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Review

Green chemistry and the evolution of flow analysis. A review

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ABSTRACT

Flow analysis has achieved its majority as a well-established tool to solve analytical problems. Evolution of flow-based approaches has been analyzed by diverse points of view, including historical aspects, the commutation concept and the impact on analytical methodologies. In this overview, the evolution of flow analysis towards green analytical chemistry is demonstrated by comparing classical procedures implemented with different flow approaches. The potential to minimize reagent consumption and waste generation and the ability to implement processes unreliable in batch to replace toxic chemicals are also emphasized. Successful applications of greener approaches in flow analysis are also discussed, focusing on the last 10 years.

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Contents

Abbreviations: FIA, flow injection analysis; FT-IR, Fourier transform infrared spectrometry; GAC, green analytical chemistry; LLE, liquid–liquid extraction; LOV, lab-on-valve; MCFA, multicommutation in flow analysis; MSFA, monosegmented flow analysis; MSFIA, multisyringe flow injection analysis; SFA, segmented flow analysis; SIA, sequential injection analysis; SIC, sequential injection chromatography; SPE, solid-phase extraction.

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1. Introduction

Green chemistry has been defined as "the use of chemistry techniques and methodologies that reduce or eliminate the use or generation of feedstocks, products and by-products that are hazardous to human health or the environment" [1]. In a short definition, "Green chemistry is using chemistry for pollution prevention" [1]. These definitions have been extended to specific areas and the terms "*Green analytical chemistry* (*GAC*)", "*Environmentally friendly analytical chemistry*" and "*Clean analytical methods*" were coined to describe analytical methodologies that eliminate or reduce the use of toxic reagents, yielding lower amounts of less impactful residues. A historical view of the development of GAC and a discussion of strategies to achieve this goal were presented in a recent overview [2].

Flow analysis has achieved its majority as a well-established tool to solve analytical problems. The evolution of flow-based techniques has been discussed from diverse points of view, including historical aspects [3], the commutation concept [4] and the impact on analytical methodologies [5]. The potential to develop greener analytical procedures is inherent to flow analysis, and changes in system design as well as the exploitation of new flow approaches have led to ingenious alternatives to minimize reagent consumption and waste generation without hindering analytical performance. On the other hand, sample processing under conditions which are difficult or unreliable to carry out using batch methods allows avoiding the use of toxic chemicals. Solvent recycling [6], reuse of reagents [7,8] and on-line waste treatment [9] are other strategies that can be exploited in flow-based systems to achieve greener procedures. These strategies were reviewed in a previous work, which established a priority order for the development of cleaner procedures and discussed successful examples of greener flow-based applications [10].

Flow-based procedures have fulfilled several of the twelve principles of green chemistry [11]. From a theoretical point of view, the reagent excess required for chemical derivatization is lower than in the batch mode in view of the kinetic characteristics of flow analysis. The first principle is also met by replacing toxic chemicals, drastically minimizing reagent amounts and waste generation or even allowing reagent recycling and reuse. Sample treatment is usually simpler and faster, thus avoiding unnecessary chemical waste, in agreement with the fifth and eighth principles. Real-time monitoring of industrial or environmental processes is also feasible satisfying the eleventh principle. In addition, according to the twelfth principle, potential risks to the analysts are reduced by sample processing in a closed system and by automation, which minimizes exposition to toxic substances and makes the analytical operation less dependent of the operator.

In the previously mentioned overview [2], new approaches and concepts in flow analysis were highlighted as milestones in the development of GAC, including flow injection analysis (1975), sequential injection analysis (1990), multicommutation in flow analysis (1994) and the lab-on-valve strategy (2000). Other potentially greener approaches that are usually implemented in flow-based systems, such as solid-phase spectrophotometry (1976), micro total analytical systems (1990) and single drop microextractions (1996), were also emphasized. A timeline with the main contributions of flow analysis to GAC is presented in Fig. 1.

This review traces a parallel between the evolution of flow analysis and GAC based on the replacement of toxic reagents as well as a minimization of the amount and toxicity of wastes. The comparison is based on reagent consumption and effluent volume generation in classical procedures. Recent examples of successful greener approaches in flow analysis are also discussed, complementing the applications considered previously [10].

2. Evolution of flow analysis towards GAC

2.1. Replacement of toxic reagents

An environmentally friendly analytical procedure should ideally avoid the use of hazardous chemicals. This condition is rarely applicable without affecting the analytical performance, but there is a trend to avoid highly toxic reagents, thus minimizing risks to the analysts and the environment. This trend can be exemplified by flow-based systems used for nitrate determination in water (Table 1).

Classical flow-based procedures for nitrate determination have exploited reduction to nitrite in a copperized cadmium column, followed by the diazo-coupling (Griess) reaction [12]. In spite of the good analytical features achieved and the reduction of the waste volume in comparison to the analogous batch methods, the waste contains residues of cadmium and copper as well as carcinogenic amines. In further studies, the reduction step was carried out photochemically [13] or using nitrate reductase obtained from corn leaves [14], thus avoiding the use of cadmium. However, these works cannot be considered environmentally friendly,

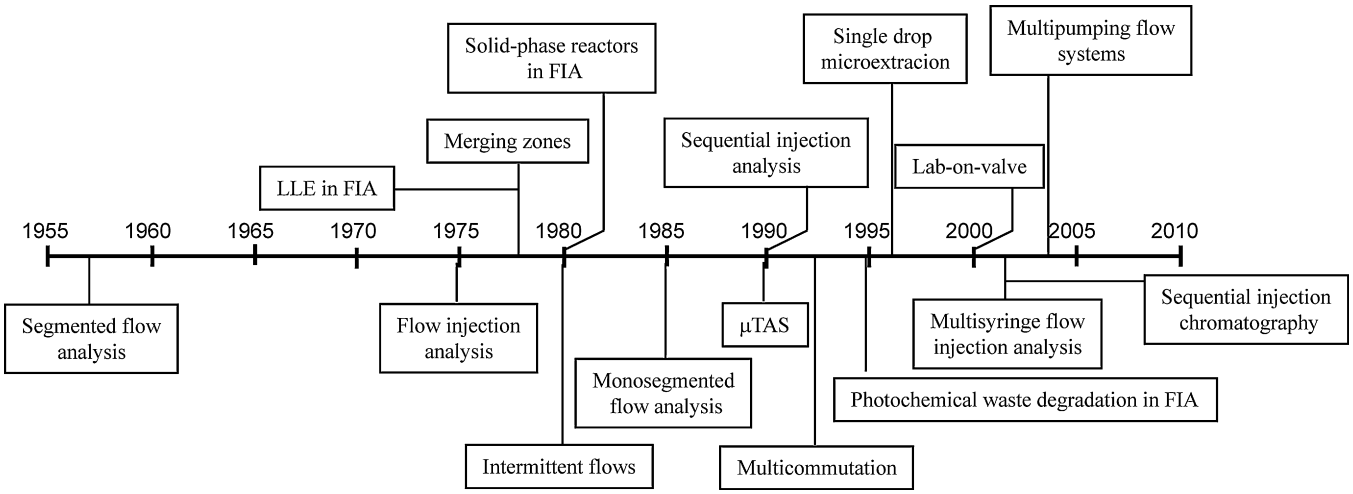

Fig. 1. Timeline with the main contributions of flow analysis to GAC.

because nitrite determination was still carried out by the Griess reaction. This reagent was further replaced by iodide, with the triiodide anion formed in the reaction detected by amperometry [15]. In another greener approach, nitrate was reduced in a mini-column filled with zinc and the formed ammonia was determined by conductimetry after separation from the sample matrix by gas diffusion [16]. A greener procedure for nitrate determination exploited direct measurements in the UV range after separation of the analyte from interfering species usually present in natural waters by ion exchange [17]. Analyte elution was carried out with a diluted perchloric acid solution, which was the only reagent employed in the analytical procedure with an amount equivalent to 18 μL per determination. In spite of avoiding the use of toxic chemicals, the analytical features were not significantly hindered, making it suitable for the proposed application.

