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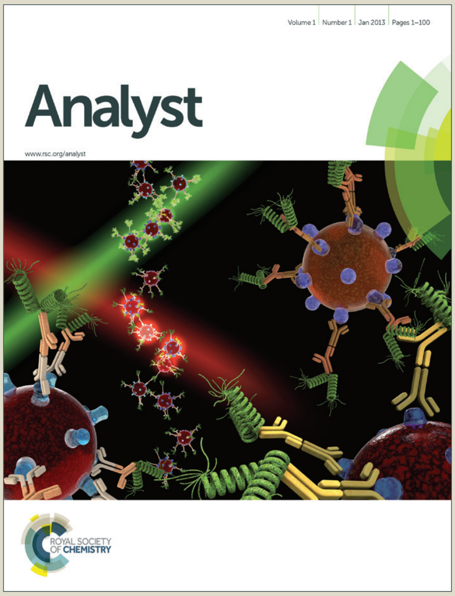

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# Flow analysis in Brazil: contributions over the last four decades

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## Abstract

The main Brazilian contributions to Flow Analysis are reviewed, with emphasis to historical developments, conceptual aspects, system design, and analytical applications. Contributions after the advent of flow injection analysis are emphasized. Novel approaches (*e.g*. zone merging, zone sampling, zone trapping, multi-site detection, and multi-commutation), flow modalities (*e.g*. monosegmented flow analysis, flow-batch analysis, multi-pumping flow analysis), as well as the pioneer implementation of different detection techniques (*e.g*. potentiometry, turbidimetry, FAAS, ICP-OES, and gravimetry), and analytical steps (*e.g*. titrations, membraneless gas diffusion, and electrolytic dissolution) are highlighted. Strategies to improve analytical figures of merit and use of the flow analyser as a tool for teaching purposes are also discussed. Brazilian contributions in the context of system miniaturization, green chemistry, analyses of complex samples, novel strategies and materials for in-line analyte separation/concentration and proposal of expert systems are also highlighted. Large scale analysis of samples of agronomical, environmental, industrial and/or clinical relevance is emphasized.

Keywords: flow analysis, flow injection analysis, multi-commutation, Brazil.

# Introduction

Flow Analysis is a branch of analytical chemistry whose development owes much to the Brazilian contribution. Indeed, the pioneering large-scale application of flow injection analysis (FIA)[1] can be regarded as an answer to the high demand for chemical analyses of natural waters and plant digests inherent to the projects carried out at the Centre for Nuclear Energy in Agriculture (CENA/USP/Brazil) sponsored by the International Atomic Energy Agency (IAEA). The seminal article coining the expression "Flow Injection Analysis"[2] was prepared at CENA in 1975, and originated the classical ten-article series[2, 3] discussing theoretical, methodological and applicative aspects of FIA. Most of these articles were prepared in Brazil, co-authored by CENA researchers or both. Focus on solving practical problems requiring large-scale routine assays culminated in several novel approaches to FIA.

Regarding the earlier developments during the seventies, relevant contributions were jointly achieved by Brazilian researchers and IAEA experts: (i) the pioneer system for large-scale analysis focusing on the determination of phosphate in plant digests,[4] (ii) the incorporation of potentiometric detection for total nitrogen determination in plant materials[5] and for rapid analysis of soil extracts and blood serum,[6] (iii) the proposal of stream splitting for wide-range determination of chloride in sample batches with high variability in analyte concentration,[7] (iv) simultaneous determinations,[8] as well as (v) the implementation of turbidimetry in flow analysis for sulphate determination[9]

These contributions involved manual syringe-based sample injection and, with this approach, the carrier flow-rate undergoes a sudden increase during injection, leading to an instantaneous instability of the flow system, especially in those exploiting confluent streams. A damper placed near the injection port minimized this effect,[10] and the use of a sliding bar injector circumvented the drawback, as demonstrated in the spectrophotometric determination of molybdenum in plants involving liquid-liquid extraction.[11] With a mild sample insertion mode, the flow stability was maintained and separation of immiscible phases was efficiently accomplished.

The double proportional injector, a development of the above-mentioned injector, allowed the sample and a reagent solution to be simultaneously inserted into convergent carrier streams, and this was the essence of the zone merging approach.[12] This device was also useful for achieving intermittent flows.[13] The potentialities of the injector-commuter were expanded with electronic operation, allowing the pioneering implementation of zone sampling, zone trapping, and system reconfiguration by relocation of devices. These latter approaches are discussed in section 3.

The need for modifying the reaction conditions in order to implement a given analytical method in a flow injection system became evident during the earlier FIA development in Brazil. A noteworthy example is the determination of boron in plant materials using Azomethine-H.[14] The original procedure was carried out in a segmented-flow analyser, the colour-forming reaction was developed under slightly acidic conditions (pH = 5.0) and about 2 h were need for reaction completion. In the FIA system, neutral conditions (pH = 7.3) were set and maximum absorbance was reached after 2 min. The modification in the reaction conditions allowed the proposal of a rugged flow injection procedure; although sensitivity was slightly reduced, a 60 $h^{-1}$ sampling rate was attained.

The main Brazilian contributions to the development of Flow Analysis, including novel concepts, system modalities, real-time manifold modifications, and applications are discussed herein. A timeline with the main contributions is shown in Figure 1.

## Scientometrics

The Brazilian scientific production has significantly increased and consequently moved from the 16th (2004) to the 13th (2012) position in scientific worldwide rankings. Presently, it is accountable for *ca.* 2.0% of the global production.[15]

Regarding chemistry, a recent overview of the Brazilian production, relying on 45 of the main international journals[16] revealed that analytical chemistry is one of the most prominent areas. Flow analysis is one of the most consolidated fields in analytical chemistry, accountable for 15.7% of the Brazilian production in the same journals. These publications refer to 30 different institutions (see some examples in Table 1)[17-50] and received more than 13300 citations, which corresponds to 21.8% of the total citations of the articles on analytical chemistry authored by Brazilian researchers. Moreover, from the 30 most cited Brazilian articles in analytical chemistry, 10 refer to flow analysis.

The annual number of publications in flow analysis authored by Brazilian researchers has been nearly constant since 1998 (*ca*. 60 publications per year, see Fig. 2). Mentions to these publications have been increasing, approaching 1500 citations in 2012. Most of the articles have been published in journals with high impact factors (Table 1), such as Analytica Chimica Acta (20.96%) and Talanta (15.44%), which also concentrates publications in FA from other countries. The Brazilian publications in The Analyst correspond to 4.81%, including highly relevant works.[9, 29, 51, 52] In spite of being a recently recent journal, 0.71% of the Brazilian production in the field has been presented in Analytical Methods.

In addition to the pioneer collaborations with Denmark and USA[5-7] flow analysis has inspired fruitful cooperation of Brazilian researchers with colleagues from Argentina,[53] Austria,[54] Canada,[55, 56] Chile,[57] Cuba,[58] England,[59, 60] Germany,[61] Greece,[62] India,[63] Italy,[64] Japan,[65] Mexico,[66] Poland,[67] Portugal,[68-70] Slovenia[40] Spain,[62, 71-74] Sweden,[75] The Netherlands[76] and Uruguay.[77]

## Real-time manifold modifications

A remarkable characteristic of flow analysers is the easy manifold reconfiguration for implementing different analytical tasks. In this regard, Brazilian researchers have presented important contributions, most of then taking advantage of the versatility of the sliding bar injector-commuter.[11] Commutation can be fully exploited with this device, aiming modification of sample processing[78], as exemplified in Figure 3. In spite of the high versatility and low-cost, the sliding bar commuter is not commercially available, which hinders its widespread use. This drawback has been circumvented by replacing it by commercially available valves, as demonstrated by Japanese researchers.[79] In fact, one section of a three-piece sliding bar commuter can be replaced by three three-way solenoid valves.[80]

## Zone sampling

Controlled dispersion is inherent to FIA, but sample dispersion cannot be increased indefinitely, as sample volume and the analytical path length are not modifiable at will. In fact, there is a minimum volume that can be reproducibly selected and the increase in wash time can be critical for long analytical path lengths. The limitation is overcome by zone sampling,[81] which is a powerful tool for sample dilutions, as pioneering demonstrated by the determination of potassium in plant digests by flame atomic absorption spectrometry.[81] A sample aliquot is introduced in the first carrier stream and, after a pre-set time interval ($t_{ZS}$), a portion of the dispersed zone is reproducibly selected and introduced into a second carrier stream, undergoing additional dispersion (Fig. 3a). The dispersion coefficient is then the product of those achieved in each step and the concentration of the re-sampled portion plays a relevant role. Dispersion coefficient values as high as $10^6$ can be attained with this innovation, without hindering neither analytical precision nor sampling rate.[82] Zone sampling is then relevant for routine large-scale analysis, in which high sample dilution and sample throughput are needed. However, the performance of the flow system with zone sampling depends on reproductive timing and minimization of pulsation of the propelling device. In addition to the sliding-bar commuter, these characteristics can be achieved in flow systems exploiting multi-commutation[83] or that designed in the sequential injection[84] configuration.

