebeverinium-

silicotungstate (Mv-ST), silicomolybdate (Mv-SM), phosphotungstate (Mv-PT), or phosphomolybdate (Mv-PM) ion-associations were dispersed in the PVC matrix for the determination of mebeverinium ions.

Flow-through electronic tongues based on miniaturized solid state electrodes for the recognition of beverage samples has been constructed [51]. The main advantages of the miniaturized system are the small samples needed and the low chemical consumption rate, which significantly reduces the cost of analysis. This system can be used to monitor foodstuff production with a fast response time and high accuracy.

2.3. Other electrochemical detection

The limitation of the batch-injection analysis concept compared to other flow methods is the lack of possibility of on-line analysis. However, some procedures of on-line sample pretreatment in BIA by using of a solid sorbent are possible [52]. This concept is employed in the voltammetric stripping determinations of trace metals using a bed of commercial chelating resin Chelex-100. The electrochemical preconcentration of analytes in the form of amalgams on the surface of mercury thin film electrodes including additional preconcentration on the packed sorbent can be achieved.

Flow injection systems with the following amperometric detection modes: sampled-DC, reverse pulse amperometry (RPA), and anodic stripping voltammetry (ASV) for determination of copper(II) have been reported [53]. A robust and fast-response flow adaptor for mercury drop electrodes (MDEs) is described. An L-shaped PTFE tube with an internal diameter of 0.5 mm is fixed on the glass capillary of a MDE with a silicone ring at a distance of about 0.5 mm. The flow of sample will be directly injected onto the mercury drop. Another automation system based on the flow injection technique with voltammetric detection using electronic tongues has been reported [54]. This system can be used to characterize wastewaters coming from the paper mill industry. A multielectrode array, consisting of platinum, gold, and rhodium electrodes, was used as the working electrode. The measurements were based on large amplitude pulse voltammetry (LAPV), which consisted of scans of pulses from 0 to 1.8 V in 0.2 V steps. The system can be used to monitor the quality of used waters in the industrial facilities.

The conductivity detector usually plays a minor role in flow injection analysis. But, an interesting contactless conductivity detector for the determination of inorganic carbon has been developed [55]. The detectors were tested in water samples by the flow injection analysis combined with a gas diffusion separator. A concentration range from 0.01 to 1 mM NaHCO₃ was tested with a linear dynamic range of up to 0.1 mM. Detection limits of 0.6 and 6 µM with RSD values of 4.1 and 4% were obtained for detectors with the electrodes placed axially and across the tube, respectively. A throughput of 15 samples per hour was attained under an experimental procedure used. Another system based on conductometric detection has been developed [56]. The stopped-flow manifold was used to assay and characterize immobilized tannase (EC 3.1.1.20). The immobilized enzyme reactor was inserted inside the tube-type electrode pair for a real-time conductometric measurement. The activities and kinetic parameters for propyl gallate, methyl gallate, and tannic acid were investigated.

3. Flow-based optical detection

3.1. Atomic absorption spectrometry (AAS)

Due to the short analysis time and feasibility for on-line system, flow-based systems have become one of the most powerful analytical tools in recent years. Various types of detection modes are applied to flow analytical techniques, and atomic absorption spectroscopy is one that is most commonly used sensors. Flow analytical techniques coupled with AAS comprise those involving flow separation and/or preconcentration.

For the ideal of on-line preconcentration, Cerutti and coworkers [57] have described an on-line flow injection preconcentration-electrothermal AAS for the determination of traces of cobalt in drinking water. The minicolumn packed with activated carbon was used as the sorbent. In this approach, an enrichment factor of 190 for a sample volume of 10 mL was obtained using 10% (v/v) nitric acid as an eluent. The detection limit for the proposed method was as low as 5 ng/L. In addition, Gil and coworkers [58], have also recently used this kind of material for on-line sorption trace speciation of chromium in drinking water before ETAAS. An enrichment factor of 35 was obtained for the same conditions as the previous work. This improvement can be attributed to the low detection limit of 3 ng/L.

