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Flow injection: The ultimate approach to automation in analytical atomic spectroscopy

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Abstract

Automation in analytical systems is reviewed, especially with regard to the preparation and analysis of solutions. Flow injection analysis (FI) lends itself to these needs as it enables many procedures such as dilution, reagent addition, derivatization etc to be automated. FI is now used even for automating chemical reactions such as solid sorbent extraction precipitation and coprecipitation. An advantage of FI is that it is used successfully even in non-equilibrium conditions and with a suitable data management package allows single reference solution calibration. It is one of the few methods available capable of addressing total automation from sample preparation to data manipulation.

INTRODUCTION

The trend towards automation is as old as mankind because automation makes life easier and people have at all times tried hard to improve their situation. This is obviously also true for analytical chemists. But besides making their life easier, well thought-out automation may also save money, increase accuracy and reliability and even offer entirely new possibilities.

In atomic spectroscopy (AS) automation has gone through various stages, and autosamplers, i.e. devices which introduce the sample mechanically into the spectrometer, have been developed already decades ago. The usefulness of these autosamplers depended very much on the type of application and the instrument they were used for. In flame atomic absorption spectrometry (F MS) autosamplers were never really accepted because manual operation was fast and easy. In electrothermal atomic absorption spectrometry (ET MS), however, where microliter samples have to be introduced into a graphite tube through a small hole, followed by 2–3 min waiting for the completion of the temperature program, autosamplers have actually stimulated the breakthrough of the technique. They took a severe burden from the operator, improved accuracy and precision and reduced problems due to contamination significantly[1].

Automation is obviously not limited to sample introduction but should include all aspects of an analysis from sample preparation to data management. There were two developments in the past two decades which had a more than average contribution to automation in analytical chemistry, viz computers and laboratory robots. The impact of computers on automation was without question revolutionary, and some of these aspects will be discussed later in this talk. The importance of robots in the analytical laboratory, however, is not as unambiguous, and a comparison with the early attempts of mankind to fly by imitating birds might be appropriate. The era of flying began only after the laws of aerodynamics have been discovered. Similarly, robotics in analytical laboratories somehow got stuck, and it became apparent that an entirely new concept was required for sample handling.

During the past decade it became more and more apparent that flow injection (FI), which was first described in 1975[2], could well be that new concept. The main reason to believe that is because FI has been shown to provide solutions for the automation of all aspects of an analysis from sample pretreatment to data management. The first commercially available FI system for AS was actually designed for automation of hydride generation (HG) MS[3]. In addition FI opens the possibility for entirely new approaches, many of which have not yet been fully exploited. One of these is the capability of performing quantitative determinations under thermodynamically non-equilibrium conditions [4] which, among other things, may involve dramatic savings in total analysis time. The scope of this paper is to demonstrate the capabilities of FI in automating the entire analytical process, highlighting the most outstanding contributions of this technique.

THE PRINCIPLE OF FLOW INJECTION

In the first edition of their book *Flow-Injection Analysis* Ruzicka and Hansen[5] defined FI as "A method based on injection of a liquid sample into a moving unsegmented continuous stream of a suitable liquid. The injected sample forms a zone which is then transported toward a detector that continuously records the absorbance, electrode potential, or any other physical parameter, as it continually changes as a result of the passage of sample material through the flow cell". In the meantime essentially all statements of this definition have been superseded by the rapid development of this technique. Taking into consideration all the essential features, Fang[4] recently proposed a new definition of FI as "A nonchromatographic flow analysis technique for quantitative analysis, performed by reproducibly manipulating sample and reagent zones in a flow stream under thermodynamically non-equilibrated conditions".

A schematic diagram of an FI system for AS is shown in Figure 1. In the basic setup a carrier solution and the reagents are pumped continuously, the sample injected into the carrier, mixed with the reagents and carried to the AS detector where the signal is recorded. Various modifications of this setup, but all based on the same hard- and software, will be described in the following chapters demonstrating the flexibility of the system and of the technique.

