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Review

Pulsed flows in flow analysis: Potentialities, limitations and applications

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ABSTRACT

In flow analysis, use of a steady and pulseless flow was considered essential for ensuring a reproducible handling of the flowing sample. To this end, peristaltic and syringe pumps have been the propelling device in the vast majority of the flow analysers. Recently, the number of applications involving pulsed flow has been increasing. Most of them refer to use of solenoid pumps, the essence of the so-called multi-pumping flow systems.

This review critically discusses the characteristics, potentialities and limitations of the pulsed flow systems, emphasizing the main advantageous characteristics of the streams involved, such as high radial mass transference and good mixing of the fluids. Diverse contributions ranging from instrumentation development to analytical applications are presented.

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1. Introduction

Investigations on the influence of flow pattern[2] in analytical chemistry started at the first quarter of the last century. The need for fast solution mixing and in-line detection was increasing, and exploitation of flow-based strategies was the right answer to it [1]. Modifications in flow pattern influenced the mixing conditions, as highlighted in 1923 by Hartridge and Roughton [2], who reported vortex movements of fluids caused by Y-shaped and cylindrical mixing devices.

Mixing chambers were further used to improve the continuous monitoring required for repetitive assays. Convergent streams of sample, reagents and carrier solutions were generally established, and steady situations analogous to that of “sample infinite volume” [3] were approached. Flow-based analytical procedures were then

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[2] Flow pattern is herein used in a broad context, encompassing flow regime, flow rate and its temporal variations, as well as the characteristics of the flowing stream.

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developed mainly for clinical assays, and the determination of glucose in blood [4] and the evaluation of kinetic parameters in enzymatic reactions [5,6] can be selected as examples. Different strategies for modifying flow pattern were applied, and the addition of air bubbles played a relevant role in the context.

Air bubbles are inherent to segmented flow analysis [7]. In fact, their addition hinders sample intermingle, minimizes sample dispersion and scrubs the tubing inner walls. Stream segmentation was then considered a rule of thumb, as the established vortices inside every flowing segment were beneficial to improve the mixing conditions. Consequently, the segmented flow analyser underwent amazing development and is still worldwide used, especially in clinical chemistry and environmental management.

Nevertheless, air segmentation is not always essential, as demonstrated in flow-based applications relying on unsegmented streams, proposed after the inception of flow injection analysis [8,9]. Without segmentation, the entire analytical channel behaves as an uncompressible liquid column, allowing different approaches such as stream splitting, flow reversal, zone circulating, zone merging, zone trapping, zone stopping and zone sampling to be efficiently implemented [10]. For improving system versatility and efficiently controlling the analytical course, discrete computer-operated devices, such as the selecting valve of the sequential injection analyser [11] or the directional valves of the multicommuted flow analyser [12] have been exploited. Development led to the proposal of several modalities of flow analysis (*e.g.* multi-syringe flow injection, mono-segmented flow, flow-batch, multi-pumping flow, sequential injection, lab-on-valve, bead injection, stop-in-loop, all injection, and simultaneous injection-effective mixing analysis), all of them assigned by an acronym. As only minor differences are generally taken into account for specifying a given modality, this policy should be strongly discouraged. The theme was already presented in a jocose editorial [13].

Regarding fluid propelling devices, the peristaltic pump has been mostly used. It delivers an almost constant flow with a slight ripple effect due to action of the rollers. This effect is beneficial for reproducible additions of the air bubbles and confluent streams [14]. A too pronounced ripple characterizes the so-called pulsating flow. Syringe pumps have been also used, specifically in unsegmented flow systems [15], and the established laminar flow regime [16] leads to a pronounced sample broadening. Although beneficial in some applications, broadening may increase the sample dispersion, thus lessening sampling rate.

In order to minimize sample broadening, several strategies such as tubing coiling, use of specially designed reactors, manifold downsizing, etc. have been proposed. Modifications in flow pattern are also beneficial for minimizing this effect, and pulsed flows, originally applied to flow analysis in 1996 [17], play a relevant role in the context. As a typical laminar flow regime is not established, interaction of the involved solutions is improved. Development of this novel strategy for solution management led to the concept of pulsed flow chemistry [18].

Pulsed flows constitute themselves in the essence of multi-pumping flow analysis [19], which involves several discretely operated pumps strategically positioned in the manifold. This architecture is an additional feature towards enhanced system versatility. The pumps may perform different tasks such as driving solutions, improving mixing conditions, selecting sample and reagent aliquots, introducing these aliquots into the analytical path, establishing tandem streams, implementing fluidized beds, stopping the sample and/or providing commuting facilities. Multi-pumping flow analysis was already reviewed [20], yet a critical discussion on use of pulsed flows in flow analysis without restricting it to a specific system modality is missing. This is the goal of the present review, which emphasizes also the characteristics, potentialities, limitations and analytical applications of pulsed flow systems.

2. Pulsed flows

A literature survey reveals that the term “pulsed flow” has been indistinctly used for specifying both a stream of successive aliquots of miscible solutions flowing at a constant flow rate (Fig. 1a) and a stream of a single solution flowing at a pulsed manner (Fig. 1b). In the present review, the term “pulsed flow” is restricted to the latter situation: the stream moves at a very high flow rate during very short successive time intervals and remains stopped during relative long periods between pulses.

In this context, a pulsed flow is established by suddenly inserting fixed solution aliquots (pulses) at a given frequency. To this end, the solenoid pump is by far mostly used, yet piston, pressure pulse [18], piezoelectric and aquarium pumps have been scarcely used.

The solenoid pump, also referred to as membrane solenoid pump, consists of a solenoid-driven diaphragm and two unidirectional check valves located at the inlet and outlet for establishing the direction of the flow. Commercially available solenoid pumps are usually operated at 12 or 24 V under direct electric current. Manufacturers recommend application of nominal voltage pulses during a fixed time span (usually 0.1–0.2 s) followed by a period of inactivation. The recommended pulse span should not be exceeded in order to avoid overheating. On the other hand, it cannot be reduced at will for decreasing the delivered volume, as a precision drop may occur. The inactivation time is set for attaining the aimed averaged flow rate.

Advantages and limitations of the solenoid pumps are highlighted as follows:

- No pumping tubes are required. Chemical inertia is then inherent to the these pumps. Moreover, drawbacks related to lifetime, maintenance and slow variations in flow rates, often reported for peristaltic pumps, are minimized.
- Downsizing is inherent to the pump. This is an additional guarantee towards system miniaturization and portability.
- Energy requirement is minimal. This is a worthwhile aspect for analyses under field conditions.