2.2. Minimization of waste amounts

In addition to the analytical features usually taken into account to evaluate an analytical procedure (*e.g.* accuracy, precision, sensitivity and selectivity), the amount and toxicity of wastes are important aspects to be considered. Minimization of reagent consumption contributes to reducing both operational costs and the toxicity of wastes, while the effluent volume defines the amount of waste to be treated and consequently the related costs. The trend of development of greener procedures in Analytical Chemistry has been highlighted [10,18,19] and the adoption of environmentally friendly procedures is mandatory for adherence to ISO 14000 guidelines.

Flow-based procedures were originally proposed for mechanization of chemical assays aiming to address the high demand for analysis. In this sense, high reagent flow rates were usual in order to improve sample throughput, a key aspect in this development stage. As a consequence, large effluent volumes were often generated, which in some applications were even higher than in the corresponding batch procedures.

The first flow systems, called segmented flow analysis (SFA), were successfully employed for routine analysis, mainly in clinical laboratories. In these systems, samples and reagents were continuously aspirated, and segmentation by air bubbles was exploited to minimize axial sample dispersion and carryover, even for long sample residence times [20]. Washing was periodically carried out by an inert solution aspirated through the analytical path. As a consequence, effluent volumes higher than 10–20 mL per determination were not uncommon.

In the second generation of flow systems, named flow injection analysis (FIA), samples were processed in an unsegmented flow under controlled dispersion conditions. Strict time control allowed measurements without achieving the steady-state condition and thus exploitation of kinetic aspects. High sample throughput was also emphasized in FIA, as can be exemplified by the former single line manifolds, in which the reagent stream, sometimes at flow rates as high as 8 mL min^{-1}, acted as both the source of the reagent for the chemical reaction and as the sample carrier. In spite of the simplicity of this flow configuration, considerable reagent consumption and difficulties in mixing sample and reagents by dispersion led to the development of flow systems with confluent streams. In this approach, reagent solutions are equally distributed in the entire sample zone, yielding a constant volumetric fraction of the reagent. The reagent flow rate can then be reduced without

Table 1
Trends to green chemistry in the flow-based procedures for nitrate determination in waters.

Publication year	Analytical method	Waste	LQ; SR; ER[a]	Reference
1980	Reduction with cadmium and diazo-coupling reaction	Cadmium and carcinogenic amines	500[b]; 90; 80%	[12]
1991	Reduction with zinc and conductimetric detection after gas diffusion of ammonia	Zinc, EDTA and sodium hydroxide	880; 60; 97%	[16]
1995	Nitrate photoreduction followed by diazo-coupling reaction	Carcinogenic amines	6; 30; 84%	[13]
1998	Nitrate photoreduction and nitrite reaction with triiodide	EDTA, iodide, triiodide	100; 25; 39%	[15]
2002	Enzymatic reduction of nitrate by nitrate reductase from corn leaves followed by diazo-coupling reaction	Carcinogenic amines	250; 90; >95%	[14]
2005	Direct UV measurements after separation of interfering species	18 μL of $HClO_4$ per determination	500; 17[c]	[17]

[a] LQ: limit of quantification (μg L^{-1}), SR: sampling rate (measurements per hour) and ER: efficiency of reduction (%).
[b] Estimate value.
[c] Procedure without conversion to nitrite.

impairing the sampling rate, because an inert solution is used as carrier. In spite of the reduction in reagent volume in relation to the single line configuration, the continuous confluent stream led to reagent consumption even when a sample was not being processed.

The merging zones approach, in which sample and reagent aliquots are simultaneously inserted in independent carrier streams, was proposed to minimize reagent consumption [21]. Sample and reagent aliquots merge at the confluence and the chemical reaction can develop suitably, without impairing the analytical features. The pioneering work demonstrated that the consumption of the reducing reagent in the molybdenum blue method was only 9% of that required in the confluent system. This potential is also presented by flow systems exploiting reagent injection in a sample stream [22]. With this strategy, reagent consumption was reduced by up to 240-fold and 4000-fold in comparison to flow systems with confluent streams and batch procedures, respectively [22].

Intermittent flows can be exploited to reduce the washing interval without affecting the sample residence time [23] or to remove solids or reaction products impregnated in the analytical path [24]. In addition to the potential to change the reaction conditions in the sampling and injection steps, reagent consumption can be reduced in a similar way to that observed in the merging zones approach. With this aim, the reagent solution can be added to the sample zone by confluence and be recycled in the sampling stage.

Monosegmented flow analysis (MSFA) combines the simplicity and high sampling rate provided by FIA with the low sample dispersion achieved by SFA [25]. In MSFA, sample is sandwiched between two air bubbles aiming to reduce axial dispersion, allowing the long residence times required for relatively slow reactions [26]. Reagent consumption can be reduced by using opto-switches to detect the air bubbles in order to confine reagent addition to the sample zone [27], by the simultaneous injection of sample and reagent aliquots [28] or by the binary sampling strategy [26]. In addition, the air bubbles make the localization of the zones containing the reagents feasible, which can be separated from the carrier stream for further treatment [10].

Sequential injection analysis (SIA) [29], multicommuted flow analysis (MCFA) [30], the multisyringe (MSFIA) [31] as well as the multi-pumping [32] approaches can be considered as an evolution of the flow injection concept towards GAC. Among other advantages, these strategies are very effective in minimizing reagent consumption and exploiting intermittent reagent addition. Thus, reagents are added to the sample zone only in the strict amounts and when required in the analytical procedure.

Sequential injection analysis was proposed as a robust alternative, aiming implementation of different flow methodologies without modification of the manifold design [29]. This process exploits a multi-port selection valve for sequential collection of the sample and reagent aliquots used in the analytical determination. These are inserted into a holding coil with aspiration by a peristaltic pump or more commonly by a syringe pump [29,31]. The process is computer-controlled and both the sampling sequence and the volume of each aliquot are defined by software. Several solutions can be handled using the selection valve with a single pumping channel, which is an advantage in comparison to the usual FIA systems. The volume of the reagent solution inserted into the analytical path is only that necessary for reaction development (few microliters). Indeed, mixing of sample and reagent aliquots usually occurs by dispersion, and an increase in reagent volumes from a threshold value does not effectively increase the availability of the reagent in the sample zone. The requirement concerning reagent saving can thus be easily attained, with an estimated 20-fold reduction in relation to FIA with continuous reagent addition [31], as confirmed by recent applications discussed in the next section.

The MCFA approach employs a set of discrete commutators (*e.g.* solenoid valves) to design flow manifolds that can be reconfigured by software, thus increasing system versatility. Each device handles a solution independently under computer control [30], resulting in more effective reagent use. In one of the possible operation modes, sample and reagent solutions are pumped through the commuting devices and are recycled to their storage vessels while the carrier solution flows through the analytical path. Sampling is carried out by switching the solenoid valves simultaneous or sequentially to insert sample and reagent aliquots by merging zones or binary sampling, respectively. All valves are then switched off, allowing the carrier solution to flow, displacing the sample zone to detection. Solutions can also be aspirated by employing a single pumping channel and sample and reagent aliquots are inserted in tandem into the analytical path (binary sampling approach). In both approaches, solution volumes are defined by the flow rate and the sampling time, which can be easily computer-controlled. Analyzing the papers based on MCFA, it is clear that low reagent consumption and low waste volumes are inherent features of this process. Recent examples of applications of this strategy to GAC are discussed in the next sections.