Simultaneous determinations involving rather different dispersion degrees are feasible by exploiting zone sampling, as demonstrated by the determination of aluminium and iron in plant digests:[85] a small aliquot of the trailing portion of the dispersed sample zone was re-sampled and inserted into a second carrier stream for the spectrophotometric determination of aluminium, whereas most of the sample zone was directed to a flame atomic absorption spectrometer in which iron was quantified under conditions of limited sample dispersion.

In zone sampling, each $t_{ZS}$ value corresponds to a different re-sampled aliquot, thus a different concentration. The entire sample zone can be examined with a $t_{ZS}$ scan.[86] By pre-setting specific $t_{ZS}$ values, analytical procedures relying on different yet known concentrations such as standard additions[87] and flow titrations[88] are efficiently implemented. Calibration with a single standard solution is also feasible. Zone sampling is also worthwhile in relation to monosegmented flow analysis. By mechanically removing the central portion of the flowing sample prior to detection, drawbacks inherent to the presence of the gas phase in the detector were avoided.[89]

An ingenious procedure that also exploited concentration gradients was proposed for implementation of the standard additions method.[90] The sample solution in infinite volume was merged with a highly reproducible dispersed zone of the analyte, being measurements carried out in definite time intervals, whose analyte concentrations were previously determined. The approach successfully compensates matrix effects in atomic spectrometry with a significant increase in sample throughput.

## Zone merging

Flow analysis is an important tool in the context of Green Analytical Chemistry[91] and one of the first attempts towards this aspect was the zone merging approach conceived in Brazil.[12] Sample and reagent aliquots are simultaneously inserted (Fig. 3b), so that reagent consumption undergoes a pronounced reduction. This advantage becomes more relevant when the reagents are toxic, hazardous and/or expensive, as highlighted when the FIA-FAAS hyphenation was conceived.[92] The efficient sample/reagent interaction typical of confluence configuration holds also for the zone merging. Alternatively, the sample and reagent solutions can be inserted into a single carrier stream aiming at partial zone overlap and a simpler manifold. This approach was recently exploited for the fluorimetric determination of bisfenol in tap waters.[93]

With zone merging, the possibility of reagent adsorption on the manifold tubing inner wall is minimized, leading to a more stable baseline, as demonstrated when ion-exchange was first incorporated to FIA:[94] by adding the Nessler reagent in a pulsed manner, the typical baseline drift verified when the reagent was continuously added was avoided. Other applications refer to simultaneous determinations[95] and chemical speciation[96] by exploiting the partial zone overlap, and implementation of the standard addition method, better performed when combined with the zone sampling.[87]

## Zone trapping

Unsegmented flow systems are very attractive for implementing procedures involving physicochemical processes demanding residence times < 30 s. Successful implementation of analytical procedures exploiting relatively slow chemical reactions depends however on a compromise between residence time, sample throughput and sample dispersion. Zone trapping was proposed in order to attain this compromise, aiming at the spectrophotometric determination of ammonium in natural waters exploiting the Berthelot reaction.[97] After mixing with the reagents, the central part of the sample zone was removed from the carrier stream and maintained inside a parallel reactor for increasing the residence time, thus improving the reaction development (Fig. 3c). During the trapping period, sample dispersion practically ceases,[98] therefore sampling rate is not critically affected by carryover effects. Zone trapping then presents some advantages in comparison to the stopped-flow approach. Increasing the temperature during the trapping period can also contribute to speed-up the chemical reactions. Potentialities of the zone trapping combined to zone merging were recently highlighted.[99]

## Intermittent flows

In procedures based on relatively slow chemical reactions, sampling rate can be enhanced by adding a confluent intermittent stream after achievement of peak maximum, thus reducing the washing time.[100] Intermittent flows under continuous pumping can be implemented by resorting from commutation, as demonstrated in the spectrophotometric determination of nitrite in natural waters involving the modified Griess reagent,[13] Fig. 3d. Reducing total flow rate improved sensitivity, while the intermittent washing stream enhanced sampling rate without affecting the other favourable analytical features, mainly sample residence time and the analytical response.

Intermittent flows were further adopted to avoid baseline drifts caused by adsorption of the reaction product in the flow cell,[101] to minimize reagent consumption[102] and for sequential determinations.[103] As a drawback, this approach can yield two distinct baselines due to the changes in composition of the solutions with and without the intermittent flow. The potential of intermittent flows is considerably increased by using independently controlled valves instead of the sliding-bar commuter,[104] which is limited to only two resting positions.

Intermittent flows were exploited by Brazilian researchers to implement zone stopping (originally named stopped-flow) by commutation (Fig. 3e), thus avoiding the drawbacks of the peristaltic pump inertia. The innovation was first demonstrated by the spectrophotometric determination of boron in plant digests.[105] Another variation of the intermittent flow approach, named alternating streams, consists in the addition of different solutions associated to the resting positions of a sliding bar commuter, as proposed to improve the turbidimetric procedure for sulphate determination in plant materials. Either the analyte or an alkaline EDTA stream was added to increase the nucleation rate of the precipitate or to improve washing.[106]

## Relocation of components

Relocation of components is an interesting approach to increase system versatility, which is easily implemented, as the flow-injection analyser operates according to a binary logistic.

For multi-site detection,[107] the flow cell is moved from one manifold site to another. The innovation permits the efficient implementation of parallel or serial monitoring aiming at simultaneous determinations by differential kinetics (two analytical channels sharing a single relocating detector), speciation (a discriminating reagent added after first measurement), standard addition (analyte added after first measurement), blank suppression (reagent added after sample passage through the first measuring site), electrochemical detector re-conditioning (measurement in one site and conditioning in the other), and improvement in MSFA (detector moved before arrival of the gas phase). All these potentialities were recently reviewed.[108]

Reactor relocation is also feasible. Two different analytical signals under two different sample handling conditions are attained with two reactors, as demonstrated in the spectrophotometric determination of cobalt and nickel in steels exploiting the time-dependent reaction of the analytes with 4-(2-pyridylazoresorcinol).[109] Relocation of filtering devices for cleaning purposes,[110] mini-columns of solid reagents (*e.g.* ion-exchangers and immobilized enzymes),[94] and sampling loops for *e.g.* zone sampling implementation[81] have also been exploited.

## Multi-commutation

During the earlier developments of flow injection analysis, Brazilian researchers realized that the sliding bar injector could be used for different tasks, and this aspect led to the inception of the flow systems with commutation[78] (see also section 3). In this regard, an ingenious manifold exploiting optional insertion of a reducing mini-column in the analytical path was designed for the spectrophotometric determination of nitrite and nitrate in natural waters.[111] The sample was inserted twice per cycle and only one of the established sample zones was allowed to flow through the mini-column, where nitrate was reduced to nitrite. Two analytical signals were then recorded, reflecting the nitrite and the nitrite + nitrate contents in the sample. In this application, the sliding bar injector can be regarded as an injector-commuter. In fact, commutation is the key point for manifold programming to perform different analytical tasks.

The versatility of the flow systems and the potential for implementation of complex analytical assays increase with the number of independently controlled commuters (n), as the number of manifold states is $2^n$.[104] In this sense, the multi-commutation concept was coined[112] to describe flow systems which can be re-configured by the control software, thus presenting potential for automation and for minimization of both reagent consumption and waste generation. This strategy has been exploited in association with tandem streams,[59, 112] as well as MSFA,[113] flow-batch,[114] multi-syringe[115] and multi-pumping[116] flow systems. Applications include expert systems for in-line analyte concentration,[117] increase of sample residence time,[118] real-time controlled dilutions,[83] sequential determinations,[103] and titrations.[20] The design of multi-purpose flow systems[116] and the in-line detection and circumvention of sources of inaccuracy[119] are also feasible. A comprehensive review on multi-commuted flow systems was presented elsewhere.[104]

## Modalities of flow analysis

The amazing development of flow analysis led to the appearance of different modalities of flow systems, and those conceived in Brazil are described as follows.

