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Chapter 2

FLOW TITRATIONS

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ABSTRACT

Titration implementation in a flow system is a consequence of the amazing development of flow analysis. This aim of this chapter is then to present historical aspects, foundations, potentialities, and limitations of flow-based titrations with emphasis to triangle-programmed, flow-injection, and batchwise titrations, involving sample continuous addition, sample insertion as a plug, or sample stopping inside a chamber-like component in the analytical path, respectively. Selected applications involving true or pseudo titrations are presented, and further developments are foreseen.

Keywords: triangle-programmed titration, flow-injection titration, batchwise flow titration, automated titration, automatic titration, types

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of flow titrators, manifolds for flow titrations, multicommutation, applications

1. INTRODUCTION

The amazing development of automated analytical chemistry is a consequence of the advantageous aspects inherent to mechanization [1, 2]. Flow analysis, "the art of playing analytical chemistry inside narrow-bored tubing" [3], is an outstanding approach to automation, relying on the transport of a continuously flowing aqueous sample through the analytical path of a flow analyzer. Controlled dilution, reagent addition, analyte separation/concentration, re-sampling, and other processes inherent to the specific analytical application are then in-line reproducibly accomplished. Passage of the handled sample through the detector yields a transient signal, which is usually recorded as a peak, ideally proportional to the analyte content.

The analytical path is a closed environment, thus sample contamination or losses, as well as analyst exposure to hazardous reagents, are minimized. Some processes inherent to flow management, such as e.g., stream splitting, reagent addition by zone merging, sample stopping, zone sampling, and sample recycling can be efficiently exploited for the development of specific analytical procedures. Timing is precise, thus the involved chemical reactions do not necessarily reach completion, and this opens the possibility of performing analysis without attainment of chemical equilibria. Although the flow-based analytical procedures are generally similar to the analogous batchwise carried out ones, there are plenty of procedures independent of any previous analogous ones.

The above-mentioned advantages of mechanization in flow analysis, as well as the reduced analyst intervention, the improved analytical figures of merit (e.g., sensitivity, selectivity, sample volume, reagent consumption, waste generation, sample throughput), the feasibility of system miniaturization, and the possibility of full computer control of the analyzer have been often pointed out [4, 5]. Finally yet importantly, the increased

capacity of the analytical laboratory, and the adherence to the 12 Green Chemistry principles [6] should be mentioned. All these aspects hold also for flow titrations.

Titrimetry is one of the few classical analytical techniques still in wide use for the determination of major and minor sample components. In a true titration (see item 4), the analytical result is generally obtained by considering the titrant amount (mass or volume of a standardized solution) needed for attaining the titration end-point, which ideally matches the equivalence point. If the titrant concentration is exactly known, and volumetric ware needs no further calibration, an analytical curve is not required. A low sample throughput and large sample/titrant consumptions are however inherent to classical titrations. With automation, these drawbacks are circumvented and precision, convenience, and affordability of titrimetric procedures are improved. In addition, other favorable characteristics such as e.g., the easy implementation of titrations with catalytic end-point detection, and titration of flowing samples should be highlighted. The feasibility of accomplishing a titration without full development of the involved reaction is another possibility.

The aim of this chapter is therefore to present the development of flow titrations, discussing historical aspects and recent advances as well as the potentialities and limitations in relation to selected applications. The main characteristics of each type of flow titrator are discussed, and specific flow manifolds are emphasized. Furthermore, concepts of pseudo and true titrations are critically reviewed.

2. HISTORICAL BACKGROUND

Controlled titrant addition is the essence of a titration. This was already verified in relation to the early manually performed titrations, which usually relied on the dropwise or continuous addition of the titrant to a precisely known sample volume. Evolution resulted in the automated titrators, considered in 1959 as “any instrument that records a titration curve and/or stops the titration at the end-point by mechanical or electrical means” [7]. In

this regard, the first titrators with automatic end-point detection [8] and with ability to record the full titration curve [9] proposed in 1914 and 1922 should be highlighted, demonstrating that titration automation was a reality about one century ago. Development led to the appearance of the titrator with automated sampling [10] and to the continuous titrator [11]. Further relevant improvements were added by Malmstadt et al. [12, 13]. The related instruments cannot however be considered as automatic titrators, as the titration step is mechanized, but the operator should prepare the sample, remove it after the titration, and clean the equipment between successive runs.

More elaborate instruments, nowadays referred to as "fully automated titrators," were further proposed to perform both the titration and other related steps such as e.g., preliminary sample handling/treatment, clean-up operation afterwards and sample replacement. The burette was gradually replaced by a syringe or a pump [14]. In fact, it was realized that titrant addition was a source of error, especially when gravity was exploited for solution displacement. This aspect held also for the manually performed titrations relying on controlled dropwise titrant additions.

Nowadays, fully automated titrators coupled to robotic workstations, titrations down to the femtoliter scale, novel hardware and algorithms for end-point detection, and consequent feedback mechanisms to assist the titrant addition are available [15]. These titrators comply with the IUPAC definition of automatic systems: "The use of combinations of mechanical and instrumental devices to replace, refine, extend, or supplement human effort and faculties in the performance of a given process, in which at least one major operation is controlled, without human intervention, by a feedback system" [16]. The key point in this context is then the exploitation of a feedback mechanism, i.e., the combination of a sensing and a commanding device to modify the system performance. This is the essence of the expert flow titrators.

Miniaturization is a reality. Moreover, the modern titrators comprise components such as pumps, valves, reactors, injectors, and tubing often used in ordinary flow analyzers. In addition, strategies such as in-line sample

dilution, reagent addition, flow stopping, etc., have been indistinctly exploited both in fully automated titrators and in flow analyzers.

As a consequence, to present the concept and initial developments of flow titrations is a hard task. In this chapter, the expression "flow titrations" is used in relation to titrimetric procedures carried out inside the manifold of a typical flow analyzer.

3. FLOW SYSTEMS TO ACCOMPLISH TITRATIONS

Flow titrations have been accomplished in different modalities of flow systems, such as:

3.1. Segmented-Flow Analysis

Flow analysis was conceived during the fifties by Skeggs [17], who proposed the segmented-flow analyzer as means of efficiently performing the repetitive assays needed in the clinical laboratory.

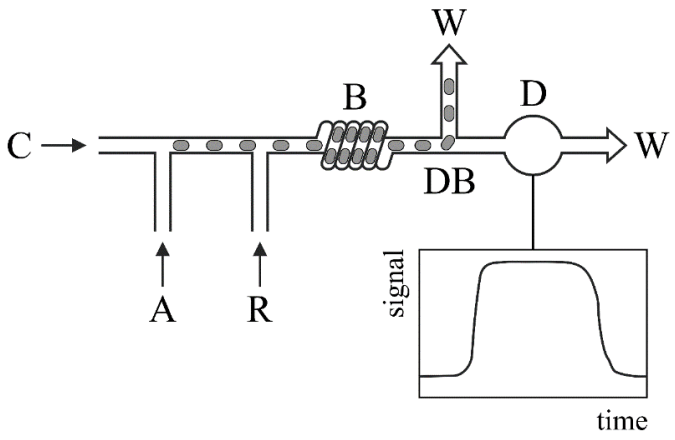

Figure 1. The segmented-flow analyzer. C: carrier or sample (from sampler); A: air; R: reagent; B: bobbin (coiled reactor); DB: de-bubbler for air removal; D: flow-through detector; W: waste disposal. Inset: typical recorded peak. For details, see text.

The sample and other convergent streams, e.g., reagents, diluent, and solvent, are continuously pumped towards the analytical path. A confluent gaseous stream (usually air) is also added to promote segmentation by air bubbles, which is beneficial to improve mixing conditions, to prevent carryover between successive samples and to scrub the tubing inner walls. The sample "infinite volume" situation [18] is then approached, minimizing sample dispersion. The recorded analytical signal (Figure 1) comprises therefore two sharp front and edge regions and a flat (plateau) region, which is generally considered as the measurement basis.