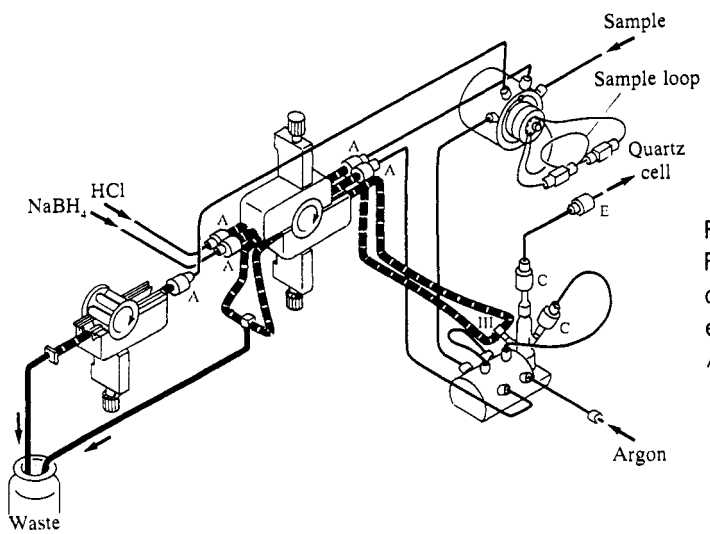

FIGURE 1
Flow injection system for the determination of hydride forming elements and mercury with AAS detection

ON-LINE SAMPLE PREPARATION

Although direct analysis of solid samples and slurries has made significant progress in ET MS[6], most AS techniques require liquid samples. In addition even liquid samples often call for an acid digestion etc. in order to convert organic compounds of the analyte element into an ionic form. Although some of these procedures have been mechanized, sample preparation is usually tedious, time consuming and often the bottle neck in the analytical laboratory.

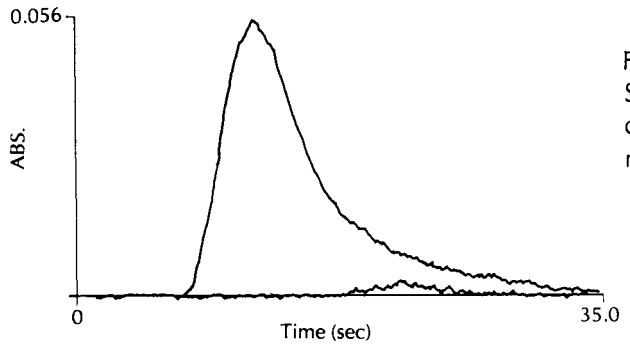

FIGURE 2
Signals for mercury from methyl mercury chloride in blood with and without on-line microwave digestion

Several attempts have been made to use FI techniques for on-line microwave assisted acid digestion. Karanassios et al.[7] for example pumped a slurry of biological materials together with an acid into a coiled PFA tube which was placed in a microwave oven. Elemental recoveries using a net digestion time of 2 min under stopped .flow conditions were comparable with those obtained using a 3 h hot-plate digestion. They have developed an FI system for on-line treatment of liquid samples such as water and urine in a microwave oven for the determination of mercury by cold vapour (CV) MS [8]. The sample was mixed with a bromination reagent and the reaction time was 45 s with an actual irradiation time of less than 10 s, so that a sample throughput rate of 20-40/h could be obtained. The system was recently modified for the analysis of blood and serum. Figure 2 shows the signals for mercury obtained from methyl mercury chloride added to blood with and without on-line microwave digestion. The recovery of mercury from a variety of organic mercury compounds added to urine was 94–111 % under optimized conditions.

ON-LINE SAMPLE INTRODUCTION

The simplest and most obvious application of FI is that of transporting the sample to, and introducing it into the atomization or excitation source of the AS instrument. But already this simple application does bring about a number of advantages over conventional operation and/or the use of conventional autosamplers. The sample is injected into a continuous flow of a carrier solution so that no air is sucked into the atomizer between samples which increases the stability of the flame[9]. The sample volume can be reduced to less than 100 µl without losing sensitivity[10] and sample solutions with high dissolved solids content can be injected over extended periods of time because the nebulizer-burner system is flushed continuously with the carrier solution[11]. An example for that application is given in Figure 3 which shows the determination of copper in a 30% (w/v) sodium chloride solution by F MS using 65 µl sample volume and a sampling frequency of about 110/h. Over a period of 80 min the sensitivity changed by only 10% and there was no obvious deterioration of the precision.