## Monosegmented flow analysis

Monosegmented flow analysis (MSFA) was conceived as an alternative to implement analytical procedures relying on relatively slow chemical reactions. The sample is inserted into the carrier stream between two air plugs, thus originating a monosegment that is directed towards detection.[120] The air phase minimizes sample dispersion, thus the mean sample residence time can be increased without lessening the sampling rate by simultaneously processing different monosegments. Sensitivity is often improved because sample is handled under limited dispersion conditions and conversion of the analyte to the detected product is improved. This modality combines the advantageous characteristics of both segmented (*e.g.* long sample residence time and limited dispersion) and unsegmented (*e.g.* high sampling rate and system versatility) flow systems, as emphasized in the pioneering work focusing on the spectrophotometric determination of phosphate, chromium and ammonium.[120] In view of its distinct characteristics, MSFA was classified by IUPAC in a specific branch in the terminology related to analytical methods based on flowing media.[121]

Besides the focus on relatively slow reactions, other ingenious strategies involving MSFA have been proposed. Gaseous samples were used instead of the air plug in the spectrophotometric determination of $CO_2$ and $O_2$ after absorption by selective reagents.[122] The indirect measurement of the sample volume before and after absorption of the gaseous analyte by the reagent was used as analytical parameter. A similar approach exploited the increase in conductance in the water acceptor taken as the liquid phase of the monosegment due to $CO_2$ absorption.[123]

Single-phase liquid-liquid extraction was implemented in MSFA by exploiting the limited interaction of the monosegment with the aqueous carrier stream, taken the spectrophotometric determination of iron as example.[124] The aqueous sample was combined with two organic solvents (methyl isobutyl ketone also containing the thenoyltrifluoroacetone chromogenic reagent, and ethanol) at suitable volumetric rates for establishing a single phase, which was inserted as a monosegment. After Fe(III) complexation / extraction, a saturated $NaNO_3$ water solution was added to promote phase separation, and the absorbance of the organic phase was measured. Biphasic liquid-liquid extraction was also implemented in MSFA, aiming at Cd determination.[125] A small plug of the extractant (1-(2'-pyridylazo) naphthol reagent in chloroform) was inserted after the sample monosegment, and the analyte was transferred to the organic phase after retention on the glass tube inner walls, and further monitored by spectrophotometry. A 75% extraction efficiency was attained.

Flow-based titrations can also be implemented in MSFA by modifying the sample and titrant volumetric fractions in the monosegment[126] or the coulometrically generated titrant amount.[127] The concepts, theory, potentialities and applications of MSFA were highlighted elsewhere.[128]

## Flow-batch analysis

Flow-batch analysis (FBA)[129] combines the advantageous characteristics of both flow and batch analysis. A mini-chamber is the main component in the manifold, into which the solutions inherent to the specific analytical application are added / removed according to a programmed routine. FBA presents ability for flow-based solution handling to implement tasks better batch-wise performed, such as those requiring long residence times, homogenization of mixtures, or liquid-liquid extraction. This modality of flow analysis has been exploited to accomplish titrations,[127, 129] in-line sample pretreatment,[130] individual sample conditioning,[131] liquid-liquid extraction,[53] matrix matching,[131] in-line preparation of solutions for standard additions or internal standard calibration,[132, 133] etc. Direct detection at the mixing-chamber[134] and gradient exploitation for non-linear calibration[135] have been also accomplished. The FBA system has been miniaturized by using deep UV-lithography on urethane-acrylate resins.[136, 137]

Regarding applications, the spectrophotometric determination of cocaine in a biphasic medium[25] (LD = 29.4 mg $L^{-1}$) and a green procedure for wide-range (0.004 – 400 mg $kg^{-1}$) determination of free glycerol in biodiesel using water as extractor solvent as well as to produce cavitation sonoluminescence quenched by the analyte could be highlighted. Concepts, potentialities and other applications of FBA were presented in a recent review.[114]

## Multi-pumping flow analysis

The versatility of the flow systems exploiting multi-commutation[112] was the driving force towards the inception of the multi-pumping flow systems (MPFS) by Brazilian and Portuguese researchers.[69] MPFS utilizes individually or simultaneously actuated solenoid pumps, which are accountable for pumping, inserting, commuting and homogenization processes. The pulsed flows delivered by these pumps are characterized by sudden and repetitive changes in the linear velocities of the fluid elements and this aspect is worthwhile for attaining good mixing conditions due to the tendency towards a turbulent mixing, *i.e*. an efficient radial mass transport.[138]

Under similar experimental conditions, sample dispersion is lower with MPFS in comparison to flow systems relying on laminar (or pulsating) flows.[139] The recorded peaks are then characterized by lower washing times, thus yielding a higher sample throughput. Reaction development can be enhanced by taking advantage of the efficient radial mass transport. Better sensitivity is then attained, especially for relatively slow chemical reactions.[140] Moreover, MPFS are effective to avoid baseline drift in flow-based turbidimetry.[141]

In order to highlight the versatility of the MPFS, a multi-purpose flow system designed with solenoid valves and solenoid pumps was proposed.[116] The independent control of each discrete device permitted the implementation of different analytical tasks, such as dilutions, calibration with a single solution, standard additions, titrations and increase of the sample residence time, all accomplished without any change in the manifold hardware.

Other favourable characteristics of MPFS were highlighted in relation to heating-dependent reactions,[142] fluidized beds,[143] gas diffusion,[144] photo-oxidation,[145, 146] speciation[147] and differential kinetics.[148] Furthermore, the favourable mixing condition inherent to the pulsed flow was exploited in the proposal of the Single Interface Flow Analysis.[149] This modality exploits the interpenetration between sample and reagent zones with consequent establishment of concentration gradients. The extension of mutual zone penetration is controlled either by the length of the analytical path or by multiple flow reversals (multiple detection) carried out at a specific site within the reaction coils.

## Detection

The pioneer implementation of some detection techniques in flow analysis were accomplished in Brazil, and the first attempt in this sense was to exploit turbidity as measurement basis in flow analysis, aiming at the determination of sulphate in natural waters and plant digests by formation of a barium sulphate suspension.[9] When carried out manually, the procedure is hindered by poor precision because it requires the strictly reproducible formation and monitoring of the suspension, and no more than about 10 samples can be run per hour. On the other hand, with the proposed flow injection system a sampling rate of 180 $h^{-1}$ was attained with a coefficient of variation of 0.85%, and results were in agreement with those obtained in batch.

Another ingenious detection strategy relying on precipitation reaction was the flow injection system with gravimetric detection.[150] Sample and reagent aliquots were injected into air carrier streams, which merged yielding a precipitate. The solid was retained by a filtration unit, placed on the plate of an analytical balance and, after partial and reproducible drying, the precipitate was weighed. Dissolution and filter conditioning were thereafter achieved by flowing intermittent washing solutions through the device. The investigated model system was based on barium oxalate formation, and handled *ca.* 30 samples per hour. The strategy was further applied to the determination of total reducing sugars in sugar-cane molasses with the Fehling reagent.[151]

Regarding spectrophotometry, an ingenious strategy was proposed for compensating the drawbacks caused by intense gradient concentrations in the sample zone (Schlieren effect). Dual wavelength spectrophotometry was pioneering applied in FIA, being the sample zone simultaneously monitored in the absorption maximum and in reference wavelengths. The differences in the absorbances effectively eliminated the perturbation even for highly concentrated solutions (*e.g.* 1 mol $L^{-1}$ sucrose).[152] Brazilian researchers have also significantly contributed to the understanding of the Schlieren effect in flow analysis,[60] including the first report of this perturbation.[9]

The first flow injection enthalpimeter was proposed[153] and exploited with different approaches (*e.g*. single line, zone merging, and reverse flow injection), as well as with different solid materials in suspension, thus avoiding the drawbacks associated with post-equilibration. The innovation was accomplished by placing manifold components as injection valve, coils and connectors in a water bath, and by using an open tube reactor. As demonstration, the neutralization of hydrochloric acid by sodium hydroxide was selected, and the results revealed enhanced detection limits (0.4 to 0.07 mmol $L^{-1}$ HCl) and sampling rate (80 to 90 $h^{-1}$).

