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Zone trapping/merging zones in flow analysis: A novel approach for rapid assays involving relatively slow chemical reactions

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ABSTRACT

A novel strategy for accomplishing zone trapping in flow analysis is proposed. The sample and the reagent solutions are simultaneously inserted into convergent carrier streams and the established zones merge together before reaching the detector, where the most concentrated portion of the entire sample zone is trapped. The main characteristics, potentialities and limitations of the strategy were critically evaluated in relation to an analogous flow system with zone stopping. When applied to the spectrophotometric determination of nitrite in river waters, the main figures of merit were maintained, exception made for the sampling frequency which was calculated as 189 h^{-1}, about 32% higher relatively to the analogous system with zone stopping. The sample inserted volume can be increased up to 1.0 mL without affecting sampling frequency and no problems with pump heating or malfunctions were noted after 8-h operation of the system. In contrast to zone stopping, only a small portion of the sample zone is halted with zone trapping, leading to these beneficial effects.

1. Introduction

The increasing acceptance of flow analysis [1] is due to the versatility, ruggedness and portability of the flow analyzers, the diversity and number of samples to be assayed and the good analytical figures of merit. The potentialities of flow analysis become more evident in relation to methods involving fast chemical reactions, thus allowing high-speed analysis. For relatively slow reactions however long sample residence times in the analytical path are required and this aspect may limit the analyser performance, especially if the analytical sensitivity is critical.

Stream segmentation is a key feature for attaining long residence times without pronounced sample dispersion, and this is the essence of segmented flow analysis [2]. Reducing the total flow rate is also advantageous for increasing the sample residence time, but this may decrease sampling frequency and/or increase carry-over. Carry-over can be mathematically compensated [3,4], but error propagation effects may hinder accuracy. Another possibility to attain long residence times in unsegmented flow analysis involves halting the sample zone, and this is efficiently accomplished by exploiting multi-commutation [5]. To this end, zone stopping and zone trapping offer interesting possibilities.

Zone stopping (Fig. 1a) – originally named as *stopped flow* – was proposed for the determination of glucose in blood serum [6]. The sample and the enzymatic reagent were simultaneously inserted into rapidly flowing convergent carrier streams, and the established zones interacted with each other as a consequence of the stream merging. After a pre-set time interval, when the sample zone was passing through the main reactor, the peristaltic pump was switched OFF and the sample zone was stopped. During the STOP period, sample broadening practically ceased while the development of the chemical reactions was not impaired. A higher degree of reaction completion was then attained, thus improving the analytical sensitivity. Thereafter, the flow was restored and the sample zone was handled and monitored as in an ordinary flow injection system. Zone stopping without switching the pump OFF has been also accomplished by exploiting commutation [7,8].

Alternatively, the sample zone can be stopped in the detector. The variation in analytical signal during the STOP period is then considered as the measurement basis and the approach is valuable for kinetic determinations. For linearly time-dependent analytical signals the slope of the recorded curve reflects the analyte content in the sample and blank running is not needed [9]. For multi-parametric determinations, multivariate calibration relying on the analytical signals associated with different monitoring times can be used [10]. In luminometric (*e.g.* chemo-luminescence, bio-luminescence, fluorimetry) analytical procedures, stopping the sample in the detector permits to increase the available time for signal integration, leading to a better counting statistics, thus enhanced analytical precision. Moreover, the STOP period can be started at different instants in order to monitor different portions of the sample zone with distinct sample/reagent volumetric

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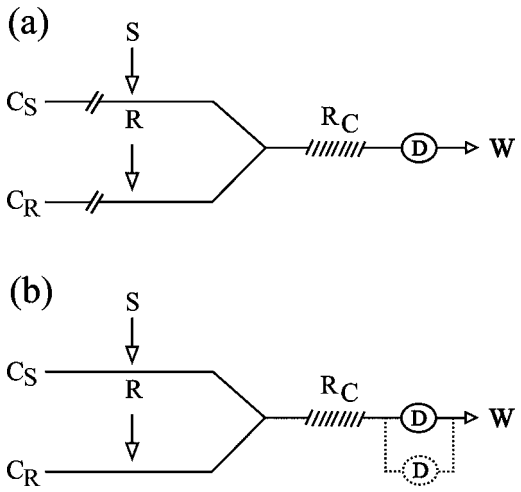

