Review on Automation using Multisyringe Flow Injection Analysis

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Abstract
In this paper, the entire publications on modern automation technique multisyringe flow injection analysis (MSFIA) of the last six years are conscientiously reviewed. MSFIA is compared to alternative flow techniques and multisyringe apparatus and its potentials are described in detail. The different injection modalities and manifold configurations as well as analytical applications according to the different detection techniques applied in MSFIA are described and compared. Features and potentials of the related analytical software AutoAnalysis are overviewed and explained on the background of automation examples. The characteristics and features of the analytical methods developed for MSFIA are summarized in a comprehensive overview table.

Keywords: Multisyringe flow injection analysis, MSFIA, Injection modalities, AutoAnalysis, Automation

1. Introduction

Since the beginnings of automation of analytical methods in liquid flows, various different techniques have been developed and used for analytical or monitoring applications. They have gained importance for clinical, industrial and environmental purposes as they allow highly reproducible and fast determinations. A comprehensive review on flow techniques has been published recently [1].

The first works were based on the principle of air-segmented continuous flow techniques (SFA) [2]. By introduction of air bubbles, full homogeneity of each liquid segment and minimization of carryover from the former sample is minimized. However, the detection has to be carried out in the state of chemical equilibrium. Besides, introduction of air-bubbles cause complex detection techniques and manifold construction and modifications of the method are time-consuming limiting the flexibility of SFA evidently. Nevertheless, SFA has become widespread for routine diagnostic purposes as in diagnostics [3] and simultaneous multi-parameter determinations are feasible.

Flow injection analysis (FIA) was introduced by Ruzicka and Hansen (1975) [4] as an innovative, non-segmented flow technique. A sample plug is inserted into a continuous, laminar flow. It disperses by radial and axial mixing forces while the analyte reacts with compounds of the carrier or downstream-added reagents. FIA stands out by simple instrumentation, facilitated adaptability of method and speed of analysis. Due to elevated reproducible flow and dispersion, detection can be performed before chemical equilibrium is reached. Limitations regarding use of solvents or aggressive reagents of FIA are caused by the need of peristaltic pumping tubes, hence, periodical recalibration of flow rates is required.

The generally high reagent consumption was overcome by the variant of multi-commutation FIA in 1994 [5]. Here, implementation of one solenoid multi-commutation valve into each channel offered the return of non-used solutions to corresponding reservoirs.

Four years before, sequential injection analysis (SIA) as an alternative technique to FIA had been proposed [6]. Precise volumes of sample and reagents are stacked in a holding coil by sequential aspiration from a rotary selection valve and propelled afterwards towards the detector by flow reversal. Radial and axial dispersion leads to an overlapping zone where the reaction product is formed. The product yield is generally reduced compared to SFA and FIA where confluence merging of reagents and sample is accomplished.

As a consequence of the sequential and discontinuous operation, the injection frequency as well as the consumption of reagents and sample is evidently reduced compared to FIA. Full computer control of all operation steps is required but implies also an elevated grade of automation and flexibility of method adaptation. By optional use of incorporated reactors, flow

modification or use of distinct reagents, sample pre-treatment and multi-parametric analysis are feasible on one single flow system. Due to the use of a syringe burette pump as liquid driver, SIA technique is characterized by elevated robustness and long-term stability [7].

Multisyringe flow injection analysis (MSFIA) was firstly described in 1999 [8,9] as a novel multi-channel technique combining advantages of FIA and SIA. By use of parallel moving syringes as liquid drivers, it overcomes the shortcomings of peristaltic pumping as pulsation, required recalibration of flow rates and limitations regarding applicable reagents. As in SIA, flow rates and propelled volumes are precisely known and defined by software-based remote control of the multisyringe device. Thus, the versatility and robustness typical of SIA assemblies are combined with the potential of advanced flow networks. This implies simultaneous or multi-parameter determination and enhanced sensitivity due to confluent mixing of reagent and sample as in FIA [1]. Periodically refilling of syringe involve a certain delay time resulting in slightly lower sampling rates as in FIA. Nevertheless, elevated injection frequencies up to 180 h^{-1} [10] and even higher ones have been reported [11]. By implementation of the multi-commutation concept, the high liquid consumption as another shortcoming of FIA was overcome.

Apparatus robustness has been proven during two monitoring experiments over each 250 h (unpublished results). In this paper, the MSFIA technique is characterized according apparatus and its potentials, injection modalities and the analytical applications published during the last six years are reviewed. Since notable benefits were taken from different features of a sophisticated analytical program in most applications, the program is described as well. An overview of the different analytical applications using MSFIA including method characteristics and applied injection modality is given in table 1. Since a former comprehensively review in 2002 [12] and a shorter communication in this journal 2003 [13], about 20 additional papers have been published on this item.

2. Device description

The essential part of MSFIA is an automated, multiple channel piston pump (Crison Instruments S.A., Allela, Spain). This module can be equipped with up to four syringes as liquid drivers. The heads of the syringes are coupled via Luer connections to three-way solenoid multi-commutation valves whereas the pistons are mounted on a common bar driven by one single step motor. Thus, all pistons are moved simultaneously and unidirectional for either delivering (dispense) or aspirating (pickup) of their corresponding solutions.