The feasibility of combining flow injection analysis with flame atomic absorption spectrometry was pioneering demonstrated in the determination of calcium and magnesium in plant digests.[92] With zone merging, low sample volumes were required, thus minimizing the consumption of the chemical modifier (lanthanum) to about 1%. The required sample dilution and reagent addition were done automatically and 300 determinations per hour were performed with a typical coefficient of variation of 0.5%.

The feasibility of exploiting flame emission spectrometry as a detection technique in flow injection analysis was concomitantly demonstrated in relation to the potassium determination in plant digests. The implementation of flow analysers for sample handling coupled to inductively coupled plasma optical emission spectrometer was demonstrated in the analysis of rock and plant materials.[154] The innovation was further exploited for implementing the generalized standard addition method in order to compensate spectral interferences in the analysis of alloys.[155] Detection by atomic spectrometry is nowadays extensively used in flow systems.

For the analysis of gaseous samples, a MSFA system relying on volumetric measurements was proposed.[122] The volume contraction of the gas plug resulting from the selective absorption of the analyte by a reagent in the liquid monosegment was evaluated. The volume of the gas phase was indirectly assessed as the time interval during the passage (at constant flow rate) of liquid-gas and gas-liquid boundaries through optical switches. The ratio of the elapsed time intervals before and after the analyte absorption was inversely related with the gaseous analyte content. The system was applied to the determination of oxygen and carbon dioxide, individually or in mixtures.

Despite usually being undesirable in flow analysis, the Schlieren effect was successfully exploited for the determination of alcoholic grade in beverages.[156] The determination involved refractive index gradient measurements when beverages were injected into an inert carrier stream (water), thus avoiding usually required laborious, slow and expensive processes such as distillation or chromatographic measurements. The reagentless procedure was applied to the alcoholic grade determination of wine, gin, rum, vodka and sugar cane-based beverages.

## Selected processes

### Liquid-liquid extraction

Liquid-liquid extraction (LLE) is characterized by the adaptability to different solutes and solvents, possibility of solvent recycling, enhancement in analytical sensitivity and/or selectivity and possibility of exploiting different extraction strategies.[157] However, LLE is often a cumbersome process, and limitations refer to operator protection, environmental safety, need for toxic, harmful, flammable and/or volatile organic solvents, formation of undesirable emulsions, solvent costs for some highly selective procedures and large volumes of sample, extractant or solvent.[158] These drawbacks are minimized by implementing LLE in a flow-injection system, as independently demonstrated by Brazilian, Japanese and Swedish researchers.[11, 159, 160]

In the Brazilian contribution, the flow system comprised a 260-cm extraction coil, a T-shaped phase-separation chamber and a sliding-bar injector, and was designed for the spectrophotometric determination of molybdenum in plant materials by the thiocyanate method.[11] Detection limit and sampling rate were estimated as 50 μg $L^{-1}$ Mo and 30 $h^{-1}$, respectively. However, the phase separator presented a low separation efficiency due to the high sample dispersion inside it. An open-phase separation device was further used to minimize this drawback in the determination of copper in plant digests[161]. An open tube vertically positioned promoted fast phase separation, driven by the difference in density of the solutions. Solenoid valves controlled the introduction of the sample zone into the tube and the volume of the extracted complex to be sent towards detection, thus reducing the organic solvent consumption. Preconcentration factor, detection limit and coefficient of variation were estimated as 10, 5.0 µg $L^{-1}$ Cu and 3.0 %, respectively.

A multi-commuted flow system exploiting liquid-liquid micro-extraction without phase segmentation was proposed for the spectrophotometric determination of diltiazem in pharmaceuticals[162]. The ion-pair formed between the analyte and bromothymol blue was extracted into 50 µL of chloroform and directly measured in a glass tube coupled to optical fibers. Other ingenious approaches for LLE have been proposed by Brazilian researchers, including single-phase[124] and two-phase[125] extractions in MSFA and in FBA systems,[53] as well as flow-based cloud point extraction.[37]

## Gas diffusion

In a pioneering work, gas diffusion (GD) was implemented in FIA to remove interferences and enhance selectivity in the spectrophotometric determination of total nitrogen in plant materials[163]. As the flow manifold comprised a membraneless GD unit, the innovation was named isothermal distillation. The main device comprised two silicone rubber sheets supported by parallel acrylic plates. The sample zone was in-line alkalinized and flowed on one sheet, the produced ammonia was released and collected by the acceptor stream flowing on the other sheet. Thereafter, the Nessler reagent was added by stream confluence and the formed product was monitored.

Different manifold architectures have been proposed in Brazil for analytical applications requiring GD, commonly through a gas permeable membrane. In this sense, an environmental friendly procedure was proposed for conductimetric determination of ammonia in Kjeldahl digests of plant tissues, fertilizers and animal feed[164]. The procedure involved diffusion of ammonia from an alkaline solution through a polytetrafluoroethylene (PTFE) membrane towards a deionized water stream, and variations in conductance were proportional to ammonia concentration. Further, determinations of sulphite and acetic acid separately in foods were described[165]: coefficient of variation of 0.2 and 0.8% and sample throughput of 120 and 80 $h^{-1}$ were reported in these applications. Moreover, a novel FIA system exploiting GD was proposed for amperometric determination of free and total $SO_2$ in wines.[166] A flow system comprising an inner gas-permeable silicone tube concentric to a polyethylene tube allowed permeation and chemical reaction to occur along the entire analytical path, thus improving system versatility and analytical sensitivity.[167]

Non-invasive sampling in a flow system relying on GD exploited a coiled tubular PTFE membrane that, immersed in the sample, acted as a sampling probe.[168] The spectrophotometric determination of ethanol in alcoholic beverages was selected as application. Ethanol diffused through the membrane and it was collected by the acceptor/reagent stream flowing continuously through the probe. The collected analyte reacted with dichromate under acidic conditions and the formed Cr(III) was monitored at 600 nm. Favourable figures of merit were attained.

Other recent applications involving GD in flow systems include exploitation of pulsed flows to improve mass transference of the analyte[144] and efficient incorporation of the process in μFIA.[36]

## Solid-phase extraction

Solid-phase extraction (SPE) was originally implemented in a FIA system for the spectrophotometric determination of ammonium in natural waters.[94] In order to accomplish the load and elution steps, a cation-exchange mini-column (Amberlite IRA-120) replaced the sampling loop in an electronically operated sliding-bar injector. In this way, concentration and elution were attained simply by displacing the mini-column between the sample and eluent channels and time-based sampling was achieved.

Brazilian researchers have investigated new sorbents for in-line separation/concentration, including biosorbents such as rice husks,[169] grape bagasse,[170] *Moringa oleifera* seeds,[171] and peat,[172] as well as alternative materials (*e.g.* polyurethane foam[18, 45,173] and modified oxides[174]). In this sense, a comparative study with C18 bonded silica, polyurethane foam and activated carbon for extraction and pre-concentration of Pb complexed with O,O-diethyl-dithiophosphate yielded enrichment factors of 166, 55 and 23, respectively.[175] Some approaches have been presented to improve selectivity of SPE process, including functionalization of sorbents with organic reagent[18, 176] and the use of molecularly imprinted polymers[21, 177] Most applications include metal extraction and detection by atomic spectrometry exploiting different flow approaches. Expert systems have also been proposed mainly to improve sampling rate[117, 178] and multi-commutation has been exploited to effective implementation of the different steps involved in SPE (sorbent conditioning, analyte retention, mini-column washing for matrix removal, and elution).[54, 172] Direct measurements in solid-phase have been exploited to improve sensitivity, selectivity and to implement kinetic discrimination, as recently discussed in a critical review.[179] Analyte separation from interfering species by SPE allowed nickel determination in silicate and alloys[173] and the development of an environmental friendly procedure for nitrate determination in freshwaters.[28]

## Electrolytic dissolution

Electrolytic dissolution (ED) is a suitable alternative for routine of large scale analysis of alloys, as the cumbersome and time-consuming sample preparation step required for traditional wet-chemical analysis of alloys is avoided. The pioneer implementation of ED in flow analysis aimed at the determination of aluminium in steels[180] and it was characterized by fast sample dissolution (few seconds) and thus high sample throughput. The solid sample was polished and placed in an electrolysis chamber: application of a precisely controlled direct current promoted the dissolution into the flowing electrolyte. The dissolved material reacted with Eriochrome cyanine R and the formed complex was monitored by spectrophotometry.

