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Flow Injection Analysis in the Undergraduate Laboratory

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Abstract: Flow Injection Analysis (FIA) is one of the most popular continuous-flow techniques and its versatility and simplicity can help to bring automation to teaching laboratories; however, the small number of educational papers about this technique indicates that the adoption of FIA procedures in the undergraduate curriculum has been scarce. In this work the fundamentals of FIA are presented using simple laboratory experiments. Some drawbacks that can hinder the employment of FIA by first-time users are discussed. Practical strategies to overcome these hindrances are suggested in order to facilitate the use of FIA for undergraduate laboratories.

Introduction

Flow Analysis (FA) encompasses an important group of analytical techniques that were classified by IUPAC in 1994 [1]. FA is extensively employed in routine and research analytical laboratories and can also be applied for fast determination of physical chemistry parameters [2–4].

Despite being one of the most popular FA techniques, the discussion of FIA in instrumental analysis books is rare. The best treatments are presented by Christian [5] and by Skoog et al. [6]. A good discussion is also presented in the textbook recently recommended by the European Community for teaching Analytical Chemistry [7]. Other classical textbooks [8, 9] practically skip this topic. A practical guide, useful for newcomers, is that written by Karlberg and Pacey [10]. FIA is an analytical technique that requires practical experience for a sound understanding. According to its creators: “Flow Injection Analysis should not be explained. It ought to be demonstrated” [11].

Several instrumental techniques can be coupled to FIA. In this sense FIA is often used for sample processing, for improving precision and sampling rate, and for implementation of less-conventional batch procedures (e.g., management of unstable reagents and products). FIA is a useful tool for carrying out chemical reactions and, as such, it makes classical procedures more attractive for students. Additionally, it can replace most of the old volumetric glassware used in undergraduate laboratories.

The success of an FIA application is strongly dependent on the manifold design and some experimental details need to be considered to overcome pitfalls. In this work fundamentals of FIA are presented through laboratory experiments. These experiments involve practical aspects useful to the optimization of FIA systems. A checklist and trouble shooting for method development is presented as supplementary material.

Experimental

The apparatus and reagents necessary to perform all the experiments are itemized below. All solutions were prepared with analytical grade reagents using distilled and deionized water. Some additional experimental details useful in the setup of the proposed manifolds are also presented in the Supplementary Material (45jn1897.pdf).

- **Apparatus.** Peristaltic pump with at least 4 channels
- Polyethylene tube (0.7 mm i.d.) and propulsion tubes with different internal diameters.
- Spectrophotometer equipped with a flow cell (a glass U-shaped flow cell with 180-µL and 10-mm path length was used)
- Potentiometer recorder.
- Injection device, a laboratory-made sliding-bar injector was used. (Any other commercial device, such as a rotary valve, could be employed.)
- Confluence points.
- A temperature-controlled water bath.
- A schematic representation of the flow cell, the laboratory-made sliding-bar injector and the confluence points are shown in Figure 1 of the Supplementary Material (45jn1897.pdf).

- **Reagents and Solutions.** *Copper(II)/PAR System.* 1.0×10^{-3} mol L^{-1} 4,2-piridylazoresorcinol (PAR), disodium salt.
- Copper(II) stock solution (100 mg L^{-1}) prepared from $CuSO_4{\cdot}5H_2O$
- Aqueous 5 mg L^{-1} or 0.5 mg L^{-1} Cu^{2+} solutions prepared by dilution of the stock.
- 0.5 mg L^{-1} Cu^{2+} in 20% (v/v) ethanol.
- 20% (v/v) ethanol.

- *Molybdenum Blue System.* R_1: 1% (m/v) ammonium molybdate in 0.5 mol L^{-1} HNO_3.
- R_2: 1% (m/v) ascorbic acid.
- S: 30 mg L^{-1} HPO_4^{2-} prepared from $Na_2HPO_4{\cdot}2H_2O$.

Procedure. The experiments were carried out employing a single-line (Figure 1a) or a confluent system (Figure 1b). Sample loops and coiled reactors were made of polyethylene tubes. The Cu(II)/PAR and the molybdenum blue complexes were measured at 520 and 690 nm, respectively. All experiments described below can be performed in a four-hour laboratory class.