For each syringe (S1-S4), both operations are feasible with the head valve activated (ON) or deactivated (OFF). Generally, the direction ON relates to the valve position connected to the flow manifold whereas direction (OFF) is used for syringe reload or to return reagent, unneeded in a particular dispense step, to the corresponding reservoir. Thus, consumption of reagents can be drastically reduced in comparison with FIA or SFA.

Often, one syringe is used for sample aspiration in ON ejecting the waste liquid to a disposal tank in OFF. A scheme of the multisyringe device and the four operation options for liquid displacement as well as the symbolic representation suggested before [9] is given in figure 1.

The three-way solenoid syringe head valves (NResearch, Caldwell, NJ, USA) withstand a backpressure of approximately 2 bars. This allows the implementation of pre-concentration columns [14-16] or enzyme reactors [17] into the manifold when moderate flow rates are applied. Due to their short response time of 35 ms, the valve position can be changed even during piston movement operation permitting a multitude of time-based injection modalities as multi-commutation [18], splitting [9] or sandwich schemes [19] or hydrodynamic injection [20].

In contrast to FIA, method optimization without manifold manipulations is possible by software-based adaptation of flow rates and volumes and analytical methods using stop-flow modality are feasible [21].

The multisyringe modules have been continuously improved and further developed. First applications were performed using a module equipped with a 5.000 step motor, providing a flow rate range of 0.185 – 7.5 ml min^{-1} for a 1 ml syringe, corresponding to 325 – 8 s for total piston displacement. However, mostly an improved module with a 16.000 step motor was used, offering higher precision and minute pulsation even at microliter volumes or reduced flow rates. In the latest module, a 48.000 step motor has been implemented. Both modules permit flow rates from 0.57 to 30 ml min^{-1} for a 10 ml syringe, corresponding to 1024 – 20 s for total piston displacement. Six different sizes of syringes (0.5, 1, 2.5, 5, 10 and 25 ml) are available, offering a great variety of combinations of about 150 different flow rates.

Since all liquid contacting parts and syringes are of chemical inert materials as PTFE, ETFE, PE or glass, there are no limitations regarding the use of solvents or aggressive reagent components. Among other reagents (see table 1) surfactants, DMSO, acetone, dioxan, alcohols [14], acetonitrile, 1-octanol and 1-chlorobutane [22] have been used without evident problems.

The MSFIA module is connected to a personal computer for remote operation control of valve positions and piston movement via serial RS232C interface. Flow networks can be enlarged by coupling several syringe pump and rotary or selection valve modules (VA 1+1, Crison) in series. Their independent operation permit sample loading and processing in parallel resulting in an increase of injection frequency [22] and counterflow operations as sample splitting [9]. By four backside ports of the multisyringe module, additional solenoid valves can be connected and controlled via the apparatus by software. This amplifies further the possibility for sophisticated flow networks.

Thus, parallel liquid film extraction of nitrophenols [22], serial dual injection for independent quantification of acidity and

Table 1: Overview about the reported applications of MSFIA up to date. The seventh column indicates the used injection modality according figure 3.

Application	Incorporated technique	Main reagents	Detection	Matrix	Working range Detection limit	Injection acc. fig. 3	Injection frequency	Ref.
Inorganic analytes								
Aluminium	--	8-hydroxyquinoline-5-sulfonic acid, HTAC	Fluorescence spectrometry	Mineral water	10 – 500 µg l^{-1} DL 0.5 µg l^{-1}	e	154 h^{-1}	[11]
Ammonium	Gas diffusion	bromothymol blue in NaOH	Spectrophotometry	Fertilizer and compost extracts	5 – 70 mg l^{-1} DL 0.03 mg l^{-1}	e	20 h^{-1}	[21]
Antimony (III), (V)	--	$NaBH_4$ for hybride generation, ascorbic acid for speciation	Atomic fluorescence spectrometry	Water, lead	0.2 – 5.6 µg l^{-1} DL 0.08 µg l^{-1}	e	80 h^{-1}	[34]
Arsenic (inorganic)	Pre-concentration (anion exchange column)	$NaBH_4$ for hybride generation	Atomic fluorescence spectrometry	Water, liver and fish muscle (calcinated)	50 – 2000 ng l^{-1} DL 0.03 µg l^{-1}	e	10 h^{-1}	[16]
Arsenic (inorganic)	--	$NaBH_4$ for hybride generation	Atomic fluorescence spectrometry	Water, liver and fish muscle (calcinated)	0.25 – 12 µg l^{-1} DL 0.07 µg l^{-1}	e	108 h^{-1}	[35]
Boron	--	azomethine-H	Spectrophotometry	Soil extracts	0.2 – 4 mg l^{-1} DL 0.05	a	15 h^{-1}	[23]
Chloride	--	selective electrode	Potentiometry	Waste- and freshwater	DL 2.3 mg l^{-1} DL 3.5 mg l^{-1}	b, c e	54 h^{-1} 62 h^{-1}	[29]
Chloride	--	selective electrode	Potentiometry	Waste- and freshwater	6-3500 mg l^{-1} DL 2.7 mg l^{-1}	f	30 h^{-1}	[40]
Cobalt (II)	--	luminol, H_2O_2	Chemiluminescence	Nitric acid containing Vitamin B12	15 – 5000 ng l^{-1} DL 1 ng l^{-1}	e	180 h^{-1}	[10]
Iron (II), (total)	--	1,10 phenanthroline, ascorbic acid for speciation	Spectrophotometry	Metallurgic liquoring	1 – 35 mg l^{-1}	g	68 h^{-1}	[18]
Iron (II), (total)	--	1,10 phenanthroline, ascorbic acid for speciation	Spectrophotometry	Soil extracts	0.5 – 10 mg l^{-1} DL 0.27	b	34 h^{-1}	[23]
Iron (III), (total)	Optional pre-concentration on chelating SPE disk	NH_4SCN, H_2O_2 for speciation	Spectrophotometry	Seawater	0.2 – 35 mg l^{-1} DL 19 ng	e	60 h^{-1} 10 h^{-1}	[26]
Iron (III), (total)	On-cell enrichment on anion exchange SPE disk	NH_4SCN, H_2O_2 for speciation	Solid phase reflectometry	Seawater, freshwater	0.4 – 37.5 ng DL 0.4 ng absolute	e	6 h^{-1}	[24]
Iron (III), (total)	On-cell enrichment on chelating SPE disk	NH_4SCN, H_2O_2 for speciation	Solid phase reflectometry	Freshwater	1 ng – 250 ng DL 1 ng	e	5 h^{-1}	[25]
Nickel (II)	--	dimethylgyloxime, KIO_3	Spectrophotometry	Metallurgic liquoring	up to 30 mg l^{-1}	g	57 h^{-1}	[18]
Phosphate (ortho-)	On-cell enrichment on polymeric resin	luminol, ammoniumvanadate ammoniummolybdate,	Chemiluminescence (Solid-phase based)	(Ground)water, steam condensate	5 – 50 µg P l^{-1} DL 2 µg P l^{-1}	f	11 h^{-1}	[39]
Phosphorous (total)	Microwave digestion	ammoniummolybdate, antimonium(III) oxide tartrate	Spectrophotometry	Wastewater	DL 0.9 mg l^{-1}	(e)	12 h^{-1}	[28]
Selenium (inorganic)	--	$NaBH_4$ for hybride generation	Atomic fluorescence spectrometry	Water, sea lettuce	0.1 – 3.5 µg l^{-1} DL 0.01 µg l^{-1}	e	84 h^{-1}	[36]