In multisyringe flow injection analysis, a set of syringe pumps is coupled to solenoid valves or a multi-port valve for solution handling [31]. The syringes have diverse capacities (*e.g.* 0.5–25 mL) and are usually moved by the same step motor. By actuation of the valves, solutions can be directed to the analytical path or delivered back to the original reservoirs. This approach combines features of SIA (robustness, low reagent volumes and versatility) with those of FIA (improved sampling rates and better analytical performance). The potential and applications of MSFIA related to GAC have been recently reviewed [19].

A multi-pumping flow system comprises a set of solenoid micropumps, each dedicated to propelling a different solution [32]. A fixed volume is dispensed per stroke, allowing the effective control of reagent amounts inserted into the analytical path. The inherent pulsed flow favors mixing and minimizes solid deposition in the analytical path. Other advantages include portability, low energy requirements and the ability to minimize reagent consumption and waste generation, making the system suitable for *in situ* measurements.

Sequential injection chromatography (SIC) is an hybrid approach which combines the versatility of SIA with the potential of analyte separation provided by HPLC [33]. This approach became possible from the development of monolithic columns with performance comparable to the usual HPLC columns and reduced backpressure. SIC has been successfully applied for separation of less complex samples, often consuming lower solvent amounts and time in comparison with HPLC. An interesting characteristic is the on-line access of reagents in all steps of sample processing, which can be exploited for sample clean up and (pre or post column) analyte derivatization in a mechanized and greener way. The determination of intracellular free amino acids in the microalgae, for example, consumed 5 and 6.5-fold less methanol and tetrahydrofuran, respectively, than the corresponding HPLC method [34].

Miniaturization is a trend in analytical chemistry, aiming the development of less invasive chemical assays, portable devices for *in situ* monitoring and low reagent consumption and waste generation. This trend is also notable in flow analysis and miniaturized flow systems have been developed for the implementation of classical methods in a greener way [35], usually with competitive costs and analytical performance. Micro-flow injection systems (μFIA) can be constructed exploiting microfluidics and microelectronics, sometimes integrating propulsion, mixing and detection units in a single device, yielding micro total analytical systems (μTAS) [36].

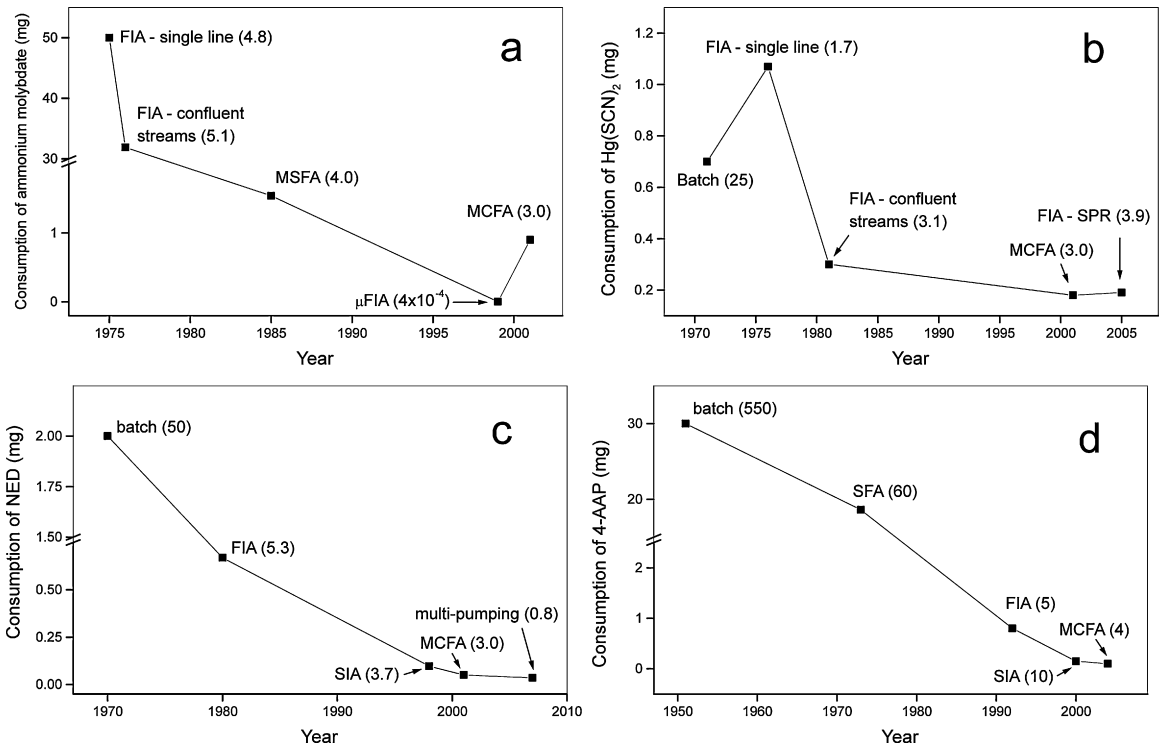

Fig. 2. Reagent consumption per determination in spectrophotometric procedures for: (a) phosphate [25,41–44], (b) chloride [42,45–48], (c) nitrite [23,45,47,49,50] and (d) total phenols [51–55]. Numbers between parentheses indicate the effluent volume per determination.

The analytical performance of μFIA is comparable with that of conventional analyzers, but flow rates on the order of nL to μL min^{-1} drastically reduce waste generation. As a limitation, these devices are usually dedicated to a specific application. The versatility has increased by the lab-on-valve (LOV) approach [37], in which injection ports, micro-channels and the detection cell are integrated in a micro-device, which is coupled to a conventional apparatus (*e.g.* optical fibers and a detection system). Solutions can then be handled by conventional flow approaches, such as SIA. The potential of LOV was formerly illustrated by the spectrophotometric determination of phosphate, in which reagent consumption and effluent generation were estimated as 10 μL and 250 μL per determination, respectively [37]. Other applications for bioanalytical [38] or environmental assays [39] have been recently reviewed. Particularly for environmental assays, the portability and low reagent demand can be exploited for *in field* monitoring, thus avoiding analyte losses and making feasible the adoption of corrective actions. This is also a greener approach by avoiding the need for sample preservatives.

The use of immobilized reagents has also contributed to the development of more environmentally friendly procedures. Solid-phase reagents can load a mini-column for analyte derivatization or for on-line generation of reagents, including unstable species [40]. The analytes can interact with an excess of the reagent, but consumes low amounts of the solid, ideally defined by reaction stoichiometry. However, the possibility of reagent lixiviation needs to be taken into account to select the suitable strategy for reagent immobilization.

A clear trend towards low amounts of chemicals and low effluent volumes can be noted by comparing different strategies for the determination of phosphate [25,41–44], chloride [42,45–48], nitrite [23,45,47,49,50] and total phenols [51–55], as demonstrated in Fig. 2.

Flow analysis has also contributed to avoiding the drawbacks of other classical procedures, such as batch liquid–liquid extraction (LLE). In flow-based systems, the operation time, solvent amounts and risks to the analyst in LLE are minimized by sample processing in a closed-system [56]. Several flow approaches have been proposed for the automation of this process, including FIA [57,58], MSFA [59], micro-extraction in MSFA [60], MCFA [61] and liquid–liquid extraction in a single 1.3 μL microdrop [62]. From these examples, the trend to reducing the organic solvent volume is evident (Fig. 3). Novel environmentally friendly extraction strategies, such as membrane-assisted LLE, cloud-point extraction, single-drop solvent extraction, and wetting-film extraction, have also been successfully coupled to flow systems [63].