The innovation was further improved and applied for the determination of molybdenum in steels[181]. A commuter was used for sample replacement, thus avoiding solution leakage when the electrolyte was not flowing through the chamber. After dissolution (200 mA direct current during 8 s), the dissolved material was mixed with the reagents, and the formed product was measured by spectrophotometry. Sampling rate, linear response range and repeatability were estimated as 40 $h^{-1}$, 0.7-2.7 % (w/w) Mo and 2.3%, respectively.

This strategy for in-line sample preparation was further exploited in relation to other detection techniques, including the simultaneous determination of nickel, chromium, manganese, silicon and iron in steels by ICP OES[182] and lead determination in high purity copper by ICP-MS[183].

## Titrations

Flow-based titrations have been continuously improved mainly aiming to increase sample throughput. However, most of them requires a calibration step and thus cannot be considered as a true titration – the term “pseudo-titration” is then often used. In this sense, Brazilian authors presented an important contribution, in which multi-commutation was exploited to change the volumetric fraction of sample and titrant aiming converging to the end-point.[20] This approach, named as binary search titration, allowed determination of analyte concentration without any calibration. The spectrophotometric titration was based on the change in the colour of the indicator caused by the variation on the volumetric fractions. By maintaining the total volume of the sample zone, the volumetric fractions were varied in order to converge to the end-point. A feedback mechanism was exploited for decision if either the sample or titrant volumetric fraction should be increased in the next step, being the accuracy level initially defined (inversely related to the sample throughput). The proposal was applied for acid-base titration, consumed less than 2 mL of titrant and reached the end-point in up to 3 min for analyte concentrations ranging within two orders of magnitude. Binary search was further applied to determination of total acidity in silage extracts[184] and red wine,[185] chloride in milk and wine,[88] and ascorbic acid in soft drinks and juices.[186] Also exploiting multi-commutation, a flow-batch titrator[129] and a multi-purpose flow system able to perform titrations[116] were presented.

The feasibility of a potentiometric acid-base titration exploiting binary search was also demonstrated.[187] The reference signal was obtained by using a diluted buffer solution as the carrier stream and the sample volume was kept through the process to avoid matrix variations that could affect the electrode response. The titrant volume was then varied by using a diluent to keep the sample zone volume constant. MSFA improved the mixing conditions and minimized sample dispersion and the procedure was successfully applied to vinegar, soft drinks, isotonic beverages and orange juices.

In view of the limited dispersion, MSFA has been exploited in several flow-based titrations. One of the most efficient approach[188] exploits a syringe driven by a step motor for precise titrant addition (± 0.5 μL) to the monosegment, allowing titration of as low as 100 μL of sample. The system allowed both complete titration curve or end-point detection in up to 8 steps in less than 2 min by a successive approximations algorithm. Other MSFA flow titrations referred to water determination in organic solvents by the Karl Fischer method[126] and coulometric generation of the titrant.[127] Acid-base titrations based on Fibonacci algorithm exploiting MSFA and tandem streams were also reported.[189]

## Multidetermination

In spite of the predominance of flow systems devoted to the determination of a single analyte, typically with high sample throughput, several approaches have been presented for simultaneous or sequential determinations. In this sense, the pioneering work focusing on determination of more than one species (nitrogen and phosphorus in plant materials) was carried out in Brazil.[8] Since then, simultaneous determination has been attained mainly by changing the reaction conditions, including kinetic discrimination.

Polyvalent flow systems have been presented for determination of several species with the same flow configuration. In this sense, a FIA manifold with confluent streams was developed for determination of macro (calcium, magnesium and phosphorus) and micronutrients (iron, copper, manganese and zinc) in digests of plant materials by changing the chromogenic reagents.[190] In other application, a flow system with reagent injection was presented for multidetermination in water samples; improved sensitivity and minimization of the consumption of chemicals were attained by inserting micro-volumes of the reagent in the sample carrier.[191] However, with these approaches, analytical determinations were carried out under compromise conditions (*e.g.* dispersion and residence times), which could impair the analytical performance. This drawback was circumvented by exploiting multi-commutation for real-time manifold reconfiguration, as demonstrated for sequential determination of anions,[117] and metal ions.[103] Sequential determinations have also been focused in MSFA, such as in the determination of glucose, creatinine and urea in clinical samples.[52]

In a particular case of multidetermination, the same analyte is quantified by two different analytical procedures aiming accuracy assessment.[192] Recoveries of the analyte spiked to the samples were also estimated for every sample aiming detection of matrix effects. When these drawbacks are absent, the mean of the obtained results yield an inherently more reliable concentration value. This strategy was first reported for chloride determination in natural waters with remarkable differences in sample matrixes based on the spectrophotometric procedure using mercury(II) thiocyanate and the turbidimetric method using silver nitrate.

## Teaching

As a consolidated research area in Brazil, flow analysis has also been discussed in several undergraduate courses mainly as a topic of instrumental analysis disciplines. Didactic articles and innovative experiments have been proposed aiming the efficient introduction of concepts. Some illustrative examples refer to the demonstration of the analytical route,[193-195] kinetic studies,[196, 197] enzymatic assays,[102] and reaction stoichiometry.[195] Practical guidelines for introduction of flow analysis in undergraduate labs[198] and demonstration of its potential for Green Chemistry[199] have also been presented. Recently, a novel strategy for video demonstrations using a CCD camera as the detector for real-time projections of the handled flowing zone on a large screen was proposed, allowing a comparative evaluation of the stability of some Cu(II) complexes.[200].

Brazilian researchers have also contributed with book chapters (Table 3)[201-209] and critical reviews focusing on different aspects of flow analysis.[91, 104, 108, 114] A comprehensive monograph about this subject was authored by researchers from Brazil and United Kingdom.[157]

## The state of art

In spite of the effervescent worldwide development of flow analysis, the recent Brazilian contributions continue in a close adherence with the overall tendency.

Following the tendency towards environmental friendly analytical chemistry, several flow-based analytical procedures have been proposed in Brazil[91] aiming at reagentless procedures,[210] replacement of toxic reagents,[146] reuse of chemicals,[211] a pronounced reduction of the reagent consumption and waste generation, as well as in-line waste treatment.[212]

In view of the benefits in portability, sample/reagent consumptions and possibility of *in situ* or *in vivo* analysis,[213, 214] miniaturization of analytical devices is also a noteworthy area in Brazil and this holds also for flow analysis. In this context, the design of micro-flow manifolds (often relying on lithography) can be highlighted; different detection techniques and processes have been then exploited in a miniaturized scale.[22,136,136] However, in some situations, microfluidics may present limitations, such as increased system complexity, low reproducibility of the manufacturing process, and the possibility of deterioration of some analytical figures of merit. Downsizing of conventional flow systems have been exploited to match the advantages of miniaturization and analytical performance.[215]

Proposal of novel strategies for *in-situ* or *in-vivo* analysis is a clear tendency and Brazil has also contributed in this aspect. The less invasive ethanol determination in alcoholic beverages[168] and the determination of aminoacids in algae[30] can be highlighted as applicative examples.

Since the pioneering works focusing on LLE and SPE, several innovations have been presented, such as novel adsorbents[18, 174] and biosorbents,[169, 170, 172] micro-extractions,[162] reversible systems,[211] strategies to improve sampling throughput,[117, 178] and to minimize the solvent amount.[125, 162]

Following the initial trend of designing flow systems to solve real analytical problems and implementing them in routine analysis, Brazilian researchers have emphasized the solution of complex analytical problems or processing of complex samples.[32, 53, 54, 216] In recent years, there is a trend to the development of flow-based procedures applied to biofuel analysis in view of their increasing incorporation in the Brazilian energetic matrix.[53]

The design of expert flow systems is a remarkable trend in Brazil, as a consequence of the development of multi-commutation and computer controlled systems. Representative examples include accuracy assessment,[192] wide range determinations relying on real-time controlled dilutions,[83] individual sample conditioning,[217] system optimization,[119] detection of inaccuracy,[119] in-line analyte concentration and separation,[117] and true titrations.[20, 188] A feedback mechanism was also exploited for prior assays,[218] aiming higher analytical productivity in routine labs. The approach was demonstrated by the determination of zinc in soil extracts conditioned to the previously determined phosphate concentration.

