Chemical Derivatization in Flow Analysis

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Chemical derivatization involves modification of the analyte for improving selectivity and/or sensitivity. It is particularly attractive in flow analysis in view of its highly reproducible reagent addition(s) and controlled timing. Then, measurements without attaining the steady state, kinetic discrimination, exploitation of unstable reagents and/or products, as well as strategies compliant with Green Analytical Chemistry, have been efficiently exploited. Flow-based chemical derivatization has been accomplished by different approaches, involving e.g. flow and manifold programming, solid-phase reagents, and strategies for sample insertion and reagent addition, as well as to increase sample residence time.

Keywords: Chemical Derivatization ; Flow Analysis ; Flow Programming ; Manifold Programming ; Multicommutation ; Green Analytical Chemistry ; UV-Vis spectrophotometry

1. Introduction

The highly versatile modern flow analyzers allow the efficient accomplishment of all steps inherent to the analytical procedure, from sample and reagent handling to flow-through detection [1]. These steps are in-line carried out in an automated manner and expert flow analyzers for managing entire sample batches without human intervention have become a reality [2].

Flow-based chemical derivatization is usual in sample processing. A species with better characteristics for detection is produced, thus improving the main analytical figures of merit, especially selectivity, sensitivity, and detection limit. The precise control of reagent addition and timing opens the way for exploiting unstable reagents and products, kinetic discrimination, catalytic reactions, and processes without attaining chemical equilibria. Chemical derivatization is carried out in a closed environment, thus minimizing risks to the analyst, sample contamination, analyte losses, and side reactions involving atmospheric components. Green Analytical Chemistry (GAC) is efficiently accomplished by saving reagents, minimizing waste generation, and exploiting diverse approaches [3]. The versatility of the flow analyzer for chemical derivatization is expanded by exploiting flow/manifold programming, often involving multicommutation [4][5][6]. However, the real-time modifications in flow rates and manifold geometry may lead to recorded signal anomalies, such as baseline fluctuations and inverted/noisy peaks of hydrodynamic and/or physicochemical origins.

2. Chemical Derivatization

Chemical derivatization involves the conversion of a compound into a product with a more favorable chemical structure, the derivative. In general, a specific functional group of an organic compound participates in the derivatization process, modifying reactivity, solubility, boiling/melting points, or the aggregation state of the product. Exploitation of these properties in analytical chemistry may improve the separation, quantification and/or preconcentration efficiencies.

In flow analysis, atomic spectrometry [7], enthalpimetry [8], amperometry [9], turbidimetry/nephelometry [10], gravimetry [11], etc have been associated to chemical derivatization. Nevertheless the process is more usual in relation to spectrophotometry and luminescence [12].

Although there is not a worldwide consensus on the definition of chemical derivatization, some statements can be mentioned, such as “the conversion of a chemical compound into a derivative (as for identification)” [13]; and “derivatization involves changing in some way the basic chemical or physical structure of a compound, usually to a single product, which may be more useful for the analysis of the original analyte” [14]. Specific definitions are also available, such as “chemical conversion of the substances to be chromatographed into more volatile derivatives” or “derivatization procedures convert non- or weakly luminescent sample molecules to highly luminescent products” for chemical derivatization in gas chromatography [15] and fluorescence [16], respectively. Here, all in-line processes (e.g., chemical, photochemical, and electrochemical) yielding a different species to be detected are considered as chemical derivatization.

2.1. Types of Flow-Based Chemical Derivatizations

2.1.1. Catalytic Methods

The analyte catalyzes an indicator reaction, which yields the species to be monitored, and the analytical signal reflects the rate of product formation, thus to the analyte content in the assayed sample [17]. These methods are typically characterized by high sensitivity, although selectivity may be a hindrance in some applications in view of the catalytic effects of potentially interfering species. As most applications exploit fixed-time methods, flow analysis is very attractive because of the rigorous time control. A pioneering application of a catalytic method in unsegmented flow analysis was focused on V(V) determination at the nanogram level relying on its effect on the chromotropic acid-bromate indicator reaction [18].