3.2. Flow-Injection Analysis

This analytical strategy was proposed independently by Ruzicka & Hansen and by Stewart et al. [19], and involves the insertion of a well defined volume of an aqueous sample into a continuously flowing unsegmented carrier stream (Figure 2), establishing a sample zone that is pushed forwards towards detection. During its transport through the analytical path, the sample zone undergoes continuous dispersion, and this is a key aspect towards the in-line development of the required analytical steps, which are reproducibly carried out. When the handled sample passes through the detector, a transient signal reflecting the analyte content is monitored and recorded as a peak.

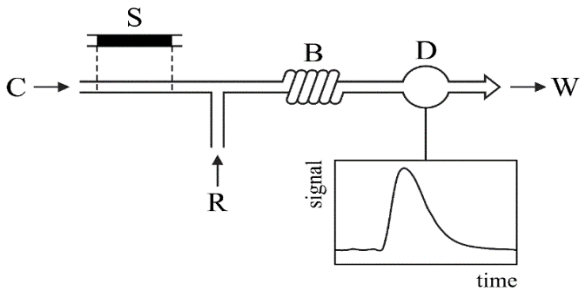

Figure 2. The flow-injection analyzer. C: carrier; S: sample; R: reagent; B: bobbin (coiled reactor); D: flow-through detector; W: waste disposal. Traced lines: towards alternative position. Inset: typical recorded peak. For details, see text.

3.3. Sequential-Injection Analysis

In 1990, sequential-injection analysis was proposed by Ruzicka & Marshall [20] aiming at a simple, rugged and computer-controlled flow system with the ability to perform different determinations without system reconfigurations. The sequential-injection analyzer (Figure 3) is similar to the flow-injection one, and operates as follows. The sample and other involved solutions (e.g., reagents, diluent, solvents) are selected by a multi-port valve to be sequentially aspirated towards a holding coil, thus establishing a stack of neighboring zones. Thereafter, the flow is reversed, and the zone stack is directed towards detection. Mixing of the zones is improved because, on transporting the zone stack, the zones penetrate each other. Similarly as above-mentioned, a transient signal reflecting the analyte content is monitored during passage of the handled sample through the detector.

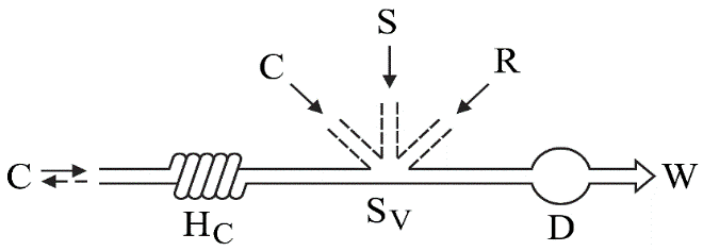

Figure 3. The sequential-injection analyzer. H_C: holding coil; C, S, R: carrier, sample, reagent; S_V: stream selecting valve; D: flow-through detector; W: waste disposal. Traced lines: towards alternative positions. For details, see text.

3.4. Discontinuous-Flow Analysis

This modality of flow system, proposed by Arnold et al. [21], exploits differential pumping [22]. It involves two syringe pumps, one for discharge and the other for suction, and the difference between suction and discharge flow rates allows the sample to be aspirated into the carrier stream. The pumps are operated by a mechanically driven interchangeable cam linked to

their pistons, so that the delivered flow rates can be constant or varied in a highly reproducible manner, depending on the cam profile, which defines the flow rate ratio. During the analytical cycle, it is then possible to convey the sample, the reagent, or a combination of both towards the flow manifold and detection. To this end, two check-valves are switched, starting the refill cycle during which one pump is refilled with the reagent whereas the other is discharged towards waste. Different cam profiles have been exploited for efficiently accomplishing single or multiple solution additions as well as automated flow titrations. An encoder is attached to the cam driveshaft, allowing the monitored analytical signal to be recorded as a function of the cam position. In this way, the entire titration curve can be gathered.

3.5. Monosegmented Flow Analysis

This modality was conceived by Pasquini & Oliveira [23], and combines the favorable characteristics of both segmented and unsegmented flow analysis. The sample is inserted between two air plugs into the sample carrier stream, and the established monosegment is directed through the analytical path towards detection. During transport, sample integrity is then maintained. The reagents are added to the sample either by confluence in the analytical path or before sample insertion. As a monosegment is involved, a laminar flow regimen is not established for the sample carrier stream, and vortices are established inside the monosegment. Mixing is then improved. Moreover, several samples can be simultaneously handled inside the analytical path, and this aspect is worthwhile mostly in relation to sample throughput. The sample passage through the detector results in a transient analytical signal analogous to that associated to segmented-flow analysis.

3.6. Batchwise Flow Analysis

Batchwise flow analysis, also termed “automated micro-batch analysis” [24] or “flow-batch analysis” [25], was proposed by Sweileh & Dasgupta in

1988 [24]. The analyzer (Figure 4) exploits the favorable characteristics of sample processing under flow and stop conditions, as well as the high flexibility and versatility of mixing chambers [26].

A well defined sample aliquot is added to a mixing chamber, where most of the steps inherent to the specific analytical procedure (e.g., reagent addition, sample dilution, analyte separation/concentration) are reproducibly accomplished. Alternatively, the sample can be continuously pumped through the chamber and stopped inside it. Detection is accomplished inside the chamber or, otherwise, the handled sample leaving the chamber is monitored. The transient recorded signal reflects the analyte content in the sample. The chamber is thereafter washed out.

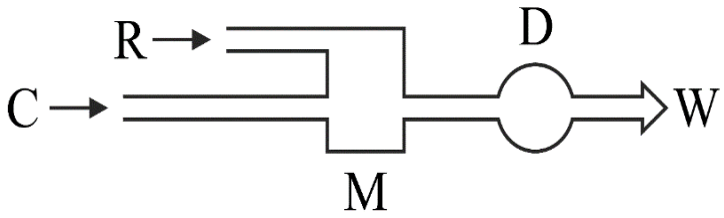

Figure 4. The batchwise flow analyzer. C: carrier/sample (from sampler); R: reagent; M: mixing chamber; D: flow-through detector; W: waste disposal. For details, see text.

3.7. Other Flow-Based Analytical Systems

Flow titrations can be also implemented in other sort of flow analysis such as continuous flow analysis, all injection analysis, cross injection analysis, multi-syringe flow-injection analysis, multi-pumping flow analysis, loop flow analysis, simultaneous injection effective mixing flow analysis, stepwise injection analysis, etc. Most of these modalities are similar to those above mentioned, and a critical classification of them, emphasizing the related analogies was recently proposed [27].

4. TITRATION TYPES IN FLOW ANALYSIS

Flow titrations can be classified in three main types, namely triangle-programmed, flow-injection, and batchwise titrations, depending on whether the sample is continuously added, inserted as a plug, or stopped inside a chamber (or a chamber-like component) in the analytical path, respectively.

4.1. Triangle-Programmed Titrations

Titrimetric analysis of flowing samples was proposed by Blaedel & Laessig in 1964 [28]. The sample and titrant solutions were pumped towards a mixing chamber, and the outlet stream was potentiometrically monitored (Figure 5, left). The sample flow rate was constant, and the titrant one was continuously increased and measured. When the electrode potential related to titration end-point was reached, the corresponding titrant flow rate was taken into account in order to evaluate the sample-to-reagent volumetric ratio, thus the analytical result [29]. As analytical standards were not required and flow ratiometry was involved, the approach can be regarded as a true titration.