Double-Peak Formation. The students assemble the single-line manifold showed in Figure 1a, employing PAR chromogenic reagent as carrier stream (C), flowing at 4.0 mL min^{-1}. A 5 mg L^{-1} Cu^{2+} solution prepared in water is used as the sample (S). Measurements are carried out with sample loops (L) of 20, 45 and 100 cm (100-, 225- and 500-µL sample volumes). A 100-cm reaction coil (B) is employed.

Effect Of Reactor Length on Sensitivity and Baseline Stability. The single-line flow manifold is modified to introduce the chromogenic

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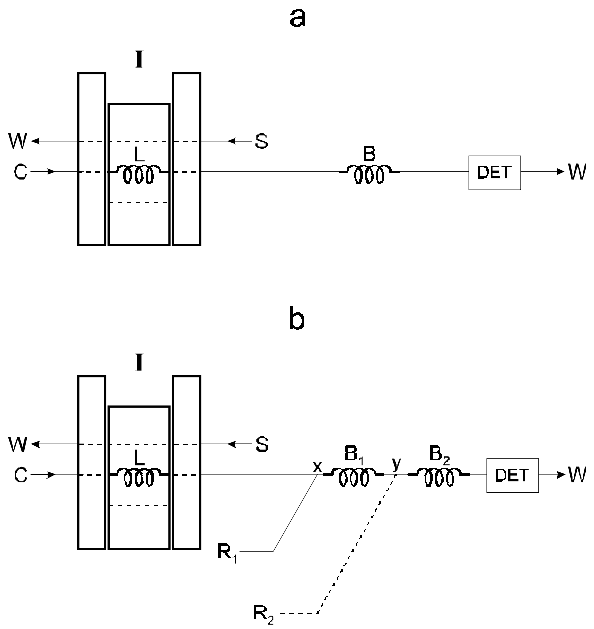

Figure 1. Flow diagrams for the systems employed in the proposed experiments: (a) single- line and (b) confluent-stream manifolds. I: sliding-bar commutator, lateral bars are fixed and the central bar is movable for sampling and sample injection; C: carrier stream; S: sample; R_1, R_2: confluent reagent streams; L: sample loop; B, B_1, B_2: reaction/dispersion coils; DET: spectrophotometric detector equipped with a flow cell; x, y: confluence points; W: waste.

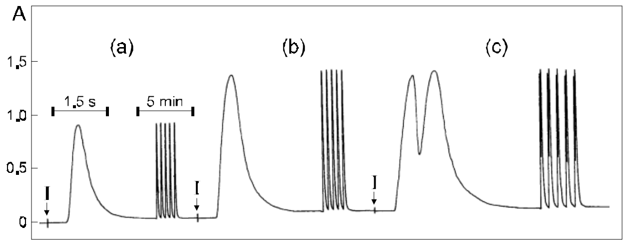

Figure 2. Double-peak formation in a single-line system (Figure 1a) employing the reaction of copper(II) with PAR. Sample loops: (a) 20 cm; (b) 45 cm and (c) 100 cm. b = 100 cm; c = 1×10^{-3} mol L^{-1} par; s = 5 mg L^{-1} Cu^{2+}. A: absorbance; I: instant of sample insertion.

PAR reagent by confluence (R_1), flowing at 1.0 mL min^{-1} (Figure 1b). Water is used as carrier stream (C), flowing at 3.0 mL min^{-1}. Measurements are made with a 5 mg L^{-1} Cu^{2+} aqueous solution (S) for reactor coil lengths (B_1) of 20, 80 and 180 cm, with a 100-cm sample loop. The confluent stream (R_2) and the reactor coil (B_2) are not employed.

Refractive Index Effect on Signal Measurements. The flow manifold with addition of PAR by confluence (Figure 1b, R_1) is employed using the same flow rates as stated previously; L is 100-cm and B_1 is 80-cm. Water is used as the carrier. Transient signals are obtained for two solutions containing 0.5 mg L^{-1} Cu^{2+} prepared in water or in 20% (v/v) ethanol and for this latter solution without Cu^{2+}. The repeatability for peak height measurements is estimated by 10 replicates on Cu^{2+} solutions in both media. This same experiment is repeated employing a 20% (v/v) ethanol solution as carrier.