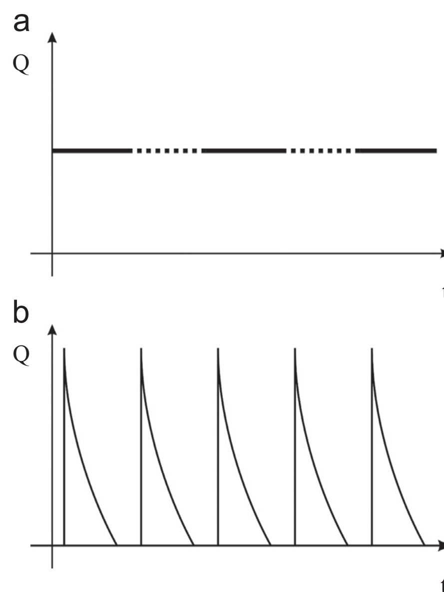

Fig. 1. Temporal variations in flow rates. Figure refers to successive aliquots of miscible solutions flowing at a constant flow rate (Fig. 1a) and to a single solution flowing at a pulsed manner (Fig. 1b). Q = flow rate; t = temporal coordinate; full and dotted lines = different involved solutions. Relaxation time too magnified for didactic purposes. See text for other details.

- The pump may undergo heating. The effect is more pronounced during continuous operation, especially for too high system backpressures, sample insertion frequencies and/or inserted volumes.
- Air bubbles can be released during the sudden solution aspiration [21]. The limitation is minimized by avoiding the use of too concentrated solutions, degassing the flowing solutions before use and/or selecting solutions less prone to gas liberation.
- Unfeasibility of flow reversal. This limitation is overcome by taking advantage of commuting facilities [22].
- Less suitability to pump highly viscous solutions [23].

The insertion speed of the aliquots depends on the elasticity of the flow conduits and the system hydrodynamic pressure. In fact, instant insertion is only feasible in the hypothetic limit situation of negligible system elasticity and zero hydrodynamic pressure. In this situation, flow rate and required time for insertion might tend to infinite and zero, respectively. In real applications however, instant insertion is not feasible and a given time interval for insertion is always needed. It should be emphasized that with a strong enough propelling device, reproducible aliquot insertions are better attained.

The flow rate during insertion is very high at the initial instant, and undergoes an exponential decrease dictated by the system elasticity and hydrodynamic pressure (see also Fig. 1b). After insertion, the normal geometry of the flow conduits tends to be restored and a low decreasing flow rate is noted during this relaxation period. Thereafter, the flow rate ceases and the stream is halted during the period between successive pulses. Concepts of STOP and GO periods in relation to pulsed flows are then meaningless.

Turbulence is approached only during the insertion times, and this transient tendency has been assigned to as “turbulent mixing” [18]. Although a typical laminar flow regime is not established, there is always a tendency towards laminarity due to the elasticity of the manifold components, especially tubing. An extreme situation refers to the placement of a damper (*e.g.* soft tubing) after the fluid propelling device aiming at a pulseless situation, which is beneficial when *e.g.* the analytical signal is affected by flow rate variations. Electrochemical detection, often dependent on the mass transport to the electrode surface, exemplifies this situation [24].

Improved radial mass transport is a consequence of turbulent mixing [25]. Other favourable aspects are the reduced sample broadening, the superior mixing conditions, and the attainment of similar linear flow velocities for all fluid elements (V. Section 3). These aspects constitute themselves as a driving force towards the worldwide acceptance of pulsed flow systems.

3. Flow systems with pulsed flows

These systems comprise several discretely operated solenoid pumps strategically positioned in the manifold, which are accountable for the enhanced system versatility, as shown in Fig. 2.

The main characteristics of pulsed flow systems are discussed as follows:

3.1. Recorded peak shape

A consequence of the efficient radial mass transport and similar mean linear flow velocities of the fluid elements in the dispersing zone is that sample broadening is reduced. This was demonstrated by comparing the shapes of peaks recorded by flow systems relying on constant or pulsed flows [25]. These systems were designed as similar to each other as possible (2.0-mL min^{-1} flow rate, 200-μL sample volume, 300-cm coiled reactor), and the only difference was the fluid propelling device, a two channel peristaltic pump or two solenoid pumps. With pulsed flows, heights of the recorded peaks underwent a 90% increase, washing time was reduced to 52% and sampling rate was almost doubled, emphasizing the beneficial influence of this flow pattern.

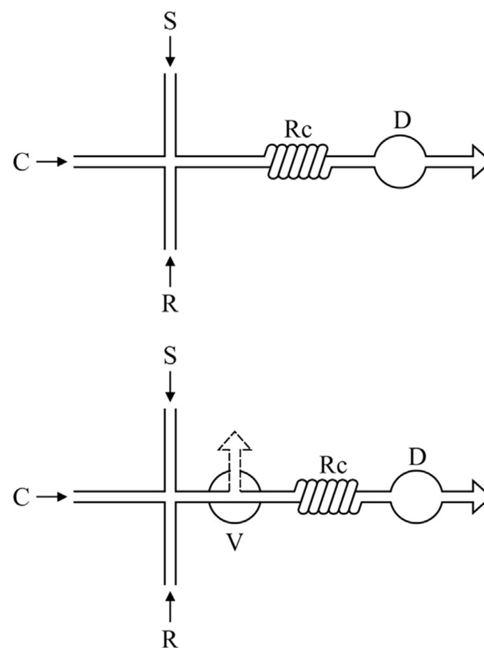

Fig. 2. Flow diagrams of typical pulsed flow systems. Black arrows = solenoid pumps; S, C, R = sample, carrier, reagent solutions; R_C = coiled reactor; D = detector. Simultaneous or sequential solution insertions controlled by the time course actuation of the solenoid pumps. Lower flow diagram = a more versatile flow system where sample replacement is facilitated by actuating the V three-way solenoid valve for directing the sample excess (and eventual gas bubbles) directly towards waste. Traced lines = alternative path.

Another characteristic of the peaks recorded in a pulsed flow system is their stair-like pattern. In fact, a continuous increase (or decrease) in the monitored signal is not noted, as there is a tendency towards a fast modification in signal during the very fast aliquot insertion and a relative steady situation between successive insertions. The stair-like pattern becomes less evident in flow systems with long analytical paths and/or presenting high tubing elasticity. A systematic investigation on this matter seems however to have not been carried out to date.

3.2. Slow reactions

A typical feature of flow analysis is that the involved chemical reactions may not necessarily reach equilibrium, and this opens the possibility of exploiting relatively slow chemical reactions. When the reaction product is the monitored species and sensitivity is critical, the degree of reaction development should be as high as possible. In the context, exploitation of the enhanced radial mass transport inherent to pulsed flows may become relevant, as demonstrated in the spectrophotometric determination of phosphate in soil extracts [26]. A comparison of performances of similar flow systems with pulsed or constant flows revealed a 30–100% sensitivity improvement (depending on the other parameters) with the former. This result reflects the better mixing conditions, lower sample broadening and lower sample dispersion involved.