Fig. 1. Didactic representation of the flow systems with zone stopping (a) and zone trapping (b). Vertical arrows = sample/reagent injections; // = intermittent streams; dotted lines = alternative position of the flow cell. Other symbols as in Fig. 2.

fractions. This potentiality may provide additional information on the reaction kinetics, thus expanding the potential of the zone stopping approach [11].

Zone trapping was proposed for the determination of ammonium in natural waters [12]. The sample aliquot was inserted into a chemically inert carrier stream [13] and the reagents were added by confluence. When the sample zone was flowing through the main reactor, the commutator was switched in order to remove this reactor from the analytical channel, thus trapping the sample zone. The sample zone was kept under static conditions during a pre-set time interval, and heating during the TRAP period was efficiently accomplished. Thereafter, the reactor was re-inserted into the main channel, and the sample zone was directed towards detection. Analogously to zone stopping, trapping the sample zone in the detector is also feasible. Development led to the proposal of the *stopped-in-loop flow analysis* [14].

With multi-commutation, the potentialities of zone trapping are expanded, as the trapped zone can be stepwise released as different aliquots, each one corresponding to a different mean concentration and a different TRAP period [5]. This innovation is useful for, *e.g.* widening the dynamic concentration range, implementing the standard addition method, attaining the analytical curve with a single standard solution, and implementing prior assays [15]. Dual zone trapping is feasible too, and the spectrophotometric determination of amiloride hydrochloride in pharmaceutical formulations [16] is a good example. The sample was inserted twice and a three-way valve selected the reactor towards which the sample zone was directed and trapped. Sampling frequency almost doubled, as when a given sample zone was trapped the other was flowing and *vice versa*. Multiple zone stopping can also be exploited [17]. To this end, a distribution valve directs the sample zone towards one of *n* parallel reactors, where it was trapped. Sampling frequency undergoes then an *n*-fold increase.

Regardless if zone stopping or trapping is concerned, the sample can be continuously aspirated towards detection thus establishing the steady situation inherent to the sample "infinite volume" [18]. Halting the sample zone can be accomplished at any instant after achievement of this situation; manual operation of the flow system is then feasible. The applicability of the approach has been however scarce, perhaps due to the low versatility of the flow system.

Zone stopping was conceived in relation to merging zones [6]. To the best of the authors' knowledge, exploitation of zone trapping/merging zones has not yet been proposed. Without merging zones, the reagent is continuously pumped towards waste and this aspect limits the applicability of the zone trapping to analytical procedures involving relatively inexpensive, harmless and/or non-hazardous reagents. This is probably the main reason why zone trapping has been less applied relatively to zone stopping.

The goal of the present work was then to critically examine the characteristics of zone stopping and zone trapping with merging zones, in regard to sampling frequency, sample inserted volume and operational aspects of the flow analyzers. To this end, the flow system for the spectrophotometric determination of nitrite in natural waters [19] was selected as a model.

2. Experimental

2.1. Solutions

The solutions were prepared with chemicals of analytical-grade quality and deionised water.

The reagent was a 2.0% (m/v) sulphanilamide, 0.10% (m/v) NED [N-(1-naphthyl)-ethylenediammonium dichloride] plus 8.0% (v/v) H_3PO_4 water solution [19]. It was stored in a refrigerator. In order to avoid the establishment of undesirable concentration gradients along the sample zone, water and an 8.0% (v/v) H_3PO_4 solution were used as the sample and reagent carrier streams (Fig. 1), respectively. The dye solution (S_D) was prepared by combining a 0.40 mg L^{-1} NO_2 solution and the above mentioned reagent in the 5:1 volumetric ratio.