A flow injection on-line preconcentration-electrothermal AAS coupled with a co-precipitation method for the determination of lead in seawater was developed by Nakajima and colleagues [59]. The preconcentration procedure was comprised of coprecipitation with iron (III) hydroxide and solid phase extraction with a lead-selective resin. This method allowed the determination of lead at the ng/kg level. For the flow-injection manifold of this method has been shown in figure 3. Dadfarnia et al. [60] have also described the on-line preconcentration technique for the determination of silver using a flow system incorporating an immobilized DDTC microcolumn coupled with flame AAS. The immobilization of DDTC on surfactant-coated alumina offers a good deposition of silver that permits effective on-line preconcentration for determination of silver at $\mu\text{g/L}$ level in different matrices. The enrichment factor of 125 was obtained and the precision at 20 $\mu\text{g/L}$ was 4%. For determination of zinc in seawater, Yebra-Biurrun and coworkers [61] have presented a field sampling preconcentration method. The sample was pumped into a minicolumn packed with a chelating resin to enrich the zinc. Then, the minicolumn was inserted into the flow injection system for on-line zinc determination by FAAS. This system showed great advantages over the other existing methods for zinc preconcentration such as analysis time are reduced because several samples can be preconcentrated at the same time.

Recently, the use of SI-LOV became more popular because of the renewable surface. The advantage of this technique over the previous works is that it has no adsorption of interferences on the sorbent surface. Miró et al. have described the sequential injection lab-on-valve coupling to the hydrophobic materials prior to ETAAS detection for the determination of cadmium [62]. C_{18} -PS/DVB beads were used to achieve the goal for a renewable surface because of the homogeneity in bead size, and the perfect shape results in improving in the reproducibility compared to the PTFE beads (3.4% via 4.3%). The potential of the renewable concept of SIA-LOV lead to future use of the C_{18} -polymeric bead. Moreover, octadecyl immobilized silica surface renewable columns in lab-on-valve have been

also used to enrich the trace cadmium and coupled to an electrothermal atomic absorption spectrometry [63].

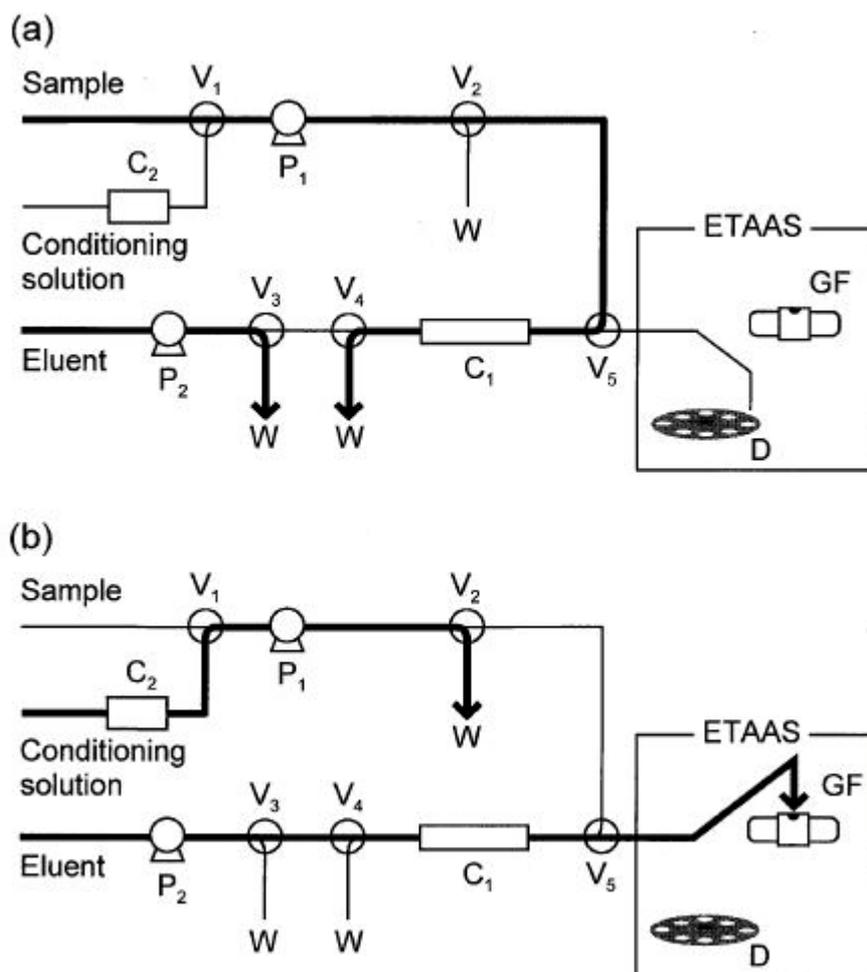


Figure 3 Flow-injection manifold for the determination of lead by an online-preconcentration – ETAAS analysis. (a) Sample loading; (b) elution, C_1 - C_2 , column; V_1 - V_5 , three-way solenoid valve; P_1 - P_2 , peristaltic pump; GF, graphite furnace; W, waste; D, autosample disk.