As a proof of concept, the determination of iron(II) involving oxidation by cerium(IV) was selected [28]. Within the 0.7-60 mmol L^{-1} Fe range, 6 min were required per determination, and precise results (0.2 - 0.5% r.s.d.) were obtained. The need for continuously measuring the titrant flow rate was however a limiting aspect, as the precision of flow rate measurements depends on a strictly constant flow, which is not always attained.

The innovation was further applied to complexometric titrations involving automatic blank correction and direct readout aiming at potentiometric determinations of total hardness and metal ions (10^{-5} mol L^{-1} magnitude order) in natural waters [30]. Enhanced analytical figures of merit were obtained. The innovation was named as “triangle-programmed titration” by Nagy et al. [31]. An analogous strategy without involving flow rate variations (Figure 5, right) was proposed by Fleet & Ho [32].

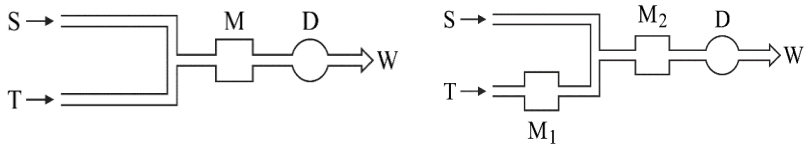

Figure 5. The original triangle-programmed titrator (left) and that operated under constant flow rates (right). S: sample; T: titrant; M_i: mixing chamber; D: flow-through detector; W: waste disposal. For details, see text.

A titrant-generating stream was continuously added to a chamber initially filled with water, so that the effective titrant concentration was linearly increased in function of time. The outlet stream and the sample stream were thereafter converged, and the resulting one was directed towards another chamber (the titration one) and detected. The time interval required for reaching the titration end-point was considered as the measurement basis. The strategy was applied to the potentiometric titration of sulfide with mercuric nitrate.

Concepts, theory, characteristics, potentialities, limitations and applications of the triangle-programmed titrators were deeper investigated by the research group headed by Pungor, who delivered a classical series of articles [33-37]. Further development involved:

- exploitation of Fourier transform [38]
- tandem stream for estimating sample-to-titrand volumetric ratio [39]
- in-line enzymatic analyte hydrolysis [40]
- multivariate curve resolution [41]

Nowadays this type of titration is still used, mainly for industrial processes.

4.2. Flow-Injection Titrations

This item deals with flow-titrations relying on flow-injection analysis and similar strategies involving sample insertion into the carrier stream. The related flow titrators are outlined as follows.

4.2.1. The Flow-Injection Titrator

Flow-injection titration was proposed by Ruzicka and co-workers [42], who used a flow system similar to that in Figure 2, yet comprising a mixing chamber which acted as a gradient forming device [43]. The sample was inserted either into the titrant stream or into a chemically inert carrier stream, and directed towards a dilution chamber. In the latter situation, the titrant stream was added by confluence. Consequently, a single-lined or a double-lined flow system was established. The inherent high dispersion involved was the basis for this kind of flow titrations, as highly reproducible concentration gradients were established along the flowing sample zone leaving the chamber [44].

In the single-lined system, each fluid element corresponded to a different sample-to-titrant volumetric fraction ratio, thus flow titration was straightforwardly implemented. The flow system was simple, but the analytical sensitivity was limited by the need for high sample dilution. Moreover, the Schlieren effect [45] could manifest itself as a potential limiting aspect in the signal-to-noise ratio.

In the confluent flow system, each fluid element leaving the chamber corresponded to a different sample volumetric fraction. As the titrant stream, at a constant flow rate, was added to the sample by confluence, a steady titrant concentration was involved. Therefore, the sample inserted volume was not restricted, and the analytical sensitivity was better in comparison to the single-lined flow titration. Moreover, the magnitude of the Schlieren effect was less pronounced.

Regardless of the system configuration, the front and trailing portions of the sample zone comprised regions characterized by different sample-to-titrant volumetric ratios, reflecting the involved direct and reverse titrations. The two specific regions associated with the corresponding end-points were present in the flowing sample, thus the recorded monitored signal comprised an almost flat central portion and two boundary portions (front and trailing) related to the direct and reverse titration curves. Two sharp modifications associated with the titration end-points were then present in the recorded signal, and the temporal distance between them reflected the titrand content

in the sample. Consequently, a linear relationship between peak width and logarithm of the titrand concentration was followed [42].

An analytical curve relying on standard solutions with different known concentrations was however required. Therefore, the innovation cannot be considered as a true titration according to IUPAC definition [46] and to Pardue & Fields arguments [47, 48]. Nowadays, there is a consensus that the innovation can be regarded as a "pseudo titration."

The flow-injection titrator was originally applied to acid-base titrations of synthetic solutions, and to calcium and magnesium determinations in natural waters using alkaline EDTA as titrant solution and spectrophotometry or potentiometry as the monitoring technique. The entire titration, including sample insertion and system washing, required less than 60 s.

A similar instrument comprising miniaturized manifold components was further designed for ultrafast flow-injection titrations [49]. The gradient-forming chamber was replaced by a small tubular reactor and the flow system was designed in the single-lined configuration, in order to provide medium sample dispersion. Consequently, a theoretical model relating the analyte concentration with the time span between end-point achievements was built-up. The capability of this high-speed titrator was demonstrated in relation to acid-base, complexometric and iodometric titrations, carried out in < 30 s.

4.2.2. The Sequential-Injection Titrator

Flow titrations can be also implemented in a sequential-injection analyzer (Figure 3), as emphasized by van Staden & Plessis [50], who selected the spectrophotometric titration of strong acid with a strong base as a proof of concept. Aliquots of the titrant (a bromothymol blue basic solution), the sample and the titrant solution again, were selected by a multiport valve, sequentially inserted into a distilled water carrier stream, and directed towards the holding coil, yielding a stack of well-defined zones adjacent to each other. After flow reversal, the mixed sample zone was directed towards the detector and spectrophotometrically (λ = 620 nm) monitored. Operation of the system was fully computer controlled. For 0.001

mol L^{-1} NaOH titrant, a linear relationship between peak width and logarithm of acid concentration was attained within 0.01 and 0.1 mol L^{-1} HCl. Other linear ranges were possible by modifying the titrant concentration. Accurate results were obtained at a sample frequency of 30 h^{-1}.

Here, it is important to point out that true titrations can also be accomplished in a sequential injection manifold (see section 3.3) comprising a titration chamber. The resulting flow set up permitted to implement a strategy analogous to the triangle-programmed titration, as demonstrated by Alerm & Bartroli [26]. The sample was inserted into a sequential injection analyzer and directed towards a micro-chamber where a titrant stream was also added. The added titrant volume was varied, allowing the entire titration curve to be gathered. The flow system was applied to acid base, complexometric, precipitation, and redox titrations. The analysis required about 5 min, accuracy and repeatability being comparable to those related to batch titrations.

4.2.3. The Discontinuous-Flow Titrator

Flow titrations can also be accomplished in a discontinuous-flow system comprising a chamber-like component, as initially highlighted by Cardwell et al. [51, 52] who determined the titratable acidity of wines and the total acidity of vinegars. NaOH was used as titrant, and the flow rates of the sample and titrant convergent streams were continuously varied, according to the cam profile. The versatility of the titrator allowed a controlled variation of sample-to-titrant flow rates ratio within 2:1 to 1:2. Different LEDs, acid-base indicators, spectral widths and cam profiles were investigated in order to reduce the interferences arising from the sample own color. The analytical signals associated to cam profile, thus to the different sample-to-titrant flow rate ratios, were considered to build-up the entire titration curve, and the first-order derivative plots were taken into account for the titration end-point estimations. With the innovation, good analytical figures of merit were obtained, and red wines and dark brown vinegars were analyzed without a prior sample dilution.