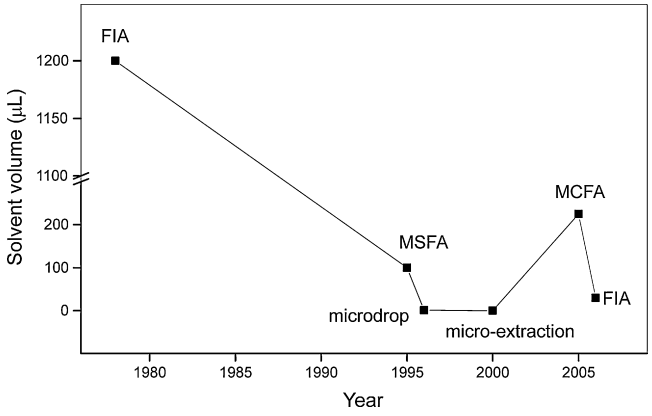

Fig. 3. Consumption of organic solvents in flow-based LLE. Volumes refer to extraction of caffeine by FIA [57], Cd/PAN complex [59] and micro-extraction of caffeine [60] by MSFA, sodium dodecyl sulfate in a microdrop [62], Pb/ditizone by MCFA [61] and thiamine by FIA [58].

3. Recent contributions of flow-based procedures to GAC

3.1. Reagentless procedures

The first principle of green chemistry establishes that prevention of waste generation is the best approach to minimize the impact of chemical activities ("*prevention is better than cure*"). This also holds for GAC, but the number of reagentless procedures is unfortunately limited. Regarding flow analysis, some examples can be found in which the ability for highly reproducible sample handling is exploited to selective analyte detection even without the use of chemicals. However, most of the applications are restricted to samples of relatively low complexity (Table 2).

A reagentless procedure exploiting the formation of falling drops was developed for the determination of ethanol in red wines [64]. A lab-made photometer with an infrared LED and a phototransistor was coupled to an FIA system in order to produce the falling sample drop between the photometric devices. The growth of the drop diminishes the IR radiation that strikes the photodetector and measurements are based on the effect of the alcohol on the size of the wine drop. A linear response was achieved from 1 to 30% (v/v) ethanol, with a sampling rate of 50 determinations per hour and a relative standard deviation of 2.5%. The procedure can be applied to colored samples (such as red wine), but other species that can modify the solution's surface tension (*e.g.* surfactants and other organic substances) can interfere.

A procedure for the monitoring of color as well as sugar and CO_2 contents of soft drinks was proposed in a SIA system without the use of chemical reagents [65]. The former parameters were determined by using LED-based photometers, with the sugar content determination based on differences in the refractive index at the water/sucrose interface in aqueous solutions (Schlieren effect). Carbon dioxide was determined after collection in an acceptor stream by measurements in a contactless conductivity detector.

3.2. Replacement of toxic reagents

Recent examples of replacement of chemicals in flow analysis are presented in Table 3. An interesting approach is the use of reagents from natural sources, such as plant extracts, animal or vegetable tissues, which can be used as chromogenic or fluorogenic reagents or as an enzyme supply. Ingenious applications in this field were recently reviewed [66], emphasizing the potential of flow systems to minimize side reactions by exploiting kinetic aspects, to increase stability by the use of the reagent in a closed system and to minimize the amounts of other chemicals required in the procedure. More reliable results can be obtained by a periodical evaluation of the analytical response and eventual recalibrations, which are feasible in mechanized procedures. However, the use of natural reagents can present some drawbacks, such as an increase of blank values and seasonal variability of the composition of the extracts or tissues. Recent applications of this strategy include the use of extracts of guava leaf [67] or green tea [68] for iron determination, porcine kidney tissue as a source of α-hydroxy acid oxidase in lactic acid determination in blood and milk [69] and polyphenol oxidase from avocado crude extract immobilized in controlled pore silica for the determination of isoproterenol in pharmaceuticals [70]. Another interesting approach is the use of natural sorbents, such as rice husks [71], peat [72] and gardening humus (vermicompost) [73]. These strategies agree with the seventh principle of green chemistry, related to the use of renewable raw materials [11].

Solid-phase extraction (SPE) is often a greener alternative in relation to LLE, because it usually avoids the use of toxic organic solvents. SPE has been successfully coupled to flow systems, resulting in better precision, high sample throughput and additional reduction in reagent consumption. On the other hand, enrichment factors are usually lower than those achieved in batch procedures. As an example of a recent application, a multisyringe flow system was proposed for speciation of nitro-phenols by exploiting solid-phase concentration and separation before spectrophotometric detection and data treatment by multiple least-square regression [74].

More environmentally friendly approaches for LLE, such as aqueous biphasic [75] and cloud point [76] extractions have also been coupled to flow systems. These processes allow replacing toxic organic solvents with low toxicity surfactants or water-soluble polymers without hindering the analytical performance. The feasibility of aqueous biphasic extraction in a flow system was first demonstrated by in-line preconcentration of a lead complex before fluorimetric detection [75]. In a recent example, CPE was implemented in a SIA system for manganese preconcentration before measurements by FAAS [76], eliminating laborious manual operations (*e.g.* heating, phase separation and removal of the surfactant-rich phase). The analyte was separated by CPE with Triton X-114 after complexation with 4-(5-bromo-2-thiazolylazo)orcinol. The surfactant-rich phase was retained in a mini-column filled with cotton and elution was carried out with a diluted acid solution. The enrichment factor was estimated as 14, attaining a detection limit of 0.5 $\mu g\,L^{-1}$.

Organic reagents and solvents are usually employed in procedures involving preconcentration of metal ions. An environmentally friendly alternative is the use of precipitation or co-precipitation reactions with low toxicity chemicals in flow-based systems. This strategy can be exemplified by cadmium determination by thermospray flame furnace atomic absorption spectrometry after retention of the corresponding hydroxide in a knotted reactor and dissolution with a 1 $mol\,L^{-1}$ HNO_3 solution [77]. The detection limit (40 $ng\,L^{-1}$) is better than that attained by other strategies for preconcentration and the procedure was successfully applied to water and rice digest samples.

The use of organic solvents for LLE in the batch spectrophotometric determination of anionic surfactants (*ca.* 45 mL $CHCl_3$ per determination) was avoided by exploiting the effect of the surfactants on ion-pair formation between methyl orange dye and cetyl pyridine cation [78]. The procedure was successfully applied to the analysis of wastewaters with a detection limit comparable to the reference procedure and advantages in relation to the sample throughput, estimated as 60 h^{-1}.

The use N,N-diethyl-p-phenylenediamine for the determination of free chlorine, was avoided in the determination of hypochlorite in bleach products in MSFIA [79]. The analytical signal was based on the native absorbance of the analyte (maximum absorption at 292 nm) with correction of the background absorption, measured after decomposition of the hypochlorite in a mini-column filled with cobalt(II) oxide. This strategy can yield greener procedures for the determination of several species by exploiting fast decomposition or selective separation of the analyte from the sample. However, because absorbance values need to be additive, application to more complex samples requires a careful evaluation and, sometimes, the use of chemometric approaches for data treatment.