Table 1 (continuation): Overview about the reported applications of MSFIA up to date. The seventh column indicates the used injection modality according figure 3.

Application	Incorporated technique	Main reagents	Detection	Matrix	Working range Detection limit	Injection acc. fig. 2	Injection frequency	Ref.
Strontium	Matrix separation by solid phase extraction	selective resin	off-line	Seawater, water, milk (calcinated), soil	usable for up to 40 µg l^{-1}	(e)	3 h^{-1}	[41]
Sulfide	Gas diffusion	N,N-dimethyl-p-phenylene diamine	Spectrophotometry	Wastewater	0.5 – 20 mg l^{-1} DL 0.03 mg l^{-1}	f	13 h^{-1}	[31]
Sulfide	--	N,N-dimethyl-p-phenylene diamine	Spectrophotometry	Waste-, sea-, ground- and freshwaters	0.2 - 5 mg l^{-1} DL 0.09 mg l^{-1}	e	45 h^{-1} 80 h^{-1}	[19]
Sulfide	On-cell enrichment on C18 SPE disk	N,N-dimethyl-p-phenylene diamine	Solid phase reflectometry	Waste-, sea-, ground- and freshwaters	20 - 200 µg l^{-1} DL 2.9 µg l^{-1}	e	8 h^{-1}	[32]
Sulfide	Gas diffusion and on-cell enrichment on C18 SPE disk	N,N-dimethyl-p-phenylene diamine	Solid phase reflectometry	Waste-, sea- and groundwater	20 - 500 µg l^{-1} DL 1.3 µg l^{-1}	e	5 h^{-1}	[33]
Sulfur dioxide (free), (total)	Gas diffusion	pararosaniline, formaldehyde	Spectrophotometry	Wine	2 – 75 mg l^{-1} 10 – 250 mg l^{-1}	h	25 h^{-1} 30 h^{-1}	[30]
Yttrium	Matrix elimination by liquid-liquid extraction	2-ethylhexylphosphoric acid on C18	off-line	Water, urine and blood (calcinated)	usable for 0.1 - 1000 mg l^{-1}	e	3 h^{-1}	[15]
Single point titration								
Acidity (free) and iron	Serial dual injection	methyl red, inoxalate buffer sodium salicylate	Spectrophotometry	Metallurgic liquoring	5 - 30 mmol l^{-1} H^+ 1 - 6.4 mg l^{-1} Fe(III)	e	78 h^{-1}	[20]
Alkalinity (total), (strong)	--	cresol red in boric acid bromocresol green & acetic acid	Spectrophotometry	Water	--	a	97 h^{-1}	[9]
Sulphuric acid (free)	On-line dilution by splitting plus open mixing tank	methyl orange in $NaSO_4$	Spectrophotometry	Metallurgic liquoring	1.5 – 6.5 mol l^{-1} Dilution 80 – 1150	a	30-36 h^{-1}	[9]
Organic analytes								
Glucose	--	luminol with Co(II), glucose oxidase (solution)	Chemiluminescence	Dietetic drinks, pharmaceuticals	90 – 2700 µg l^{-1} DL 72 µg l^{-1}	e	20 h^{-1}	[37][38]
Glucose	Enzyme reactor (Glucose oxidase)	luminol with Co(II) as well as luminol with peroxidase	Chemiluminescence	Urine, dietetic soft drinks and lemonade	0.45 – 180 mg l^{-1} DL 0.16 / 0.23 µg l^{-1}	(e)	12 h^{-1}	[17]
Nitrophenols	Dual wetting film extraction Multi-component analysis	octanol, 1-chlorobutane, acetonitril	Spectrophotometry	Fresh-, ground- and seawater	DL 0.07, 0.11, 0.46 µmol l^{-1}	a	11 h^{-1}	[22]
Phenols (total)	--	4-aminoantipyrine, $K_3[Fe(CN)_6]$	Spectrophotometry	Water	DL 0.17 mg l^{-1} DL 0.34 mg l^{-1}	b c	70 h^{-1} 85 h^{-1}	[29]
Warfarin	Pre-concentration (C18 column)	DMSO, HTAC	Fluorescence spectrometry	Ground- and freshwaters	50 – 6400 ng l^{-1}	f	12 h^{-1}	[14]