3.3. Heating

Some chemical reactions are speeded up by heating, and immersion of the main reactor in a temperature-controlled water

Table 1
Selected applications. PP = pharmaceutical products; UV–vis = spectrophotometry (conventional, LED-based or diode array detection); CL = chemiluminescence; FAAS = flame atomic absorption spectrometry; FAES = flame atomic emission spectrometry (flame photometry); AFS = atomic fluorescence spectrometry; Turb. = turbidimetry; F = fluorimetry or photoluminescence; Cond. = conductimetry; ASV = square-wave anodic stripping voltammetry; SPE = solid phase extraction; LLE = liquid–liquid extraction; BCA = 2,2′-biquinoline 4,4′-dicarboxylic acid; MPA = mercaptopropionic acid; PDBA = *p*-dimethylaminobenzaldehyde; MO = methyl orange; NTU = nephelometric turbidity unit [59]; NA = not available information.

Analyte	Sample	Chemical reactions	Detection technique	Dynamical range	Detection limit	Remarks	Ref.
Ambroxol	PP	Reaction with PDBA	UV–vis	10–200 mg L^{-1}	NA	–	[60]
Americium; plutonium	Soils, plants, and biological fluids	In-line SPE for analyte separation/concentration	Off-line α spectrometry	NA	0.004 Bq mL^{-1}	Multi-pumping/multi-syringe hyphenated flow system	[61]
Ammonium	Sea waters	Reaction with *o*-phthaldialdehyde and sodium sulfite in alkaline medium	F	NA	13 nmol L^{-1}	UV–LED as the light source; modified SIMPLEX for system dimensioning	[62]
Ammonium	Natural waters	Analyte conversion to NH_3, gas diffusion, and chemiluminescence inhibition of the luminol/$HClO^-$ system	CL	0.3–5.0 mg L^{-1}	0.02 mg L^{-1}	Improved reagent addition with a connector with sintered glass particles	[63]
Ammonium	Estuarine, coastal, and shelf waters	Analyte conversion to NH_3, gas diffusion and acceptor stream monitoring	Cond.	1–10, 1–10, and 0.020–0.125 μmol L^{-1}	0.2, 0.2, and 0.014 μmol L^{-1}	In-situ analyser for <3 m depths	[41]
Ascorbic acid; dipyrone; tryptophan	Synthetic solutions	Oxidant scavenging by the analyte and inhibition of the chemiluminescence of the luminol/singlet oxygen system	CL	NA	IC_{50} values: 3.36×10^{-5}; 7.84×10^{-5}; 1.28×10^{-2} mol L^{-1}	In-line generation of singlet oxygen involving dismutation of H_2O_2 catalysed by Mo(VI)	[64]
Ascorbic acid	Powdered material for fruit juices	Analyte oxidation by H_2O_2, and luminol/H_2O_2 oxidation catalysed by $[Fe(CN)_6]^{3-}$	CL	0.8–1.5 mmol L^{-1}	0.17 mmol L^{-1}	Chemiluminescence inhibition proportional to the H_2O_2 consumption by the analyte	[65]
Ascorbic acid	PP	Chemiluminescence inhibition of the photo-degradation products/luminol system by the analyte	CL	5.0×10^{-7}–5.0×10^{-6} mol L^{-1}	3.05×10^{-7} mol L^{-1}	In line photo-activation of glutathione-capped CdTe quantum dots	[66]
Bromhexine	PP	Reaction with 3-methyl-2-benzothiazolinone hydrazine and Ce(IV)	UV–vis	Up to 400 mg L^{-1}	2 mg L^{-1}	Exploitation of two consecutive reactions	[67]
Bromine (speciation)	tap, mineral waters	Br^- oxidation to BrO_3^- with $K_2S_2O_8$; reaction with 5-Br-PADAP plus SCN^-	UV–vis	5.0–100 (BrO_3^-); 20–400 μg L^{-1} (Br^-)	2.0 (BrO_3^-); 7.5 μg L^{-1} (Br^-)	Off-line UV-assisted oxidation; multivariate optimization	[68]
Buspirone	PP	Folin–Ciocalteu reaction	UV–vis	Up to 60 mg L^{-1}	2.8 mg L^{-1}	Parallel reactors for implementing kinetic determinations relying on fixed time	[69]
Cadmium; lead	Natural, tap, and waste waters	Electrodeposition of a bismuth film on a screen-printed electrode; further Cd(II) and Pb(II) determinations	ASV	6.30–75.6; 3.20–38.4 μg L^{-1}	0.60; 0.10 μg L^{-1}	Flow-batch analyser; thermostated flow cell	[43]
Calcium; magnesium	Natural waters	Reaction with *o*-cresolphthalein complexone; optional EGTA or 8-hydroxyquinoline addition for masking	UV–vis	0–40; 0–25 mg L^{-1}	0.7 mg L^{-1}, 1 mg L^{-1}	Manifold imprinted on polymeric substrates by direct-write milling	[70]
Captopril	PP	Reaction with iodate, further KI addition	UV–vis	10–60 μg mL^{-1}	NA	Kinetic determination relying on initial reaction rate	[71]
Captopril	PP	Chemiluminescence enhancement of tris(2,2-bipyridyl) ruthenium(II)/Ce(IV) system	CL	0.002–0.15 mmol L^{-1}	0.001 mmol L^{-1}	Reaction mechanism presented	[72]
Carbaryl	Natural waters	Reaction with *p*-aminophenol	UV–vis	5–200 μg L^{-1}	1.7 μg L^{-1}	In-line photochemical degradation of the outlet stream	[73]
Carvedilol	PP	Chemiluminescence inhibition of the luminol/ClO^- system	CL	1.2×10^{-7}–3.0×10^{-6} mol L^{-1}	8.7×10^{-9} mol L^{-1}	Analytical strategy relying on carvedilol antioxidant activity	[74]
Chemical oxygen demand	Waste water	Chemiluminescence inhibition of the photo-degradation products/luminol system	CL	1–35 mg L^{-1}	NA	Single interface flow system; in line photo-activation of MPA-capped CdTe quantum dots	[75]
Chloride	Urine, waters	Analyte photo-conversion to chlorine promoting MO discoloration	UV–vis	2.0–20 mg L^{-1}	0.7 mg L^{-1}	Sample stopping for improving the conversion efficiency	[46]
Chloride; sulphate	Natural waters	Analyte precipitations with silver and barium ions	Turb.	NA	0.7; 1.3 mg L^{-1}	Expert system; real time decision on the need for next assay	[76]
Chlorine (free)	River, lake, and tap waters	Reaction with N,N-diethyl-p-phenylenediamine	UV–vis	10.0–100.0 μg L^{-1}	6.8 μg L^{-1}	Sample stopping for improving sensitivity	[77]
Chromium	Natural waters	Reaction of Cr(VI) with 1,5-diphenylcarbazide	UV–vis	10–200 μg L^{-1} Cr(III) or Cr (VI)	2.05 [Cr(III)]; 1.0 μg L^{-1} [Cr(VI)]	Speciation analysis of chromium	[78]