4.2.4. The Monosegmented Flow Titrator

The feasibility of implementing flow titrations in the monosegmented flow system (see item 3.5), was demonstrated by Honorato et al. [53] in relation to the determination of vinegar acidity. The sample monosegment behaved as a titration vessel to which exact titrant amounts were added. This unique feature, associated with the other inherent characteristics of monosegmentation, expanded the potentialities of flow titrations. The innovation was also implemented in the sequential injection analyzer [54] and in the lab-on-valve [55], a miniaturized version of this system, aiming at to evaluate acidity of vinegar and soft drinks, and of fruit juices, respectively.

Further development of flow-injection titrations and similar involved:

- controlled sample dilution with zone merging [56]
- tandem stream for estimating sample-to-titrand volumetric ratio [57]
- artificial neural network for data treatment [58]
- pseudo titration using an indicator mixture aiming at simultaneous titrations [59]
- Gran-plot exploitation [60]

Flow-injection titrations exploiting flow-injection and sequential injection analyzers, are nowadays largely used for routine assays, especially in relation to industrial processes.

4.3. Batchwise Flow Titrations

This flow titrator (Figure 4) was proposed by Alerm & Bartroli [14, 26] and constitutes itself in an ingenious approach to mimic the classical manually performed titration. A mixing/reaction chamber is the heart of the system, thus both true and pseudo titrations are readily implemented.

For true titrations, a precise known sample volume was inserted in the "infinite volume" situation [18] into an aqueous carrier stream or, otherwise,

a gas carrier stream was used [14] to prevent sample dispersion. When passing through the chamber, the undispersed sample was stopped, and the titrant was continuously added by an automatic burette until end-point reaching.

The entire titration curve was then obtained. The innovation was initially applied to acid-base volumetric analysis and precise and reproducible results in adherence with those obtained with discontinuous titrations, were obtained.

For pseudo titrations, the sample is inserted into a carrier stream and handled as mentioned in section 3.2, the chamber acting then as a mixing element. This potentiality was emphasized by Medeiros et al. [61] in relation to the determination of metronidazole in pharmaceutical formulations.

Further developments of batchwise flow titrations involved:

- exploitation of a monosegmented flow [62]
- design of a miniaturized multicommuted setup [63]
- non-aqueous pseudo titrations [64]
- hyphenation with a sequential injection analyzer [65]
- Karl Fischer true titration [66]

Batchwise flow titrations are presently not so intensively exploited probably due to the unavailability of a commercial flow analyzer dedicated to this end. In spite of this hindrance, the scientific contributions in the field have been expressive, as assessed by analyzing the item 6, where the applicative potential of this type of flow titration is emphasized.

4.4. Specific Approaches to Flow Titrations

Ingenious approaches for accomplishing flow titrations have been proposed, some of them outlined as follows.

4.4.1. Zone Merging

The sample and reagent aliquots are generally inserted into independent carrier streams [67]. Insertion into the same carrier stream aiming at a single-lined flow system is also feasible, especially in relation e.g., the sequential injection analyzer (Figure 6). For accomplishing flow titrations, several titrant solutions with different concentrations and the sample (without modifications) are successively inserted via the titrant and sample injection ports [56]. Coupling zone sampling and zone merging leads to an analogous, yet powerful approach to accomplish titrations without the need for different titrant solutions.

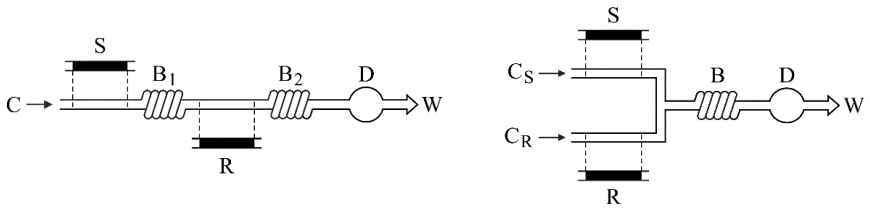

Figure 6. Single-lined (left) and confluence flow systems with zone merging. C: carrier; S: sample; R: reagent; B_i: bobbins (coiled reactors); D: flow-through detector; W: waste disposal. Traced lines: towards alternative position. For details, see text.

4.4.2. Zone Sampling

The sample aliquot is inserted into a first carrier stream, establishing a first sample zone which is handled and directed towards a second injection port (Figure 7). After a predefined t_{zs} time interval, a specific portion of the sample zone is re-sampled and introduced into a second carrier stream [68] for further processing. By setting several t_{zs} values, sample aliquots with different concentrations are selected. The approach has been often exploited for attaining e.g., large sample dilution, simultaneous determinations, analytical curve relying on a single standard solution, standard additions, and titrations. Regarding this later potentiality, the approach is accountable for selecting different titrant aliquots with known concentrations to be added to the sample zone [69], thus expanding the potentialities of flow titrations.

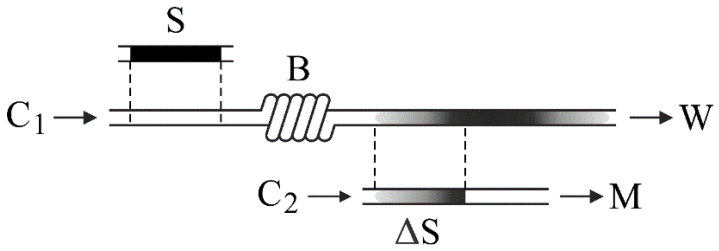

Figure 7. Flow system with zone sampling. C_1, C_2: first, second carrier streams; S: sample; B: bobbin (coiled reactor); W: waste disposal; ΔS: resampled aliquot; M = towards manifold. For details, see text.

4.4.3. Successive Flow Reversals

Flow reversal is inherent to sequential injection analysis, yet can also be exploited in flow-injection analysis [70] and related techniques. Successive flow reversals permit mixing improvement without excessive sample dispersion, thus the approach constitutes itself in an important aspect for improving flow-injection titrations.

4.4.4. Coulometric Titrations

The titrant is in-line coulometrically generated, usually inside the titration chamber, and its amount is defined in terms of the electric current and elapsed time [71]. The approach is particularly attractive in relation to true titrations carried out in microfluidic flow systems, and the determination of diffused ammonia and carbon dioxide involving enzymatic reactions [72] can be highlighted as an example.

4.4.5. Tandem Streams

Small aliquots of different miscible solutions are sequentially added as alternate neighboring plugs. This unique stream comprises then a series of parabolic interfaces between the plugs, which are efficiently mixed together. Coalescence of these plugs leads to the homogenization of the sample zone, thus improving mixing conditions. A tandem stream is therefore especially attractive in relation to single-lined flow systems, where mixing tends to be a more critical aspect. This stream is useful for controlling sample dilution (see item 5.2). Several plugs of sample and diluent solutions are successively

added, mixed together and directed towards detection. Although undulations in the detector output have been reported [3], the involved good mixing conditions yield an almost steady situation.

Another possibility for establishing a tandem stream is to use a fast switching three-way valve to mix two different streams. This innovation, "binary sampling" [73], can be also implemented by using a reciprocating pump [74] or by adding the sample/reagent plugs at a high frequency through nozzles [75]. A tandem stream can also be established inside the sampling loop of a flow-injection system [76]. The sample/reagent interaction starts during the sampling step, thus increasing the mean available time for reaction development without affecting the sampling rate.

Regarding flow titrations (Figure 8), the sample-to-titrant volumetric ratio, defined in terms of number and length of carrier, titrant and titrand plugs, is in-line efficiently modified in order to reach the titration end-point [39]. To this end, concentration-orientated feedback mechanisms relying on an extrapolative algorithm have been useful. The approach, "binary search" [77], proved to be relevant for accomplishing true flow-based titrations [75].

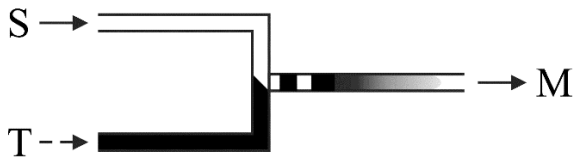

Figure 8. Establishment of a tandem stream. S, T: sample/titrant alternating convergent streams; M: towards manifold. For details, see text.