Flow-based systems allow monitoring of unstable products, expanding the analytical application of low-toxicity chemicals. As examples, greener procedures have been developed for the determination of chromium(VI) [80] and manganese(II) [81]. In the former, the unstable blue intermediate CrO_5 which is produced in the reaction of the analyte with H_2O_2 in acid medium was measured in a SIA system, which also contributed to reducing the reagent consumption (145 μL per determination). The procedure thus avoided organic reagents, such as the 1,5-diphenylcarbazide usually employed for the spectrophotometric determination of Cr(VI). As a drawback, the procedure showed low sensitivity, being

Table 2
Recent examples of reagentless procedures and reuse of chemicals in flow-based procedures.

Analyte	Sample	Strategy for GAC	Reagents	Reference
Lead	Gasoline	Reagent reuse after regeneration on a cation-exchange resin	Arsenazo III	[7]
Iron	Natural waters	Reversible retention of the analyte in C_{18}-TAN and measurements by solid-phase spectrophotometry	TAN	[8]
Ethanol	Wines	Photometric detection of a falling drop whose size is affected by ethanol	Reagentless	[64]
Sugar	Soft drinks	Detection based on differences of refractive index at the sucrose/water interfaces	Reagentless	[65]
Carbon dioxide	Soft drinks	Contactless conductometric detection after collection in an acceptor stream	Reagentless	[65]
Glucose	Human blood serum, solutions for perfusion	Optosensor with immobilized thionine for monitoring of enzymatic oxidation reactions	$K_2Cr_2O_7$, glucose dehydrogenase, NAD+, phosphate buffer	[90]

applied to alloys, sewage sludge and wastewaters which present significant amounts of the analyte. Manganese was determined after on-line oxidation to Mn(III) and complex formation with EDTA in a flow system designed with solenoid micro-pumps. Threshold limits established by health and environmental regulations for natural waters were achieved by the increase in sensitivity provided by long pathlength spectrophotometry.

Photochemical reactions are ingenious alternatives for the development of greener procedures, in which light replaces toxic reagents for (i) analyte derivatization [82,83], (ii) sample treatment for destruction of interfering species [84] or (iii) waste decomposition [50,85,86]. Typically, UV radiation (*e.g.* from a low-pressure Hg lamp) is used for breaking chemical bonds of the analyte or precursors of oxidizing radicals. This strategy is suitably implemented in flow systems in view of the sample processing in a closed system under highly reproducible control of time and reaction conditions. Drawbacks caused by incomplete analyte conversion and instability of intermediate products formed in the photochemical reaction are thus avoided.

A number of greener procedures have exploited UV photolysis for analyte derivatization followed by detection by chemiluminescence or fluorescence. The principles of photo-induced luminescence were discussed in recent review articles [82,83] and applications have been mainly focused on pesticides [82] and pharmaceuticals [87] determination. In this sense, an ingenious procedure was proposed for the determination of chemical oxygen demand without using oxidant reagents [88]. The photochemical reaction yields free radicals which are detected by the chemiluminescence provided by the oxidation of luminol, used in low concentrations. As an additional advantage, the procedure is free from the interference of chloride. However, drawbacks can occur due to the differences in the oxidation efficiencies of different organic compounds and in the presence of large amounts of metal ions in the samples.

A flow system with solenoid micro-pumps has been used to significantly reduce waste toxicity in cyclamate determination in table sweeteners [89]. The analyte reacts with nitrite in acid medium, with the excess of the reagent determined by the reaction with

Table 3
Recent examples of replacement of toxic chemicals in flow-based procedures.

Analyte	Sample	Strategy for GAC	Reagents	Reference
Lead	Gasoline	Reagent reuse after regeneration on a cation-exchange resin	Arsenazo III	[7]
Iron	–	Guava leaf extract as natural reagent	Acetate buffer	[67]
Iron	Pharmaceuticals	Green tea infusion as natural reagent	Acetate buffer	[68]
Lactic acid	Blood and milk	Porcine kidney tissue as source of α-hydroxy acid oxidase	Luminol and potassium ferricyanide	[69]
Isoproterenol	Pharmaceuticals	Polyphenol oxidase from avocado crude extract	Phosphate buffer	[70]
Cadmium and lead	Water, wine, animal and vegetal tissues	Rice husks as natural sorbent	Diluted HNO_3	[71]
Copper	Water	Peat as natural sorbent	Acetate buffer and diluted HNO_3	[72]
Cadmium	Fuel alcohol	Vermicompost as natural sorbent	Tris(hydroxymethyl)aminomethane and diluted HNO_3	[73]
Nitro-phenols	Waters	Liquid–liquid extraction replaced by solid-phase extraction	Diluted HCl, NaCl and NaOH	[74]
Lead	–	Aqueous biphasic extraction	Polyethylene glycol, inorganic salts and 8-hydroxyquinoline-5-sulfonic acid	[75]
Manganese	Rice flour, infant formula and corn flour	Cloud point extraction	Triton X-114 and 4-(5-bromo-2-thiazolylazo)orcinol	[76]
Cadmium	Water and rice	Preconcentration by precipitation of cadmium hydroxide	Diluted NaOH and HNO_3	[77]
Anionic surfactants	Wastewater	Ion pair formation to avoid organic solvents	Methyl orange dye and cetyl pyridine chloride	[78]
Hypochlorite	Bleach products	Native absorbance of the analyte with correction of the background absorption	Solid Cobalt(II) oxide	[79]
Chromium(VI)	Alloys, sewage sludge and wastewaters	Formation of the intermediate CrO_5 in the reaction of the analyte with H_2O_2	H_2O_2	[80]
Manganese(II)	Waters	Formation of the Mn(III)/EDTA complex	EDTA and immobilized PbO_2	[81]
Chemical oxygen demand	Water	UV photolysis and chemiluminescence detection of the formed radicals	Luminol in buffered medium	[88]
Cyclamate	Table sweeteners	Replacement of carcinogenic amines by iodide and waste minimization with a multi-pumping flow system	$NaNO_2$, KI	[89]

iodide. The procedure thus avoids the hazardous chemicals (*e.g.* $Pb(NO_3)_2$ and N-(1-naphthyl)ethylenediamine) needed in previous studies and consumed only 3.0 mg KI, 1.3 μg $NaNO_2$ and 125 μmol H_3PO_4 with a waste volume of 2 mL per determination. As the most toxic chemical (NO_2^-) was completely decomposed in acid medium containing an excess of iodide, the waste was a solution containing only triiodide and iodide ions.

3.3. Reuse of chemicals

Ingenious strategies in flow-based systems make the reuse of chemicals feasible in sequential measurements. These exploit a reversible reaction with the analyte and regeneration of the reagent, which is then separated from the sample matrix and employed in another measurement cycle. The effectiveness of the approach thus depends on the number of measurement cycles that can be implemented without affecting the analytical performance.

A simple strategy was adopted for reagent reuse in a single line (closed loop) flow system for the spectrophotometric determination of lead in gasoline [7]. A cation-exchange resin was inserted after the detection cell for regeneration of the chromogenic reagent (Arsenazo III). After analyte determination, the metal ion was retained in the mini-column and the released reagent was directed back to its original reservoir. In addition, the heavy metal was accumulated in the mini-column, making the residue treatment feasible. System performance was evaluated with 50 mL of the same recirculating reagent solution for 7 days, yielding reproducible analytical curves without baseline drift. Similar approaches could be adopted to develop greener procedures in applications in which the affinity of the metal ion for the solid adsorbent is higher than that to the ligand reagent. However, a careful evaluation of both blank values and the occurrence of memory effects are necessary.