DL = detection limit

Fe(III) or intelligent manifold variation for speciation [23-25], and including optional sample dilution or enrichment of iron [26] were reported as exemplary applications.

The provided power (12 V, 0.5 A) of each port is sufficient for the supply of up to three, in-parallel-connected discrete solenoid valves. Besides, this configuration also allows the remote control of other, direct or via-relays-coupled instruments.

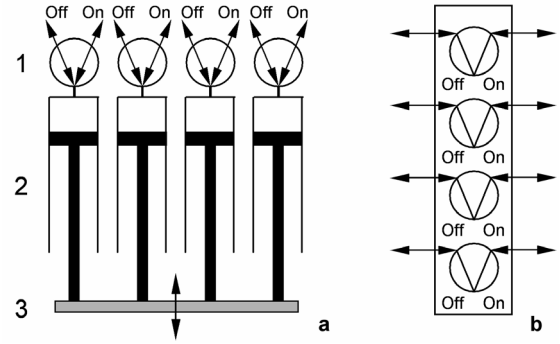

Figure 1: a) Scheme of the frontal view of the Multisyringe device with solenoid commutation head valves indicating the four flow options pickup and dispense, both in ON or OFF (1), connected syringes (2) motor driven piston bar (3). b) Suggested schematic representation of the Multisyringe for flow manifolds drawings [9]

3. Control software

The need of remote computer control can be regarded as one of the main drawbacks of modern flow techniques involving syringe or solenoid micro-pumps or valves [3]. This might explain the evident lower number of applications of these techniques whereas several thousand papers have been published on FIA since its first description [1].

Often, individual software solutions are used for the exclusive control of pump devices, whereas for data acquisition from the detector, the accompanying software has to be used. For some MSFIA applications, the language QuickBasic was used for operation control and data acquisition. However, re-programming is required for each instrumentation modification demanding for a profound user skill. Only a few commercial programs have been developed and are often limited to specific flow instruments.

For automation and monitoring applications, specific software features are required to assure the reliable operation and to fulfill the users demands. Here, the possibility of real-time data evaluation, intelligent operation as on-demand cleaning, sample dilution or pre-concentration or user interaction can be named.

In most of the reported MSFIA applications, operation control of instrumentation, data acquisition and processing was carried out using the software package AutoAnalysis from Sciware S.L. (Palma de Mallorca, Spain). A fundamental description was given by BECERRA ET AL. (1999) [27]. It is written in Delphi (5.0) and Visual C++ (6.0) and can be run on all 32-bit operation systems from Microsoft cooperation.

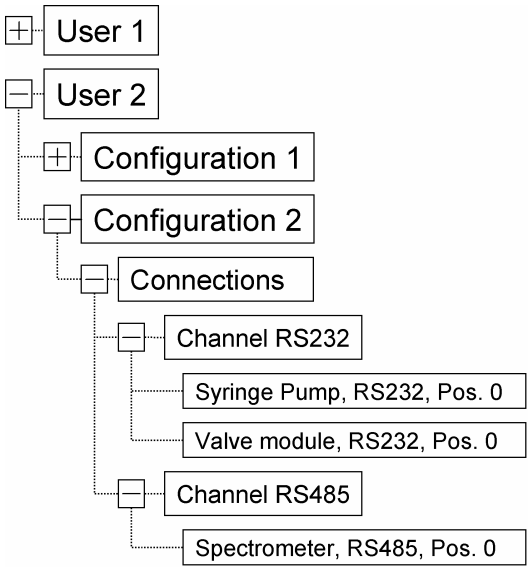

Figure 2: Structure scheme of software AutoAnalysis. Each user can define different configurations of instruments, which might communicate via different communication protocols and interfaces and are incorporated into the program via DLL-files.

The main program offers a window-oriented, graphical surface for the creation and running of operation protocols. These include the instructions for connected instruments and programming features as loops, waiting steps, variables, procedures and conditional inquiries. Procedures offer clearer structures of the operational instruction protocols. They can be influenced by variables, which might specify a particular volume or valve positions of required solutions. Variables can be changed by input during method execution allowing specified users inventions. They can further be used for loop and peak indexing, to store time or peak data for on-line processing as calibrations or for conditional inquiries. Conditional inquiries were the essential software feature for a lately published MSFIA application for iron specification including optional, automated processing of in-line pre-concentration on a chelating solid phase extraction disk [26]. Finally, the software allows data and spectra recording, evaluation and peak detection during or after method execution.