2.1.2. Photochemical and Electrochemical Derivatization

A particular type of chemical derivatization involves photochemical processes, typically carried out under ultraviolet irradiation [19]. In general, it relies on either the formation of a radical species or reactions involving radicals, yielding a species which is more efficiently monitored.

Analogously to photochemical reactions, electrochemical processes may also yield species more appropriate for detection [20]. The approach involves redox processes under controlled potential at the electrodes, associated to spectrophotometric detection of the products, as demonstrated in the determination of pharmaceuticals in blood plasma [21]. The coulometric generation of reagents for chemical derivatization was also described [22].

2.1.3. Discolorimetry

Some applications rely on the consumption of a monitored reagent by the analyte, and a typical example is discolorimetry. The reagent is a colored species and the reaction with the analyte yields a colorless (or less absorbing) chemical species. In flow-based procedures involving the reagent as the sample carrier or added as a confluent stream, the recorded inverted peak reflects the reagent consumption and constitutes itself the analytical signal. Intermittent additions of sample and reagents are also feasible, as exemplified in the determination of acid dissociable cyanide [23].

2.1.4. Analyte Volatilization

Volatilization of the analyte is a kind of derivatization accomplished for segregating it from the sample matrix. To this end, the analyte is converted to a gaseous species, separated from the sample, and further processed [24]. The chemical species to be monitored seldom mimics the analyte. A diversity of alternatives is available for gas-liquid separation in flow analysis, including gas diffusion, pervaporation, and membraneless (e.g., isothermal distillation) approaches [25]. The determination of ammonium involving conversion to ammonia under alkaline conditions, collection into a neutral or acidic stream and directioning towards detection is an example [26]. Volatilization is also useful for hydride and cold vapor generation in flow-based atomic spectrometry, sometimes assisted by photochemical processes [27].

2.1.5. Other Approaches

Liquid–liquid and solid–liquid microextractions often involve chemical derivatization of the analytes by a suitable reagent dissolved in the extractant or adsorbed on the solid support. In flow analysis, several microextraction techniques have been exploited and approaches have been proposed to ensure the proper interaction of the species [28][29]. Although phase transference seldom reaches equilibrium conditions, precise results are obtained in view of the reproducible sample handing conditions and timing.

The processes involved in the product formation in a chemical derivatization are also of interest in some applications, such as in kinetic analytical methods. Moreover, variations of physicochemical properties, such as the heat release in enthalpimetry [8] and light emission in chemiluminescence [12], may also provide useful information.

2.2. Derivatization in Liquid Chromatography

Chemical derivatization is often exploited in liquid chromatography, with the aim of reducing matrix interferences, enhancing the analytical selectivity and detectability, as well as improving the analyte separation.

Off-line derivatization is time-consuming and laborious as it requires completion of the involved processes, and often large volumes of samples and reagents. This hinders its applicability, especially for large-scale assays, and can be circumvented by exploiting in-line derivatization.

For in-line post-column derivatization, a confluent reagent stream merges with the eluent immediately after the chromatographic column, and the monitorable product is formed inside a reactor just before detection. The involved reactions should be fast, and band broadening due to dilution by the reagent and dispersion inside the reactor needs to be minimized. An advantage is that post-column derivatization is not limited by hydrodynamic pressure, as the eluent stream has already left the chromatographic column.

Pre-column derivatization is beneficial especially regarding to compounds difficult to be separated by modifying chemical and physical properties of the species and improving the analyte-stationary phase interaction. An adverse aspect, however, is that in-line pre-column derivatization may be limited by pressure effects, as it takes place between the sample insertion port and the chromatographic column. To circumvent this limitation, the derivate may fill a sampling loop under ambient pressure, which is thereafter inserted into the pressurized eluent flowing towards the chromatographic column.

3. Flow-based Approaches for Chemical Derivatization

A diversity of modalities has been proposed in flow analysis, in part motivated by novel possibilities to implement chemical derivatization, i.e. the way the reagents are added, the strategies for sample/reagents mixing and to achieve reproducible timing.