3.2. ICP-AES, ICP-OES, and ICP-MS

ICP-AES and ICP-OES ICP-MS techniques have some limitations because of the error caused by the matrix effects. Moreover, the direct determination of trace elements in saline sample is limited by the highly dissolved solid elements in the sample. To overcome these problems, sample preparation procedures are required. Solvent extraction is an effective method for the preconcentration and removal of the sample matrix, moreover, the presence of solvent may improve the nebulization and transportation efficiencies. Xu et. al. [64] have designed the glass gravity phase separator for flow injection liquid-liquid extraction to determine rare earth elements. The proposed method was applied for the simultaneous determination of multi-rare earth elements in geological samples with good accuracy and reproducibility.

Hydride generation coupled to ICP-MS is a sensitive method for the determination of various elements. For example, FI-HG-ICP-TOFMS was used for the simultaneous determination of As, Bi, Ge, Sb, Se, and Sn with the pre-reduction treatment [65]. The pre-reduction treatment was used to avoid the problem from the differences between the oxidation states of the element of interest in the standard and sample. In the system, the pre-reduction was conducted to reduce analytes with different oxidation states to the same form. A detection limit of the 0.08-0.54 ng mL⁻¹ was obtained. However, the method based on the chemical hydride generation provided a large amount of hydrogen that is extinguished or destabilized in the plasma. To overcome these drawbacks, electrochemical hydride generation was introduced. As, Bi, Ge, Hg, Sb, and Se were determined using electrochemical hydride generation inductively coupled plasma time-of-flight mass spectrometry [66]. The new design for low sample volume electrolysis cells was used. The method successively evaluated the 12 isotopes in the single transient signal. Under the optimum conditions, the detection limit of 0.07-5.6 ng mL⁻¹ was obtained.

Cloud point method is also used for the preconcentration of metals. Sombra et. al.[67] have combined the cloud point preconcentration-flow injection coupled plasma optical emission spectrometry for trace aluminum determination. The preconcentration procedure was based on the cloud point extraction of aluminum with non-ionic surfactant polyethyleneglycolmono-p-nonylphylether (PONPE 7.5). The detection limit was 0.25 µg L⁻¹, and a linear range up to 200 were obtained for the preconcentration of aluminum in 50 mL parenteral solution. Martinez and coworkers have reported the on-line preconcentration of copper [68] and chromium [69] in parateral solution using a minicolumn packed with activated carbon and subsequently determination using inductively coupled plasma optical emission spectrometry. The detection limit for the preconcentration of 25 mL of sample was 5 µg L⁻¹ and 29 ng L⁻¹ for copper and chromium, respectively. The linear range was near the detection level up to at least 200 µg L⁻¹ for copper and 60 µg L⁻¹ for chromium.

Speciation of chromium (III) and Cr(VI) in fresh water was introduced based on the on-line preconcentration of small-sized thin solid phase (STSP) column resin reactions coupled to flow injection ICP-OES [70]. In this study, anion-and cation-exchange resins (disk type) were packed in the line-filters. Then, they were fixed and serially connected to the loop of each six-way valve. Cr(III) was collected on the cation-exchange while anionic chromate ion, Cr(VI), was collected on the anionic-exchange. Detection limits of 0.04 and 0.02 µg L⁻¹ were obtained for Cr(VI) and Cr(III), respectively. However, the ion-exchange cannot be employed to accumulate chromium in seawater due to the high concentration of seawater matrix. Therefore, the dual columns containing imidodiacetate chelating resin as the on-line preconcentration systems for the speciation of Cr(III) and Cr(IV) in seawater were introduced [71]. Cr(III) was collected on the first column; and the second column was used to collect the chromium in the effluent from the first column after passing through a reduction agent, or not, for the reduction of Cr(VI) to Cr(III). Cr(VI) was calculated as the difference between the concentration of pre-reduced Cr(VI) and Cr(III) in the effluent from the first column. A detection limit of 0.04 and 0.09 µg L⁻¹ were obtained for Cr(III) and Cr(VI), respectively.

Speciation of vanadium was developed using the alkaloid quinine modified resin micro-column separation/preconcentration [72]. The selectivity of vanadium (V) and (VI) depended on the pH value. The adsorbed species was eluted and evaluated by fluorination assisted electrothermal vaporization (FETV)-ICP-OES. PTFE was used as a chemical modifier to eliminate the memory effect and avoid

the formation of the refractory carbides for improvement in the analytical performance of the procedure.