Configuration and communication with the assembled analytical equipment is performed by specific dynamic link libraries (DLL), which are installed and loaded on users demand. Thus, change of connected instrumentation requires a minimum of adaptation effort due to the hardware-independent main program.

Currently, DLL files for seven communication protocols and 30 instruments can be provided including fluorimetric, spectrometric, atomic fluorescence and electrochemical detectors, autosampler, syringe-, peristaltic- and solenoid-pumps, valve

modules and digital I/O, A/D or D/A PC cards for integration of further instruments [1].

4. Injection modalities

The use of the multisyringe and solenoid valves controlled via the same device allows the aggregation of advanced flow networks and offers various possibilities for sample introduction into the manifold. The eight so-far applied injection modalities are schematically shown in figure 3.

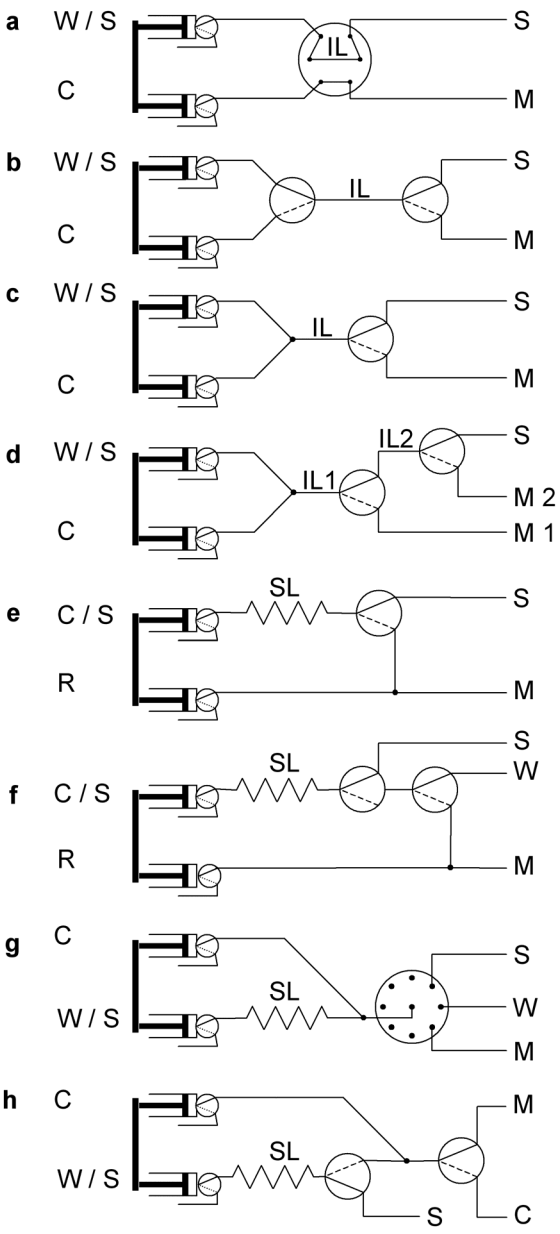

Figure 3: Reported injection modalities in MSFIA. For simplification, only two syringes are given, propelling reagent (R), carrier (C), and (S) sample to waste (W) or the manifold lines (M). Injection loops are marked with IL, sampling loops (holding coils) with SL

One syringe is always used for sample loading and injection, whereas the others impel reagent and carrier solutions. Manifold configurations implying injection of a pre-fixed or a variable volume have to be differentiated. The variation of the injection volume can be furthermore achieved by time-based change of solenoid valve positions (multi-commutation) or by propelling of particular volumes as in SIA (stacking).

FIA-like manifolds with fixed injection volumes were described using a rotary low-pressure injection valve (a) [9] or two solenoid valves instead (b) as a faster and economical alternative [23] or one solenoid valve (c) performing hydrodynamic injection [20]. A serial arrangement of the solenoid valves (d) allowed dual injection after a single sample aspiration step into for analysis of iron (manifold 1) and free acidity (manifold 2).

Most frequently, injection of variable volumes with manifold configurations (e) and (f) was performed. Here, a large sample volume is aspirated into the sampling coil and used for multiple injections by repeated dispense of certain sample aliquots. Last, unused sample is discharge to waste in syringe head valve position OFF (e) or ON (f). This injection modality permits the adaptation of method sensitivity by varying the injected sample volume and optional confluence sample and reagents, improving mixing and sensitivity [18,19].

The first MSFIA work applying variable sample volumes by time-based injection was analogous to figure 3 g, used for the quantification of nickel(II) in metallurgic liquoring samples. The configuration presents a variant of (c) and (e), which both can be used for variant-volume or hydrodynamic injection. Additionally, the selection valve permits the prior aspiration of an air segment to diminish sample dispersion [22,28]. Configuration (h) is similar to (f), it also allows the flushing of the tube between the solenoid valves with carrier.

The insertion of sample can be defined by the activation time of a particular solenoid valve and the applied flow rate (time-based) or the dispense volume. Time-based operation becomes faster if sample and reagent slugs are impelled in an alternating way (slicing, binary sampling), since the flow has not to be stopped for valve position change. An application was reported for the determination of iron [23], where two reagents were merged by such slicing procedure to minimize the contribution of Schlieren effects to the analytical signal. Time-based and fixed volume injections modalities were investigated recently in a comparison study and similar values of repeatability, sample frequency and limits of detection have been found [29].