Ciprofloxacin; norfloxacin	PP	Analyte oxidation by N-bromosuccinimide in acidic medium	UV–vis	5–70 mg L^{-1} for both	0.99; 0.27 mg L^{-1}	Tandem stream for improving mixing conditions	[79]
Copper	Serum, urine	Not pertinent	FAAS	Up to 5 mg L^{-1} (serum), up to 300 μg L^{-1} (urine)	0.035 mg L^{-1} (serum), 0.67 μg L^{-1} (urine)	In-line SPE for urine analysis	[44]
Copper	Waters, multi-vitamins, and animal tissues	Oxidation of the reduced form of 2,6-dichlorophenol indophenol by H_2O_2 catalysed by Cu(II)	UV–vis	0.4–35.0 μg L^{-1}	0.12 μg L^{-1}	Syringe and solenoid pumps coupled to a thermostatised micro-chip	[80]
Cyanide (acid-dissociable)	Waters	Decomposition of the Cu(I)–BCA complex by HCN	UV–vis	5–200 μg L^{-1}	2 μg L^{-1}	In-line gas diffusion; 100-cm pathlength flow cell	[45]
Cyclamate	Table sweeteners	Reaction with nitrite in acidic medium, iodometric determination of the excess of nitrite	UV–vis	Up to 3.0 mmol L^{-1}	30 μmol L^{-1}	Tandem stream for improving mixing conditions	[81]
Diazepam	Alcoholic beverages, PP	Sample irradiation for generating fluorescent products, exploitation of organized micellar medium for enhancing the fluorescence emission	F	5–40 mg L^{-1}	2.02, 0.97 mg L^{-1}	Sample stopping for improving in-line photo-degradation	[82; 83]
Dichromate; salicylic acid; hydrogen peroxide; starch	Milk	Reactions with 1,5-diphenylcarbazide; Fe(III); V_2O_5; iodine	UV–vis	1.0–10.4; 103.6–414.3; 10.0–200.0; 12.5–150.0 mg L^{-1}	0.12; 2.58; 6.14; 0.29 mg L^{-1}	Solenoid pumps after the detection unit; sequential analytes determination without system reconfiguration	[48]
Dipyrone	PP	Reaction with PDBA	UV–vis	10–400 mg L^{-1}	1 mg L^{-1}	Tandem stream for improving mixing conditions	[84]
Fluoxetine	PP	Chemiluminescence inhibition of the luminol/ClO^- system	CL	Up to 10 mg L^{-1}	0.31 mg L^{-1}	Tandem stream for improving mixing conditions	[85]
Gabapentin	PP	Chemiluminescence inhibition of the luminol/ClO^- system	CL	60–350 μmol L^{-1}	40 μmol L^{-1}	Zone merging for reducing the reagent consumption	[86]
Gabapentin	PP	Reaction with 1,2-naphthoquinone-4-sulphonate in alkaline medium	UV–vis	Up to 150 mg L^{-1}	NA	Pioneer use of piezoelectric pumps	[87]
Glibenclamide	Teas	In-line SPE, elution with ethanol + HCl + cetyltrimethylammonium bromide	F	Up to 50 mg L^{-1}	0.81 mg L^{-1}	Reversed flow elution	[88]
Glibenclamide	PP, alcoholic beverages	Native fluorescence in acidic medium	F	Up to 75 mg L^{-1}	2.75 mg L^{-1}	Organized micellar medium for improving sensitivity	[89]
Gliclazide; glipizide	PP	Reagent scavenging by the analytes, chemiluminescence inhibition of the sulphite/Ce(IV) system	CL	18.0–100.0 mg L^{-1}	2.9 mg L^{-1}; 6.3 mg L^{-1}	CdTe quantum dots as sensitizers	[90]
Glucose; fructose	Molasses	Analyte oxidation by periodate, reaction of the produced iodate with potassium iodide	UV–vis	0.50–2.00% w/v (both analytes)	NA	Novel sampling strategy; samples collected in soluble capsules and handled in a flow-batch analyser	[91]
Glycerol (free)	Biodiesel	Analyte oxidation by periodate, reaction of the produced formaldehyde with acetyl acetone	UV–vis	5–50 mg L^{-1}	1.0 mg L^{-1}	Off-line sample preparation procedure; sample stopping for improving sensitivity	[92]
Glycerol (free; total)	Biodiesel	Analyte oxidation by periodate; reaction of the produced formaldehyde with acetyl acetone	F	5–70 mg L^{-1}	0.5 mg L^{-1}	Different off-line sample preparation procedures	[93]
Glyphosate	Waters	Reaction with p-dimethylaminocinnamaldehyde in acidic medium	UV–vis	0.5–10 μg mL^{-1}	0.17 μg mL^{-1}	100-cm liquid waveguide capillary cell	[94]
Indapamide	PP	Reaction with 3-methylbenzothiazolin-2-one hydrazone in the presence of Ce(IV)	UV–vis	Up to 50 mg L^{-1}	1 mg L^{-1}	Optimization through a Plackett–Burman factorial design and a central cubic faces design	[95]
Indomethacin	PP	Alkaline hydrolysis in micellar medium	F	Up to 1×10^{-5} mol L^{-1}	1.6×10^{-8} mol L^{-1}	Sequential injection analyser	[96]
Inorganic carbon (total); ammonium	Natural waters	Analyte conversion to CO_2 and NH_3, gas diffusion, acceptor stream monitoring	Cond.	0.08–9.0 mmol L^{-1}; 0.5–25 mol L^{-1}	50; 0.27 μmol L^{-1}	Random reagent selection for accomplishing two determinations in the same manifold	[97]
Iodate	Table salts	Reaction with iodide yielding iodine that reacts with N, N-diethyl-*p*-phenylenediamine	UV–vis	0.01–10.0 mg L^{-1}	0.004 mg L^{-1}	Micro flow-batch analyser	[98]
Iron	Natural waters, biological materials	Reaction with 1-(2-thiazolylazo)-2-naphthol	UV–vis	10–200 μg L^{-1}	5 μg L^{-1}	Cloud point extraction	[99]
Iron (speciation)	River waters, dog-fish muscle digests	In-line SPE, Fe(II) oxidation to Fe(III) with H_2O_2, complexation with SCN^-	UV–vis	0.05–10 [Fe(III)]; 0.2–15 mg L^{-1} (total Fe)	0.05 [Fe(III)]; 0.2 mg L^{-1} (total Fe)	Chelating discs as solid phase	[100]
Iron (speciation)	River waters	Fe(II) oxidation to Fe(III) with $K_2S_2O_8$, complexation with SCN^-	UV–vis	0.1–2.0 mg L^{-1}	0.08 [Fe(II)]; 0.06 mg L^{-1} [Fe(III)]	Tandem stream for improving mixing conditions and reducing the reagent consumption	[101]
Iron (total)	Ground waters	Oxidation to Fe(III), reaction with SCN^-	UV–vis	Up to 10 mg L^{-1}	0.15 mg L^{-1}		[102]