Temperature Effects. The flow manifold with addition of two confluent solutions is assembled (Figure 1b) and the chromogenic reagents for phosphate determination using the molybdenum blue method are introduced as R_1 and R_2 streams, both flowing at 1.8 mL min^{-1}. Carrier flow rate is adjusted to 5.0 mL min^{-1}; 20-cm and 200-cm reactors are employed as B_1 and B_2, respectively. A 20-cm sample loop (100-µL sample volume) is used to insert the phosphate solution as a sample. The reactor B_2 is immersed in a temperature-controlled water bath. The bath temperature is adjusted to achieve 25, 40, and 50 °C in the sample zone after passing through the heated reactor.

Results and Discussion

Double-Peak Formation. Single-line FIA manifolds are simple and applicable for systems without chemical reactions, such as those employing potentiometry [12] or atomic absorption spectrometry [13]. They can be unsuitable, however, when chemical reactions are involved owing to insufficient mixture between sample and reagent. This is demonstrated using the reaction between Cu^{2+} and PAR and the results are shown in Figure 2. Sample dispersion in FIA can be reduced by increasing the sample volume, which results in sensitivity improvement. This effect can be noted by comparing the signals obtained with the 20-cm and the 45-cm sample loops (100 µL and 225 µL, respectively); however, when a 100-cm loop (500 µL) was used, a double peak was generated due to the lack of reagent in the center of the sample zone. This is typical in single-line systems because the mixing of sample and reagent occurs only by dispersion. This drawback is critical because the signal corresponding to the center of the sample zone, which usually is the signal maximum, is often employed as the analytical parameter. In conclusion, single-line systems are not suitable to design low-dispersion flow systems with large sample loops due to the incomplete overlapping between sample and reagent zones.

When chemical reactions are involved, systems with reagent addition by confluence are more suitable. In these systems (e.g., Figure 1b), an inert solution is employed as carrier and the reagent is continuously added to each segment of the sample zone. Consequently, sensitivity can be improved by increasing the sample loop without perturbation of the transient signals.

Effect of the Reactor Length on Sensitivity and Baseline Stability. In FIA systems the size of the reactor affects both sensitivity and baseline stability. The former is influenced by the residence time and by sample dispersion. The dimension of the reactor is a compromise between reaction development and sample dispersion. A large reactor increases the residence time and avoids baseline instability; however, it should not be larger than needed because this may cause sensitivity loss due to excessive sample dispersion. Reaction kinetics should be considered when choosing residence time. The effect of the reactor length is demonstrated using the fast reaction between Cu^{2+} and PAR, as is shown in Figure 3. An 80-cm reactor was suitable as a compromise between sensitivity and baseline stability (Figure 3a). A 20-cm reactor caused an unstable baseline due to irregular mixing between the colorless carrier and the orange chromogenic reagent (Figure 3b) and an 180-cm reactor caused a loss of sensitivity due to excessive sample dispersion (Figure 3c).

Effect Of Refractive Index on Signal Measurements. Chemical and physical differences between sample and carrier can affect transient signal measurements in FIA [14, 15]. The experimental strategy generally adopted is to use a carrier with chemical and physical properties as similar as possible to the samples. This can be easily implemented when samples present similar matrixes.

