Exploiting Zone Trapping to Avoid Liberation of Air Bubbles in Flow-based Analytical Procedures Requiring Heating

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In flow-based analytical procedures requiring heating, liberation of air bubbles is avoided by trapping a sample selected portion into a heated hermetic environment. The flow-through cuvette is maintained into a temperature-controlled aluminium block, thus acting as the trapping element and allowing real-time monitoring. The feasibility of the innovation was demonstrated in the spectrophotometric catalytic determination of vanadium in mineral waters. Air bubbles were not released even for temperatures as high as 95°C. The proposed system handles about 25 samples per hour, requires only 3 mg p-anisidine per determination and yields precise results (r.s.d. = 2.1%), in agreement with ICP-MS. Detection limit was evaluated (3.3 σ criterion) as 0.1 μg L⁻¹ V.

Keywords Flow analysis, zone trapping, heating, spectrophotometry, vanadium, natural waters

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Introduction

Air bubbles in the analytical path are inherent to segmented flow systems, as they play a positive role in lessening sample axial dispersion (thus tailing effects), improving the mixing conditions and scrubbing the manifold tubing inner walls. In unsegmented flow systems, however, the presence of air bubbles should be avoided, as they may affect sample dispersion and detection. Although worldwide recognized as a nuisance, this drawback has been scarcely mentioned. Several situations may lead to the presence of gas bubbles in unsegmented flow analyzers:

a) Inadequate sealing of the connectors; aspect more pronounced in flow analyzers relying on solution aspirations.

b) Air inlet in the analytical path during sample replacement. This drawback is circumvented either by stopping the aspirating flow when the sampling arm is moving from one sample cup to another (time-based insertion) or by avoiding the air retention inside the sampling loop (loop-based-insertion). In either case, complexity of system operation is increased.

c) Presence of dissolved gases in the flowing streams. This aspect is more severe when highly concentrated solutions and/or large gas dissolved amounts are involved. Solution de-gassing is generally used but the remediation is temporary, as the involved gas/liquid equilibrium tends to be re-established.

d) Gas formation during reaction development; aspect more pronounced in relation to in-line sample preparation.

e) Heating of the reaction medium.

Bubble release associated with situations (c – e) can be minimized by increasing the hydrodynamic pressure of the reaction medium. To this end, a restrictor coil built up with a long and thin tube can be placed after the detection unit. High temperatures can be adjusted without release of air bubbles, and immersing the main reactor in a thermostatic bath with the temperature adjusted to up to 140°C is feasible. The spectrophotometric determination of sulphate in river waters can be selected as an example: presence of bubbles in the analytical path were avoided by using a 10-m long restrictor coil with an inner diameter of 0.5 mm. The innovation has been scarcely used, however, probably because of the increased possibility of system blockage by suspended material in the sample zone and the need for a high performance fluid propeller device.

Another possibility refers to exploitation of zone trapping. The sample is inserted into its carrier stream, confluent reagents are added and the sample zone is directed towards a heated reactor. When the most concentrated sample portion is flowing through this reactor, it is removed from the main channel, trapping the selected sample portion during a pre-set trapping period (t₁). As both ends of this reactor are closed, heating promotes an increase in hydrodynamic pressure, thus release of air bubbles inside the trapping element is not expected. In fact, the principle of the pressure cooker is involved.

Zone trapping was originally proposed for the spectrophotometric determination of ammonium ions in natural waters. The trapping element was a coiled reactor immersed in a 38°C water bath. Bubbles were eventually released, however, leading to a sharp recorded peak (Fig. 1 in the above quoted reference). The bubble release probably occurred inside the transmission line between the heated reactor and flow cell, where the hydrodynamic pressure and temperature were lower.

The drawback can be minimized by trapping the selected sample portion inside a heated flow-through detector. The feasibility of using the flow cell as a trapping device was recently demonstrated in the spectrophotometric determination of nitrite in natural waters but heating of the reaction medium was not needed.

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The purpose of the present work was to demonstrate the feasibility of using a heated flow cell as the trapping element (Fig. 1). We also sought to compare the strategies involving heated reactor or heated flow cell.

As an application, the spectrophotometric catalytic determination of vanadium in mineral waters was selected. The method involves the V(V)-catalysed oxidation of p-anisidine by bromate, activated by Tiron, and carried out under heating and acidic conditions. As the innovation is compatible with zone merging, this approach was also exploited in order to reduce the consumption of p-anisidine.

**Experimental**

**Samples, standards, reagents**

The solutions were prepared with distilled-deionized water (specific conductance < 0.1 μS cm⁻¹) and reagents of analytical grade quality. Mineral water samples were purchased in a local supermarket and analyzed as received.