Table 1 (*continued*)

Analyte	Sample	Chemical reactions	Detection technique	Dynamical range	Detection limit	Remarks	Ref.
						Novel direct-injection procedure; paired emitter-detector diodes	
Iron; nitrite; phenol; carbaryl	Waters	Reactions with SCN^-, iodide, nitroprusside, *p*-aminophenol	UV–vis	NA	22; 60; 25; 60 ng mL^{-1}	Single detection unit with RGB LEDs	[103]
Iron; vanadium	Alloys	Iodide oxidation by Cr(VI) catalysed by Fe(II) and V(IV)	UV–vis	0.0–15.0 mg L^{-1}	NA	Multivariate calibration	[104]
Ketoprofen	PP	Formation of photo-degradation products	F	Up to 50 mg L^{-1}	1.45 mg L^{-1}	In-line UV photo-degradation	[105]
Lansoprazole	PP	Reactions with chloranilic acid (a) or 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (b)	UV–vis	2.17×10^{-4}–8.12×10^{-4} (a); 2.71×10^{-4}–8.12×10^{-4} mol L^{-1} (b)	NA	Two methods in the same manifold for accuracy assessment	[106]
Levodopa; benserazide	PP	Chemiluminescence inhibition of the benserazide/luminol system by levodopa	CL	5–30; 2.5–20 mg L^{-1}	NA	Multivariate calibration	[49]
Manganese	Natural waters	Mn(II) oxidation to Mn(III) by immobilized PbO_2; Mn(III) reaction with EDTA yielding a coloured complex	UV–vis	25–1500 μg L^{-1}	6 μg L^{-1}	Solid-phase packed reactor	[107]
Mannitol	PP, urine	Chemiluminescence inhibition of the luminol/ myoglobin system by the analyte	CL	0.025–1.0 mol L^{-1}	NA	Single interface flow system	[108]
Mercury	Synthetic solutions	Cold vapour generation	AFS	0.05–2 μg L^{-1}	0.02 μg L^{-1}	Pioneer use of solenoid pumps for adding sample and reagent aliquots to the AFS phase separating chamber	[109]
Metformin	PP	Chemiluminescence inhibition of the luminol/H_2O_2/Cu (II) system through Cu(II) scavenging by the analyte	CL	5–15 mg L^{-1}	0.94 mg L^{-1}	Pioneer chemiluminometric procedure with pulsed flows	[37]
N-acetyl-L-cysteine	PP	Reaction with MPA	F	50–750 μmol L^{-1}	1.6 μmol L^{-1}	MPA-capped CdTe quantum dots for fluorescence enhancement	[110]
Nilutamide	PP	Reaction with NaOH in ethanolic medium	UV–vis	Up to 100.0 mg L^{-1}	2.26 mg L^{-1}	Sample stopping for improving sensitivity	[111]
Nitrate; silicate; phosphate	Deep sea waters	Griess–Ilosvay reaction after analyte reduction to NO_2; molybdenum blue formation involving ascorbic and oxalic acids; molybdenum blue formation involving stannous chloride	UV–vis	Up to 40; 150; 5 μmol L^{-1}	0.1; 0.5; 0.1 μmol L^{-1}	Autonomous *in situ* nutrient analyser	[54]
Nitrite	Exhaled breath condensate	Griess–Ilosvay reaction	UV–vis	Up to 500 ng mL^{-1}	3.8 ng mL^{-1}	Dual wavelength spectrophotometry for compensating the Schlieren effect	[112]
Nitrite	Natural waters	Griess–Ilosvay reaction (a) or reaction with KI (b)	UV–vis	Up to 1.0 (a) or 2.0 mg L^{-1} (b)	8 (a) or 25 μg L^{-1} (b)	In-line photo-degradation of the residues	[113]
Nitrite; nitrate	Waters	Griess–Ilosvay reaction for nitrite, nitrate reduction to nitrite by hydrazine sulphate	UV–vis	0.026–5; 0.039–7 mg L^{-1}	0.013; 0.039 mg L^{-1}	Stream splitting/merging; three-way valve for selecting alternative analytical paths	[114]
Paracetamol	PP	Reaction with hypochlorite yielding *N*-acetyl-*p*-benzoquinoneimine that reacts with salicylate producing a blue indophenol dye	UV–vis	5.0–125.0 mg L^{-1}	0.4 mg L^{-1}	Solenoid pumps after the detection unit; pinch valves for stream selection	[115]
Paraquat	Natural waters	Reaction with dehydroascorbic acid	UV–vis	0.10–5.0 mg L^{-1}	22 μg L^{-1}	10-cm optical path flow cell	[116]
Phenols	Waters	Reaction with sodium nitroprusside and hydroxylamine hydrochloride	UV–vis	50–3500 ng mL^{-1}	13 ng mL^{-1}	Sample stopping for improving sensitivity	[117]
Phenotiazines (trifluoperazine; fluphenazine; thioridazine; perphenazine; chlorpromazine)	Drugs	Chemiluminescence induced by the analyte oxidation by Ce(IV) in acidic medium	CL	Up to 100; 200; 5000; 2000; 2500 μg L^{-1}	0.2; 0.3; 2; 3; 3 μg L^{-1}	Sample stopping for improving sensitivity	[118]
Phenylglyoxylic acid	Urine	Reaction with vanadate, affecting the H_2O_2/vanadate reaction in acidic medium	UV–vis	Up to 700 mg L^{-1}	37 mg L^{-1}	Differential kinetic analysis exploiting sample stopping for phenylglyoxylic and mandelic acid determinations	[119]
Phosphate	Natural waters	Formation of yellow vanadomolybdophosphoric acid	UV–vis	Up to 20 mg L^{-1}	0.2 mg L^{-1}		[120]