The stock standard solution (100.0 mg L^{-1} NO_2) based on $NaNO_2$, was standardized against potassium permanganate, preserved with a few drops of chloroform and kept in a refrigerator [19]. Working standards covering the 0.00–0.40 mg L^{-1} NO_2 range were daily prepared in water.

River water samples were collected into polyethylene containers, filtered through 0.6 μm cellulose membrane, preserved with sulphuric acid and analyzed immediately [20].

2.2. The flow systems

The main components of the flow systems were a model IPC-4-V2.00 Ismatec peristaltic pump, a three-piece injector-commutator [21] and a USB 2000 UV-Vis Ocean Optics spectrophotometer with an Ultem Z-shaped flow cell (inner volume = 70 μL, optical path = 10 mm). The control software provided by the spectrophotometer manufacturer was used for data acquisition and treatment. The in- and out-let ends of the flow cell were attached to the movable central portion of the injector-commutator (Fig. 2b), and the transmission lines were kept as small as possible (10 cm). PEEK connectors and accessories were also used. Sampling loops, coiled reactors and transmission lines were made of polyethylene tubing (i.d. = 0.8 mm) of the non-collapsible wall type. Wavelength and integration time were set as 540 ± 1 nm and 80 ms, respectively.

The system in Fig. 2a exploits the zone stopping approach. In the situation specified, the sample and reagent solutions are filling their sampling loops that define the inserted volumes. The sample excess is wasted, and the reagent excess is stored in the reagent recovery vessel. Switching the injector-commutator inserts the sample and reagent aliquots into their corresponding carrier streams. The established zones merged at confluence site, and the modified Griess reaction [22] proceeds inside the main reactor yielding a coloured chemical species. When the most concentrated portion of the sample zone is flowing through the detector, the peristaltic pump is switched OFF, stopping this sample portion inside the detector during a pre-selected STOP period. Thereafter, the flow is restarted and the sample zone is directed towards waste. The absorbance related to the end of the STOP period is proportional to the nitrite concentration in the sample.

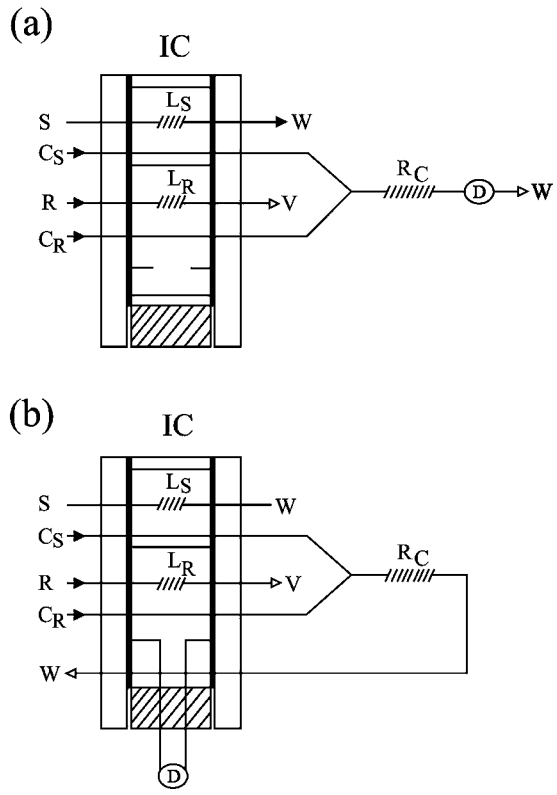

Fig. 2. Flow diagrams of the flow systems with zone stopping (a) and zone trapping (b). *S* and *R* = sample and reagent solutions; C_S and C_R = sample and reagent carrier streams (5.0 and 1.0 mL min^{-1}); L_S and L_R = sample and reagent loops (100 cm); IC = injector-commutator; R_C = coiled reactor (25 cm); *D* = spectrophotometric flow cell; *V* = reagent recovery vessel; *W* = waste; black arrows = sites where pumping is applied; empty arrows = flow directions; dashed area = alternative position of the IC central sliding bar. For details, see text.