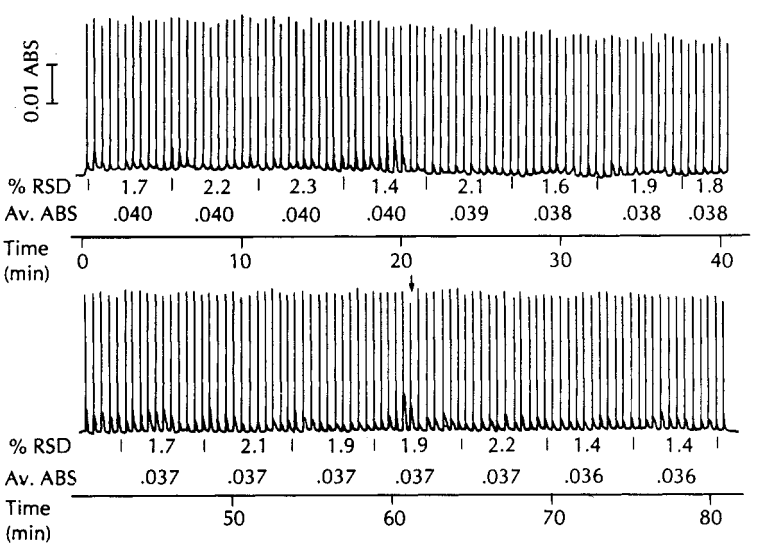

FIGURE 3
Repetitive F AAS determination of 1 mg/l Cu in 30% (W/v) NaCl solution using 65 µl sample solution and water carrier

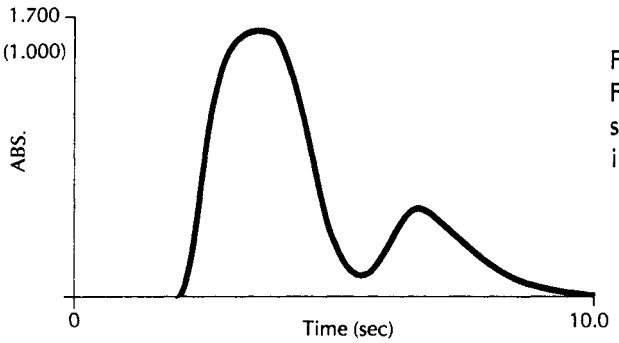

FIGURE 4
FI zone penetration dilution using 40 mg/l Ca solution. Sample loops 100 µl and 20 µl, intermediate loop 150 µl

FI systems also permit automatic on-line addition of spectroscopic buffers, reagents etc. and automatic on-line dilution, a frequent source of errors if done manually. Figure 4 shows 10 superimposed signals obtained for a 40 mg/l calcium standard solution using the so-called FI zone penetration dilution[12]. Three dilution levels were obtained in this example in one injection of two volumes of a standard solution using the two peaks and the valley for evaluation. The reproducibility (in RSD) at the two peaks was about 0.6% and about 2% in the valley, which corresponded to a 20 fold dilution(13).

ON-LINE SEPARATION AND PRECONCENTRATION

A variety of on-line or quasi on-line separation and preconcentration techniques using FI techniques have been described, including liquid-liquid extraction, solid sorbent extraction, gas-liquid separation, coprecipitation etc. A very good example for the numerous improvements brought about by FI is the coupling of solid sorbent extraction with ET MS[14,15]. A schematic diagram of the FI manifold and the sequence of operation is shown in Figure 5. Sample and complexing agent (dimethyl dithiocarbamate, DDTC) are mixed on- line and the metal chelates collected on a bonded silica reversed phase sorbent with octadecyl functional groups (RP-C 18) in a micro-column of only 15 µl volume which was installed on the injection valve replacing the sample loop. In a second stage the column is washed with dilute nitric acid in a direction counter to that of sample loading in order to remove residual matrix, excess