Isotope dilution coupled with flow injection ICP-MS was developed for the determination of traces of lead [73]. The removal of the matrix by the chelating reaction on the 8-hydroquinoline-5-sulfonic acid (8-HQS) column was employed. This method can be applied for trace lead determinations in the presence of high salt concentrations.

3.3. Spectrophotometry

Spectrophotometry is widely used as the detection technique for the flow injection, sequential injection, MCFIA, MSFIA, and SI-LOV for the on-line determination of either inorganic or organic species in a wide range of applications. For example, spectrophotometry based on the Griess-Saltzman reaction was applied to determine nitrile and nitrate concentrations [74],[75] or flow injection/stopped flow coupled to kinetic catalytic spectrophotometry [76]. For the kinetic catalytic spectrophotometry, the catalytic reaction was utilized to improve the sensitivity and capability for the trace or ultratrace level determinations.

Ohno, et. al. [77] have presented the determination of copper and iron in river water, tap water, and natural water using the oxidation coupling of p-anisidine with N,N-dimethylaniline in the presence of hydrogen peroxide. Zhengjun et. al. [78] have proposed the method using the acceleration effect of Se(IV) on the reaction of ethelenediamanine tetraacetic acid disodium salt and sodium nitrite with ammonium iron (II) sulfate hexahydrate in acidic media for the determination of trace amounts Se(IV) in seawater. Moreover, the total and dissolved amounts of iron based on the catalytic reaction of Fe(III) and Fe(II) in the oxidation of N,N-dimethyl-p-phenylenediamine in the presence of hydrogen peroxide was described by Lunvongsa [79]. The LOD and LOQ were 0.02 and 0.06 $\mu\text{g L}^{-1}$, respectively.

The combination of kinetic catalytic reactions with stopped-flow is also suitable because it allows the rapid and automatic mixing of sample and reagents and a short time for obtaining measurements. Wang, et. al. [80] have employed the procedure based on the reaction of nitrite with perphenazine using kinetic spectrophotometry coupled to stopped-flow technique for the determination of nitrite in natural and drinking water.

Another interesting flow-based technique related to spectrometry is absorptiometry[81, 82]. For example, silicic acid in highly purified water was successful determined by Yoshimura et al [83] using on-line and gel-phase absorptiometry with molybdate and malachite green. This group also used ion-exchanger phase absorptiometry combined with flow system to determine trace amounts of iron in highly purified water[84]. Flow analysis and solid phase absorptiometry was also used to analyzed micro amounts of chromium(III) and (IV) in natural water[85].

Sequential injection is another system that is widely used. The conjugation of sequential injection with spectrophotometry has been used for many applications. Van Steden and colleagues have published the determination of speciation of Mn(II) and Mn(VII) using on-line spectrophotometry sequential injection analysis [86]. The method is based on the reaction between Mn(II) with 4-(2-pyryndylazo) resorcinol (PAR) to form a red complex in alkaline media. In addition, sequential injection has been applied for the titration for the determination of a number of analytes. Fletcher and

van Staden [87] have purposed this method for the determination hydrogencarbonate by acid-base titration using sequential injection. The results were agreement with the potentiometric titration. Sequential injection based on acid-base titrations was also applied to evaluate the acidity of fruit juices using sodium hydroxide and phenolphthalein [88].

Monolithic column is the new tool for the separation. The incorporation of a monolithic column into a SIA system or SIC system with spectrophotometry has been introduced for online sample pretreatment. A monolithic column was utilized for separation and determination of some pharmaceutical components including ambroxol hydrochloride, methylparaben, and benzoic acid [89].

In the past few years, a number of studies focused on the use of MCFIA and MSFIA for the on-line determination of various samples. Prior et. al. [90] have developed the method for determination of trimipramine in pharmaceutical preparations by a multicommutated reverse-flow system. This method employed the sample/reagent intermixing by combining binary sampling with flow reversal. The homogenization was improved even with highly viscous solutions. Recently, the solenoid micro-pump controlled by microprocessor has been introduced as the flow propelling system and used to replace the peristaltic pump. Lavorante et. al. [91] have proposed the method using a multicommutated flow system for the spectrophotometric determination of paracetamol in pharmaceutical formulations. They used the pinch solenoid valve which functioned simpler than the conventional three-way solenoid valve and two solenoid micro-pumps to employ the multicommutation system resulting in the reduction of reagent consumption and waste generation. This method had a linear range of 5.0 to 125.0 mg L⁻¹ with a detection limit of 0.4 mg L⁻¹.