5. Applications according detection technique

5.1. Spectrophotometry

Spectrophotometry is the most frequently applied detection principle in FIA and related techniques since the required instrumentation is simple, widely available and robust regarding

most sample matrixes. The absorbance of a reaction product whose concentration is linearly related to the analyte of interest is measured. Finding a sufficiently selective reaction for the analyte and matrix effects as sample hue, turbidity, distinct refraction indices and absorbance of sample components on the quantification wavelength are the mayor limitations for a particular task. Thus, devices for analyte separation are often integrated into the flow manifold or reference wavelength is used to compensate unselective effects on the analytical signal.

Superiority of MSFIA over other flow techniques was demonstrated in several works applying gas-diffusion for separation of a volatile analyte from the sample matrix, such as the quantification of ammonia [21]. Conversion of ammonia into its volatile base was done by introduction of sample (S1) and NaOH (S2) into water carrier flow (S3) which afterwards passed the donor side of a gas diffusion membrane. A neutral carrier (S4) containing the acid–base indicator bromothymol blue (BTB) was used as acceptor flow and the intensity of color alteration of BTB was quantified. Low consumption of solutions and time and high sensitivity by parallel merging of sample and alkaline carrier was achieved. However, most noteworthy were the variation of sample volume and repeated reversal of the donor for better gas diffusion yield, which allowed to adapt the sensitivity of the method. Before, a similar work has been reported for the determination of sulfur dioxide in wine using pararosaniline (S3) and formaldehyde (S4) as acceptor mixture after gas diffusion [30]. For the quantification of sulfide in waste water, gas diffusion was applied performing Fischers reaction to generate the detectable dye methylene blue in the acceptor flow [31]. By doing this, high selectivity was obtained in respect of a former work [19], where metal ions interfered significantly. In this former work, different injection modalities were compared and by MSFIA, a high injection frequency of 80 h^{-1} was feasible.

A drawback of photometric detection is, that the sample matrix can considerably contribute to the analytical signal, as observed for the quantification of boron in soil extracts [23]. Here, an additional solenoid valve was placed between the reagent-driving syringe and the corresponding confluence with the sample analogous to figure 3(e). Hence, either water (in OFF) or the color generating reagent azomethine-H (in ON, via the solenoid valve) could be aspirated for the quantification procedure corresponding to sample blank or boron concentration of the sample.

Several works have been reported on the photometric determination of iron including specification by either quantification of ferrous ions with phenanthroline and reduction with ascorbic acid [18,23] or ferric ions with salicylate [20] or thiocyanate [26] and oxidation with hydrogen peroxide. The last one is especially worth mentioning due to the application of smart conditional inquiries. On the basis of the former peak data, the adequate analytical procedure is automatically chosen either including or omitting an in-line pre-concentration on a chelating membrane disk for both ferrous ions and total iron with a detection limit of 19 ng l^{-1}. By applying hydrodynamic injection, a sample frequency of up to 85 h^{-1} was achieved [20].

At last, MSFIA has been used for single point titration applications as determination of free acidity, and total and strong alkalinity in metallurgic liquoring samples [9,20]. The sample was introduced into a buffered, acid-base-indicator containing carrier flow, where the dispersion lead to the detectable, partial color conversion of the indicator. The versatility of the technique was demonstrated in the first MSFIA application, where the sample was diluted by splitting and dilution in an open mixing chamber with variable dilution factors between 80 and 1150 [9].

5.2. Reflectometry

Four MSFIA applications were reported applying diffuse light reflectance. By enrichment of the reaction product on an active, illuminated solid phase extraction (SPE) surface, the resulting light attenuation can be quantified as an apparent absorbance. In contrast to spectrophotometry, the analytical signal is not affected by the Schlieren effect and selectivity and sensitivity are improved due to on-cell concentration of the analyte, whereas long-term stability and back-pressure were the main shortcomings of the proposed applications.

A laboratory-made optosensor was used in all works, equipped with the active SPE disk. For determination and specification of trace amounts of iron in waters, the use of an anion-exchange [24] and a chelating [25] SPE disk was compared. In both cases, iron(III) was determined as its thiocyanate complex. For quantification of total iron, iron(II) was first oxidized with hydrogen peroxide. An additional syringe module was required for sampling, whereas carrier, reagent, oxidant and eluent for re-generation of the SPE disk surface were driven by the four syringes of the first module. Similar mass detection limits (0.4 ng and 1 ng) and injection frequencies (6 h^{-1} and 5 h^{-1}) were achieved.

The second analyte quantified in trace amounts using reflectometry was sulfide, which was converted into methylene blue by direct merging with the reagents N,N-dimethyl-p-phenylene diamine and iron as catalyst [32] or after gas diffusion [33] to obtain higher selectivity. On-cell enrichment was feasible using a C18 SPE disk as the active surface. As well, similar detection limits (2.9 µg l^{-1} and 1.3 µg l^{-1}) and injection through-put (8 h^{-1}, 5 h^{-1}) were achieved, respectively. In this works, up to eight syringes on two devices were successfully controlled by software, used for sampling (S3), carrier (S8), reagents (S1, S2), eluent (S6), SPE disk conditioner (S7), acid (S4) and base (S5).

5.3. Chemiluminescence

Chemiluminescence (CL) detection is a highly sensitive quantification technique and linear working range can reach several orders of magnitude. MSFIA can be regarded as an optimal flow technique for automation of CL detection and

offers evident advantages over FIA or SIA techniques, as discussed in detail recently [3].