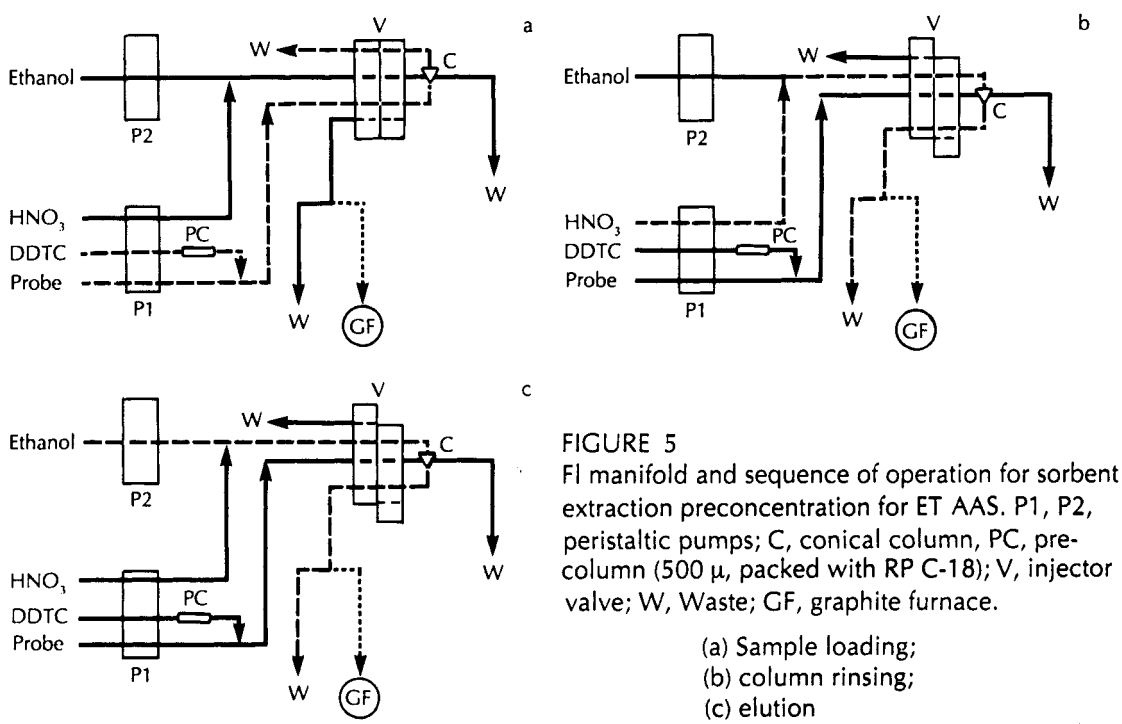

FIGURE 5
FI manifold and sequence of operation for sorbent extraction preconcentration for ET AAS. P1, P2, peristaltic pumps; C, conical column, PC, pre-column (500 µ, packed with RP C-18); V, injector valve; W, Waste; GF, graphite furnace.

(a) Sample loading;
(b) column rinsing;
(c) elution

reagent and less stable metal complexes which might have been absorbed as well. The analyte element is finally eluted with ethanol directly into the graphite tube of the electrothermal atomizer. The principle of elute zone sampling[14], in which the early and the late part of the elute is discarded and only the 40 µl containing about 50% of the analyte element are introduced into the graphite tube, is used in order to avoid any sub-sampling in a separate container. A 20–25 fold sensitivity enhancement was typically obtained using 1 min preconcentration, making possible a sampling frequency of more than 20/h[16].