A flow-through reversible optosensor with immobilization of the redox indicator thionine on gel beads was proposed for monitoring enzymatic oxidation reactions [90]. The glucose oxidation mediated by glucose oxidase was used as a model and a sequential injection system was explored to minimize reagent amounts. As a consequence, the consumption of the most toxic chemical (potassium dichromate), used for regenerating the sensor phase, was reduced to 50 μg per determination. This proposal is a greener alternative to the monitoring of the products of the enzymatic reactions by chemiluminescence or spectrophotometry. Another example of the reuse of chemicals due to reversible reactions is solid-phase spectrophotometry, as discussed in the next section.

3.4. Waste minimization

Recent examples of waste minimization in flow analysis are presented in Table 4.

3.4.1. Immobilized reagents

Direct measurement on a solid phase is an alternative to increasing the sensitivity of procedures with optical detection [91]. In spite of several batch applications, the process is more efficiently carried out in flow-based systems, in which the solid support is deposited in the measurement cell and analyte retention and detection occur simultaneously. This approach is also less time-consuming and saves the solid support, which is usually employed for 100–300 sequential measurements. Exploitation of kinetic aspects and the proper choice of the conditions for analyte retention (*e.g.* solid support, pH and polarity of the eluent solution) results in better selectivity in comparison to measurements in solution. Thus, analyte determination can be sometimes carried out without chemical derivatization, yielding environmentally friendly analytical procedures. A number of applications can be found in the literature, including multianalyte determination, especially for less complex samples, such as pharmaceutical preparations [18,91]. However, when chemical derivatization is involved, greener procedures can be obtained by reversible retention of the analyte, allowing the reuse of the immobilized reagent. For example, this strategy was adopted for iron determination based on reversible sorption on a solid support with 1-(2-tiazolylazo)-2-naphthol (TAN) immobilized on C_{18}-bonded silica [8]. The analyte was eluted with a low volume of a diluted acid solution without removing the immobilized reagent, which was used for at least 100 determinations. As a consequence, the reagent consumption was lower than 1 μg per determination. Sensitivity was 10-fold higher than that achieved by measurements in solution, which consumes greater amounts of sample and reagent. Further applications have been presented in recent review articles [18,91].

A recent application of a solid-phase reactor for the development of a cleaner procedure was the chloride determination using $Hg(SCN)_2$ immobilized in an epoxy resin [48]. The thiocyanate anions released by the reaction of the analyte with the solid reagent were quantified after complex formation with iron(III). This strategy reduced the amount of Hg generated per determination from mg to μg level.

3.4.2. Multicommuted flow systems

The potential of MCFA to minimize reagent consumption was exploited for the environmentally friendly spectrophotometric determination of total phenols based on oxidative coupling with 4-aminoantipyrine in alkaline medium [51]. This resulted in a 200-fold reduction in reagent consumption in comparison to the reference batch method. Sensitivity was 80-fold higher due to the use of long pathlength spectrophotometry, thus avoiding the need for liquid–liquid preconcentration of the reaction product, which would require *ca.* 50 mL of chloroform per determination [45].

The carbamate pesticide asulam has been often determined by diazotization of its amino group and subsequent coupling with N-(1-naphthyl)ethylenediamine (NED). This method is poorly selective and uses a carcinogenic reagent. Chivulescu et al. [92] exploited a flow system with three solenoid valves for determination of the analyte by chemiluminescence, substantially saving reagents (a few microliters consumed per determination). In addition, the procedure is greener in view of the replacement the toxic reagent (NED) by a glycine buffer and potassium permanganate. In another approach, carbamate pesticides were determined in vegetable samples after liquid–liquid microextractions with a hollow fiber filled with the organic solvent (dodecanol), whose consumption was estimated at 50 μL per determination [93]. Other examples of waste minimization in ELL are shown in Table 5.

Replacement of hazardous reagents and minimization of reagent amounts were also exploited for the determination of acid-dissociable cyanide in natural waters [94]. The reference batch procedure is based on a reaction with barbituric acid and pyridine followed by spectrophotometric detection. The flow system was designed with four solenoid valves in order to implement the reaction with o-phthalaldehyde (OPA) and glycine which yields a highly fluorescent isoindole derivative. The proposed procedure consumes 6 μg OPA and 7 μg glycine per determination, which is up to 230-fold lower than the amounts required in a FIA procedure with continuous reagent addition.

MCFA was also employed for dilutions, external calibration and standard additions for the determination of benzene in gasoline by FT-IR [95]. The analytical features were comparable to those achieved by FI-FT-IR, but the consumption of the organic solvent (hexane) was three-fold lower. MCFA also reduced the effluent volume by six-fold in the determination of mercury in milk [96]. In this procedure, on-line waste treatment by coprecipitation with

Table 4
Recent contributions of flow-based procedures for waste minimization.

Analyte	Sample	Strategy	Reagent (mg per determination)	Reference
Iron	Natural waters	Reversible sorption of the analyte	TAN (1×10^{-3})	[8]
Amino acids	Microalgae	SIC with pre pre-column derivatization	OPA (0.2)	[34]
Chloride	Natural waters	$Hg(SCN)_2$ immobilized in epoxy resin	$Hg(SCN)_2$ (0.12)	[48]
Nitrite	Natural waters	Waste minimization and in-line photodegradation	NED (0.05); sulfanilamide (1)	[50]
Phenol	Water	MCFA with a 100-cm optical path flow cell	4-AAP (0.10) and $K_3[Fe(CN)_6]$ (0.12)	[51]
Carbaryl	Natural waters	Minimization of waste generation, cloud point extraction and photodegradation of the residue	PAP (2×10^{-3}); KIO_4 (6×10^{-3})	[86]
Asulam	Natural waters	MCFA and waste photodegradation	$KMnO_4$ (0.012)	[92]
Cyanide	Natural waters	MCFA and fluorescence	OPA (6×10^{-3}); glycine (7×10^{-3})	[94]
Benzene	Gasoline	MCFA coupled to FT-IR	Hexane (1.2 mL)	[95]
Mercury	Natural waters	Cold vapor fluorescence spectrometry with a MCFA	$SnCl_2$ (41.8)	[99]
Tellurium	Milk	Atomic fluorescence spectrometry with MCFA	$NaBH_4$ (96)	[100]
Chlorine	Bleach	Automatic falling drop system on based MCFA	DPD (0.055)	[101]
Paraquat	Natural waters	MCFA and replacement of toxic reagents	$C_6H_8O_6$ (0.4); KIO_4 (0.16); EDTA (0.16)	[103]
Chlorine	Natural waters	MCFA and increase of sensitivity by long pathlength spectrophotometry	DPD (0.026)	[105]
Orthophosphate	Soils and sediments	MSFIA system coupled to a selection valve	Ammonium molybdate (0.5), $SnCl_2$ (0.02) and oxalic acid (0.1)	[106]
Mercury	Fish muscle and waters	Six-fold reduction of the effluent volume by MCFA with on-line treatment of the waste	$SnCl_2$ (26)	[108]
Chloride	Natural waters	MSFIA hyphenated with liquid core waveguide	Hg(II) (2×10^{-4}); Fe^{3+} (0.028)	[109]
Cromium(VI)	Wastewater and alloys	μFIA manufactured on urethane–acrylate	Diphenylcarbazide (9×10^{-3})	[114]
Copper	Wastewater from electroplating	μFIA based on a planar glass chip with poly(dimethylsiloxane) top plate	Zincon (1.6×10^{-3})	[117]
Copper	Natural waters	μFIA fabricated by laser ablation on a polymethyl methacrylate chip	Nitroso-R salt (0.08)	[118]
Zinc	Pharmaceuticals	PMDS microfluidic device with an integrated optical sensor	Xylenol orange (0.04)	[119]
Zinc	Seawater	μSI-LOV with fluorescence detection	FluoZin-3 (5×10^{-4})	[122]
Iodate	Table salts	Downscaled MCFA with a LED-based photometer	DPD (7×10^{-3})	[124]
Riboflavin	Foodstuffs	On-line sample clean-up by bead injection of MIP in a LOV system	Methanol (856 μL) and MIP (11.2)	[126]
Sulfur dioxide	Indoor air	Microchip coupled to fluorescence detection	Triethanolamine (0.09)	[128]

iron(III) and sodium hydroxide also contributed to a greener procedure.