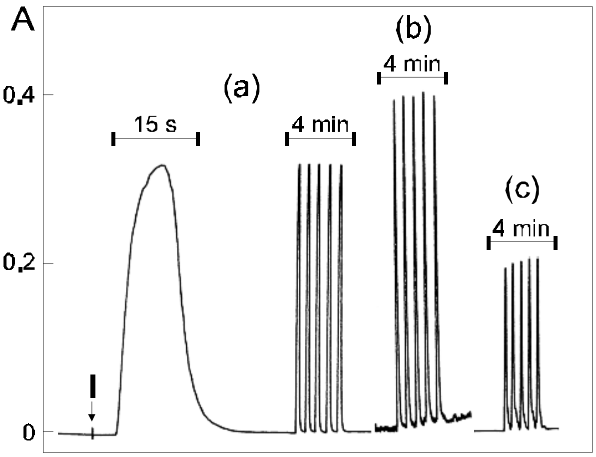

Figure 3. Effect of reactor length on sensitivity and baseline stability. Flow system with PAR addition by confluence (Figure 1B). Transient signals obtained for (a) 80-cm, (b) 20-cm, and (c) 180-cm reactor coils. c = water; r = 1 × 10^{-3} mol L^{-1} par; s = 5 mg L^{-1} Cu^{2+} ; l = 100 cm. A: absorbance; I: instant of sample insertion.

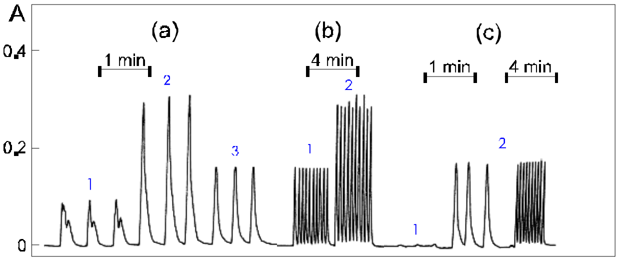

Figure 4. Refractive index effect on signal measurements. Flow system with PAR addition by confluence (Figure 1B) with an 100-cm sample loop and an 80-cm reactor. **a**: Transient signals for 20% (V/V) ethanol (1), 0.5 mg L^{-1} Cu^{2+} in 20% (V/V) ethanol (2), and 0.5 mg L^{-1} Cu^{2+} aqueous solution (3) obtained with water as carrier stream. **b**: Repeatability study for a 0.5 mg L^{-1} Cu^{2+} solution in water (1) and in 20% (V/V) ethanol (2) employing water as the carrier stream. **c**: Signals for 20% (V/V) ethanol (1) and 0.5 mg L^{-1} Cu^{2+} in 20% (V/V) ethanol (2) obtained by using 20% (V/V) ethanol as carrier. A: absorbance.

The deleterious effect caused by refractive index on spectrophotometric measurements is demonstrated by injecting ethanol solutions in a water carrier stream (Figure 4). The effect can even be visually observed in the flow cell, owing to the formation of liquid interfaces such as those observed when a concentrated solution is added to water. The accuracy is affected by refractive index gradients and a positive error can be observed in the signals obtained for a Cu^{2+} solution in 20% (v/v) ethanol. When the signals are corrected using the blank, the net absorbance is not equivalent to that achieved for an aqueous Cu^{2+} solution with the same concentration. In addition to this inaccuracy, the repeatability also deteriorates (coefficients of variation of 3.8% and 0.4% for Cu^{2+} solutions prepared with and without ethanol, respectively). These perturbations can be avoided by adopting the matrix-matching procedure, as demonstrated in Figure 4c employing a 20% (v/v) ethanol solution as carrier.

Signal perturbations are also observed when carrier and confluent streams have different physical or chemical characteristics, notably when mixing is poor. This is frequently observed with reagents prepared in organic medium or in concentrated acids and bases.

The presence or passage of air bubbles through the flow cell can also affect the baseline stability and the signal measurement. Baseline instability is observed when an air bubble remains trapped into the flow cell. On the other hand, if an air bubble passes through the flow cell an abrupt peak will be formed due to the sharp change in the refractive index.

Baseline Drift. Measurement in FIA are generally based on the maximum peak height of the transient signal and the net signal is determined by the difference between the maximum and the baseline. Thus, a stable baseline is desirable to make an accurate determination of the analytical signal feasible. In addition to the baseline instability caused by unsuitable mixing between carrier and confluent streams, the baseline can also be disturbed by the continuous retention of solids or adsorption of dyes in the flow cell. The retention of solids in the analytical path can cause both memory effects and leakage of solutions by increasing the backpressure. These drawbacks can be usually circumvented by changing the material used to build the flow cell and the analytical path or by performing the reaction in a different medium (e.g., by adding a surfactant). An alternative is the use of an intermittent washing stream to dissolve the retained solid or remove the adsorbed dye. A typical example is the turbidimetric determination of sulfate [16] in which an alkaline EDTA solution is employed as an intermittent washing stream to remove the barium sulfate accumulated in the analytical path and in the flow cell.