The flow system in Fig. 2b exploits the zone trapping approach. When the central portion of the sample zone is flowing through the detector, the injector-commutator is switched, allowing the main stream to bypass the detector towards waste and the sample zone inside the detector to be trapped. After the TRAP period, the injector-commutator is switched again, re-inserting the trapped portion of the sample zone into the main carrier stream that pushes it towards waste.

2.3. Procedure

The flow systems in Fig. 2 were used for a critical examination of zone stopping and zone trapping. The manifold geometry, flow rates and reagent concentrations were the same as in the original article [19] and the components associated with the determination of nitrate were not used.

Initially, the mean sample residence time, t_r (time interval between instants of sample insertion and peak maximum achievement [23]), the wash time, t_w (time interval between peak maximum achievement and baseline restoration [23]), sampling frequency, *F*, analytical sensitivity and measurement repeatability were estimated for the original flow system aiming at a comparison with the systems including sample halting. The original flow system was similar to that in Fig. 2a, did not involve sample stopping and comprised a 150-cm coiled reactor.

For the 2a and 2b systems, the influence of reactor length, sample inserted volume and halting period was investigated between 10 and 200 cm, 120 and 1000 μL ($25 < L_S < 200$ cm) and 0–60 s, respectively. The working standard solutions were run in triplicate and the reagent loop (L_R) was maintained as 100 cm to avoid the need for L_R replacement after every modification in L_S. In these experiments, the length of the main reactor was 25 cm and the STOP/TRAP period was 15 s. Under all the investigated conditions, measurement repeatability ($n = 10$), t_w and t_r values, the interaction between the merging zones and sampling frequency were evaluated. Evaluation of zone interaction involved successive insertions of the S_D coloured solution via L_S and then via L_R and analysis of the recorded signals.

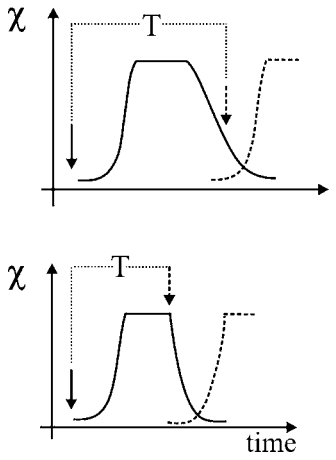

Fig. 3. Didactic representation of successive analytical signals recorded for the flow systems with zone stopping (upper) and zone trapping (lower). Figure refers to the S_D dye solution. χ = volumetric fraction (dimensionless concentration); arrows = insertion instants; full, traced lines = present, next sample; *T* = analytical period [23].

For estimating sampling frequency, the t_w value, the halting period and/or the t_r value were taken into account (Fig. 3). For the original system, this parameter [$F = 3600 \times t_w^{-1}$] was estimated as in ordinary flow injection systems [23], and expressed in h^{-1}. For the 2a system, only the STOP period, t_{STOP}, and the t_w value were considered, as wasting the trailing edge of the sample zone was done after the STOP period. The t_r value was not considered as it was shorter than t_w and the related processes occur simultaneously. Sampling frequency was then estimated as [$F = 3600 \times (t_{STOP} + t_w)^{-1}$]. For the 2b system, sampling frequency could be estimated as [$F = 3600 \times (t_{TRAP})^{-1}$] if sample plus reagent insertions were independent of zone trapping; the approach would require two commutators with discrete operation. As a single injector-commutator was available, these processes were simultaneously performed after every commutator switching, and sampling frequency was reduced to [$F = 3600 \times (t_{TRAP} + t_r)^{-1}$]. The t_w value was always shorter than t_r (see also Table 1), therefore did not influence the sampling frequency.