4.4.6. Tracer Monitored Titrations

Regardless of the type of flow titration (triangle-programmed, flow-injection or batchwise), the involved procedure can be simplified by exploiting the "tracer-monitored titration" [78] innovation. The innovation was proposed for estimating the sample-to-titrant volumetric ratio without the need for volume, mass, charge or peak width measurements. It was initially applied to the classical spectrophotometric determination of total alkalinity in seawater. A dye tracer was added to the titrant solution for real-

time estimation of the sample-to-titrant volumetric ratios after each titrant addition. In this way, the entire titration course was followed.

The approach was recently implemented in flow analysis by Sasaki and co-workers [79] and the spectrophotometric determinations of acidity in vinegars in flow systems relying on the triangle-programmed and flow-injection titrations was selected as an application. Good figures of merit were attained, and no physically metering of volumes, masses, flow rates or electric currents were needed.

Another important aspect refers to the exploitation of multicommutation to improve the main characteristics of a flow titrator.

5. MULTICOMMUTATION IN FLOW TITRATIONS

Multicommutation in flow analysis involves the exploitation of discretely operated commuting elements to add/remove specific manifold components, stream directing devices, and go/stop components for fluid delivery establishment. Aspects such as commutation vs multicommutation and timing are presented, in order to permit a better evaluation of the potentialities of multicommutation in flow titrations.

5.1. Commutation

Exploitation of commutation in flow analysis was initially discussed by Krug et al. [80], who emphasized the beneficial aspects arising from the presence of commuting devices in the manifold of a flow system. The authors pointed out that different injection procedures (e.g., loop-based, time-based, hydrodynamic, sequential, nested injection) intermittent/ alternating streams and modifications in the manifold architecture could be efficiently accomplished with a simple commutator, often manually operated.

With computer-controlled operation, the commutator could be used also for performing some time-dependent steps such as zone sampling, zone trapping, solid-phase extraction, sample stopping under continuous

pumping, etc. Some of these steps were interdependent, thus should be performed together. To this end, the commutator was designed with several commuting sections. However, it was operated between two resting positions, thus only two manifold status were available. This constituted itself in a negative aspect because, with solidary commutation, further improvements in the flow analyzer were hindered.

It was soon realized that versatility of the flow analyzer could undergo a remarkable improvement by resorting from multiple discretely operated commuting devices, and this was the essence of multicommutation [73].

5.2. Multicommutation

Multicommuted flow systems comprise several discrete commuting devices strategically positioned in the manifold to provide enhanced system versatility, hence good analytical figures of merit.

Regarding historical aspects of multicommutation in flow analysis, some pioneering contributions should be mentioned. Malcome-Lawes & Pasquini [81] proposed a flow system exploiting reagent random access for simultaneous determinations in natural waters; Israel et al. [82] implemented a tandem stream for attaining high and variable degrees of sample dilution prior to inductively coupled plasma optical emission spectrometry or mass spectrometry; Reis and co-workers [73] established the basis of this emergent innovation.

Most of the multicommuted flow systems rely on unsegmented flows, due to low costs of installation and operation, inherent flexibility, easy operation and control, low consumption of reagents and short analysis times. As any commuting device is associated to a modification in the manifold architecture, multicommutation usually permits the establishment of a number of manifold status, and this is a positive factor towards the improvement of the flow analyzer performance [83].

5.3. Timing

The flow analyzers are characterized by three cornerstone features: sample insertion into the analytical path, reproducible sample handling, and precise timing [3]. This latter feature is of utmost relevance, as it permits the analytical steps to be developed during precisely defined time intervals. In this way, highly reproducible conditions for sample handling are attained, thus improving the analytical figures of merit. In this context, internal and external timing should be highlighted.

Internal timing is inherent to the system design and predominates in simpler flow analyzers. This feature permits the analytical steps to be developed during precisely defined time intervals. In more advanced flow analyzers, external timing associated with, e.g., time intervals for sample stopping or trapping, valve switching and timing schedule associated with flow rate variations, can also be exploited to improve system versatility. The external timing is usually set by taking advantage of discretely computer-operated commuting devices. Regardless of the timing involved, most flow analyzers operate in a passive manner, as the conditions for sample handling are set prior to sample insertion into the system.

Alternatively, flow analyzers can operate in an active manner: the sample handling conditions are real-time modified according to a concentration-oriented feedback mechanism. As a rule, a prior assay is carried out and the analytical result is used to feed the software with information for real-time decisions. The need for in-line modification of the degree of sample dilution, sample replacement, activation of pumps or stream directing devices, as well as the need for accomplishing further optional analysis, are then real time defined. This aspect is inherent to the expert systems, often referred to as smart or intelligent ones.

According to IUPAC definitions [16], automatic flow analyzers are expert systems. In fact, the presence of discretely computer-operated commuting devices (e.g., valves and pumps) constitutes itself in a powerful approach for improving the performance of the flow analyzer, and the inception of a multi-purpose valve, “magic valve” [84], should also be mentioned in the context.

With the advent of expert systems, a paradigm shift in the practice of analytical chemistry was experienced. This shift was also noted in flow analysis, especially with regard to multicommuted flow systems. In addition to monitoring the sensor response, the innovation required computer interfacing and discrete actuation devices in the manifold, aiming at the design of analytical procedures involving response-oriented real-time decisions based on preliminary results (prior assays).

6. APPLICATIONS

Acid-base, precipitation, chelatometric and redox titrations have been efficiently implemented in flow systems. There are situations where the prompt availability of the sample to titrant volumetric ratios associated to every titrant addition are required or, in other words, the entire titration curve should be available. When the entire titration curve is taken into account for e.g., evaluating pK values, accomplishing simultaneous determinations, or improving analytical reliability through chemometric strategies, true titrations have been preferred. On the other hand, pseudo titrations have been mostly applied to real assays, probably because of their inherent simplicity, robustness and expeditious aspects. Moreover, the analytical results are obtained at a high frequency, and the sample and titrand volumes are usually small.

All types of flow titrations, namely triangle-programmed, flow-injection and batchwise titrations have been applied, and selected applications related to analytical determinations are presented in Tables 1 - 3. Other specific situations are presented as follows.

In this regard, the stability constant of Cd^{2+}/glycine complex was potentiometrically evaluated and the related data treatment relied on non-linear least squares regression [85]. The innovation was applied to complex total concentrations higher than 0.1 mmol L^{-1}. Analogously, a mono-segmented flow system involving micro-volumes of sample was proposed for evaluating the pKa values of weak acids within 3 and 6 [86]. NaOH as

titrant, and multiple linear regression or partial least squares for data treatment were used.

A flow system for accomplishing pseudo titrations was designed for the potentiometric evaluation of pKa values of phthalic acid, phosphoric acid and EDTA [87]. Range of pH values, direction of titration (direct or reversal), total flow rate and weak acid concentrations were investigated. The pKa-values, determined with a 0.01-0.05 standard deviation, were in agreement with literature data.

A pseudo titrator able to evaluate acidity constants of acetic and benzoic acids was designed for a class demonstration in an undergraduate course on instrumental analysis [88]. The evaluation involved monitoring of the H_3O^+ concentration inside the gradient chamber as a function of time. This was indirectly accomplished by spectrophotometrically monitoring the change in absorbance, thus concentration, of the basic form of bromocresol green. The innovation proved to be worthwhile for in-class demonstrating the implementation of automated procedures at the microscale level and the rigorous analysis of an acid-base titration.