Real-time flow programming is a worldwide tendency in designing expert flow systems.[209] Brazilian research has expanded it by implementing the concept of manifold programming through multi-commutation. As a future trend, efficient exploitation of both flow and manifold programming is foreseen.

## Abbreviations

| | |
|---|---|
| ED | Electrolytic dissolution |
| FBA | Flow-batch analysis |
| FIA | Flow injection analysis |
| GD | Gas diffusion |
| LLE | Liquid-liquid extraction |
| MPFS | Multi-pumping flow systems |
| MSFA | Monosegmented flow analysis |
| PTFE | Polytetrafluoroethylene |
| SPE | Solid-phase extraction |
| SIA | Sequential injection analysis |

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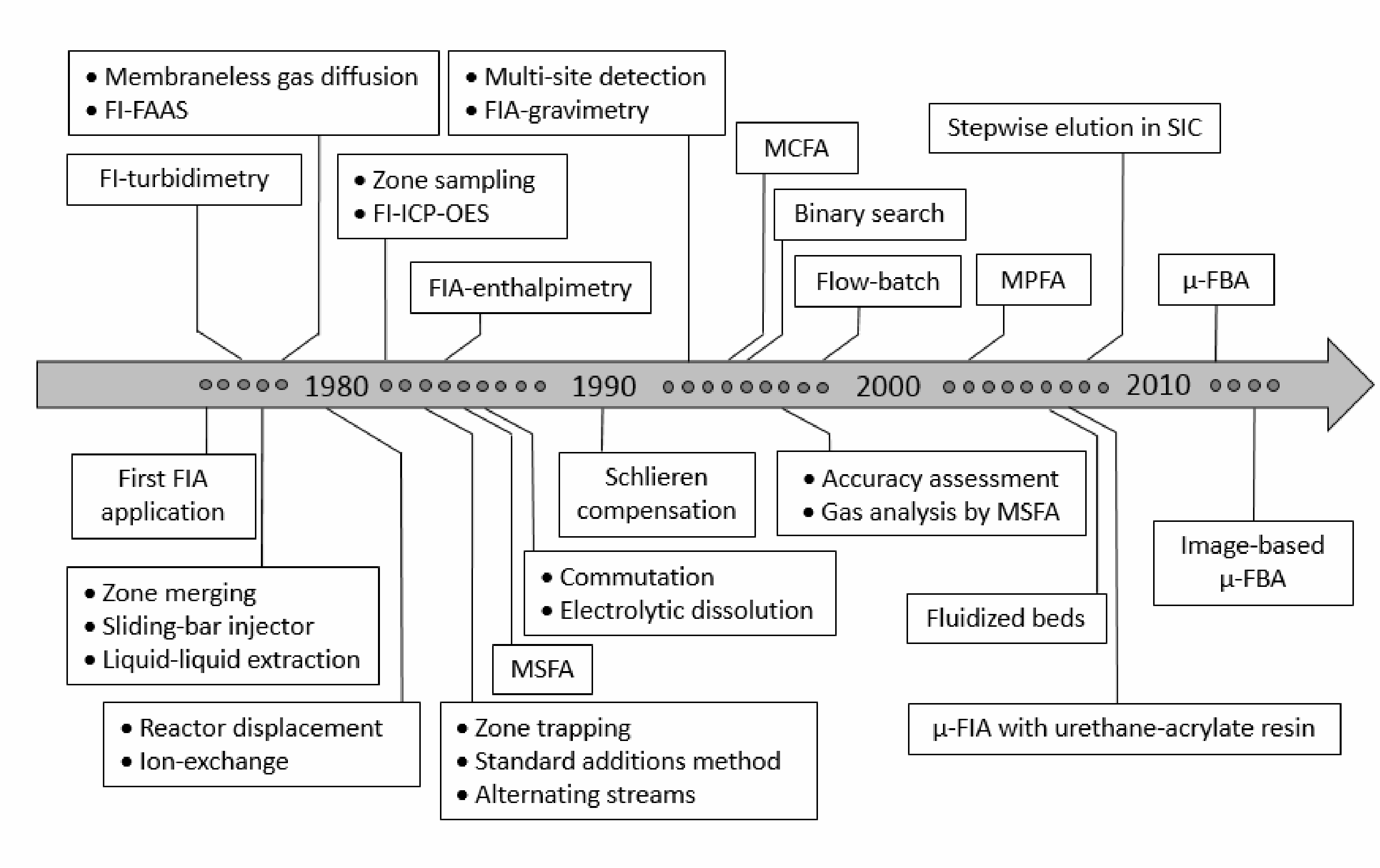
• Membraneless gas diffusion
• FI-FAAS
• Multi-site detection
• FIA-gravimetry
MCFA
Stepwise elution in SIC
FI-turbidimetry
• Zone sampling
• FI-ICP-OES
Binary search
FIA-enthalpimetry
Flow-batch
MPFA
μ-FBA
1980
1990
2000
2010
First FIA application
Schlieren compensation
• Accuracy assessment
• Gas analysis by MSFA
Image-based μ-FBA
• Zone merging
• Sliding-bar injector
• Liquid-liquid extraction
• Commutation
• Electrolytic dissolution
Fluidized beds
MSFA
• Reactor displacement
• Ion-exchange
• Zone trapping
• Standard additions method
• Alternating streams
μ-FIA with urethane-acrylate resin

**Figure 1**

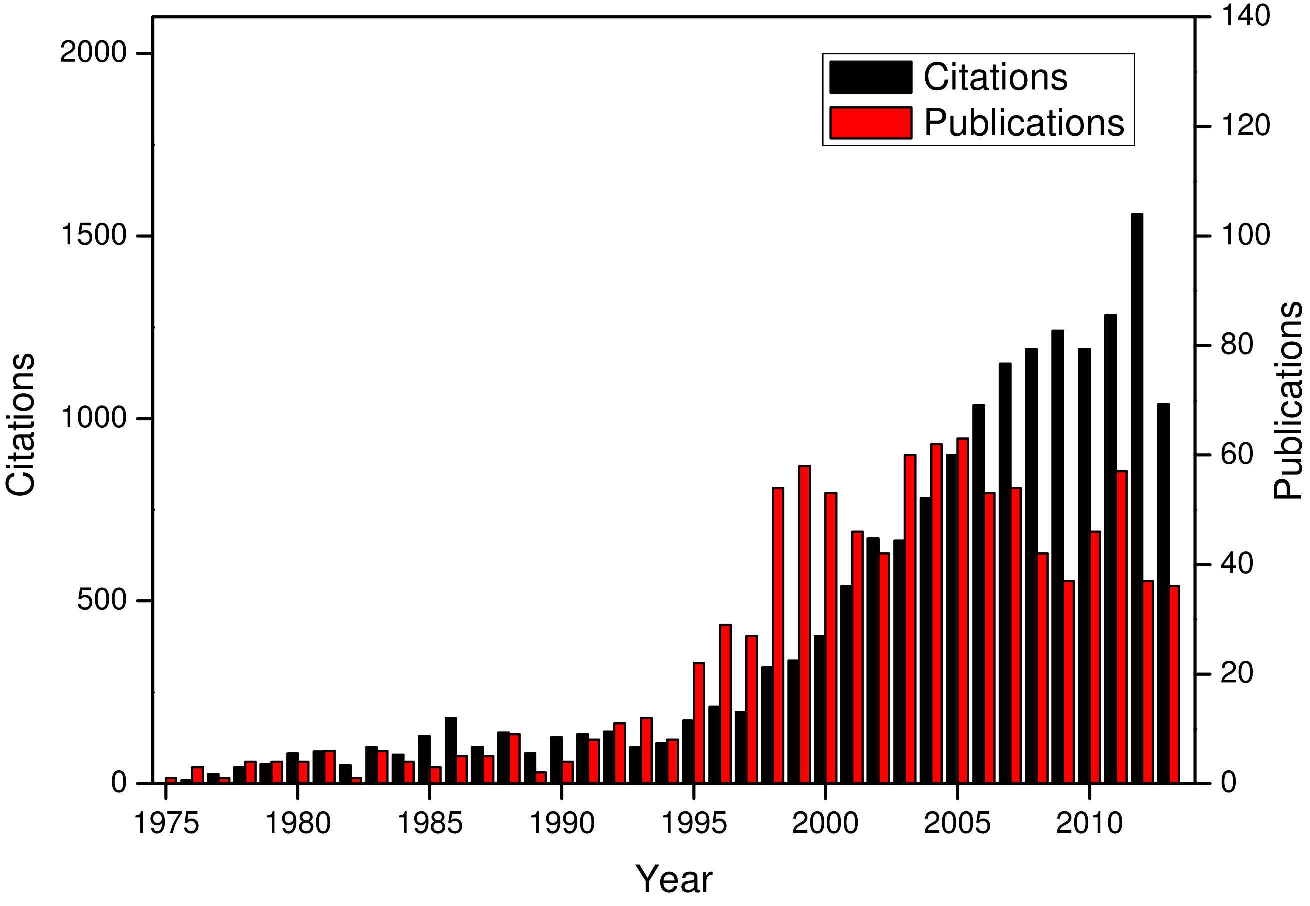
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Figure 2