The state-of-art of flow analysis involves computer-control for implementing all steps of sample processing without the analyst intervention. This includes the capability for modifying sample/reagent volumes and flow rates, the addition of different reagents, flow reversal, stream redirecting, flow stopping, zone sampling, etc. It is the essence of flow and manifold programming [4], which has been widely exploited for e.g. implementing complex assays, kinetic methods, wide-range analysis, and simultaneous determinations [25].

3.1. Strategies for Reagents and Sample Additions

Reagent addition is a cornerstone in flow-based chemical derivatization, as it may affect the analytical figures of merit and the system reliability. Therefore, different possibilities for reagent (and sample) additions into the analytical path have been proposed (**Figure 1**).

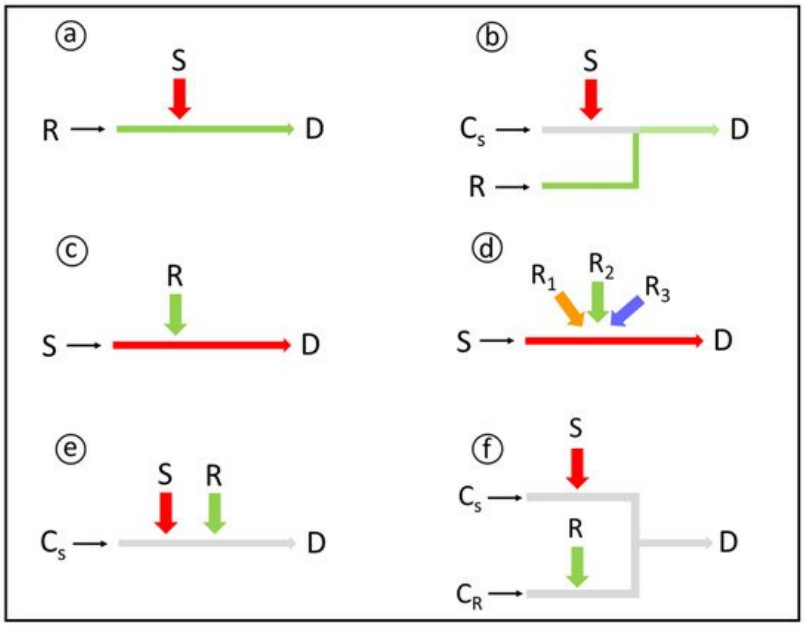

Figure 1. Configurations for sample and reagent insertions in flow analysis. S: sample; R, R_1, R_2, R_3: reagents; C_S: sample carrier stream; C_R: reagent carrier stream; D: detection; Large arrows: time-based, loop-based, hydrodynamical or syringe insertions; Small arrows: flow directions; Flow diagrams: **a**—single line, **b**—confluent, **c**,**d**—reverse flow injection, **e**—single line merging zones, **f**—confluent merging zones. Further sample processing may occur before detection.

In the first flow injection systems, the sample was inserted into a reagent carrier stream, featuring a single-line flow analyzer [25]. This configuration (**Figure 1**a) is mostly suitable for relatively low sample inserted volumes, because the sample–reagent mixing depends mainly on the axial dispersion.

Alternatively, the sample aliquot is inserted into a chemically inert carrier stream, which mimics the major components of the sample matrix in order to minimize the Schlieren effect [30], and the reagent additions occur by stream confluences.

This configuration (**Figure 1**b) is inherent to segmented flow analysis, and especially attractive in relation to large sample inserted volumes in unsegmented flow analyzers.

A less usual variant of single-line flow analyzers involves injection of the reagent into a continuous flowing sample, featuring a reverse flow injection system, rFIA [31]. The resulting configuration (**Figure 1**c) minimizes the reagent consumption and maximizes the amount of the analyte in the sample zone associated with the analytical signal. It is also a simple alternative for sequential determinations, accomplished by successively injecting different reagents into the flowing sample[32]. The main limitations refer to the high sample consumption and the time intervals for sample replacement and system washing, the latter being critical for large-scale assays. Aiming at system simplicity and ruggedness, the different reagents are usually inserted with a single injector (**Figure 1**c). Alternatively, system versatility can be expanded by using one injector for each reagent (**Figure 1**d).