A typical set of signals obtained for lead is shown in Figure 6, revealing some of the key advantages of this technique. Firstly, the blank values are extremely low due to online purification of reagents, working in a closed system, and elution directly into the graphite furnace which reduce contamination to a minimum. Secondly, a very high sensitivity with detection limits in the low ng/L range can be obtained with only 1 min preconcentration. This sensitivity can be further improved by at least one order of magnitude by using accordingly longer preconcentration times[17]. Thirdly, there is essentially no background signal visible even in the extracts from seawater samples which indicates the complete separation of the analyte elements from the concomitants such as sodium chloride. It has been shown that accuracy and precision of the preconcentration and determination for a number of trace elements is the same in deionized water and in seawater samples[15,18]. Due to the complete separation of the analyte element from the matrix detection limits in real samples are as much as three orders of magnitude better than for direct determination.

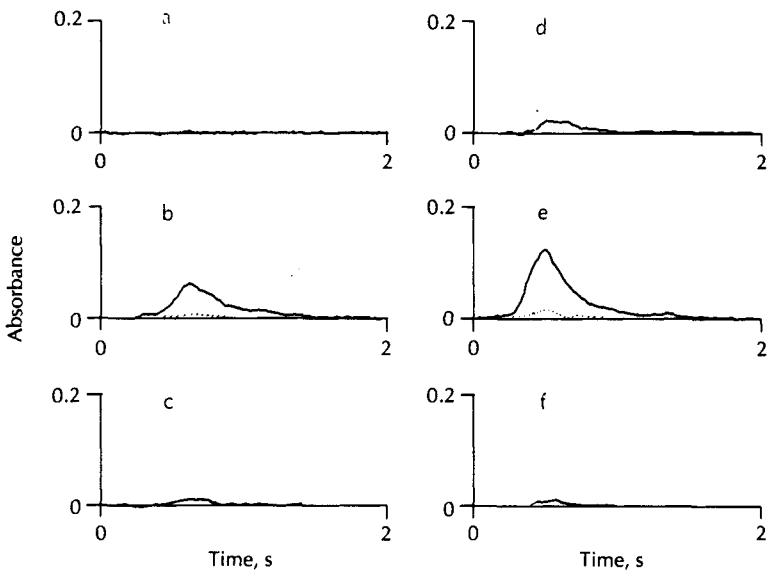

FIGURE 6. Atomization signals for Pb after FI sorbent extraction preconcentration ET AAS. Sample loading period 60 s. (a) Blank; (b) 100 ng/l Pb standard solution; (c) deionized water; (d) NASS-2 open ocean seawater; (e) CAAS-1 coastal seawater; (f) CASS-2 coastal seawater. Solid lines analyte absorbance; dotted lines background absorbance

ON-LINE SPECIATION

Many elements exist in more than one oxidation state and it is frequently of interest to determine the concentration of individual oxidation states rather than the total concentration of an element. The above-described preconcentration system can be used directly for that purpose because complex formation and sorption on the solid phase is usually significantly different for the different oxidation states. The advantage of the DDTC - RP-C 18 system is that the most toxic species forms the most stable complex and can hence be preconcentrated and determined directly. Applications have been described for the determination of trivalent arsenic(19) and hexavelant chromium(20). The total concentration of these elements can be determined in the same system after on-line or off-line reduction of the higher and oxidation of the lower oxidation state, respectively.

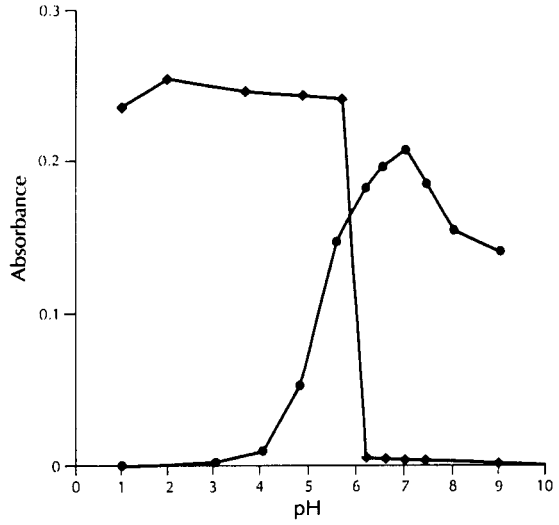

FIGURE 7
Effect of the pH of various buffer solutions on retention and peak height absorbance of 200 µl Cr(III) and Cr(VI)