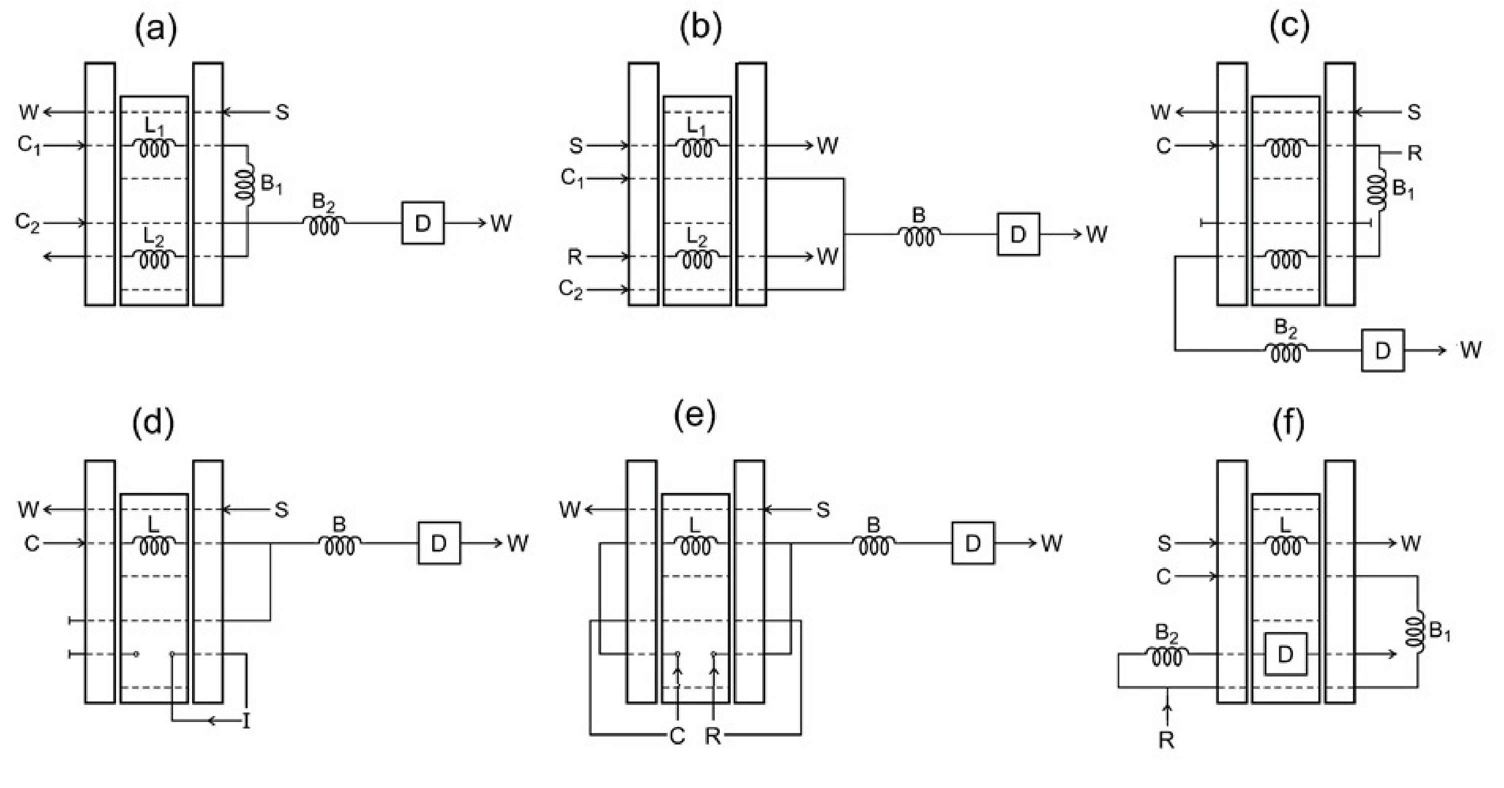
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Figure 3

## Figure captions

**Fig. 1** Timeline with the main contributions of Brazilian researchers to flow analysis.

**Fig. 2** Evolution of publications and citations of Brazilian researchers.

**Fig. 3** Different system designs achieved with the sliding-bar commuter: (a) zone sampling; (b) zone merging; (c) zone trapping; (d) intermittent flow for minimizing washing time; (e) stopped-flow and (f) multi-site detection. S: sample; R: reagent; I: intermittent flow (washing stream); C, $C_1$, $C_2$: carrier; B, $B_1$, $B_2$: reaction coils; L, $L_1$, $L_2$: sampling loops; D: flow-cell; W: waste vessel.

**Table 1** Distribution of the contributions of Brazilian researchers to flow analysis in scientific journals.

| **Journal** | **Impact factor (2013)** | **Articles (%)** |
|---|---|---|
| Anal. Chim. Acta | 4.344 | 20.96 |
| Talanta | 3.498 | 15.44 |
| Quim. Nova | 0.737 | 8.58 |
| J. Braz. Chem. Soc. | 1.283 | 7.26 |
| Analyst | 3.969 | 4.81 |
| Anal. Lett. | 0.965 | 4.50 |
| Electroanal. | 2.817 | 3.48 |
| Microchem. J. | 2.879 | 3.37 |
| Anal. Sci. | 1.569 | 2.97 |
| J. Pharma. Biom. Anal. | 2.853 | 1.94 |
| Anal. Bioanal. Chem. | 3.659 | 1.84 |
| J. Anal. At. Spectrom. | 3.155 | 1.84 |
| J. Autom. Meth. Manag. Chem. | 0.565 | 1.53 |
| Food Chem. | 3.334 | 0.92 |
| J. Agric. Food Chem. | 2.906 | 0.92 |
| Anal. Meth. | 1.855 | 0.71 |
| Others | — | 18.93 |