Other configurations involve sample and reagent insertions by exploiting different flow modalities [25]. If a single carrier stream is used (**Figure 1**e), the interaction between sample and reagent zones is restricted to axial dispersion, and the analyzer presents the advantages and limitations inherent to single-line configuration, such as poor sample-reagent mixing, restricted exploitation of high inserted volumes, and a high susceptibility to the Schlieren effect. If the sample and reagent are inserted into convergent carrier streams, a flow system with merging zones [25] is established (**Figure 1**f), presenting the characteristics of the confluence configuration, although with a significant reduction of the reagent consumption.

Solid-phase reagents can be exploited for chemical derivatization in flow analysis, either as packed mini-columns [33], open tubular reactors [34], or suspensions [35]. Advantages include a low reagent consumption, increase of the reaction rate due to the reagent excess, possibility of using slightly soluble reagents, avoidance of dilution effects, and manifold simplification. On the other hand, strategies for minimizing reagent lixiviation (e.g. reagent chemically bound to the solid support) and backpressure effects (e.g. suitable particle size and/or porous materials) should be applied.

3.2. Mixing Conditions

Suitable mixing is essential for a successful chemical derivatization in flow analysis, especially considering the short sample residence time available. The process relies on diffusion (both axial and radial) and convection, which may be maximized or minimized according to the involved analytical application. Besides the sample and reagent volumes and flow rates, the dimensions and geometry of the reactors (e.g., coiled, knotted, or packed) are relevant [25]. Mixing is highly affected by the characteristics of the flowing stream, thus pulsed flows [36], particles fluidization [37], and flow reversal [38] have been exploited to maximize the analyte–reagent interaction. The latter is typical in sequential injection flow analyzers [39].

A mixing chamber (with or without stirring) is useful mainly in derivatizations involving several reagents. It is inherent to the flow-batch [40] and the lab-in-syringe analyzers [41], being also worthy for increasing sample residence time in derivatizations relying on relatively slow chemical reactions.

3.3. Reproducible Timing

The reproducible timing characteristic of flow analysis favors the exploitation of processes even when full completion of the involved chemical reactions is not attained. In addition, it is inherent to flow and manifold programming aiming to increase the system versatility, to develop expert flow analyzers, and to enhance the productivity of the analytical laboratory.

Specific strategies, such as flow stopping, zone trapping, flow rate variations, and zone recycling [25], are worthy to increase sample residence time. The aim is to minimize the impact of the increased residence time on sample dispersion and sampling rate.

Flow stopping (**Figure 2**a) is implemented either by switching the pumping off or by commutation, typically when the sample zone reaches the flow cell. Formation or degradation of the product is then efficiently monitored. Zone recycling [25] is a variant of this process: the sample zone is kept flowing in a closed loop, including the flow cell, for detection at different sample residence times. Zone trapping (**Figure 2**b) relies on the removal of the central part of the sample zone, and stopping it inside a trapping reactor, in which the components are allowed to react further. After a predefined time interval, the zone is reinserted into the carrier stream, flowing towards detection.