Recently a rapid and sensitive method was described for the selective determination of chromium(III) and chromium(VI) in water samples by F MS using on-line preconcentration on a micro-column packed with alumina in the acidic form(21). The retention behaviour of the two chromium species depending on the pH is shown in Figure 7. Sequential species-selective sorption was possible using a buffer of pH 7 for Cr(III) and of pH 2 for Cr(VI). The preconcentrated species were eluted directly from the column into the nebulizer-burner of the F MS using 1.0 mol/l nitric acid for Cr(III) and 0.5 mol/l ammonia for Cr(VI). Detection limits of 1.0 µg/l and 0.8 µg/l were obtained for Cr(III) and Cr(VI), respectively, using a 35 s preconcentration time which resulted in a sampling frequency of 60/h.

For many of these applications which include complexation and separation it is of vital importance that FI is capable of performing quantitative determinations under thermodynamically non-equilibrium conditions and that FI permits very fast operation. On-line acidification, complexation, sorption etc. may be carried out within a distance of only a few cm in narrow-bore tubing which means within fractions of a second at the flow rates typically used in FI. This enables the analyst to use reagents under conditions where they are not stable and/or to form complexes which decompose rapidly under batch conditions.

In speciation analyses speed of operation may be even more important as it directly contributes to accuracy. It is well known that species distribution for example in natural aquatic systems is governed by complex redox equilibria etc. and there are many pathways of interconversion. These equilibria are affected and interconversion is initiated often already upon sampling and sample storage, but most seriously if one of the components is removed by extraction etc. If these extraction-removal processes are slow the natural equilibria will change during the analytical procedure and the result will become a function of the time involved. It is obvious that these changes are minimized and hence the results most accurate if the reaction times are in the millisecond range as they are in FI.

PROCESS CONTROL

FI is extensively used for process control purposes because it is ideally suited for unattended on-line operation. Although AS detectors are not yet in use very frequently there are undoubtedly numerous applications for this technique. One recently described example is the control of the cleaning process of aircraft engine blades[22]. The manufacturing process requires the blades to be fixed using a low-melting Bi/Sn alloy. This must later be removed as bismuth attacks the blade material at the high engine operating temperatures. The blades are therefore cleaned with nitric acid and the content of Bi in the acid vessels must be controlled on a regular basis. The completely automatic system for cleaning and control of bismuth content is shown schematically in Figure 8. The system was tested by measuring the signal difference between two high Bi concentrations, 200 and 220 µ/l, over a period of 20 days. The

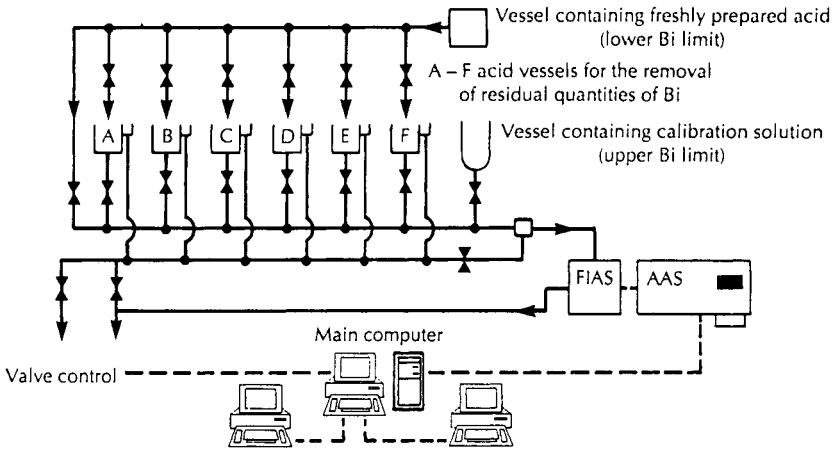

FIGURE 8. On-line control of the cleaning process of aircraft engine blades monitoring the bismuth content in nitric acid

mean value and standard deviation of the difference in integrated absorbance was 0.35 ± 0.03 s. This means that the difference could be measured with a precision better than 10%, a figure sufficiently low for production process control purposes.