						Only two solenoid pumps required	
Phosphate; dissolved organic phosphorus	Waste waters	Formation of yellow vanadomolybdophosphoric acid	UV–vis	Up to 20; up to 40 mg L^{-1}	0.08; 0.5 mg L^{-1}	In-line photo-degradation of organic matter	[121]
Phosphate; organic phosphorus	Freshwaters	Molybdenum blue formation	UV–vis	10–75 μg L^{-1}	2.0 μg L^{-1}	In-line photo-conversion of organic phosphorus; tandem streams for improving mixing conditions	[50]
Phosphate; organic phosphorus	Cereals	Molybdenum blue formation	UV–vis	5–40 mg L^{-1}	0.5; 1.2 mg L^{-1}	In-line photo-conversion of organic phosphorus; tandem streams for improving mixing conditions	[51]
Phosphorus	Biodiesel	Molybdenum blue formation	UV–vis	0.10–10.0 mg L^{-1}	0.014 mg Kg^{-1}	In-line sample preparation; micro-flow-batch system	[122]
Phytic acid	Foodstuffs	Analyte extraction; reaction with Fe(III)-salicylate	UV–vis	5.0–100.0 mg L^{-1}	1.0 mg L^{-1}	Parallel array of SPE mini-columns	[123]
Polyphenols	Wines	Chemiluminescence inhibition of the luminol/$HClO^-$ system through scavenging by gallic acid	CL	10–100 mg L^{-1}	6.6 mg L^{-1}	Zone merging for reducing the reagent consumption	[124]
Protein	Certified serum materials	Reaction of protein adduct with Coomassie Brilliant Blue (UV–vis) or with fluorescamine (F)	UV–vis, F	0–500 μg mL^{-1} (F)	NA	Paired emitter-detector diodes	[125]
Pyrazinamide	Drugs	Chemiluminescence inhibition of the luminol/H_2O_2 system through scavenging of the Cu(II) catalyst	CL	10–70 mg L^{-1}	5.79 mg L^{-1}	Zone merging for reducing the reagent consumption	[126]
Quinine	Urine	Exploitation of native fluorescence	F	12.5–150 ng mL^{-1}	NA	In-line SPE	[127]
Radium	Natural waters	In-line analyte separation/concentration	Off-line α spectrometry	NA	0.05 Bq L^{-1}	Multi-pumping/multi-syringe hyphenated flow system	[128]
Reducing sugars (total)	Sugar-cane juices	In-line hydrolysis of sucrose, alkaline degradation of the reducing sugars at about 98 °C	UV–vis	0.0–2.0% w/v	0.018% w/v	Improved heating	[27]
Selenium	Natural waters	Reaction with iodide yielding iodine, oxidation of Variamine Blue	UV–vis	0.010–0.500 mg L^{-1}	0.004 mg L^{-1}	100-cm liquid waveguide capillary cell	[129]
Simvastatin	PP	Inhibition of MO bromination by the analyte	UV–vis	5–35 mg L^{-1}	1.2 mg L^{-1}	Additional three-way valve for sample interchange	[130]
Sodium; calcium; magnesium	Urine, hair	In-line sample dilution; optional La addition	FAAS (Ca, Mg) FAES (Na)	NA	NA	Pioneer application involving flame atomic spectrometry	[39]
Sulphate	Natural waters	Precipitation with barium ions	Turb.	7–16 mg L^{-1}	150 μg L^{-1}	100-cm liquid waveguide capillary cell; sample stopping for improving crystal growth	[131]
Sulphide	Natural waters, wines	Quenching of MPA-capped CdTe nanocrystals that act as photo-luminescent probes	F	0.25–5.0 mmol L^{-1}	0.19 mmol L^{-1}	In-line gas diffusion	[132]
Surfactants (anionic; cationic)	Waters	Formation of ion pair with MO (anionic) or ternary complex with Fe(III) and Chromazurol S (cationic) in micellar medium	UV–vis	0.35–10.5 mg L^{-1}	0.06 mg L^{-1}	LED based detector	[133]
Surfactants (cationic)	Natural waters	Ternary complex formation involving the analytes, Fe(III) and Chromazurol S	UV–vis	0.34–10.2 mg L^{-1}	0.035 mg L^{-1}	Tandem stream for improving mixing conditions	[134]
Tannins	Teas, wines, and bee	Folin–Denis reaction	UV–vis	2–100 mg L^{-1}	0.3 mg L^{-1}	Zone merging for improving the sample/reagent interaction	[135]
Titanium	Lake sediments, sunscreens, and natural waters	Reaction with chromotropic acid	UV–vis	Up to 100 μg L^{-1}	0.4 μg L^{-1}	1.0-m liquid waveguide capillary cell	[136]
Trimipramine	PP	Reaction with ammonium monovanadate in acidic medium	UV–vis	Up to 50 mg L^{-1}	1.15 mg L^{-1}	Pioneer demonstration of the solenoid pump robustness in relation to highly viscous and concentrated acidic solutions	[23]
Trolox; ascorbic acid; resveratrol	PP, tea extracts	Chemiluminescence inhibition of the luminol/H_2O_2 system	CL	Up to 3.2×10^{-4}; 1.1×10^{-3}; 8.8×10^{-8} mol L^{-1}	NA	Antioxidant capacity; data for lucigenin/H_2O_2 also presented	[137]
Turbidity	Water	Not pertinent	Turb.	Up to 160 NTU	0.1 NTU	LED-based detection	[59]
Uric acid	Urine	Cu(II) reduction by the analyte, Cu(I) reaction with BCA	UV–vis	10–100 μmol L^{-1}	3.0 μmol L^{-1}	In-line 100-fold sample dilution	[138]
Volatile fatty acids	Anaerobic treatment processes	Reaction with N-(1-naphthyl) ethylenediamine	F	19–1000 mg L^{-1}	5.1 mg L^{-1}	In-line LLE	[139]

Table 1 (*continued*)

Analyte	Sample	Chemical reactions	Detection technique	Dynamical range	Detection limit	Remarks	Ref.
Zinc	Plant digests	Formation of zinc chloro-complexes; in-line SPE, reaction with Zincon	UV–vis	Up to 2.50 mg L^{-1}	0.1 mg L^{-1}	Novel strategy for use of solid reagents in flow analysis	[31]
Zinc	River waters	In-line SPE; reaction with pyridyl-azo-naphthol	UV–vis	30–3000 µg L^{-1}	30 µg L^{-1}	In situ analysis; battery-powered submersible flow analyser	[21]

bath has been exploited since the sixties. In a streamlined flow, the concentric solution layers near the tube wall travel at lower mean linear velocities being then more heated, whereas the inner ones are less heated, thus acting as a coolant. Heating transfer is therefore impaired. On the other hand, the enhanced radial mass transport inherent to pulsed flows promotes a better temperature homogenization, thus improving heating.

This beneficial effect was demonstrated in the spectrophotometric determination of total reducing sugars in syrups and molasses [27]. The method relied on alkaline sugar degradation, which is enhanced at higher temperatures [28]. For an 80 °C water bath temperature, height of the peak recorded with the pulsed flow system was about 0.02 absorbance, whereas no analytical signal was recorded for the system with constant flows. Raising the temperature to 85 °C led to appearance of the analytical signal related to the flow system with constant flow, but this signal was only about 20% of that related to the pulsed flow system. At 98 °C (boiling water), 0.355 and 0.285 peak height absorbance were obtained in relation to pulsed and constant flows.