Multisyringe FIA was interfaced with in-line gas-diffusion to determine volatile analytes such as sulfides [92]. They used the gas-diffusion method for the separation of volatile and gas evolving species from the solid and liquid matrices in conjugation with a multisyringe flow injection system. The method based on the analyte releases hydrogen sulfide from the donor channel of the gas-diffusion module and reacted with N,N-dimethyl-p-phenylene diamine in the presence of Fe(III) or Fischer's reagent to form methylene blue against the oxidative coupling. This species was downstream to the detector. The implementation of MSFIA was accompanied with spectrophotometric detection for the determination of iron and boron in soil extracts [93]. The sample was directly introduced into the flow system where the pH was controlled. Both analytes were determined based on the formation of ferroin complex (for iron) and azomethine H-reaction (for boron) in the same configuration but by changing the reagent in the syringes.

Bead injection is a technique that used the beads for sample pretreatment, such as to trap, preconcentration, isolation, and separation the analytes of interest [94-107]. The coupled bead injection is suitable for large amounts of samples and reagents. The method was applied to analyze for zinc and copper [102]. The Saphadex QAE bead was loaded with the chromogenic agent, 2-carboxyl-2-hydroxy-5-sulformazylbenzene which is complexed with Cu(II) and Zn(II). In the system, the sample was injected into the first carrier where Cu(II) was selectively reacted with Zincon on the bead. The complexes were decomposed with hydrochloric acid. Then, the pH is adjusted to 11 which is suitable for the reaction of Cu(II) and Zn(II) with Zincon on the bead. The two complexes decomposed. The beads were regenerated with the strong acid solution used to decompose the complexes. Beads are automatically discarded from the reverse flow. The trace amounts copper and iron were successively determined using SI-LOV format [108]. The method was based on the

complex formation of 2-5(-bromo-2-pyridylazo)-5-[N-n-propyl-N-(3-sulfopropyl)amino]aniline (5-Br-PSAA) with copper(II) and/or iron(II) at pH 4.6. Copper reacts with 5-Br-PSAA to form a complex while the iron was only complexed with 5-Br-PSAA in the presence of reducing agent. The large amount reagent blank was suppressed using spacer, which contained 5-Br-PSAA. In the system, there are two determination zone from holding coil 1 give the first peak and holding coil 2 give the second peak. Manifold of the SIA system is shown in figure 4.

