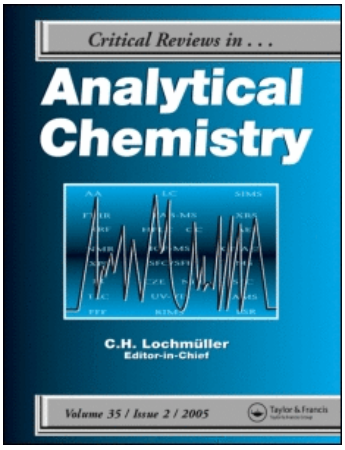

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Sample Handling and Pretreatment Using Flow Injection Analysis

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To cite this Article Clark, Gregory D. , Whitman, David A. , Christian, Gary D. , Ruzicka, Jaromir and Zielinski Jr., Walter L.(1990) 'Sample Handling and Pretreatment Using Flow Injection Analysis', Critical Reviews in Analytical Chemistry, 21: 5, 357 — 375
To link to this Article: DOI: 10.1080/10408349008050849
URL: http://dx.doi.org/10.1080/10408349008050849

Sample Handling and Pretreatment Using Flow Injection Analysis

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ABSTRACT

Flow injection analysis (FIA) has been demonstrated to be an ideal tool for the handling and pretreatment of samples prior to their analysis. Sample handling may be as simple as the use of FIA for the introduction of the sample into a detector or as complicated as extraction of an analyte followed by back extraction prior to reaction and detection. In this survey, it is shown that FIA can encompass a large diversity of sample handling and pretreatment techniques. By employing FIA methods, one is able to significantly increase the sample throughput and, in many cases, increase the overall precision compared with batch style sampling, pretreatment, and analysis schemes. FIA sample handling and pretreatment techniques reviewed here include injection, dilution, membrane techniques, solvent extraction, and sorbent extraction/preconcentration.

I. INTRODUCTION

Flow injection analysis (FIA) is based on the concept of controlled dispersion, which allows reproducible development and subsequent chemical reactions on an injected sample zone. It is essential for the success of this technique that each sample experiences the same conditions as the previous one. The sample plug on injection disperses into the carrier stream, with concomitant dispersion of the reagent into the sample zone. An example of a FIA manifold and resulting FIA readout is shown in Figure 1. Through this mutual dispersion, a sample profile is developed which is unique to the FIA system employed.[1] The magnitude of the response curve for similar samples is usually a linear function of the sample's concentration.

FIA originated as a method for rapid assays.[2-7] However, it soon became apparent that FIA, through virtue of its reproducible sample development, could be used for and even enhance those pretreatment steps which are typically performed manually.[32,49,53] Reproducibility is improved through removal of human intervention and bias. Additionally, pretreatment steps performed using FIA are rapid. The pretreatment and analysis, including chemical conversion, usually require less than a minute. Furthermore, when samples are toxic or pathogenic, as are many of industrial or clinical origin, FIA offers the advantage of removing, or at least minimizing, human exposure.

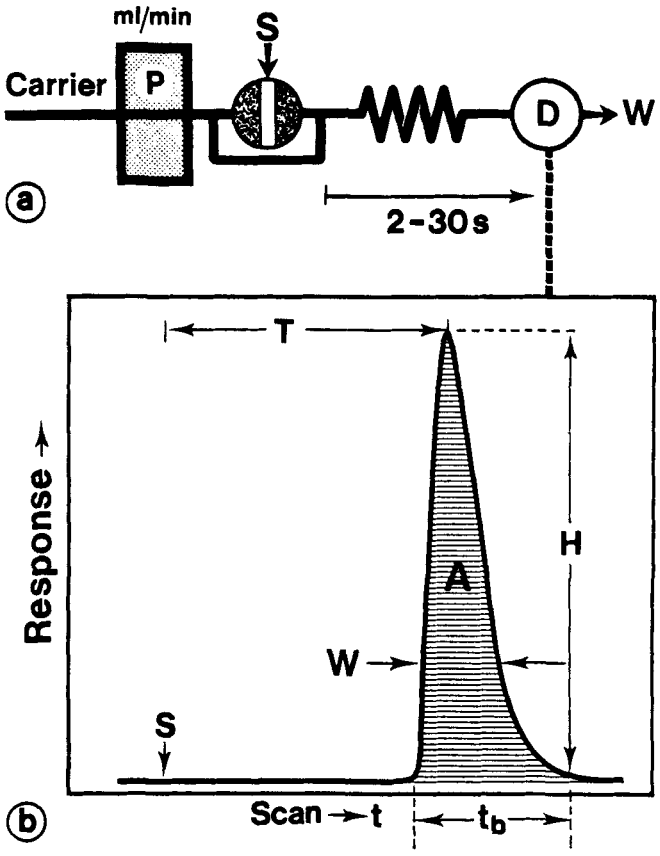

FIGURE 1. (a) Single-line FIA manifold where sample (S) is injected into a carrier (R) stream propelled by an appropriate pump (P) and detected in a flow through detector (D). (b) Readout from and some of the parameters used to characterize a single line flow injection system. The measured parameters may include peak width (W), height (H), area (A), the residence time (T), and peak width at the baseline (t_b).