3.4. Solid reagents

Solid reagents (*e.g.* metallic powders, slightly soluble salts, resins, enzymes and biological materials such as fibres and straws) have been often used in flow analysis. The solid material is generally immobilized and/or confined inside a cartridge or a mini-column. Solid-phase reactors present some advantages in comparison to traditional open tubular reactors. Better development of the involved reactions and lower sample dispersion are attained, due to the channelling effects inside the reactor and the development of the reactions at the solid/liquid interface, where the reagent is present at its highest possible concentration. Sensitivity and sample throughput are then improved. The manifold is usually simpler, with fewer confluent streams. Drawbacks associated with the reagent excess reaching the detector are avoided, allowing solid-phase spectrophotometry and fluorimetry to be efficiently implemented. Reactor stability and lifetime are in general high [29]. Wasted reagent amount is reduced, as the reagent is ideally consumed only in the presence of the flowing sample. A minute mass of reagent is then enough for a large number of samples, and use of toxic reagents becomes less restrictive. This is a favourable aspect towards the Green Chemistry [30].

Use of mini-columns as solid-phase reactors may however lead to some drawbacks such as backpressure and swelling effects, establishment of preferential pathways and limited accessibility to active sites [31]. These drawbacks can be overcome by using open tubular reactors with the solid reagent immobilized on the inner wall [32]. The strategy may however limit the reagent/analyte interaction as, with a streamlined flow, only the outer concentric solution layers are close to the tubing wall.

This limitation is circumvented by exploiting pulsed flows, as recently demonstrated in the spectrophotometric determination of ascorbic acid in pharmaceutical products using a MnO_2-coated open tubular reactor [33]. The analyte was in-line oxidized by Mn (IV), and the released Mn^{2+} reacted with the chromogenic reagent forming the coloured complex to be monitored. System ruggedness, sampling rate, reagent consumption and analytical precision were superior in comparison to a similar flow system with constant flows. Sensitivity enhancement was however not so pronounced (*ca.* 2.1%) because rate of the involved redox reactions was more influenced by the intrinsic reaction characteristics than by geometric predisposition of the involved reactants dictated by the exploitation of pulsed flows.

Another possibility to circumvent the drawbacks of packed bed solid-phase reactors is to implement fluidized beds [31], a novel strategy for utilization of solid reagents in flow analysis. Particles of

solid reagents are maintained in constant floating, reflux, and circulating motion inside a mini-chamber, and this diffuse and reproducible geometry is established by resorting from pulsed flows. Potentialities and limitations of the approach were discussed in relation to the spectrophotometric determination of zinc in plants [31]. The solid phase extractor was a Dowex 1-X8 anionic resin freely maintained inside a mini-chamber. The analyte was retained as chlorocomplexes and, after elution, derivatized with Zincon. Enhanced figures of merit were attained.

3.5. Tandem streams

This unique stream was conceived in relation to flow systems with constant flows [34] and has been also implemented in pulsed flow systems (Table 1). Segments of different miscible liquids (*e.g.* samples, carrier, reagents) are successively introduced into the manifold establishing a moving stream of segments with different compositions, referred to as a binary string. Mixing conditions are improved by using smaller segments, and this aspect is especially attractive in flow systems designed in the straight configuration. With tandem streams, the manifold architecture becomes simpler, as demonstrated in the fluorimetric determination of aminocaproic acid in pharmaceutical formulations [35]. Other potentialities of tandem streams are discussed elsewhere [36].

The establishment of tandem streams requires an inserting unit for fast and time controlled actuation, allowing reproducible volumes of each liquid to be successively inserted into the manifold in a pre-set order. Two distinct situations, involving constant or pulsed flows, should be taken into account. Flow systems relying on constant flow rates resort from pulse-width control in order to determine the time during which a valve is activated and hence the volume of the inserted segment. On the other hand, size of the inserted segments in flow systems with solenoid pumps is determined by the pump stroke, which is usually fixed. A critical comparison of tandem streams with constant or pulsed flows seems to have not been carried out to date.

4. Applications

Use of pulsed flows in a variety of analytical applications involving a wide array of analyte–matrix combinations has been increasing. Moreover, the number of applications exploiting tandem streams is expressive in view of the beneficial aspects inherent to this unique stream (see Section 3.5) and its easy implementation in a pulsed flow system.

Most applications involve analyses of environmental and/or pharmaceutical relevance (Table 1 and Fig. 3), as they were proposed by research groups dedicated to these kinds of analyses. About 30% of the applications refer to water analysis, as they are often required for environmental control, which is better performed when *in situ* assays are attainable. To this end, pulsed flow systems are potentially suitable.

Analyses of pharmaceutical products are also of high concern, and almost 25% of the applications refer to these samples. As the analyte is often a major constituent, procedures for selectivity and/or sensitivity enhancement are generally not needed. The flow manifold is then simple and generally comprises 2–3 solenoid pumps. This is the main reason for the increasing use of pulsed flow systems in drug dissolution studies. Other applications, involving *e.g.* foodstuffs, biological materials, environmental matrices other than natural waters and process monitoring, have been also reported, demonstrating the worldwide acceptance of pulsed flow analysis.

Regarding detection techniques, UV–vis spectrophotometry, chemiluminescence and fluorimetry are most widely used. The large utilization of UV–vis spectrophotometry is justified by recalling that

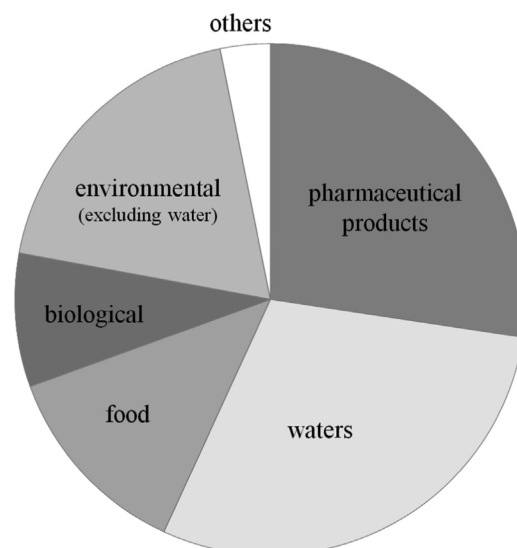

Fig. 3. Application fields. Figure refers to a literature survey (Institute for Scientific Information, ISI – Web of Science) relying on the key-words multi-punping flow analysis, multi-pumping flow systems, solenoid pumps, micro-pumps and pulsed flows.

this technique is widely used in flow analysis, and presents favourable characteristics of simplicity, robustness, versatility, portability, low-cost and possibility of miniaturization. The expressive number of applications involving chemiluminescence and fluorimetry is a consequence of the improved mixing conditions inherent to the pulsed flow systems and the precise timing involved. Mixing is often the limiting factor in analytical sensitivity and repeatability, especially when a fast sample/reagent interaction is required, as generally occurs in luminometric analytical procedures. The improvement in some analytical figures of merit attained by exploiting pulsed flows was evident in the pioneer exploitation of chemiluminescent detection in a multi-pumping flow system aiming at the determination of metformin in pharmaceutical formulations [37].