Another approach potentially applicable to class demonstrations, involved digital movie-based images gathered in a batchwise flow titrator [89]. A web cam recorded the images during addition of the titrant to the mixing chamber. During recording, the images were decompiled into frames ordered sequentially at a constant rate of 26 frames per second. The first frame was used as a reference to define the region of interest of 28 x 13 pixels and the R, G and B values. The titration curves were real-time plotted, and the end-point was estimated by the second derivative method. The feasibility of the method was assessed in relation to acidity evaluation in edible oils.

Flow titrations have been also exploited for drug dissolution studies, and the dissolution kinetics of promethazine and ampicillin from pharmaceutical preparations [90] is an illustrative example. To this end, successive in-line fast titrations involving hypobromite as titrant were carried out in a triangle-programmed titrator with spectrophotometric detection. Dissolution occurred inside a titration vessel placed in a thermostated bath.

Regarding simultaneous determinations, most of the titrations involve two or three end-points associated to the sharp inflections of the entire titration curve (see Tables 1-3). Some innovations specific to flow titrations are outlined as follows.

I. The Mg + Ca concentrations in pharmaceutic products were spectrophotometrically determined (Table 2) by using a flow-injection titrator exploiting zone merging [56]. The dynamical concentration range was then expanded. An alkaline EDTA solution was used as titrant, eriochrome black T as indicator, and area of the recorded peak was taken into account for end-point estimation. Results were precise (r.s.d. < 0.6%) and in agreement with those obtained by flame atomic absorption spectrometry, as well as with those declared by the producers of pharmaceutics. As the results reflected the summation of Ca plus Mg concentrations, the innovation was potentially applicable to evaluating water hardness, as confirmed by running some synthetic solutions.

II. Hydroxide and carbonate were determined in industrial sodium aluminate solution [91] by using a batchwise flow titrator. The sample was inserted into the sample carrier stream, a sulfosalicylic acid plus barium chloride plus phenolphthalein solution, and the established sample zone was directed towards the titration cell. Sulfosalicylic acid acted as titrant and complexing agent for aluminum to prevent formation of the aluminum hydroxide precipitate. Before reaching the end-point indicated by the phenolphthalein, hydroxide was titrated and carbonate remained as suspended barium carbonate. Thereafter, the additional sulfosalicylic acid acted as a dissolution agent for barium carbonate. Turbidity lessening was then taken into account for evaluating the carbonate content. In-chamber detection was accomplished by a LED photometer and excellent figures of merit were reported (Table 3).

Table 1. Selected applications of triangle-programmed flow titrations

Analyte	Sample	Titrant	Detection technique	Titration range or detection limit (mmol l^{-1})	Remarks	Ref.
acetylcholine	SS	OH^-	pot (pH)	8×10^{-2}	in-line enzymatic analyte hydrolysis; acetic acid monitoring; coulometric titrant generation; acid-base pseudo titration	40
Acidity	Vinegar	NaOH	UV-Vis	-	tracer-monitored flow titrations; triangle-programmed technique vs FIA	79
acidity	beer, vinegar, wines, juices	NaOH	UV-Vis	-	titrant variable flow rate; indicators mixture; titration of strong and weak monoprotic acids, alone or mixtures, or polyprotic acids	94
acid dissociation constants	SS	NaOH	pot (pH)	-	detection of the end-point by feedback-based flow ratiometry and the subsequent estimation of the half equivalence point	95
glucose	SS	NaOH	fluor	0.1 - 0.25	true titrations; flow-rate gradients; wide concentration range by exploiting zone sampling; short capillary tubes	96
hydrazine, ammonium	tap, river water	Br_2	CL, amp	20, 0.5	true bromimetric titration; coulometric titrant generation	97
iron(II), chromium(VI)	alloy reference material	Ce(IV), Fe(II)	pot	0.5 - 2.0 (Fe), 0.1 - 1.0 (Cr)	true titration exploiting ratios of sample (constant) and titrant (increasing) flow rates	98
nicotinic acid	PF	OH^-	L-photo	0.36 - 0.44	sample preparation: grind, dissolution, filtration, dilution; mixture of indicators; coulometric titrant generation; acid-base pseudo titration	99
oxidability	waste waters	MnO_4^-	UV-Vis	13 - 110	multicommuted flow system; tandem stream; calibration step not required	100
penicillin compounds	SS	OH^-	pot (pH)	0.05 - 0.5	in-line enzymatic analyte hydrolysis yielding penicilloic acid; coulometric titrant generation; acid-base pseudo titration	101

SS: synthetic solutions; PF: pharmaceutic formulations; pot: potentiometry; UV-Vis: spectrophotometry; fluor: fluorimetry; CL: chemiluminescence; amp: amperometry; L-photo: LED-based photometry; FIA: flow-injection analysis.

Table 2. Selected applications of flow-injection titrations

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
acetic acid	Vinegar	NaOH	pot	SIA	10 - 90 g L^{-1}	no sample preparation; pseudo titration	102
acidity	Beverages	NaOH	pot	FIA	5.0 - 100 mmol L^{-1}	multicommuted mono-segmented flow system; no sample preparation; ionic strength inside the chamber adjusted to 0.1mol L^{-1} NaCl; successive approximation approach to determine end-point; true titration	103
acidity	fruit juices	NaOH	UV-Vis	SIA	0.2 - 1.0, 0.5 - 2.5% w/v citric acid	sample preparation: filtration and dilutions; pseudo titration involving peak area	104
acidity	fruit juices	NaOH	UV-Vis	SIA	0.2 - 1.2% w/v citric acid	sample preparation: filtration; mono-segmented flow system; pseudo titration; lab-on-valve SIA system	55
acidity	fruit juices, vinegar, wines	NaOH	L-photo	MCFA	0.036 - 0.176 mol L^{-1}	sample preparation: dilution and optional filtration; binary search approach; true titration	105
acidity	oils, biodiesel	NaOH	UV-Vis	SIA	0.0 - 26.0 mg L^{-1} oleic acid	sample preparation: dilution; multivariate curve resolution-alternating least squares for second-order data treatment	106
acidity	olive oil	KOH	UV-Vis	FIA	0.1 - 1.3% w/v linoleic acid	no sample preparation; non aqueous (n-propanol) pseudo titration involving peak areas	107
acidity	silage extracts	NaOH	UV-Vis	MCFA	0.001 - 0.1 mol L^{-1}	sample preparation: extraction, filtration and dilution; true titration exploiting ratio of sample (constant) and titrant (increasing) volumes	108
acidity	soft drinks	NaOH	pot	SIA	0.1 - 0.6% w/v citric acid	sample preparation: degassing; pseudo titration	109

Table 2. (Continued)

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
acidity	Vinegar	NaOH	UV-Vis	FIA	-	controlled sample dilution with zone merging; pseudo titration involving peak areas	56
acidity	Vinegar	NaOH	UV-Vis	FIA	-	tracer-monitored flow titrations; also triangle-programmed technique	79
acidity	vinegar, soft drinks	NaOH	UV-Vis	FIA	-	sample preparation: degassing (soft drinks); controlled dilution; pseudo titration	110
acidity	vinegar, soft drinks	NaOH	UV-Vis	SIA	2.61- 13.1 mmol L^{-1} citric acid	sample preparation: degassing (soft drinks); mono-segmented flow system with controllable sample dilution; pseudo titration	54
acidity	vinegar, soft drinks	NaOH	pot	FIA	-	no sample preparation; mono-segmented multicommuted flow system; binary search; true titration	111
acidity	wine and vinegar	NaOH	pot	DFA	-	continuous variation of sample to titrant flow rate ratios; calibration based on peaks of first-order derivative plots	112
acidity	Wines	NaOH	pot	FIA	0.5 - 12.5 mmol L^{-1} malic acid	sample preparation: purge with N_2 for CO_2 elimination, dilution; pseudo titration involving peak areas	113
acidity	wines, vinegar	NaOH	L-photo	DFA	-	controlled variation of sample to titrant flow rate ratios; calibration based on first-order derivative plots	51
ammonia, hydrogen sulphide	leachate from waste landfill	BrO^-	biamp, CL	FIA	0.002 - 1.5, 0.002 - 5.0 mmol L^{-1}	continuous flow system; gas diffusion; coulometric titrant generation; analysis before and after sample bio-oxidation; pseudo titration	114