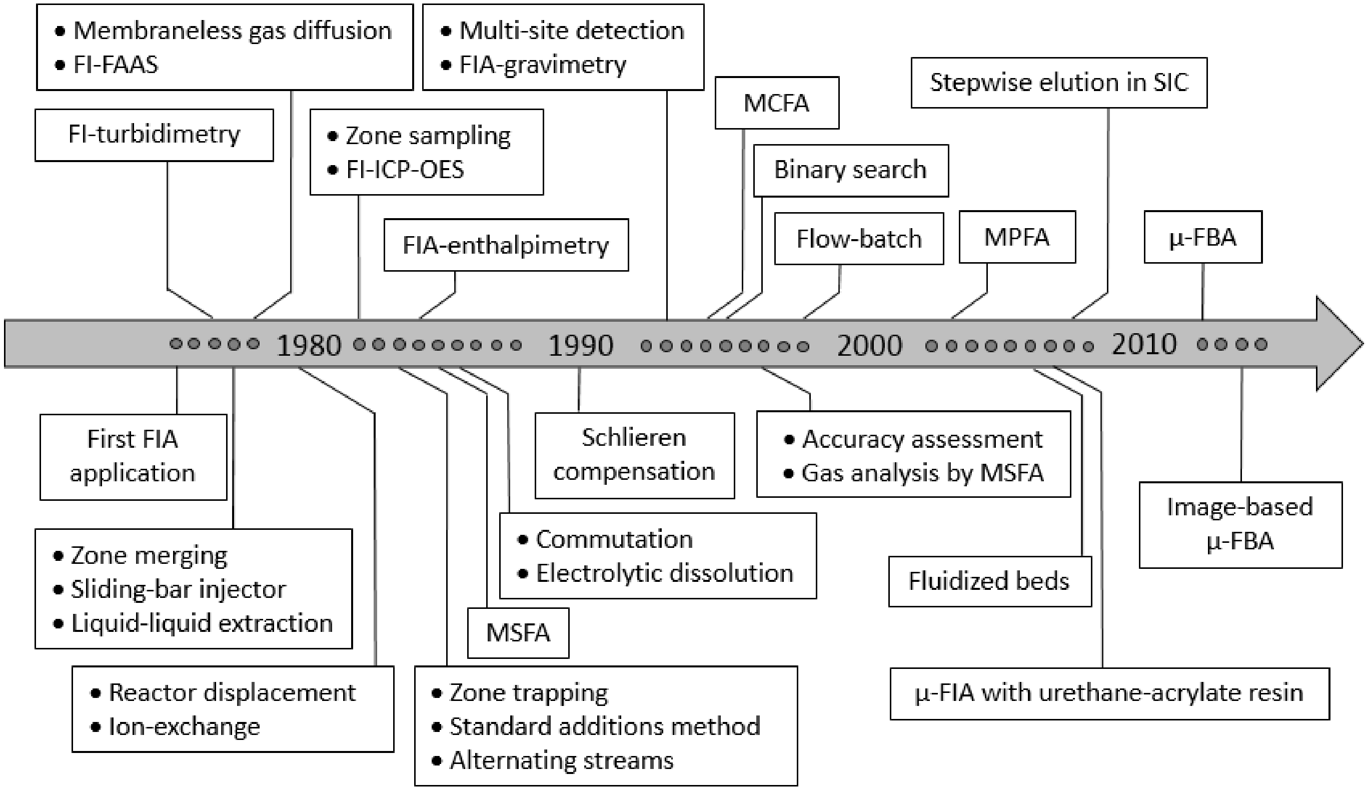
**Table 2** Selected contributions of different Brazilian researcher groups to flow analysis.

| **Application** | **Flow modality** | **Detection** | **Remarks** | **Ref.** |
|---|---|---|---|---|
| In-line Cd concentration with multiwall carbon nanotubes | FIA | TS-FF-AAS | Sensitivity 640-fold better in relation to FAAS | 17 |
| In-line Pb concentration with polyurethane foam loaded with BTAC | FIA | FAAS | Enrichment factor = 26; sampling rate = 48 $h^{-1}$ | 18 |
| Determination of total organic carbon | FIA | Turb | Microwave-assisted digestion; sampling rate = 120 $h^{-1}$ | 19 |
| Binary search titration | MCFA | UV-Vis | True titration in < 3 min; coefficient of variation = 0.01% | 20 |
| SPE with molecularly imprinted polymer | MCFA | UV-Vis | Selective extraction of catechol | 21 |
| Microfluidic device manufactured by deep-UV lithography | FIA | Fluor | Manifold internal volume < 38 µL | 22 |
| Inexpensive gold electrodes from recordable CDs | FIA | Voltam | LD = 250 ng $L^{-1}$ Hg for 5 min deposition time | 23 |
| Pioneer application of internal standard method in flow analysis with amperometric detection | FIA | Amp | Compensation of variations in instrumental parameters, unsuitable matrix matching and gradual electrode passivation | 24 |
| Cocaine determination in forensic samples | FBA-SIA | UV-Vis | In-line LLE with $CHCl_3$ | 25 |
| In-line As concentration and speciation | FIA | ETAAS | Complexation with DDTP; sorption on C-18 bonded silica | 26 |
| Supressing Pb and Cu interferences for Cd determination | MCFA | UV-Vis | Electrolytic deposition of the interfering species and analyte SPE as $[CdCl_4]^{2-}$ | 27 |
| Green procedure for nitrate determination | FIA | UV-Vis | In-line separation of the analyte from interfering species by SPE | 28 |
| Determination of L-dopa and carbidopa | FIA | UV-Vis | *Polyphenol oxidase* from crude extract of sweet potato roots | 29 |
| Determination of intracellular free amino acids in microalgae | SIA | Fluor | Stepwise solvent elution in sequential injection chromatography | 30 |
| Microfluidic device with photocatalytic decomposition of organic matter using a UV LED | FIA | Voltam | *Anatase* immobilized in a gold matrix from recordable CDs | 31 |
| Chloride determination in electrolyte of lead-acid batteries | FIA | UV-Vis | Large sample volumes to minimize the Schlieren effect | 32 |
| Determination of isoniazid in urine | FIA | Amp | Screen-printed carbon electrode modified with silver hexacyanoferrate nanoparticles | 33 |

| | | | | |
|---|---|---|---|---|
| In-line coulometric generation of chemical species | FIA | UV-Vis | Generation of reagents, standards and titrant solutions | 34 |
| Sequential determinations of Cu and Hg exploiting bead injection | SIA | UV-Vis | Retention of the analytes on Chelex-100; chemical derivatization and direct measurement in solid phase | 35 |
| Integration of a gas diffusion unit in a micro-flow analyser | FIA | UV-Vis | PTFE membrane adapted in a manifold prepared by deep ultraviolet photolithography on urethane-acrylate | 36 |
| In-line CPE for Mn concentration | SIA | FAAS | Complexation with BrTAO, CPE with Triton X-114 and retention in a mini-column filled with cotton | 37 |
| Determination of orthophosphate, phytate and total phosphorus in cereals | FIA | UV-Vis | In-line hydrolysis exploiting *phytase* immobilized on silica beads | 38 |
| Enzymatic determination of hydrogen peroxide in rain water | FIA | UV-Vis | *Peroxidase* immobilized on Amberlite IRA-743 | 39 |
| Pb determination in medicinal plants | FIA | FAAS | In-line SPE on AG-50W cationic ion exchange resin | 40 |
| Redox speciation of chromium in waters | FIA | FAAS | Complexation of Cr(III) and Cr(VI) with xylenol orange and 1,5-diphenylcarbazide and sequential retention on C-18 bonded silica | 41 |
| Determination of L-dopa | FIA | Amp | Thin film of oxovanadium-salen complex on a graphite-polyurethane electrode | 42 |
| Sulphite determination in wines and fruit juices | FIA | UV-Vis | Analyte reduction to $H_2S$ by Zn in acidic medium; gas diffusion | 43 |
| Green determination of free glycerol in biodiesel | FBA | SonoLum | Quenching of water sonoluminescence by glycerol | 44 |
| Al determination in certified silicate and iron ore materials | FIA | UV-Vis | In-line interference suppression using a polyurethane foam mini-column | 45 |
| Iodide determination seawater | FIA | Amp | Screen-printed electrodes | 46 |
| In-line Sb(III) and Sb(V) separation and concentration | FIA | ICP OES | SPE with l-proline immobilized on controlled pore glass | 47 |
| In-line Mn(II) separation and concentration | FIA | FAAS | SPE with poly(protoporphyrin-co-vinylpyridine) | 48 |
| Determination of phytic acid in foods involving SPE | SIA | UV-Vis | Aspiration of AG1-X8 ion exchange resin to build a mini-column | 49 |
| Characterization of metal availabilities in humic hydrocolloids | FIA | ETAAS | *In-situ* monitoring | 50 |

**Table 3**. Monographs and book chapters authored by Brazilian researchers on FA

| **Year** | **Topic** | **Reference** |
|---|---|---|
| 1989 | Applications in agricultural and environmental analysis | 201 |
| 2005 | Overview on Flow analysis: | 202 |
| 2007 | In-line sample preparation | 203 |
| 2008 | Multi-commutation | 204 |
| 2008 | Industrial and Process Analysis Applications | 205 |
| 2008 | Flow Voltammetry | 206 |
| 2008 | Flow Injection Mass Spectrometry | 207 |
| 2010 | Green analytical chemistry | 208 |
| 2012 | Spectrophotometric and luminometric detection | 157 |
| 2012 | Expert systems | 209 |



**Graphical abstract: Timeline with the main contributions of Brazilian researchers to flow analysis**