Chemiluminescence (CL) is mostly applied for quantification of enzymatic-generated oxidizing species as hydrogen peroxide using luminol in alkaline solution. Thus, incorporation of enzyme cartridges into the manifold lines demands on satisfying backpressure stability and robustness typical for syringe pumps. Besides, automation by MSFIA permits sophisticated operation protocols as periodic washing of the enzyme cartridge in order to prolong their stability. In contrast to FIA technique, economic use of enzyme and coenzyme solutions in MSFIA is possible since minute volumes can be delivered on demand and with high precision by small-size syringes assuring a minimum consumption.

For chemiluminescence reaction with short relaxation time, the detection should succeed directly upon merging of the reactants. Since in SIA merging of solutions occurs at flow reversal by dispersion of the stacked solution stacked, its feasibility is restricted to reactions with moderately slow kinetics [3]. On the other hand, the flow rate in FIA is limited by the resulting back-pressure.

By use of the multisyringe device, excellent method performance was achieved for luminol based CL quantification of hydrogen peroxide. Detection was performed directly after confluence merging of solutions and a 4 cm reaction coil at a flow rate of 72 ml min^{-1} [37,38]. The method was successfully applying to the quantification of glucose in dietetic drinks and pharmaceuticals. Hydrogen peroxide was generated with dissolved glucose oxidase (GOD), prior the merging with luminol and Co(II) catalyzing solution. Using a laboratory developed detector and low volume flow cell [3], log-log linearity from 90 to 2700 µg l^{-1} was achieved at an injection frequency of 20 h^{-1}.

A similar approach was done using a cartridge with immobilized GOD instead [17]. Horseradish peroxidase and Co(II) have been compared as catalysts for luminol procedure. Both systems have been applied for soft drinks and urine with similar but evidently higher and extended working range compared to the use of dissolved GOD (0.45 – 180 mg l^{-1}) at a slightly reduced injection frequency of 12 h^{-1}.

Trace determination of cobalt(II) in the range of 15 ng l^{-1} to 5000 ng l^{-1} was performed with a sample throughput of 180 h^{-1} [10]. Although the method suffered form the interference of other metals, the tolerance levels were sufficient for the aimed subject of degradation study of vitamin B_{12}.

Trace determination of orthophosphate with a detection limit of 2 µg l^{-1} was performed by on-cell enrichment of the analyte as vanadomolybdophosphate on a polymeric resin filled into the spiral channel of the detection flow cell [39]. At propulsion of alkaline luminol and methanol to the detection cell, luminol was oxidized under light emission and simultaneous elution and re-conditioning of the flow cell. The method showed a 500-fold tolerance against silicate, which exceed the tolerance level of the method by two orders of magnitude.

5.4. Fluorescence spectrometry

The limited potential of analyte separation as in chromatography presents an evident shortcoming of all flow techniques. By quantification of fluorescence emission of the analyte of interest or its reaction product, sensitivity and selectivity can generally be improved considerably, often accompanied by extension of the linear working range.

The advantageous coupling of fluorescence detection with MSFIA allowed the selective quantification of the rodenticide warfarin in waters after pre-concentration on octadecyl-modified silica beads [14]. A simple system of only two syringes were used, one for sample pickup and propelling to the bead-containing, laboratory-made column and a second for dimethyl sulfoxide as eluent. Addition of 0.024 % of the cationic surfactant hexadecyltrimethyl-ammonium chloride (HTAC) to the eluent was found to improve both, sensitivity and reproducibility of quantification. By an enrichment factor of 155, a linear working range of 50 to 6400 ng l^{-1} and an injection frequency of 12 h^{-1} were achieved.

The high potential of MSFIA for fast analytical methods was demonstrated by a three-syringe system for fluorimetric quantification of aluminum in mineral water [11]. Hydroxyquinoline sulfonic acid was applied as fluorogenic ligant. Higher sensitivity with detection limit down to 0.5 µg l^{-1} was achieved by cationic micellar medium using HTAC. An injection frequency of 154 h^{-1} was achieved by aspiration of an enlarged sample volume into a holding coil and triple, time-based injection of reagent and sample into a carrier flow at flow rates of 9 ml min^{-1}.

5.5. Atomic fluorescence spectrometry

Four works on MSFIA have dealt with the automated quantification of the hydride forming semimetals antimony [34], arsenic, [16,35] and selenium [36] by atomic fluorescence spectrometry. This technique is rather inexpensive, robust and shows higher sensitivity compared to atomic adsorption spectrometry.

The hyphenation with MSFIA offered fast and automated quantifications, reproducible sampling, and in-line sample treatment. The hydrides were generated by parallel mixing of sample, hydrochloric acid and sodium tetrahydroborate and stripped with argon in a miniature separator.

The syringes were used for sampling and handling the HCl and $NaBH_4$ solutions. Total inorganic arsenic was determined using a mixture of the reductive reagents potassium iodide and ascorbic acid for the conversion of As(V) to As(III). To determine total inorganic antimony, a similar composed reagent was used for reduction of Sb(V) to Sb(III), but omitted for exclusive quantification of species Sb(III).

By implementation of a laboratory-made column filled with anion-exchange resin, the limit of linear working range could be lowered four-fold to 50 ng l^{-1} [16] with an injection frequency of

30 h^{-1}. The other MSFIA methods reached injection frequencies in the range 80 h^{-1} to 108 h^{-1}.