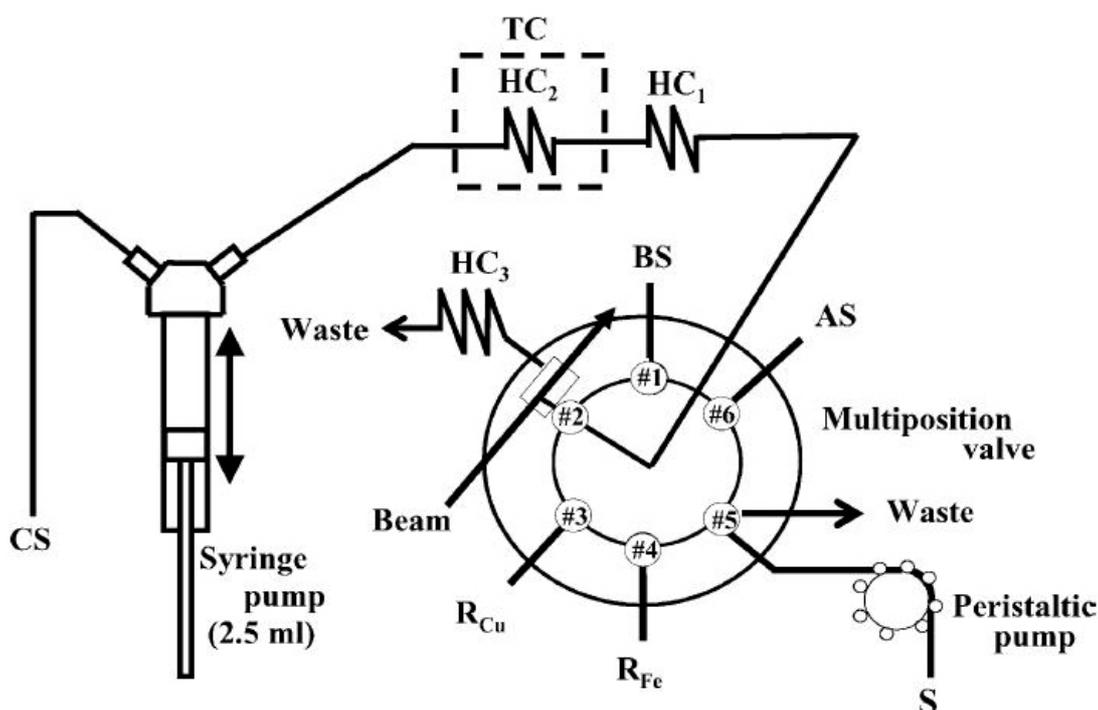


Figure 4 Manifold of the SIA system in the proposed method. HC₁, holding coil₁ (0.5mm i.d., 60 cm); HC₂, holding coil₂ (0.5mm i.d., 55 cm); HC₃, holding coil₃ (1mm i.d., 50 cm); TC, temperature controller (45 .C); CS, $5 \times 10^{-5} \text{ mol l}^{-1}$ 5-Br-PSAA in 0.01 mol l^{-1} HCl; R_{Cu}, $4 \times 10^{-4} \text{ mol l}^{-1}$ 5- Br-PSAA; R_{Fe}, $7 \times 10^{-4} \text{ mol l}^{-1}$ 5-Br-PSAA; S, sample containing copper and/or iron; AS, $5 \times 10^{-4} \text{ mol l}^{-1}$ ascorbic acid; BS, 0.2 mol l^{-1} acetate buffer at pH 4.6.

3.4. Fluorescence

Fluorescence is a luminescence that is more sensitive and selective than UV-vis spectrophotometry. Fluorescence detectors can be used as sensors for flow systems. For example, fluorescence determination of the pesticide asulam was carried out by flow injection analysis [109]. This study focused on the influence of surfactants on the native fluorescence of asulam, as an effect of ultraviolet irradiation on the fluorescence of asulam by forming photo-fragments with high intensities. Moreover, Chen et. al. [110] has determined tannin in green tea infusions using the quenching effect of 3-aminophthalate without interference from the ascorbate.

For immunoassay applications, Yan et. al. [111] developed time-resolved fluoroimmunoassays coupled with flow injection analysis for the determination of carcinoembryonic antigens. In addition, Tsukatani and Matsumoto [112] presented a method using co-immobilized gluconate kinase (GK) and 6-phosphogluconate dehydrogenase (PGDH) reactors for the determination of total D-gluconate.

Bead injection can provide the advantage of a renewable surface. This system coupled with fluorescence detection was applied to determine vanadium(V) [113]. V(V) was reacted with a fluorogenic agent 1,2-dihydroxyanthraquinone-3-sulfuric acid (Alizarin Red S) immobilized on the bead to form the fluorescent chelate. Then, the bead was subsequently discarded from the flow cell by reversing the flow. To increase the selective adsorption, flow injection renewable drop spectrofluorimetry [114] with Sephadex CMC-25 was used in the sequential determination of vitamins B₁, B₂, and B₆. Also simple fluorescence coupled to sequential injection analysis can be used for determination of drugs in pharmaceutical preparations [115],[116],[117]. To obtain rapid analysis and reduce the reagent, innovative stirring actions of magnetic beads in the sequential injection system was proposed [118]. The magnetic beads were immobilized with antibiotics and injected in the microreactor where they were vigorously agitated by a magnetic field of several hundred gauss. Furthermore to enhance of sample/reagent mixing, a controlled pulsed flow was applied to implement SIA methodology to determine indomethacin [119]. In order to accomplish the separation and preconcentration/detection, a multicommuted fluorescence-flow based sensing system was employed for the determination of vitamins B₂ and B₆ [120]. This method achieved a linear range of 0.01-0.4 $\mu\text{g mL}^{-1}$ for B₂ and 0.15-3 $\mu\text{g mL}^{-1}$ for B₆ with detection limit of 0.003 for B₂ and 0.045 $\mu\text{g mL}^{-1}$ for B₆.

To reach the lower detection limit, micro-sequential injection or lab-on-a-chip was developed [121]. The low sample consumption and miniaturized system were demonstrated. The system was employed for the quantification of small amounts of nucleic acids, particularly dsDNA (double stranded DNA) with laser-induced fluorescence detection. A detection limit of 0.009 $\mu\text{g mL}^{-1}$ was achieved.