A multicommuted flow system was exploited for liquid–liquid microextraction of the anti-hypertensive diltiazem after ion-pair formation with the bromothymol blue dye [97]. The sample zone was sandwiched by chloroform aliquots and extraction occurred at the interfaces and through the film of the organic solvent formed at the hydrophobic tube walls. An extraction efficiency of 61% was achieved with a 200-fold lower consumption of the organic solvent (50 μL per determination) and a waste volume of 380 μL per determination. The procedure is therefore a greener alternative to those previously described in the literature, which use large amounts of organic solvents or concentrated acids and bases. MCFA also allowed a 64-fold reduction in the consumption of chloroform and an 80% higher sensitivity in comparison to the reference batch procedure for the determination of anionic surfactants by the methylene blue method [98].

Greener procedures based on the MCFA approach have also presented improved analytical performance. This aspect can be exemplified by mercury determination by cold vapor fluorescence spectrometry [99]. In comparison to the batch method, the sampling rate was increased by a factor of 3.6 and the precision was 10-fold better, without affecting the sensitivity. Simultaneously, reagent consumption and waste generation were 8.4-fold and 2.4-fold lower, respectively. Other recent environmentally friendly applications of MCFA include tellurium determination in milk by hydride generation atomic fluorescence spectrometry with a 4-fold lower reagent consumption and 4-fold higher sample throughput [100]; the reduction in the consumption of DPD to 55 μg in chlorine determination in a falling reagent drop system [101], and 5-fold lower consumption of $CHCl_3$ in nicotine determination by FT-IR [102].

3.4.3. Multi-pumping flow systems

Two procedures for nitrite determination in natural waters based on solenoid micro-pumps were compared in relation to the analytical features and waste toxicity [50]. The former was based on Griess diazo-coupling reaction and the low amount of

Table 5
Recent contributions of flow-based procedures for waste minimization in LLE.

Analyte	Sample	Strategy	Reagent per determination	Reference
Carbamate pesticide	Vegetable	Liquid–liquid microextraction with dodecanol immobilized on the hollow fiber	42 μg of dodecanol	[93]
Diltiazem	Pharmaceuticals	Liquid–liquid microextraction without segmentation in MCFA	30 μg of bromothymol blue and 50 μL of chloroform	[97]
Anionic surfactant	Natural waters	Liquid–liquid microextraction in MCFA	90 μg of methylene blue and 70 μL of chloroform	[98]
Nicotine	Tobacco, cigarette filters and tobacco ash	FT-IR spectroscopy in MCFA	2 mL of chloroform and 3 mg NH_4OH	[102]
Carbamate pesticides	–	Extraction of the reaction product in toluene (nL) and detection by thermal lens spectrometry in μFIA	300 nL of toluene	[115]

waste generated was on-line treated by the photo-Fenton reaction ($Fe^{2+}/UV/H_2O_2$). The second procedure exploited the formation of iodine from nitrite and iodide in acid medium, avoiding the use of toxic reagents. Better sensitivity and selectivity were achieved by the Griess method and the consumption of the most toxic reagent (N-(1-naphthyl)ethylenediamine) was reduced by 55-fold and 20-fold in comparison to the batch procedure and flow injection with continuous reagent addition, respectively. A colorless residue was obtained after in-line photodegradation of the waste also reducing in 87% the total organic carbon content.

Replacement of a toxic reagent (sodium dithionite by dehydroascorbic acid) and a 12-fold reduction of the reagent amounts were adopted in a greener analytical procedure for the determination of the paraquat pesticide [103]. In each determination, 400 μg of ascorbic acid, 160 μg of potassium iodate and 160 μg of EDTA were consumed, with a corresponding effluent volume of 2 mL. In view of the increase in sensitivity, analyte preconcentration was not required, thus avoiding additional waste generation.

An environmentally friendly and highly sensitive analytical procedure was recently proposed for the determination of the pesticide carbaryl in natural waters [86]. Improved mixing conditions and a reduction in both reagent consumption (2 μg of p-aminophenol and 6 μg of potassium metaperiodate) and effluent volume (2.6 mL per determination) were provided by a multi-pumping flow system. Long pathlength spectrophotometry was exploited to increase sensitivity, thus making analyte preconcentration unnecessary and minimizing waste generation. A green procedure based on cloud point extraction was employed to remove interfering organic species present in the natural water samples. This step replaces the LLE recommended by EPA method 8318, which consumes 90 mL of methylene chloride per sample [104]. The waste of the analytical procedure was treated with potassium persulfate and ultraviolet irradiation, yielding a colorless residue and decreasing the total organic carbon content by 94%. The residue after treatment was not toxic to *Vibrio fischeri* bacteria. Despite the relevance of toxicity tests, this evaluation is not common in studies focusing on the degradation of wastes from analytical procedures.

Coupling of a flow system with solenoid micro-pumps was also exploited for the determination of low concentrations of free chlorine in natural waters [105], reducing by 20,000-fold the consumption of the most toxic reagent (N,N-diethyl-p-phenylenediamine) in comparison to the batch method.

3.4.4. Sequential injection analysis and multisyringe flow injection systems

A MSFIA system coupled to a selection valve was proposed for phosphorus fractioning in soils and sediments with detection by the molybdenum blue method [106]. The solid sample was directly deposited on a mini-column and the sequential extraction of labile P and P-species bound to Fe, Al and Ca was performed on-line with 1.0 mol L^{-1} NH_4Cl, 0.1 mol L^{-1} NaOH and 0.5 mol L^{-1} HCl. The proposed flow assembly presented several advantages as compared to fractionation in the batch mode such as decrease in reagent consumption of *ca.* 92% and a reduction in the analysis time (from days to hours), thus minimizing energy expenditure. Sample treatment under mild conditions was also achieved by on-line coupling of a high-intensity focused ultrasound probe to a sequential injection system [107]. The potential of the approach was demonstrated by developing a greener procedure for the determination of total and inorganic mercury fractions by cold vapor atomic absorption spectroscopy.

A flow system with two syringe pumps coupled to a cold vapor atomic fluorescence detector was used for mercury speciation, after in-line sample clean-up and preconcentration [108]. Speciation was feasible by separation of the inorganic form from the organic mercury as a tetrachloro complex by retention on an anion-exchange membrane. Recoveries of the analyte spiked in the samples were close to 90% and results agreed with the reference values of a fish muscle certified reference material. The system reduced reagent consumption, while membrane separation replaced the classical LLE with toluene and dichloromethane, thereby reducing toxicity and amount of waste.

MSFIA was also hyphenated to a flow-cell based on a liquid core waveguide for the determination of chloride in waters [109]. The classic analytical methodology exploiting the reaction with $Hg(SCN)_2$ was improved by reducing reagent consumption on the order of 3400-fold and 600-fold in comparison to that required in a single line flow system or with the reagent immobilized in epoxy resin [48], respectively. This example illustrates the development of greener procedures even with the use of a highly toxic chemical.