The standard stock solution (1000 mg L⁻¹ V) was prepared by dissolving 0.8925 g V₂O₅ in 10 mL of a 1+1 (v/v) HCl solution and filling the volume to 500 mL with water. Working standard solutions, also 0.01 mol L⁻¹ HNO₃, were prepared to cover the 0.00 – 6.00 μg L⁻¹ V range.

The reagent stock solution (0.25 mol L⁻¹ p-anisidine) was prepared by dissolving 6.16 g of p-anisidine (FW = 123.16, Aldrich) in a 3.0-mol L⁻¹ HCl solution and filling the volume to 200 mL with this acidic solution. The R₁ working reagent (Fig. 2) was a daily-prepared 0.1 mol L⁻¹ glycine solution; the pH was adjusted to 2.5 with a 10-mol L⁻¹ NaOH solution. The R₂ masking reagent was a 0.3-mol L⁻¹ NH₄F solution prepared by dissolving 0.55 g NH₄F in 50 mL of water. The R₃ oxidising reagent was a 0.05-mol L⁻¹ bromate solution prepared daily by dissolving 0.418 g KBrO₃ in 50 mL of water. The Tiron reagent (R₄) was a 0.1-mol L⁻¹ Tiron solution prepared by dissolving 1.66 g of 1,2-dihydroxy-3,5-benzenedisulfonic acid disodium salt monohydrate (FW = 332.22, Aldrich) in 50 mL of water.

The S and R₁ carrier streams were 0.01 mol L⁻¹ HNO₃ and water, respectively.

**Apparatus**

The main components of the flow system were a Model USB 2000 Ocean Optics UV-Vis spectrophotometer furnished with a Model 178 OS Hellma flow cell (10 mm optical path, 80 μL inner volume), the sliding bar injector-commuter used in earlier work and a Model IPC 08-R Ismatec peristaltic pump furnished with Tygon pumping tubes. Wavelength was set at 503 nm.

Sampling loops, coiled reactors and transmission lines were built up with 0.8 mm i.d. polyethylene tubing. The inlet and outlet ends of the RC₃ reactor (Fig. 2a) or flow cell (Fig. 2b) were attached to the movable central portion of the injector-commuter in order to permit leaping. Perspex connectors and accessories were also used.

The RC₃ reactor (Fig. 2a) was built up by winding 200 cm of polyethylene tubing around an aluminium rod immersed in a tubular-shaped (i.d. = 7 cm; height = 10 cm) thermostated bath. The flow cell (Fig. 2b) was placed inside a cuboidal aluminium block (Fig. 3) with 6-cm height, 4-cm width and 6-cm length. The block temperature was maintained by a Model TIC-17RGTi electronic temperature controller (Full Gauge, Brazil). Optical fibre cables were attached to both sides of this block for radiation transmission from the source to the flow cell and from there to the detector. Heating of the aluminium block was accomplished by a 100 W, 220 V cartridge heater (Dennex, Discetic).
The feasibility of the novel approach for heating in flow analysis was demonstrated by designing the flow system in Fig. 2a. After addition of all reagents, the handled sample zone reached the leaping flow cell. When its most concentrated portion was flowing inside it, IC was switched, trapping this portion under heating conditions. During the \( t_1 \) period, a continuous increase in absorbance was monitored (Fig. 1, inset). The absorbance at the end of the trapping period constituted the measurement basis. Next IC switching re-inserted the handled sample zone into the main stream that directed it towards waste.

**Procedure**

The flow system in Fig. 2a was dimensioned to provide limited sample dispersion.\(^{14}\) Flow rates of the sample and R₁ reagent carrier streams were selected as 3.0 and 0.75 mL min\(^{-1}\), respectively, in order to avoid a pronounced sample dilution at the \( x \) confluence site. Flow rates of the R₂ - R₄ confluent reagent streams were set at 0.4 mL min\(^{-1}\), as preliminary experiments confirmed that for lower flow rates (thus more concentrated reagents) their addition became erratic,\(^{15}\) deteriorating the mixing conditions. Sampling loops were set as 150 and 50 cm (approximately 750 and 250 \( \mu \)L). Their ratio were slightly higher than the flow rate ratio (4.0) in order to guarantee a good overlap of the sample and R₁ reagent zones. With larger inserted volumes, conditions tend to become more steady in the central portion of the flowing zone, allowing for the use of a manually operated commuter without deteriorating the measurement repeatability.

The RC₁ reactor selected length as 50 cm in order to avoid carry-over. On the other hand, the RC₂ reactor length was not critical, as this reactor was outside the analytical path; a 100-cm reactor was then set. The RC₃ reactor length was set as 200 cm in order to avoid significant dispersion inside the \( T \) transmission line and flow cell. In this regard, the transmission line was set as short as possible (15 cm). A 150-cm restrictor coil (P - Fig. 2a) was selected. In this situation, a glycine bath temperature of 80°C could be set without the release of gas bubbles, as demonstrated in preliminary experiments.

