Brazilian Journal of Analytical Chemistry
doi: 10.30744/brjac.2179-3425.letter.ezagatto1

LETTER

A Critical Look at the Evolution of Flow Analysis

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The inception of flow analysis (FA) is closely related to the need for a better understanding of chemical reactions. At the beginning of the last century, these reactions were intensively investigated, and the main involved parameters were the reactant amounts and the available reaction time.[1] It was soon realized that solution mixing and detection were often time-dependent, so they were better accomplished on a fluidic basis.[2] This stimulated the development of simple flow-based instruments[3] that allowed chemical reactions to be more efficiently investigated. The availability of commercial colorimetric detectors in the 1930s[4] increased the demand for chemical analysis and, thus, the laboratory workload. This motivated the proposal of the AutoAnalyzer®, the first commercial air-segmented flow analyzer.[5] It was intensively used for large-scale assays, persisted for several decades, and involved only a few manufacturing companies. As the implementation of official, recommended, or tentative methods was preferred, the AutoAnalyzer underwent a relatively slow evolution yet an amazing worldwide acceptance.

Later on, flow analyzers without segmentation were proposed.[6] An example is the flow injection analyzer, which involves precise sample insertion, controlled dispersion, and reproducible timing. Exploitation of these features improved the simplicity, versatility, and flexibility of the analyzer. Further evolution led to the emergence of the sequential injection analyzer and derived ones, which occasionally involved segmented and unsegmented streams.

Nowadays, flow analysis is approaching maturity, as evidenced by the number of applications, books, book chapters, tutorials, thesis, academic courses, workshops, scientific articles, events, seminars, commercially available instruments (some of them specially designed for educational purposes), patents, etc. Intensive development of expert flow systems,[7] usually exploiting manifold programming,[8] is underway. Micro-flow analyzers are also being proposed, especially for large-scale assays under laboratory, *in-situ* and *in-vivo* conditions. Compliance with the principles of Green[9] and White[10] Analytical Chemistry has always been a positive factor for FA development.

For a FA healthy evolution, inertia or setback should be avoided, and synergy rather than divergence should be ensured. To this end, flashback and consensus are relevant. Flashback permits the evaluation a particular situation in the past, whereas consensus is more related to future developments.

FLASHBACK

In order to get a good evaluation of the past, it is advisable to critically examine the following phrases:

Too much love may kill

Repeated emphasis on a characteristic may be dangerous if it does not apply to all aspects. To increase acceptance and convince others, it is better to keep a critical distance and be realistic. In this context, the philosopher G.F. Lichtenberg said: “Schwächen schaden uns nicht, wenn wir sie kennen” (weaknesses do not harm, as long as we know that they exist). Courtesy: W. Frenzel.

Cite: Zagatto, E. A. G. A Critical Look at the Evolution of Flow Analysis. *Braz. J. Anal. Chem.* (Forthcoming). https://doi.org/10.30744/brjac.2179-3425.letter.ezagatto1

This letter was submitted to the BrJAC special issue celebrating the achievement of a Journal Impact Factor.

Automation is only one way to improve flow analysis. An enthusiastic flow analyst should always consider alternative ways as well. The writer K. Kraus said: "Eine Methode ist keine Methode. In Zweifelsfällen entscheide man sich für das Richtige" (freely translation: One method is no method. In case of doubt, make the right choice). Courtesy: W. Frenzel.

These aspects have sometimes hindered the development and acceptance of flow analysis.

Acceptance of novelty outside the affected community tends to be low

Has flow analysis been fully accepted by the community of flow analysts and by other communities? Have the innovations been used in practical applications? Do flow analysts see things that others do not? Have they failed to convince others? A look at these questions is pertinent, as *the laboratory flow systems cannot be under threat*.[11]

In fact, some unsegmented flow analytical procedures seem to be a conservative translation of the analogous segmented-flow ones. The advantages of the former (e.g., sample throughput, reagent economy, fluidic manipulation, timing control, and detector design) have not always been fully exploited.

Regulation of analytical methodologies by official agencies has been overzealous, so that there are too few recommended and accepted unsegmented flow-based analytical methodologies. Exceptions are those suggested by The Japanese Association for Flow Injection Analysis and by catalogues from some companies.

Acronyms are not always beneficial

A flash back shows 107,000,000 SIA Google entries in 2006[12] and 472,000,000 in 2015,[11] most of them not related to sequential injection analysis.[13] IUPAC recommends FIA for "Flow Immune Assay".

Dedicated flow analysts are well acquainted with SIA and FIA modalities.[14] Are these acronyms easily recognizable by the majority of flow analysts? Maybe not. Does this mean that flow analysis is not active enough? It does not seem so.