A dual-valve sequential injection system has been proposed for liquid–liquid extraction in an entirely closed system [56]. The first valve is used for segmentation of organic and aqueous phases, while a second device is used to direct the organic phase towards the detection cell. Flow-reversals [56] or air bubbling [110] were exploited to improve extraction efficiency in the determination of picric acid or copper, respectively, by ion-pair formation. In spite of the solvent consumption larger than the required in other flow approaches, the exposition of the analyst to hazardous solvent vapors is minimized, illustrating other important feature of flow systems in accordance to the twelve principle of green chemistry.

Dispersive liquid–liquid microextraction is an ingenious approach for ELL, in which a disperser solvent, miscible in both aqueous and organic phases, is used to form micro-drops of the extraction phase, thus yielding a superb increase in the surface area and minimizing extraction time. The process was recently adapted to SIA systems [111], aggregating the advantages provided by the automation of the extraction process in a closed system, as well as the consumption of micro-volumes of the extraction solvent and the eluent without hindering the extraction efficiency [112,113]. In addition, SIA makes phase separation feasible (*e.g.* using a micro-column filled by a hydrophobic material) and extend the application of the proposal to solvents less dense than water. Some recent applications include the preconcentration of cadmium and lead before GFAAS measurements [112] and of thiocyanate before spectrophotometric detection [113].

3.4.5. Miniaturization

Reagent consumption and effluent generation have been drastically reduced in miniaturized flow systems (μFIA). Recent developments have focused on inexpensive microfluidic devices exploiting polymeric materials, such as the μFIA analyzer constructed by deep UV lithography on two layers of urethane–acrylate polymers [114]. The device presented a total volume of 7 μL, integrating micro-channels and LED-based photometric detection, and it was applied for the determination of chromium(VI) and chloride. For the halide determination by the reaction with $Hg(SCN)_2$, the reagent consumption (2 μg Hg(II)) was *ca.* 300-fold lower than in the batch procedure, generating *ca.* 20 mL of effluent after an eight-hour working day. The proposed procedure can be thus considered environmentally friendly even though it uses a hazardous chemical. More complex sample processing has also been implemented as demonstrated by μFIA for the determination of the carbaryl pesticide by thermal lens spectrometry after derivatization and LLE [115]. Two glass micro-chips were used to carry out pesticide hydrolysis, diazotization and extraction of the colored product in toluene. The enrichment factor was 50, yielding a detection limit of 70 nmol L^{-1} with a toluene volume lower than 300 nL per determination. An ingenious microfluidic device was developed for sulfur dioxide determination in indoor air, including analyte diffusion through a porous glass, absorption in a triethanolamine solution, chemical derivatization and fluorimetric detection on an integrated

microchip [116]. Other recent examples include micro-flow analyzers fabricated by photolithography and wet etching technique on a planar glass chip [117] or by laser ablation on a polymethyl methacrylate chip [118], both using a molded polydimethylsiloxane (PDMS) as the top plate and applied for copper determination. The PDMS substrate was also employed for developing a low-cost microfluidic device used for the determination of zinc in pharmaceuticals [119]. This work exploited a conventional peristaltic pump to propel the fluid at 16 μL min^{-1}.

One of the main challenges in miniaturization is coupling to the detection system without affecting the sensitivity in view of the low sample volume. Typical situations are the decreasing sensitivity of spectrophotometric measurements due to the short optical path (usually less than 1 mm) and in some electrochemical techniques in view of the smaller electrode area. Miniaturized devices then require small-sized detectors with good sensitivity and a low noise level. Optical detection has usually been accomplished with LEDs as the radiation source or optical fibers to transport the radiation. Miniaturized electrodes are usually microfabricated in a fluidic device or coupled to the channels of a LOV system. Successful examples include spectrophotometric [114], fluorimetric [116], potentiometric [120] and voltametric [121] detection, as well as application of LOV for trace analysis [115,122].

The lab-on-valve and lab-on-chip approaches were critically compared in relation to the potential for environmental chemical assays [39] and advantages of the former approach were highlighted from a practical viewpoint. These include higher versatility for implementing different chemical assays, facilities for coupling to different detection systems, pretreatment of complex samples (including those with solids in suspension) and in-line analyte concentration. A recent work reviewed applications of the LOV approach for metal analysis by electrochemical or optical detection [123]. An interesting feature is the use of one of the valve microchannels as a multi-purpose flow cell for spectrophotometric or fluorimetric detection, highlighting the versatility of the device.

Downscaled inexpensive lab-made apparatus have also contributed to minimization of reagent consumption, even with flow-channels of dimensions considerably higher than the lab-on-chip devices. As an example, a flow system with a LED-based photometer with a total volume of 170 μL allowed the reduction of the reagent consumption to 7 μg and the waste volume to 600 μL in the determination of iodate in table salts [124].

Sample pretreatment is usually time-consuming and generates large waste amounts. These drawbacks have been overcome by integrating sample treatment and analytical detection in microsystems. In this sense, an ingenious approach exploited the immobilization of TiO_2 (anatase) in a gold matrix from CD-Rs for the photocatalytic decomposition of organic matter using an UV-LED (maximum emission at 365 nm) as the radiation source [84]. The sample treatment in the micro flow cell (volume of *ca.* 19 μL) resulted in quantitative decomposition of the Cu(II)/EDTA complex before voltammetric measurement of the metal. The LOV approach also shows potential for more environmentally friendly sample clean-up or analyte derivatization before chromatographic or electrophoretic separations [125]. A recent application exploited bead injection of a molecularly imprinted polymer (MIP) for clean up of foodstuff samples aiming riboflavin determination by liquid chromatography [126]. Sample preparation was carried out in parallel with the chromatographic separation, enabling the analysis of six samples per hour and requiring a 72% lower volume of methanol and a 55% lower mass of the MIP sorbent.

Technological innovations and new approaches have contributed to the diffusion of micro-flow systems and, consequently, the incorporation of the advantages of GAC in analytical procedures. For example, drawbacks for fluid propulsion [127] and reproducible manipulation of solid beads in microfluidic devices [128] have been circumvented, and low-cost strategies for lab-made construction of miniaturized manifolds [114] and microfabricated reactors for photochemical sample treatment [129] have been presented. In addition, specialized companies supply devices for miniaturized systems, such as valves and micro-pumps (*e.g.* http://www.nresearch.com, http://www.biochemfluidics.com and http://www.theleeco.com), microfluidic chips and pumps (*e.g.* http://www.dolomite-microfluidics.com), LOV systems (*e.g.* http://www.flowinjection.com) and optical detectors (*e.g.* http://www.oceanoptics.com).

4. Conclusions

The present overview has shown the evolution of flow analysis towards green analytical chemistry by means of replacement of hazardous chemicals and the trend towards waste minimization. This development has been successfully accomplished without hindering the analytical features that could impair analyte determination. Indeed, better analytical performances have been achieved in some circumstances, as the improvement in sensitivity which makes time-consuming and not environmentally benign preconcentration steps unnecessary. In this sense, the potential of flow approaches such as MCFA, SIA and MSFIA as well as the use of immobilized reagents was demonstrated by recent examples. Ingenious strategies such as reagentless procedures, green analyte extraction and sample clean-up as well as the reuse of chemicals have been feasible using flow systems. A recent trend is the development of miniaturized procedures coupling all the stages of sample processing (including sample treatment and preconcentration) with minimal waste generation. It is a general-use strategy and the devices have allowed the implementation of well-established methods without a significant impact on the environment.

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