After dimensioning, the flow system in Fig. 2a was used for investigating the influence of the main parameters involved in the vanadium determination (reagent concentrations, pH of the reaction medium, glycine bath temperature and timing). Selectivity was evaluated by handling a 5.00-\( \mu \)g L\(^{-1}\) V solution (also 0.1% v/v HNO\(_3\)) prepared with a potential interfering chemical species (Fe(III), Mn(II), Cu(II), Zn(II), Ni(II), Co(II), Al(III), Pb(II), Ti(IV), Si(IV), based on Fe₂O₃, MnO₂, CuSO₄·5H₂O, ZnSO₄, NiSO₄·7H₂O, CoSO₄·7H₂O, AlCl₃, CH₃COOH, Pb, TiO₂ or Na₂SiO₃·5H₂O respectively). For each potential interfering species, the highest tested concentration was that yielding a ≥ 10% deviation in the recorded peak height.

Thereafter, the flow system was simplified in order to permit zone trapping inside the heated flow cell and the main advantageous characteristic of the resulting flow system (Fig. 2b), namely the feasibility of using a high bath temperature in a less complex manifold was demonstrated.

The influence of temperature was then re-investigated under a wider range (25 – 90°C). As the inner volume of the analytical path in the 2b flow system is smaller in relation to the 2a system, an investigation on the influence of the sample volume (50 – 150 cm) was carried out.

Thereafter, the flow system in Fig. 2b was applied to the analyses of mineral water samples. The main figures of merit (sensitivity, repeatability, detection limit, reagent consumption, sampling rate) were evaluated, and accuracy was assessed by running some samples already analyzed by ICP-MS.\(^{16}\)
Results and Discussion

Flow system with zone trapping inside the main reactor

Regarding the influence of the main parameters related to the vanadium determination, timing is accountable for selecting the most concentrated portion of the handled sample zone to be trapped and for defining the mean available time for reaction development. To this end, the time interval between instants of sample insertion and zone trapping ($t_2$) was varied from 5 to 55 s in 5-s steps. As best sensitivity was noted for the $20 < t_2 < 35$ s, the $t_2$ value was selected as 25 s. The $t_1$ trapping period was varied between 1.0 and 6.0 min and sensitivity underwent a continuous improvement as this parameter increased (Fig. 4a). As a compromise between sensitivity, sampling rate and expected vanadium content in the samples, the $t_1$ value was selected as 2.0 min. A noteworthy feature of the proposed procedure is that sensitivity can be adjusted by properly modifying the $t_1$ value.

Temperature manifested itself as a relevant parameter, as a pronounced improvement in sensitivity was noted by increasing the glycerine bath temperature (Fig. 4b). This parameter could not be increased at will, however, as air bubbles were released under higher temperatures. Without the P restrictor coil (Fig. 2a), bubbles affecting the measurement repeatability were noted for $T > 60$ °C. In order to circumvent this drawback, the 150-cm restrictor coil (i.d. = 0.5 mm) was placed after the detection for increasing the system backpressure, thus reducing the possibility of bubble release inside the T transmission line and flow cell. The restrictor coil was efficient, allowing bath temperatures as high as 80 °C to be set.

Acidity of the reaction medium manifested itself as a relevant parameter (Fig. 4c). As pH measurement on the trapped sample portion was awkward, we decided to estimate the acidity of the stream towards waste (W - Fig. 2). As sensitivity was less dependent on pH for $2.3 < \text{pH} < 2.7$, the pH of the R$_1$ reagent was adjusted to 2.5, meaning a pH = 2.7 for the stream towards waste.

Sensitivity of the proposed analytical procedure was improved also by increasing the reagent concentration. Influence of the $p$-anisidine concentration was evaluated in the 0.01 – 0.20 mol L$^{-1}$ range. Increasing this parameter up to about 0.1 mol L$^{-1}$ led to a pronounced increase in the rate of the catalyzed indicator reaction, thus improving sensitivity (Fig. 4d). Beyond this value, the improvement in sensitivity became less pronounced. The uncatalyzed reaction was less influenced by variation of this reagent concentration. Considering this aspect and ratio of the catalyzed-to-uncatalyzed reaction rates, the $p$-anisidine concentration was selected as 0.1 mol L$^{-1}$.

Influence of the bromate concentration, evaluated in the 0.01 – 0.10 mol L$^{-1}$ range, was analogous to that noted for $p$-anisidine. Sensitivity underwent a significant improvement by increasing the bromate concentration up to about 0.04 mol L$^{-1}$, which demonstrated that the Mass Action Law is
the main aspect altering the reaction kinetics. As this aspect was less pronounced for > 0.05 mol L⁻¹ BrO₃⁻, and for the uncatalyzed reaction, this concentration was selected.