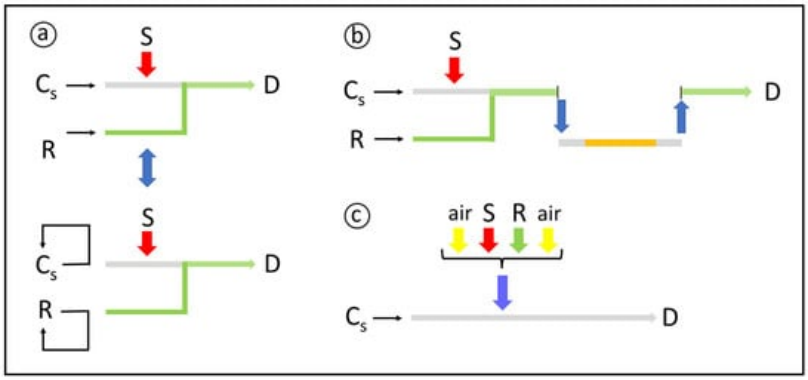

Figure 2. Flow manifolds related to strategies for increasing sample residence time without significantly incrementing sample dispersion. **a**—flow stopping, **b**—zone trapping, and **c**—monosegmented flow analysis. S: sample; R: reagent; C_S: sample carrier stream; D: detection; Large arrows: time-based, loop-based, hydrodynamical or syringe insertions; Small arrows: flow directions.

Monosegmented flow analysis [42] (**Figure 2**c) is an alternative for efficiently implementing relatively slow chemical derivatizations. It is compatible with the different modalities of flow analysis, allowing also to accomplish, e.g., liquid–liquid microextractions, flow titrations, and determinations of gaseous species. The sample–reagent zone is sandwiched between two air plugs to restrict dispersion, so that high sample residence times can be efficiently attained. Simultaneous sample processing is also feasible, with beneficial effects on sample throughput. Similar effects can be attained by using air-carrier flow systems [43].

4. Highlights of Chemical Derivatization in Flow Analysis

4.1. Kinetic Methods

Segmented flow analysis typically involves reactions approaching the steady state, which is less usual in unsegmented flow analysis, where the implemented methods are characterized as fixed-time ones, established by the proper choice of flow-rates and dimensions of the analytical path. The strict and reproducible time control permits also to exploit other kinetic approaches by continuously monitoring the product formation. To this end, the flow is stopped when the sample zone is at the flow cell [44] or otherwise, the central portion of the sample zone is trapped inside a coiled reactor [45]. The former approach is particularly useful for evaluating kinetic parameters and for attaining a more selective analyte determination, including compensation of the radiation absorption by colored samples. Moreover, different approaches for exploiting two residence times for each injected sample, such as sample splitting [46], detectors in series [47], relocation of reactors [48], and multi-site detection [49] have been proposed. Analytical determinations without attaining the steady state are typically characterized by high sample throughputs, but the inherent sensitivity lessening may be a hindrance in some applications.

Kinetic discrimination is also feasible, especially when the reaction product is measured without a significant formation of a potential interfering species via a side reaction. The strategy improves selectivity and may be exploited for simultaneous determinations [49][50][51].

4.2. Unstable Reagents and Products

Another favorable aspect, inherent to the reproducible and controlled timing and reaction conditions, is the reproducible generation of unstable species in flow systems, paving the way for the exploitation of novel reagents and products [52]. Strong oxidizing, e.g., Ag(II), Co(III), and Mn(III), or reducing, e.g., Cr(II), U(III), and V(II) agents, can be in-line generated from stable species by exploiting e.g. electrochemical processes [53] or a mini-column filled with the Jones reductor [54]. Unstable reagents can be also chemically produced in solution, as exemplified by in-line generation of bromine from bromide and bromate [55]. Applications involving unstable reagents benefit from their generation in a closed system, without contact with the atmosphere. The ability to detect unstable yet reproductively formed species allows the proposals of novel analytical methods and represents a clear advantage of flow analysis [56][57]. Moreover, the instability of the measured species can be exploited to improve selectivity, as demonstrated in the ingenious determination of ascorbic acid relying on its intrinsic UV absorption and further decomposition [58].

4.3. Optosensing

A particular application of solid reagents/sorbents in flow analysis refers to solid-phase spectrophotometry or optosensing [59], involving a suitable sorbent placed placed in the flow-through detector. The reagent is immobilized on the solid support, where chemical derivatization occurs directly, or the derivative is formed in solution and further retained on the support. Both approaches allow measurements to be carried out simultaneously with the analyte/derivative retention. The approach is worthy for improving sensitivity as the reaction product is accumulated at the support, and the dilution inherent to analyte elution before measurement is avoided. Selectivity may also be improved due to changes in reactivity of the immobilized reagent or kinetic discrimination [60]. Reversible retention of the analyte and exploitation of the same immobilized reagent for successive measurement cycles is also feasible [61], yielding more environmental friendly procedures.