Other detection techniques such as *e.g.* turbidimetry [38], atomic absorption (or emission) spectrometry [39], atomic fluorescence spectrometry [40], conductimetry [41], alpha spectrometry [42] and anodic stripping voltammetry [43] have been also reported in specific applications.

The increasing number of applications reflects that important features of flow systems with pulsed flows such as system versatility and portability, efficient implementation of tandem streams, and suitability for carrying out biochemical studies have been essential in the design of novel applications. The following characteristics of the pulsed flow systems are accountable for the overall acceptance of pulsed flow analysis.

4.1. System versatility

Versatility of the pulsed flow system has been the key aspect in several applications. In this context, in-line analyte separation/concentration for improving sensitivity and/or selectivity was efficiently accomplished, and the atomic absorption spectrometric determination of copper in serum and urine involving solid phase extraction [44], as well as the spectrophotometric determination of acid-dissociable cyanide in natural waters involving gas diffusion [45] can be selected as examples.

Implementation of sample stopping for increasing the mean sample residence time without increasing the sample dispersion, thus improving sensitivity, is straightforwardly attained, as demonstrated in the spectrophotometric determination of chloride in natural waters and urine exploiting in-line photo-induced oxidation

[46]. The procedure adhered to the main requirements of Green Chemistry, as hazardous reagents were not required. Critical comments on the potential of pulsed flow systems in relation to Green Chemistry were given elsewhere [47].

Multi-determinations can be efficiently accomplished by taking advantage of the system versatility to provide commuting facilities, as demonstrated in the spectrophotometric determinations of dichromate, salicylic acid, hydrogen peroxide and starch in milk involving multi-commutation [48]. Multivariate calibration can be also exploited in the context, as highlighted in the simultaneous chemiluminometric determinations of levodopa and benserazide in pharmaceutical formulations involving artificial neural networks [49].

Versatility of the flow systems with pulsed flows is a key aspect to implement in-line sample preparation. Phosphorus fractionation in waters [50] and cereals [51] exploiting photochemical conversion can be selected for illustrative purposes. Inorganic phosphorus was directly determined whereas the determination of total (*i.e.* organic plus inorganic forms) required an in-line oxidation step for mineralization of the organic fraction. The optional addition of the persulfate oxidant allowed then the differentiation of the inorganic and organic fractions with the same reactor. Ultraviolet radiation was needed for the oxidation step, lamp turn-off was not required, heating for accelerating the involved reactions was not needed and the amounts of required reagents were reduced.

The possibility of precise additions of known analyte amounts to a given flowing zone permits to obtain the analytical curve with a single standard solution [52]. This task was similarly implemented in a multi-purpose flow system able to perform also in-line dilutions, standard additions, titrations and strategies to increase sample residence time without modifying the manifold architecture [53]. Regarding in-line dilutions, dispersion coefficients ranging from 1 to 7800 were efficiently achieved simply by changing the sample volume and exploiting zone sampling: precise results were always obtained.

4.2. Portability

With discretely operated small solenoid pumps, manifold downsizing is efficiently attained, allowing the proposal of rugged analytical procedures for *in situ* and eventually *in vivo* assays, thus opening the possibility of improving environmental monitoring. In this context, the NH4-Digiscan analyser [41] could be mentioned, as its performance in relation to ammonium determination in seawaters led to expressive figures of merit. Other relevant applications refer to the proposal of an autonomous nutrient analyser for biogeochemical monitoring of deep seawater [54], and a dedicated flow-based analyser for zinc determination in natural waters [21].

Flow systems with pulsed flows are also useful for ecotoxicological studies, and the flow system designed for evaluating acute toxicity bioassays involving the *Vibrio fischeri* bacteria [55] can be selected as an example. It was able to perform in-line sample dilutions and, as good mixing conditions were involved, the short lifetime of the bacteria became less restrictive. EC_{50} values for emerging contaminants (parabens, caffeine, acetaminophen, diclofenac, salicylic acid) were in agreement with those obtained with a commercial kit in a microplate.

4.3. Suitability for biochemical studies

Flow systems with pulsed flows have been often used for investigating the interaction of analytes of biochemical interest with reactive chemical species. These reactive species are generally in-line formed and their possibility of degradation is minimized due to the improved mixing conditions associated with the use of pulsed flows. In general, the involved oxidant (or reducing) agent is scavenged by the biochemical analyte allowing the efficient estimation of the IC_{50} values.

The peroxynitrite scavenging activity by lipoic acid, dihydrolipoic acid, cysteine, glutathione or sulindac were evaluated in terms of the chemiluminescence inhibition of the luminol/peroxynitrite system [56]. Analogously, interactions of melatonin and selected melatonin precursors with reactive oxygen and nitrogen species were evaluated by exploiting their scavenging effects [57]. To this end, the inhibition of the chemiluminescence arising from the luminol oxidation by H_2O_2, hydroxyl radical or peroxynitrite system was taken into account. The results demonstrated that 5-hydroxytryptophan is the most potent scavenger, followed by melatonin and tryptophan.

5. Trends

As a consequence of the beneficial aspects arising from the exploitation of pulsed flows, a pronounced tendency towards application of this unique flow is foreseen.

Flow analysers relying on this flow pattern would not constitute themselves in a novel modality, as flow modalities and acronyms are not sustainable (see Section 1). A didactic classification of flow analysers would then rely solely on flow pattern: constant or pulsed flows, air-segmented or not.

Regarding the use of pulsed flows, recent research mostly carried out by Ibero-American researchers has been generally focused on the adaptation of ordinary flow-based analytical procedures to pulsed flow manifolds. The advantageous characteristics of these manifolds such as ease of programming and assembling, incorporating facilities of wireless transmission of analytical results, and suitability to miniaturization open a window towards the development of automated analysers for *in situ* measurements. In this regard, the work carried out in Ireland and Denmark on the design of pulsed flow systems applicable to environmental samples is outstanding, and the phosphate analyser using a microfluidic lab-on-a-chip format [58] is a typical example. It contains all the required chemical storage, pumping and electronic components to carry out a complete assay. The system is self-calibrating and self-cleaning, thus able to long-term operation. This *in situ* analyser is characterized by similar sensitivity and linear range relatively to the commercially available monitor, and presents ability to operate over extended periods of time.

This confirms that the paradigm shift "send the analytical laboratory to the site where the sample is" is becoming true.

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