Several activators such as oxalic, citric and sulfosalicylic acids, oxine, tartrate and Tiron have been used in catalytic methods for vanadium determination¹³ aiming at sensitivity and/or selectivity enhancement. Tiron was selected in the present application and the influence of its concentration was investigated within 0.025 and 0.20 mol L⁻¹. Results were analogous to those found for p-anisidine and bromate, and 0.1 mol L⁻¹ Tiron was selected.

Regarding selectivity, Fe(III) and Ni(II) at concentrations > 2.5 and 1.0 mg L⁻¹, manifested themselves as the main interfering chemical species (Fig. 5). For selectivity improvement, different masking agents (tartrate, citrate, thioacetamide, triethanolamine, thiocyanate, pyrophosphate, oxalate and fluoride) at different concentrations (0.003 – 0.3 mol L⁻¹) were tested as the R₂ reagent (Fig. 2). The 0.3-mol L⁻¹ NH₄F solution was selected, as it suppressed these interferences.

Flow system with zone trapping inside the heated flow cell

The flow system in Fig. 2a was simplified, resulting in the 2b system, which permits the implementation of zone trapping inside the heated flow cell. As high hydrodynamic pressure was not required, the restrictor coil was not used; moreover, the T transmission line was removed.

The 2b flow system is very rugged, and baseline drift was not noted after extended (4 h) periods of operation. Release of air bubbles were not noted even for temperatures as high as 95°C. As the zone merging configuration was adopted, the consumption of the R₁ reagent was only 3 mg of p-anisidine per determination. Results were precise, as r.s.d. was estimated at 2.1% after 10-fold successive handling of a 5.0-μg L⁻¹ V solution.

Table 1  Vanadium content in mineral waters, as determined by the proposed procedure and by ICP-MS¹⁶ (data, in μg L⁻¹ V, refer to three replications)

| Sample | Proposed procedure | ICP-MS |
|--------|--------------------|--------|
| 1³     | 2.14 ± 0.06        | 2.12 ± 0.02 |
| 2      | 3.00 ± 0.03        | 3.03 ± 0.01 |
| 3      | 2.51 ± 0.09        | 2.89 ± 0.02 |
| 4⁵     | 5.73 ± 0.24        | 5.59 ± 0.01 |
| 5⁵     | 4.35 ± 0.18        | 4.13 ± 0.04 |
| 6      | 1.72 ± 0.02        | 1.69 ± 0.03 |
| 7      | 1.66 ± 0.02        | 1.38 ± 0.02 |
| SRM⁺   | 3.65 ± 0.03        | 3.73 ± 0.17 |

a. 100-fold sample dilution with a 0.1% (v/v) HNO₃ solution prior to analysis.
b. 20-fold sample dilution with a 0.1% (v/v) HNO₃ solution prior to analysis.
c. NIST SRM 1643e, trace elements in water, diluted 10-fold. Certified value = 37.86 ± 0.59 μg L⁻¹ V.

Sampling rate was 25 h⁻¹ at the 1% carryover level. The detection limit was estimated at 0.1 μg L⁻¹ V (3.3 σ criterion). A typical equation for the analytical curve is:

\[ Y = 0.2403 + 0.151 [V] \]

where: \( Y \) = absorbance at the end of the \( t₁ \) trapping period; and \([V] = \) vanadium concentration in μg L⁻¹. The linear and angular coefficients reflect the developments of the uncatalyzed and vanadium-catalyzed indicator reaction, respectively.

The proposed innovation can be also implemented by using six-way rotary valves instead of the injector-commuter, in a geometry analogous to that used for implementing the stopped-in-dual-loop configuration.¹³ This aspect was confirmed in parallel experiments, and expands the exploitability of the proposed innovation.

The flow system with zone trapping inside the heated flow cell (Fig. 2b) was applied to mineral water analysis, and results are presented in Table 1. The paired \( t \)-test was applied and no statistical differences between results obtained by the proposed procedure and by ICP-MS were found at the 95% confidence level (experimental \( t \) value = 0.40; tabulated \( t \) value for 7 freedom degrees = 2.36).

Conclusions

The proposed strategy for heating in flow analysis is promising, especially when sensitivity is critical and the involved reactions are speeded up under higher temperatures. Air bubbles are not generated, as the trapped portion of the sample zone is maintained in a sealed environment. Furthermore, implementation of the innovation leads to a simplified flow system. A noteworthy aspect is that the innovation is compatible with zone merging, and the favorable characteristics inherent to the resulting system were recently highlighted.¹⁸ The innovation enables the efficient exploitation of novel analytical procedures carried out under heating conditions, whose implementation in analytical flow systems is currently awkward.

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