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
ammonium, creatinine	Urine	BrO^-	amp	FIA	0.002 - 2.0 mmol L^{-1}	sample preparation: dilution; continuous flow system; gas diffusion; coulometric titrant generation	115
ascorbic acid	fruit juices, soft drinks	DCPI	UV-Vis	MCFA	0.6 - 6.0 mmol L^{-1}	sample preparation: dilution; tandem streams; feed-back mechanisms for real-time processing titration course (sample/titrant volumetric ratios); true titration	57
ascorbic acid	PF	MnO_4^-	UV-Vis	SIA	up to 1200 mg L^{-1}	sample preparation: grind and dissolution; pseudo titration involving peak areas	116
ascorbic acid	PF	IO_3^-	pot	MCFA	7.5 - 15.0 mmol L^{-1}	sample preparation: grind and dissolution; iodide ISE; pseudo titration	117
ascorbic acid	PF	DCPI	UV-Vis	FIA	0.1 - 10.0 mmol L^{-1}	pseudo titration; pneumatically actuated injection valve	118
atrazine (triazine pesticide)	outcome from atrazine production plant	K4E7	UV-Vis	FIA	0.5 - 100 µg L^{-1}	pseudo immuno-titration; antigen/antibody binding; zone merging for widen dynamical concentration range; numerical calculations involving neural network algorithm	119
calcium	natural waters	EGTA	pot	MCFA	0.1 - 5.0 mmol L^{-1}	tandem stream; single channel pump and four electronically-operated valves; spectrophotometric flow-injection phosphate determination with the same flow set-up	120
calcium	natural waters	EDTA	UV-Vis	FIA	0.5 - 50.0 mmol L^{-1}	pseudo titration; pneumatically actuated injection valve; titrant: magnesium interference minimized by replacing the ordinary EDTA by Mg-EDTA complex	118
calcium, magnesium	mineral, river waters	EDTA	pot	FIA	1.0 x 10^{-5} mol L^{-1} (both analytes)	determination of analytes sum, and calcium only; pseudo titration involving peak areas	121

Table 2. (Continued)

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
carbonate, bicarbonate	soda, water	HCl	UV-Vis	SIA	0.8 - 10 mmol L^{-1}	use of two indicators; pseudo titration	122
chloride	Milk	$AgNO_3$	pot	SIA	0.01 - 0.25 mol L^{-1}	no sample preparation; pseudo titration	123
chloride	milk, wines	$AgNO_3$	pot	SIA	0.8 - 30 mmol L^{-1}	mono-segmented flow system; no sample preparation; successive approximation approach to determine end-point; true titration	124
chloride	Serum	$AgNO_3$	pot	FIA	8.0 - 12.0 mmol L^{-1}	sample preparation: dilution; pseudo titration	125
chloride	soil extracts	$AgNO_3$	pot	FIA	1 - 100 ppm	sample preparation: analyte extraction from soil, treatment with activated charcoal and filtration; pseudo titration	126
citric-malic acid mixtures	orange juice	NaOH	pot	r-FIA	-	sample preparation: analyte precipitations as barium salts, filtration; precipitate dissolution and dilution; artificial neural network for data treatment	58
conc. HCl	outcome from a HCl production plant	NaOH	pot	SIA	5.93 - 8.99 mol L^{-1}	no sample preparation; in-line sample dilution; pseudo titration	127
ephedrine	PF	TPB	pot	FIA	0.2 - 2.0 mmol L^{-1}	sample preparation (tablets): powdering, dissolution, dilution; pseudo titration	128
Fe(II)	PF	MnO_4^-	UV-Vis	FIA	1.0 - 10 mmol L^{-1}	mono-segmented flow system; simultaneous multiple injection; the same manifold for spectrophotometric determination of Cr(VI) in waters by the standard addition method	129

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
Fe(II), Fe(III)	artesian well waters	EDTA	UV-Vis	r-FIA	0.1 - 3.0 mg L^{-1} Fe(II) 0.9 - 3.5 mg L^{-1} Fe(III)	pseudo titration using an indicator mixture; sulfosalicylic acid/peak width measurements for Fe(III) and 1,10-phenanthroline/peak heights for Fe(II)	59
H_2O_2	commercial products	MnO_4^-	UV-Vis	FIA	-	sample preparation: dilution to minimize viscosity; mono-segmented flow titrator; true titration; use of relatively slow reaction; algorithm for reaching end-point	130
H_2SO_4	1-butanol	NaOH	UV-Vis	SIA	0.44 - 5.50 mmol L^{-1}	titration without mixing or dilution; agarose bead suspension; automated packing and disposal in a flow cell	131
phenothiazine derivatives	PF	acetous $HClO_4$	pot	FIA	2.0 - 20.0 mg mL^{-1}	sample preparation: crushing, homogenization, and issolution in acetic acid; pseudo titration	132
sulfate	ground, drinking waters	$BaCl_2$	pot	FIA	5 - 400 mg L^{-1}	in-line cation-exchange column for interfering species removal; pseudo titration	60
sulfuric acid	products from zinc refinery	$Na_2B_4O_7$	UV-Vis	DFA	35 - 75 g L^{-1}	sample preparation: dilution; calibration based on first-order derivative plots; pseudo titration	133
sulphide, cysteine, thiol-containing species	PF	ClO^-	CL	FIA	1.0 - 100 mmol L^{-1}; lower limit for sulphide: 0.1 mmol L^{-1}	redox pseudo titration; two different redox processes	134

Table 2. (Continued)

Analyte	Sample	Titrant	Detection technique	Sort	Titration range or detection limit	Remarks	Ref.
vitamin C	PF	Ce(IV)	UV-Vis	SIA	30 - 200 ppm	factorial design for optimization; pseudo titration	135
zinc	products from zinc refinery	EDTA	pot or UV-Vis	DFA	3.5 - 8.0 (pot.) and 2 - 8 (UV-vis) g L^{-1}	sample preparation: dilution; calibration based on first-order derivative plots; pseudo titration	133

AA: ascorbic acid; PF: pharmaceutic formulations; BrO^-: hypobromite; DCPI: 2,6-dichloroindophenol; MnO_4^-: permanganate; IO_3^-: iodate; K4E7: monoclonal atrazine antibodies; TPB: tetraphenylborate; SIA: sequential injection analysis; MCFA: multicommuted flow analysis; DFA: discontinuous flow analysis; r-FIA: reverse flow-injection analysis. Other abbreviations as in Table 1.