After dimensioning, the flow systems in Fig. 2 were applied to the analysis of river water samples and the main analytical figures of merit were evaluated. A comparison with a reference

Table 1
Influence of sample inserted volume (*V*/μL) on the analytical signal (*A*/absorbance) and sampling frequency (*F*/h^{-1}). Data refer to a 0.20 mg L^{-1} NO_2 solution inserted into the 2a (STOP) and 2b (TRAP) flow systems with a 25-cm coiled reactor; t_w and t_r = wash time and mean sample residence time in s. For details, see text.

V	*A*	STOP		TRAP	
		t_w	F^a	t_r	F^b
120	0.223	4	189	4	189
250	0.523	7	164	4	189
500	0.728	10	144	4	189
1000	0.838	14	124	4	189

[a] [$3600 \times (t_{STOP} + t_w)^{-1}$].
[b] [$3600 \times (t_{TRAP} + t_r)^{-1}$].

method was not needed, as the conditions for reaction development (reagent concentrations, sample dispersion, timing) were maintained for the different flow systems.

3. Results and discussion

3.1. Influence of the main parameters on the system performance

The t_r value in a flow system is strongly dependent on total flow rate and manifold dimensions. The original system [19] was designed with a 6.0 mL min^{-1} total flow rate and a 150-cm reactor length, thus this parameter was determined as 9.7 s. Accordingly the t_w value was measured as 31 s, meaning a sampling frequency of 116 h^{-1}. This frequency could be improved by adding an intermittent wash stream at a high flow rate immediately after peak maximum attainment [24], but the system would become less rugged and more susceptible to the Schlieren noise.

Alternatively, halting of the sample zone in the main reactor or in the detector can be exploited. As several zone stopping or trapping were not aimed at, the later possibility was selected, namely sample halting in the detector. The available time for reaction development was then closely related to the halting period. Thus, the main reactor should be as short as possible in order to minimise broadening of the sample zone, yet long enough to permit suitable mixing conditions. Increasing this parameter deteriorated sensitivity as a consequence of the higher dispersion involved and also increased the t_w value, thus impairing sampling frequency. The aspect was more limiting for zone stopping, as this strategy involves passage of the entire sample zone through the detector. With zone trapping, the front and trailing edges of the sample zone are not trapped, but directly discarded during the TRAP period. Consequently, t_w is shorter and sampling frequency is higher relatively to the zone stopping strategy.

Sensitivity and signal-to-noise ratio proved to be also dependent on the length of the main reactor. For too short reactors (<15 cm) good mixing conditions were not attained and a drop in repeatability of the analytical signal was noted (peak height r.s.d. >5%). On the other hand, a slight decrease in analytical signal was noted for too long (>150 cm) reactors, as the increase in the degree of reaction completion was not enough for compensating the reduction in peak height due to the sample dispersion. As a compromise between sensitivity, sampling frequency and mixing conditions, length of the main reactor in the 2a and 2b flow systems was selected as 25 cm. In this situation, a thin baseline (<0.001 absorbance) was recorded and Schlieren noise was not relevant.

The halting period is an important parameter for the 2a and 2b flow systems (Table 1). Without halting of the sample zone, the degree of reaction completion was only 37.2%, due to the low t_r value (ca 4 s). Consequently, a pronounced increase in absorbance was noted during the halting period, for both zone stopping and zone trapping. The temporal increase in absorbance during the halting period followed an asymptotical function, approaching the maximum value after about 20 s. As about 95% of this value was already attained for a 15-s halting period, this value was selected. The available time for reaction development was then ca 19 s, therefore a higher degree of reaction completion relatively to the original system was attained, and this is a favourable aspect towards system ruggedness.

Although the degree of reaction completion and sample dispersion were similar for the 2a and 2b flow systems, a pronounced difference in the t_w value was verified, and parameter was always higher for the flow system with zone stopping (Table 1). This result can be explained by recalling that the trailing edge of the sample zone was directly discarded during zone trapping in the 2b flow system, but discarded through the detector after the zone stopping in the 2a flow system.