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Discussions of such pretreatment steps that are automated by FIA have contributed substantially to the FIA literature. It is the opinion of the authors that FIA will continue to develop dramatically in its use as a sample handling and pretreatment tool. The eventual goal is the ability to develop FIA systems that sample process streams or chemical reaction vessels directly and are robust enough to handle difficult sample conditions such as high or low concentrations, particulates, elevated temperatures, pressure, etc. By coupling FIA to the process stream or reactor, improvements in process analysis, as illustrated in Figure 2, become possible. In addition to protecting the analysts, automated pretreatment techniques would greatly enhance the speed of the analysis and hence control of the process.

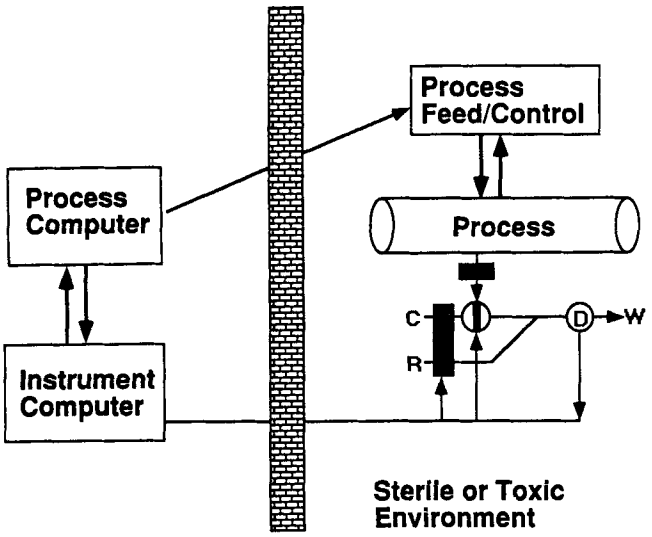

FIGURE 2. Role of FIA in process control. FIA apparatus directly samples, pretreats, and analyzes process materials. The FIA apparatus may be controlled remotely and transmit the data to the process computer for more direct and expedient control.

Most of the common FIA sample handling and pretreatment methods are reviewed in this article. Emphasis is placed on the techniques themselves. Much has been presented, however, on the theory behind these FIA applications. The reader is referred to review articles on theory[1,8,9] and two monographs[10,11] for these topics. Additionally, the reader is referred to theoretical discussions of a given topic, where appropriate in this paper.

II. METHODS OF SAMPLE HANDLING AND PRETREATMENT

A. Injection Techniques

Sample handling and pretreatment can be achieved by using many techniques in FIA. The first opportunity for sample manipulation, however, begins with the mode of injection. The purpose of sample injection in FIA is to reproducibly deliver a well-defined sample bolus into a continuously flowing carrier stream. The importance and utility of sample injection in sample handling and pretreatment by flow injection analysis should not be underestimated. In fact, as **Rule 1** in the monograph of Ruzicka and Hansen[11] states, the easiest way to affect the output of a flow injection system is to vary the injected sample volume:

> Changing the injected sample volume is a powerful way to change dispersion. An increase in peak height — and in sensitivity of measurements — is best achieved by increasing the volume of the injected sample solution. Conversely, dilution of overly concentrated sample material is best achieved by reducing the injected volumes.

Methods of injection may be divided into two main categories: time- and volume-based injection. In time-based injection, a sample is loaded for a precise time at a given flow rate. Volume-based injection techniques are, however, the more popular methods of injection. In the volume-based techniques, the sample fills a geometrically defined volumetric cavity. This well-defined sample bolus is then inserted into the carrier stream. Volume-based injections are performed using rotary valves, commutators, and hydrodynamic injection. Methods for time- and volume-based injection are discussed next; however, the reader is referred to two excellent reviews on injection.[11,13]

1. Time-Based Injection

Precise sample volumes can be delivered into a flow injection manifold by loading a sample stream for a precise time and at a precise flow rate. The injected volume can be calculated as the product of flow rate and loading time. Time-based injections have the obvious advantage that sample volume may be changed at will, thus providing an increased dynamic range of the analysis. Such an injection system is shown in Figure 3. This method is, however, dependent on reproducible flow rate of the sample solution which makes frequent calibration necessary when using peristaltic pumps.