The original air-segmented flow analyzer was not linked to an acronym. Later, CFA and SFA started to be used to specify continuous flow analysis and air-segmented flow analysis, respectively. With the amazing development of unsegmented flow analysis, especially after the 1990s, many acronyms were proposed to represent flow modalities:[15] FA, flow analysis (general); AIA, all injection analysis; BIA, bead injection analysis or batch injection analysis; CIA, cross injection analysis; DCFM, dispersion-convection flow method; FBA, flow batch analysis; LAV, lab-at-valve; LOV, lab-on-valve; MCFA, multi-commuted flow analysis; MPFS, multi-pumping flow system; MSFIA, multi-syringe flow injection analysis; SIAMCh, sequential injection analysis with a mixing chamber; SIC, sequential injection chromatography; SIEMA, simultaneous injection effective mixing flow analysis; SWIA, stepwise injection analysis; and ZF, zone fluidics. It is therefore recommended that the number of acronyms be reduced by using more rigorous criteria to represent the flow modalities. Note: SIA, SIEMA, and SWIA are too similar to each other.

CONSENSUS

Consensus-seeking is essential for the future evolution of flow analysis. To this end, the following topics should be considered:

Expressions: stopped-flow or zone stopping, merging zones or zone merging, flow-batch or batch-wise flow, flow technique or flow method, commutation or zone fluidics, micro-pump or pump, etc.

Flow diagrams: presently a big mess.

Standardized components: important to promote convergence of ideas, improve the acceptance and dissemination of novel proposals, make the flow analyzer more educational, and improve its robustness. This will certainly increase the number of official flow-based methods of analysis.

Instrumentation: pump life time, stability of flow rates, tubing clogging by microparticles, adsorption of compounds on the tubing inner surface and detector walls, air-bubble entrapment. With a rudimentary instrumentation, there are different component categories, which are selected by choice. The wrong choice may dominate. The number of procedural variants, methods and techniques is another disadvantage.

Flow-based techniques

There are several ways to perform a given sample processing step, most of them utilizing different flow-based analytical techniques, e.g., electrochemistry, chromatography, molecular and atomic spectroscopy, mass spectrometry, chemometrics, speciation, titration, enthalpimetry, thermal lens spectrophotometry, inductively-coupled argon plasma optical emission spectrometry, and capillary electrophoresis. Some of these are not considered typical flow-based techniques, and a consensus to clarify this aspect is strongly suggested.

Nowadays, most flow-based techniques rely on micro-techniques, and the progress in μ-FA in different communities is remarkable. Diffusion is an important aspect of μ-FA, whereas dispersion is more related to FA. Interestingly, the dimensions of the flow analyzer do not always characterize μ-FA or μ-sensors.

The advent of so many flow-based techniques signaled some paradigm shifts. Sampling rate has often become less relevant, and the importance of some analytes has changed, mainly to attain better adherence with the principles of Green and White Analytical Chemistry.

Manifold modalities

There are many modalities, yet the conceptual differences between them are often too small to justify a specific name. They do not stand on their own. This policy has been an obstacle to scientific interactions.

A reduction in the number of acronyms by more conscientious criteria for representing the different flow analyzers is advisable. To this end, consensus is essential. A good alternative is to present the advances in flow analysis in terms of algorithms.[16] As any step of the analytical procedure can be performed in different ways, there are always several facets associated with it.

Dissemination

National and international conferences, merging of existing databases and homepages, overview of technical progress, inclusion of disciplines in the academic curricula, and creation of permanent discussion forums ("Web facilities") are typical strategies for disseminating flow analysis. The organization of an "International Institute for Flow Analysis" is strongly recommended. Consensus attainment is of utmost relevance as these aspects depend on different community sectors.

University/manufacturing company relationships

Relevant achievements generally do not become commercially available. Why? Need for a strong consensus. Flow analysis does not play the role it deserves! It deserves better. What to do? The optimism and enthusiasm of flow analysts, as well as the wide variety of flow-based techniques and options, may lead to numerous innovations, usually not implemented in practice. It is, therefore, advisable to face more clearly the competition from alternative methods and automation concepts. The standardization of flow-based procedures is a crucial prerequisite for their broad acceptance in routine analysis. For some challenging tasks, flow analysis may offer very suitable, often unique solutions.

An important task of the university is to sensitize those working in flow analytics to the importance of these aspects, convince them that research and routine analysis can be better carried out by the same staff, and, above all, ensure the personnel formation.

The perspectives for fruitful cooperation of scientific societies and institutions, training of scientific staff, permanent employment of young scientists, and development of thematic networks certainly will avoid the presence of some partners interested only in using the facilities.

Robustness

As robustness is the focus of this letter, and there is a consensus that flow analysis needs to be more robust, the phrase "If anything can go wrong, it will" (Murphy's law) is pertinent.

The robustness and maturity of the flow analysis can be evaluated by considering some indicators, such as the number of innovations, companies involved, novel proposals, overall acceptance, application to large-scale assays, etc. Workshops tend to be meaningless, as they usually refer to well-established techniques. Moreover, manufacturers are interested in publicity, especially when expensive instruments are involved. Workshops taking place in parallel to scientific events can be mischaracterized.

In short, maturity is important to anticipate the trend of future FA developments. Typically, the more mature technique is associated with a more robust analyzer.

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