4.4. Simultaneous Determinations and Chemical Speciation

Chemical derivatization is also involved in most simultaneous determinations in flow analysis, either by adjusting the reaction conditions or by adding selective reagents. To this aim, flow and manifold programming [4] have been often exploited. Other strategies involve kinetic discrimination, multi-site detection [49], multi-purpose flow systems [62], asynchronous merging zones [63], sandwich techniques [64], and reverse flow analysis [32].

A noteworthy application relies on chemical speciation, exploiting the differences in reactivity of the species resulting from the chemical derivatization. Selective determination of a species followed by total determination after sample pretreatment becomes then feasible. Classical examples are the determinations of Fe(II)/Fe(III), Cr(III)/Cr(VI), and NO_3^-/NO_2^- [65]. The determination of clinical iron parameters (serum iron, unsaturated iron binding capacity, and total iron binding capacity) in human serum, by exploiting sample processing under different acidities and complexation with ferrozine exemplifies a more recent application [66]. Overall, the approach is usually more simple, cost-effective, and faster than the chromatographic ones, although the scope is limited by the number of analytes determined, typically restricted to 2–3 species.

4.5. Green Analytical Methods

While in GAC there is a premise to avoid chemical derivatization whenever possible [67], several approaches have been proposed to minimize its impact in the environmental friendliness, relying mainly on the use of less hazardous chemicals and solvents, and more effective energy sources [68]. In this sense, flow analysis is a powerful tool especially because of its potential to minimize reagent consumption and waste generation. This is inherent to modalities involving the intermittent addition of reagents in the chemical derivatizations, such as sequential injection, multipumping flow, and multisyringe flow analysis, as well as those involving solid-phase reagents. This goal is also successfully attained by miniaturization, including μ-FIA and lab-on-valve. However, the potential of flow analysis to GAC is significantly wider, involving e.g. reagentless procedures [69][70], replacement of toxic reagents [71][72], reuse of reagents [61][73], vegetable natural extracts as source of reagents [74] and enzymes [75], as well as in-line waste treatment [72][76].

4.6. Expert Systems

The potential of chemical derivatizations in flow analysis is significantly expanded in expert flow systems [2]. This encompasses e.g. in-line optimization of the reaction conditions, in-line adjustment of the medium and flow/manifold programming, aiming to avoid matrix effects, and to perform multi-analyte determinations. Accuracy assessment relying on analyte determination by different analytical methods, typically involving different approaches for chemical derivatization, is also feasible [77].

5. Final Remarks

Chemical derivatization benefits itself from the favorable characteristics of flow analysis, allowing a better exploitation of chemical reactions without attaining equilibrium, the possibility of kinetic discrimination, the exploitation of unstable reagents and products, and the compliance with the GAC principles.

Most flow-based applications involve a simple mechanization of well-established analytical methods. Nevertheless, the advantages are increased when chemical derivatization exploits the characteristics inherent to flow analysis. This is a fertile field for further development, also when the flow analyzer is a front-of-end for chromatography.

The term “derivatization” is typically associated to chromatography, mass spectrometry, UV-vis spectrophotometry, and luminescence. However, its relevance to flow analysis has been increasingly emphasized. This also holds for µFIA and microfluidic devices.

In spite of the outstanding current development of flow analysis, an intense human labor is still required to minimize systematic errors and to ensure reliable analytical results and safer working conditions to the analysts. In this context, the development of expert systems plays an important role.

Several environmentally friendly innovations have been proposed for chemical derivatizations in flow analysis and the flow-based systems paved the way for making chemical derivatization a useful strategy for GAC. This is a counterpoint to the less realistic statement of GAC principle that chemical derivatization should be avoided [67].

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