SIGNAL EVALUATION AND CALIBRATION

Among the fundamental characteristics of FI are the controlled dispersion of the injected sample in the carrier solution and the reproducible timing of its transport from the point of injection to the detector[5], producing transient signals which can be characterized by peak height, peak area and peak width. In addition to the use of these values for calculating the analytical result, a reproducible reading can also be taken at any defined delay time on the ascending or descending part of the gradient peak. By introducing the dispersion as a new predictor variable and using the total information contained in the transient signal caused by this dispersion, the zerothorder AS technique is enhanced to a first-order technique.

Sperling *et al.*[23] proposed an algorithm for 'gradient ratio calibration' which can apparently overcome two of the most notorious limitations of F MS, the limited linear working range and matrix interferences. By use of all the information provided over time from the transient signal recorded by a computer, sample concentration can be calculated by comparing the sample pulse with only one calibration pulse even in the presence of multiplicative interference effects. The algorithm is based on the fact that calibration curves are linear at low absorbance values (high dilutions). This means that the concentration ratio between a reference solution and a sample solution can be determined at high dilution, i.e. at the wings of the transient signal. This information can be used to correct for deviations from this ratio at high absorbance values in the peak apex.

The same principle can be used to correct for interference effects in case they disappear at high dilution. This is shown in Figure 9 for the well known interference of phosphate on the calcium determination in an air-acetylene flame. A concentration of 0.01 mol/l of phosphoric acid causes a decrease in the calcium signal of 43% in peak height absorbance. If the ratio between the calcium signal without phosphate (reference solution) and with phosphate is plotted against the absorbance readings of the sample solution (Figure 9b) and extrapolated to zero absorbance, the curve arrives at the ratio 1, i.e., no interference.

Even though the precision of the extrapolation may not be extremely high, the accuracy of the correction is quite good. But the algorithm can not only correct for interferences, it can detect the presence of an interference and set a flag which might be even more important in fully automatic operation which is typical for FI.

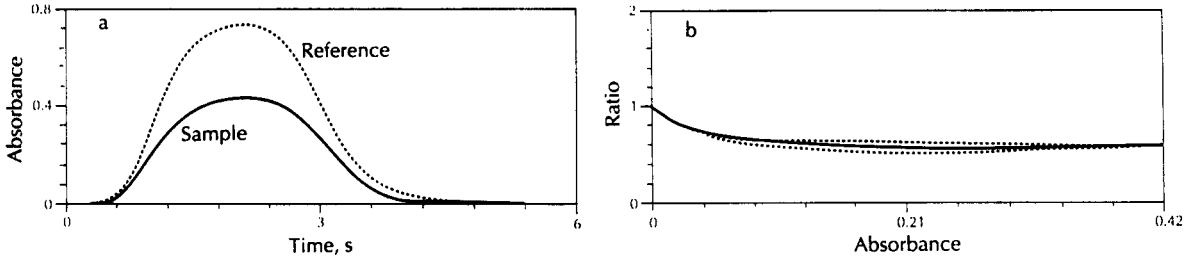

FIGURE 9. Automatic control of interferences in F AAS using peak ratio calibration
(a) signals for 16 mg/l Ca with and without 0.01 mol/l phosphate
(b) signal ratio versus absorbance for 16 mg/l Ca with 0.01 mol/l phosphate

TOTAL AUTOMATION BASED ON FI

Widespread application of FI with AS detection has started only recently with the introduction of the first dedicated instrument for that combination. The full evaluation of many approaches is therefore still ahead of us. Nevertheless it is obvious already now that FI will have a much more significant contribution to automation of AS - and most probably also of other techniques - than any other development until now. FI can be considered a sample management system offering the possibility of a totally automated analysis starting with automatic sample pretreatment such as acid digestion and finishing with data management including control of interferences, automatic warning and call for corrective measures. FI will set new standards in automation and at the same time increase accuracy and reliability of the data produced by the equipment.

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