3.5. Chemiluminescence

Chemiluminescence (CL) detection plays an importance role for flow injection systems due to its high sensitivity and selectivity. When chemiluminescence was coupled to flow injection systems, the obtained results were reproducible. Moreover, this method was cheap, rapid, and simple. In recent work Haertas-Peréz et. al. [122] studied flow injection chemiluminescence for the determination of carbaryl in vegetables and natural water. The enhancement in CL intensity was linear for carbaryl over the concentration range of 5-100 ng mL^{-1} with a detection limit of 4.9 ng mL^{-1} . Yue and Song [123] also presented the method for the determination of nitrite at the femtogram level using flow injection chemiluminescence based on luminol-myoglobin catalyzed by nitrite. Linear enhancement of the chemiluminescence intensity was observed in the presence of concentrated of nitrite ranged from 0.05 pg mL^{-1} to 10 ng mL^{-1} and had a detection limit of 20 fg mL^{-1} . Moreover, Fernández-Ramos [124] described the CL of the peroxyoxalate reaction for the detection of gentamicin in pharmaceutical formulation. In addition, Waseem and coworkers [125] reported the use of the tris(2,2'-bipyridyl)ruthenium(III)-NADH system to increase the CL signal for the determination of thyroxine. A limit of detection of 1.0×10^{-10} was achieved.

Specific detection of some compounds can be achieved by enzymatic chemiluminescence methods coupled to flow injection analysis. Nozaki and Kawamoto [126] presented the micro-flow injection horseradish peroxidase-catalyzed chemiluminescence method for the determination of hydrogen peroxide. The detection limit of hydrogen peroxide using ethyleneurea (100 mmol L^{-1}) was 0.6 pmol per injection and is still sufficient for the determination of hydrogen peroxide in clinical laboratories.

In recent years, a sequential injection analysis using micro-magnetic beads was introduced for many immunoassay studies. Soh, et. al. [127] developed a sensitive immunoassay method for the determination of vitellogenin. The system involved a sequential injection analysis system coupled with a chemiluminescence detector and a samarin-cobalt magnet. The micro-magnetic bead and sequential injection was also used for the determination of anionic surfactants [128]. A schematic diagram of the sequential injection system using magnetic microbeads is shown in figure 5. Moreover, Kradtap Hartwell et. al. have also utilized the sequential injection-ELISA based system for online determination of hyaluronan [129].

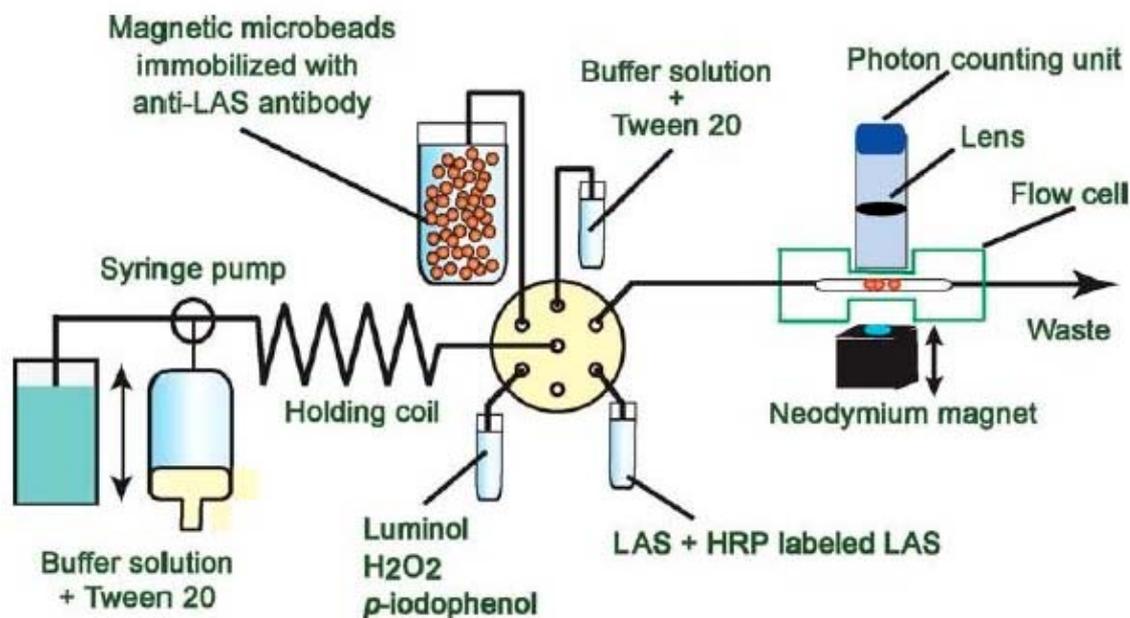


Figure 5 Schematic flow diagram of the sequential injection system using magnetic microbeads.