Table 3. Selected applications of batchwise flow titrations

Analyte	Sample	Titrant	Detection Technique	Titration range or detection limit	Remarks	Ref.
AA, iron(II)	feed supplement	I_2, Ce(IV)	biamp, pot	0.5 - 10 mmol L^{-1} AA, 2.0 - 10.0 mmol L^{-1} Fe	grind, suspension in water, filtration and dilution as sample preparation; monosegmented flow system; coulometric titrantion; extrapolation of the linear segments before and after the equivalence point (ascorbic acid), and second derivative of the titration curve (Fe) for end-point determinations	62
Acidity	fruit juices, vinegar	NaOH	L-photo	-	sample preparation: centrifugation and filtration; twin LED-based photometer; feed-back mechanisms for real-time modifying the titration course; true titration	136
Acidity	olive oil	KOH in n-propanol	L-photo	0.1 - 1.5% (w/v)	no sample preparation; multicommuted system; in-chamber detection; true titration	63

Analyte	Sample	Titrant	Detection Technique	Titration range or detection limit	Remarks	Ref.
Acidity	red wine	NaOH	L-photo	5.70 - 8.50 g L^{-1} tartaric acid	sample preparation: Ar bubbling for CO_2 removal; feed-back mechanism for real-time processing titration course (titrant volume variations); true titration	137
Acidity	Vinegar	NaOH	UV-Vis	47.1 - 53.4 g L^{-1} acetic acid	monosegmented flow system; end point estimated after successive titrant volumetric variations, selected by a Fibonacci algorithm	53
Acidity	Vinegar	NaOH	pot	0.15 - 1.2 mol L^{-1} acetic acid	multicommuted flow system; sequential additions of increasing titrant and decreasing titrand volumes to a mixing chamber; end-point determined by second derivative method; true titration	138
Acidity	white wine	NaOH	UV-Vis	5.2 - 7.3 g L^{-1} tartaric acid	no sample preparation; end point estimated after successive titrant volumetric variations, selected by a Fibonacci algorithm	25
alkalinity	wastewater treating plants	HCl	pot	-	titration to pH 5.75 and 4.3 (partial and intermediate alkalinities) for distinguishing buffering contributions of bicarbonate and volatile acids in anaerobic digesters; no prior calibration; automatic titrator	139
amines, their hydrochloride salts	PF	$HClO_4$ plus $Hg(CH_3COO)_2$	UV-Vis	1 - 20 mmol L^{-1}	non-aqueous pseudotitration	64
Br_2 index, Br_2 number	liquid hydrocarbons	Br_2	biamp	50 - 100; 500 - 1000 mg Br_2/100 g sample	no sample preparation; pseudo titration; coulometric titrant generation; signal threshold for end-point determination	140
Br_2 number	olefins, petroleum distillates	Br_2	UV-Vis	-	sample preparation: dissolution in organic solvent; in-chamber coulometric titrant generation; in chamber monitoring; FIA and SIA systems	141

Table 3. (Continued)

Analyte	Sample	Titrant	Detection Technique	Titration range or detection limit	Remarks	Ref.
calcium	Water	EDTA	UV-Vis	-	SIA system for successive sample volumetric variations; first order derivative signals for data treatment	65
chloride	bottled waters	$AgNO_3$	pot	2 - 110 mg L^{-1}	true precipitation titration; multicommuted system; sequential additions of increasing titrant and decreasing titrand volumes to mixing chamber; Gran plot for end point determination	142
hydroxide, carbonate	$NaAlO_2$	sulfosalicylic acid plus $BaCl_2$	L-photo	50 - 300 g L^{-1} NaOH; 0 - 50 g L^{-1} CO_3	in-chamber monitoring; end-point for hydroxide: acid-base indicator, end point for $BaCO_3$: attainment of quantitative dissolution	91
metabisulfite, starch	corn syrups	I_2	UV-Vis	0.35 - 29.0 mmol L^{-1} metabisulfite	coulometric titration; in chamber monitoring; single or conjunct (with starch) determinations; pseudo titrations involving elapsed times (metabisulfite) and slopes (starch) of the monitored signals	143
metronidazole	PF	$HClO_4$	UV-Vis	-	open chamber; pseudo titration; monitoring of flow leaving the titration chamber; fully computer-controlled flow system	61
nickel	outcome from metallurgy	Zn	L-photo	10 - 70 g L^{-1}	in-chamber monitoring; back titration (Ni reaction with EDTA and titration of EDTA excess with Zn)	144
water	ethanol, methanol	Karl Fisher	biamp	0.02 - 0.5% w/w	monosegmented flow; true titration	66

Abbreviations as in Tables 1 and 2.

Other specific applications refer to the estimation of enzyme activities, and the potentiometric pseudo titration to determine enzyme activity of lipase type B from *Candida Antarctica* [92] can be selected as an example. Triacetin and tributyrin substrates were hydrolyzed in a buffered phosphate environment, and the concentrations of the buffer base component at different hydrolysis times were titrimetrically evaluated.

To this end, successive aliquots were sampled and inserted into the carrier stream (hydrochloric acid plus potassium chloride) of the flow titrator, which was calibrated with butyric acid and acetic acid. For a 15 min hydrolysis, the detection limit of enzyme activity was determined as 0.07 U mL^{-1}. The specific activities of lipase B for the hydrolysis of tributyrin and triacetin were determined as 16 ± 2 and 2.0 ± 0.2 U per mg of commercial lipase preparation.

It should be emphasized that flow-based titrations are analogous to some flow-based calibration strategies relying on successive controlled additions to the sample [93], such as the standard addition method (SAM), as both titration and SAM can be implemented in the flow set-up. As a more robust analytical calibration is aimed at, a generalized calibration strategy is welcome. Thus, research taking advantage of the potential of this hyphenation is nowadays experiencing an amazing development.

7. TRENDS

Miniaturization has been often stressed as an outstanding tendency in flow analysis, as the smaller the flow analyzer, the better adherence to the 12 Green Chemistry principles. In fact, the reduced consumptions of sample and other required solutions (reagents, diluent, solvents), the efficient cost-benefit ratio, the system portability, the enhanced operator safety, and the improvement of some analytical figures of merit are profitable aspects inherent to miniaturized flow analyzers. These aspects led to the amazing development of micro-flow analysis [145, 146].

In flow titrations, however, extreme miniaturization is not common [145]. In fact, the flow regime associated to micro-flow analyzers tends to be strictly laminar (very low Reynolds numbers), thus adjacent fluid elements may present pronounced differences in sample-to-tritant volumetric ratio. Monitoring a larger fluid element and, eventually, improving the mixing conditions circumvents this drawback. For attaining good mixing conditions, chamber-like components exploiting stirring, ultrasound irradiation, pulsed flows, flow reversals, etc., have been exploited. These strategies are typical to ordinary flow analyzers [3].

In spite of these aspects, micro-flow titrations have been proposed, and a rapid and simple acid-base titration [147] accomplished in a novel microfluidic paper-based analytical device (μPAD) can be selected for illustrative purposes. The μPAD comprised several reservoirs for reaction and detection, to which different titrant amounts, and a constant amount of phenolphthalein were added. The alkaline sample dropped onto the μPAD center spread towards the reaction reservoirs, where the titrant neutralized it. When the sample alkalinity stoichiometrically surpassed the titrant in the reaction reservoirs, non-neutralized hydroxide ion penetrated the detection reservoirs, promoting color formation with the phenolphthalein. The number of detection reservoirs with no color change was associated with the titration end-point. Titration was accomplished within 1 min, and end-point was visually determined. Acidic samples were also analyzed by using sodium carbonate as titrant. The μPADs were stable for more than one month when stored in darkness at room temperature. Acidic hot spring waters were in-field analyzed and results agreed with those obtained by a classical acid-base titration.

An analogous strategy was recently proposed for the complexometric titration of calcium and magnesium in natural waters [148]. Other microflow titrators involving manifold architectures typical from e.g., μTAS, μFIA, lab-on-chip, etc., [149, 150] should be mentioned in the context.

Further developments and applications of flow titrators with the architecture of ordinary flow analyzers tend to be more pronounced, in view of the excellent characteristics of this analyzer, the adherence to the 12

Green Chemistry principles, the feasibility for *in situ* assays, and the easy handling of organic solvents.

Other developments will probably be focused on simultaneous determinations involving titrimetric and non-titrimetric methods, as well as on wide-range titrations. This later trend might exploit titrant and/or sample resampling (zone sampling), peak height measurements for analysis of more diluted samples and pseudo titrations for the more concentrated ones, etc.

The advantages arising from the exploitation of kinetic aspects in flow analysis are well known. Thus, one can foresee that applications of flow titrations with catalytic end-point will experience an amazing growth. Moreover, studies on the feasibility of using relatively slow titrand/titrant reactions will probably be of relevance in the near future.

These tendencies will certainly include expert flow systems with multicommutation for real-time flow and manifold programming.

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