Carryover and Sampling Rate. Sampling rate (or sample throughput) can be defined as the number of samples that can be analyzed per hour. By using FIA, the time consumed for sample analysis can be notably reduced in comparison with batch methods, and flow rates higher than 100 h^{-1} are often attained. Nevertheless, carryover effects between successive samples can limit the sampling rate. The compromise between carryover and sampling rate can be experimentally demonstrated by increasing the sampling rate in successive injections of a dye solution. Carryover can deteriorate the repeatability of the results, and this should be kept in mind. The system dead volume caused by a large flow cell, unsuitable connections, etc [10, 11] can also reduce sampling rate.

Temperature Effects. With the advent and dissemination of FIA analytical chemistry gained a new kinetic perspective. Measurements are often carried out before reactions are complete and thus temperature effects become more perceptible. Temperature affects the sensitivity mainly for slow reactions; thus, the temperature can be increased to favor the formation of the reaction product. This is a straightforward strategy to increase sensitivity, but difficulties caused by the decrease of the gas solubility in the heated solution can occur. Gas evolution can be observed and this can disturb signal measurements.

These effects are demonstrated with an experiment carried out using the phosphomolybdate reduction by ascorbic acid, which results in the formation of molybdenum blue (Figure 5). Ascorbic acid is often used as a reducing agent in systems used for phosphate determination by this method, but the redox reaction is relatively slow. The measurements are performed at solution temperatures of 25, 43, and 51 °C measured after the reactor is heated. The signal obtained at 43 °C (Figure 5b) is 25% higher than that attained at 25 °C (Figure 5a).

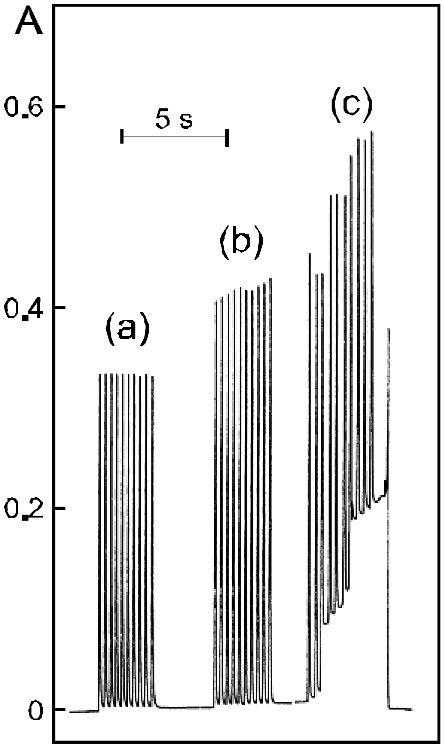

Figure 5. Temperature effect on the sensitivity and formation of air bubbles. Results for molybdenum blue system obtained at (a) 25 °C (b) 43 °C, and (c) 51 °C. The flow diagram shown in Figure 1B was used with L = 20 cm, B_1 = 20 cm, and B_2 = 200 cm. A: absorbance.

Nevertheless, despite the trend towards better sensitivity observed at 51 °C, serious drawbacks due to air bubble formation were observed (Figure 5c).

Even for systems without an external heating source, gas evolution can affect measurements in laboratories without temperature control. In some seasons the temperature can vary as much as 15 °C during a workday, causing a pronounced effect on gas solubility. This problem can usually be avoided by degassing the solutions before use.

Conclusions

FIA can be easily introduced in the undergraduate laboratory using low cost instrumentation. It can be used to bring the automation used in the real world into the analytical chemistry laboratory. This can help students learn about the multidisciplinary character of the modern analytical science. Kinetic aspects of chemical reactions can be brought to light and can be exploited for selectivity enhancement. In this sense, it is valuable to focus on some pitfalls that can slow the dissemination of FIA in undergraduate laboratories and to point out simple and practical alternatives that overcome these experimental difficulties.

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