Table 2
Comparative results. Nitrite concentrations in natural waters as determined by the flow systems with zone stopping (STOP) and zone trapping (TRAP). Table refers to a 500-μL sample inserted volume. Data in mg L^{-1}; uncertainties based on three replicated measurements.

Sample	STOP	TRAP
1	0.103 ± 0.002	0.103 ± 0.003
2	0.200 ± 0.009	0.222 ± 0. 001
3	0.134 ± 0.002	0.137 ± 0.001
4	0.150 ± 0.001	0.154 ± 0.002

The sample inserted volume proved to be an important parameter too. Increasing this volume improved sensitivity due to the lowering of the sample dispersion, but increased the t_w value thus deteriorating sampling frequency (Table 1). This later effect was noted only for the 2a system, because the trailing portion of the sample zone was stopped inside the reactor and the related absorbance increased during the STOP period; moreover, the entire sample zone passed through the detector. As a compromise between sensitivity and sampling frequency, length the L_S for the 2a and 2b systems was selected as 100 cm, meaning inserted volumes of about 500 μL. In this situation, the sample volumetric fraction, χ, was determined as 0.86. For the 2b system, this loop could be increased up to 200 cm, reaching $\chi = 0.98$, as the wash time was unaffected by its variations. Moreover, 240 samples could be run per hour with sample and reagent insertions independent from the zone trapping. The higher sampling frequency of the 2b flow system is evident in Fig. 3.

The experiments involving insertion of the dye via L_S or L_R revealed that overlap between the sample and reagents zones was attained for all the investigated sample volumes. It should be stressed that, as the confluence configuration was involved, variations in the sample inserted volume did not influence the available time for reaction development [13].

It should be stressed that a distortion in recorded signal due to switching the peristaltic pump ON/OFF was observed for the 2a flow system. It was probably caused by the plasticity of the manifold (and pump) tubing, often referred to as an inertial effect, and may limit the signal-to-noise ratio. This drawback was not noted for the 2b system which involves continuous pump operation.

3.2. Application

The investigated systems present similar figures of merit, exception made for the sampling frequency that is better for the system with zone trapping (Table 1). As both the 2a and 2b system exploit the merging zones approach, a very low reagent amount (500 μg NED) was consumed per determination. No statistical differences on the sensitivities related to the 2a and 2b systems were found at the 95% confidence level, confirming that sample dispersion caused by analyte diffusion during the STOP period is negligible [6]. The sensitivity inherent to the 2b system could be slightly improved by increasing the sample inserted volume, as χ was 0.86 and the t_w value was almost independent on this volume. This possibility however did not hold for the 2a flow system, where use of larger sample volumes would cause a drop in sampling frequency (Table 1).

A typical analytical curve for both 1a and 1b flow systems is described by:

$$y = 1.36x - 0.0345 \quad (r = 0.9974; n = 5)$$

where y = analytical signal, in absorbance; x = concentration in mg L^{-1} NO_2.

With both flow systems, precise results were obtained in the analyses of river water samples (Table 2). No statistical difference between the involved procedures at the 95% confidence level were

found. Moreover, baseline drift was not noted during extended operation periods.

A noteworthy feature of the system with zone trapping is the continuous operation of the peristaltic pump. In this way, no heating or other malfunction was noted after 8 h of system operation.

4. Conclusions

A noteworthy advantage of the flow systems with zone trapping/merging zones is that sampling frequency tends to be independent from the sample inserted volume. The operational aspects of these flow systems permit larger sample inserted volumes to be used and a more complete development of the chemical reactions. As a consequence, sensitivity of flow-based procedures relying on relatively slow chemical reactions is then improved and stopping the peristaltic pump is not required. Another favourable characteristic arising from the continuous pump operation is that its life time is probably longer, as ON/OFF turning and eventual heating are not concerned.

Parallel experiments revealed that six- or four-way rotary valves can be used for building up the flow system with zone trapping. The related flow diagram is available upon request.

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