5.6. Potentiometry

Potentiometric determinations generally require a rather simple manifold for repeatable sample injections and provision of background electrolyte for ionic strength adjustment. For cleaning of electrode surface and manifold lines and optional sample pre-treatment, a multi-channel technique as MSFIA can nevertheless offer some advantages. Twofold higher injection throughput was achieved in comparison with SIA technique for the potentiometric determination of chloride with a selective electrode due to sampling via solenoid valves with syringe 1 and manifold cleaning with syringe 2 of the multisyringe device. [40]

A further duplication of injection throughput to 62 h^{-1} was achieved using three syringes for time-based sampling, sample propelling and confluence parallel mixing with the ionic strength adjustment electrolyte [29].

Finally, multiple SIA systems are feasible by coupling each of the syringes to a particular manifold, detector and sampling system. An unpublished work deals with the provision of four different types of ionic strength buffer, one for each syringe and line, optimized for the incorporated selective electrodes for total eight different analytes, read periodically against one common reference electrode by a multiplexer. An additional single syringe pump was used for sampling and sample provision to the four single SIA lines by means of solenoid valves. [1]

5.7. Automated sample treatment

Separation of analytes from sample matrix components, elimination of incompatible sample components and enrichment of analyte to achieve required limits of detection and quantification are the main purposes of sample treatment. As a pressure stable, robust, and easily adaptable and fully computer controlled multi-channel technique, MSFIA has a high potential for the automation of laborious sample treatments. Cleaning-up, pre-concentration on solid phase or by liquid-liquid extraction, in-line sample dilution, acidification, and digestion of organic compounds has been successfully applied.

In-line dilution of strong acid metallurgic liquoring samples with variable dilution factor and subsequent single-point titration was performed. By propulsion of the sample by a single syringe pump and simultaneous pickup from the multisyringe, splitting at a confluence junction was performed before the remaining sample fragment was further diluted in an open mixing chamber. Dilution factors from 80 to 1150 were feasible in respect to fit the range of the moderate acidic standards [9].

For the determination of free acidity in similar samples with elevated Fe(III) content, spiking of acid was performed to repress the hydrolytic contribution of iron to the acidity and resulting quantification error [20].

Liquid-liquid extraction was automated with MSFIA for determination of nitrophenols in water samples. For each determination, the inner walls of a PTFE coil were coated with a chlorobutane-octanol film for extraction of the nitrophenols from the acidified sample. After back-extraction with sodium hydroxide solution and spectrometric detection, the wetting film was removed with 90 % acetonitrile. By performing parallel extraction in two alternately loaded coils, a high sample throughput of 11 h^{-1} with considerable enrichment of analytes was achieved. Two single syringe pumps were used for sample loading, whereas the multisyringe was used for back-extractant, tube coating and film removal. Multi-component analysis was performed by multi-linear regression using the spectra data from each peak maximum acquired, treated and exported with former described software AutoAnalysis.

Exclusive use of the MSFIA device retention of the analyte for matrix removal was made for isotope quantification of strontium [41] and yttrium [15]. Strontium was retained on an analyte selective resin. The syringes channels were used for sample, carrier, and nitric acid of different concentrations for elution and manifold rinsing and conditioning. Yttrium was retained in a film of selective extractant di-2-ethylhexylphosphoric acid brought on a C18 capped resin. The liquid film was renewed within every procedure. Simplification of cleaning-up of samples with considerable saving of time, reagents and waste as well as high reproducibility was achieved. The methods were applied to waters and digestions of blood, milk, urine and soil.

In-line digestion using miniature digestion vessel placed into a commercial microwave devise was performed for determination of total phosphorus in waste water [28]. A system aggregated of a multisyringe and a peristaltic pump was used, the later device used for liquid and air propelling to the digestion vessel for digestion, displacement and cleaning. Fast and fully automated quantification of phosphorus was achieved and the high potential of hyphenating different pumping systems for sample handling was demonstrated.

The multisyringe devise can also be used for feed-back control of process parameters. Adjustment of pH was carried out using the software AutoAnalysis by impelling of proper volumes of acids or bases (unpublished).

5. Conclusion

Apparatus, characteristics and applications of versatile analytical technique multisyringe flow injection analysis (MSFIA) are described. The technique is based on the combination of FIA and SIA technology in one pumping device including up to four syringes of six optional sizes for simultaneous liquid driving. Solenoid multi-commutation valves on the head of each syringe permit the selection of the flow directions leading to minimum consumption of sample and reagents. Up to twelve additional solenoid valves or micropumps can be controlled via ports of the multi-syringe device.

Thus, complex flow networks are feasible, in which reagent and sample can be impelled simultaneously at variable flow rates. Robustness according pressure or used solvents, high volumetric precision and method flexibility due to fully instrument automation are portrayed by examples. The different injection modalities applied so far are given, including binary sampling, splitting, hydrodynamic and time-based insertion.

The automation software AutoAnalysis was used in most experimental works for instrument operation control, data acquisition and treatment and smart monitoring. Its potential and corresponding features are stated including examples.

Useful combinations of the multisyringe technique with additional valve or pumping devices are stated. The different applications are shortly described according to the applied detection technique. Single sample treatment, monitoring, control and smart applications and combination with chromatography will be future tasks in this field of work.

6. References

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