To design portable and low cost equipment, a flow injection system consisting of a set of solenoid micropumps coupled to a chemiluminescence detector was developed by Rocha, et. al. [130]. The system was applied for the determination of hydrogen peroxide using the oxidation of luminol. The linear range of 0.1—80 mmol L⁻¹ with a detection limit of 400 nmol L⁻¹ was achieved. Guan et. al. also developed a micro-flow injection [131] coupled to chemiluminescence using a micropump. The luminol/hexacyanoferrate/hydrogen peroxide CL reaction was used as the model for testing the performance of the system. A schematic diagram of the microfluidic flow injection system is shown in figure 6. The results provided a linear range of 0.2-1.4 mM.

To facilitate the automation system, a multicommutated flow system was coupled to chemiluminescence detection for the determination of clomipromine [132]. This method is based on the CL oxidation of sulphite by Ce(IV) and sensitized by clomipramine using the single pumping channel. The method is simple, fast, precise, and suitable for the routine analysis of clomipramine in bulk drug and pharmaceutical preparations.

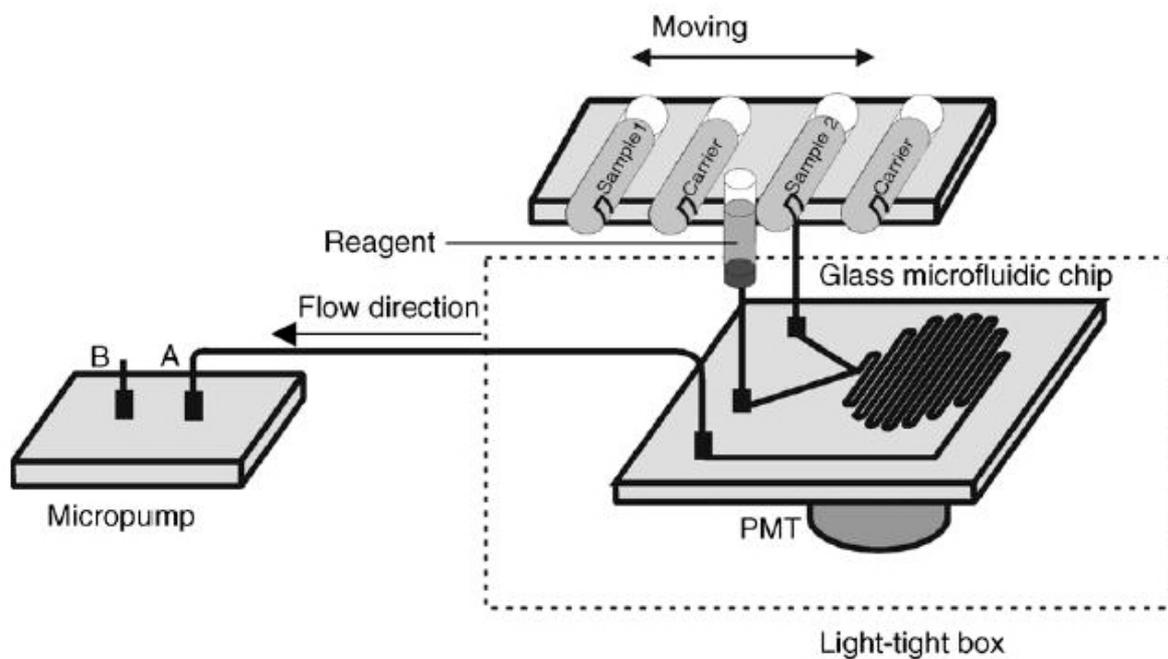


Figure 6 Schematic diagram of the microfluidic flow injection CL system.

Pizà, et. al. [133] proposed the multisyringe flow injection chemiluminescence for the determination of ultratrace glucose in sugar. This method is well suited for the enzymatic-based CL methodologies in a homogeneous (renewable) solution. The method involved the on-line glucose oxidase catalyzed oxidation of β -glucose in a homogeneous phase to β -glucono- δ -lactone and hydrogen peroxide.

4. Conclusion remarks

Sensor based on electrochemical and optical detection coupled with flow injection system have been tremendous popular for many applications. This article reviews the technical status of flow-based electrochemical and optical devices and addresses works of different flow technologies for the detection of both inorganic and organic substances. The reports cited in this overview show the advantages of sensors in flow systems including high sensitivity and selectivity, low cost of equipment, less amount of reagents used, easy automation, and multianalyte determinations. After all, the future researches in flow-based sensors are focus on the development of small portable, automated and low cost systems for various species, including metals and functional molecules to satisfy the clinical and environmental requirements.

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