Riley and co-workers have described a variant of FIA which they labeled controlled-dispersion flow analysis, in which they used a novel time-based injection method.[14,15] A sample probe on a robotic arm is positioned in a sampling cup for a predetermined time. The pump is stopped and the robotic arm arcs to a new position to aspirate the carrier. The pump is restarted to draw carrier, thereby injecting the sample. Hungerford described a similar method for use in a miniaturized FIA system where injection of submicroliter quantities of solution is necessary.[16] Jorgensen et al.[17] described a method to achieve a similar time-based injection using a two-position valve. The valve is opened to the sampling position for a precise time, then is switched to the carrier position. The advantage cited for using a two-position valve is that the pump never stops, thus the injected volumes are more precise.

A clever time-based injection technique was developed by Krug et al. that utilizes a microcolumn on which the analyte is completely adsorbed from a continuously flowing sample

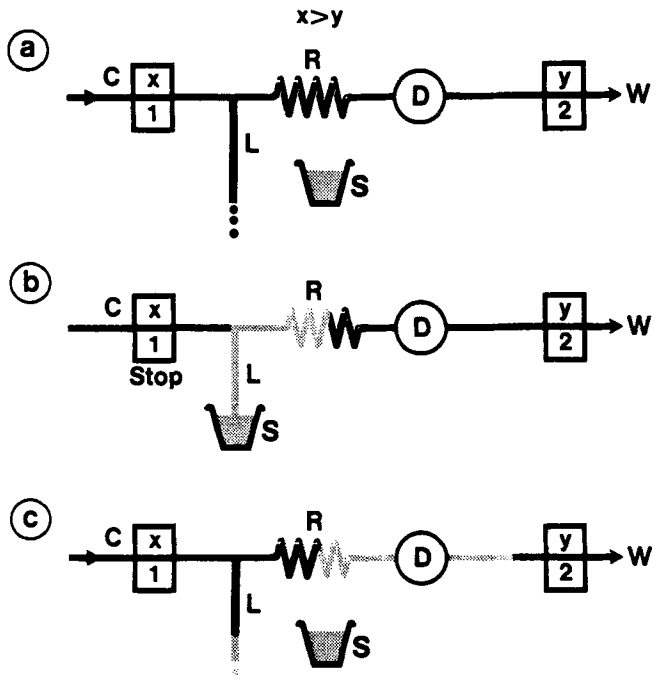

FIGURE 3. Time-based injection FIA system comprising two pumps, 1 and 2, with volumetric flow rates x and y, respectively, where x > y. In the standby position (a), both pumps are activated, propelling carrier stream solution (C) through the manifold, excess of carrier being expelled via channel L. For sampling (b), the sample container is contacted by channel L, pump 1 is stopped while the action of pump 2 is maintained, whereby a defined zone of sample solution is aspirated into the manifold. In the injection step (c), the sample cup is removed and pump 1 is restarted, the carrier stream thereby forcing the sample zone through the FIA manifold and into the detector, while the surplus of carrier is wasted through channel L.

stream.[18] The microcolumn is placed in the sample loop. After an appropriate loading time, the valve is switched to the inject position, and the eluent stream elutes the sample. This injection method serves three pretreatment steps: the analyte is removed from the bulk sample matrix, it is preconcentrated, and is then reproducibly "injected" into the carrier by complete elution from the column. This technique is limited to only those analytes which are adsorbed onto the column. Furthermore, an eluent stream must be chosen to completely desorb the sample, otherwise sample carry over is likely.

2. Volume-Based Injection

Use of the term *injection* in FIA comes from the oldest volume-based technique in flow injection analysis. In the first paper by Ruzicka and Hansen on FIA, injection was made directly into the carrier stream through a rubber septum with a syringe equipped with a hypodermic needle.[19] There are, however, two severe limitations in the use of syringe injections. The volume of material injected depends on the position of the plunger. The shape of the sample bolus depends on the rate of injection. Both limitations are very dependent on the skill of the analyst. Because these limitations are easily overcome by rotary or slider injection valves, syringe injections are now only of historical interest.

a. ROTARY VALVES

There are many designs for rotary valve injectors.[20,21] Because FIA, analytical liquid chromatography, and HPLC are of approximately the same volumetric scale, commercially available chromatographic equipment (fittings, tubing, flow cells, pumps, and valves) may also be used in FIA. The six-port rotary valve is probably the most popular system of injection today. This type of valve is shown in Figure 4. Some of the many uses of the six-port valve design have been described by Erikson et al.[21] There are two positions in most rotary valves, load and inject. In the load position, a geometrically defined external volumetric cavity is filled with the sample. The carrier stream flows through the valve in a bypass channel. When the sample cavity is filled, the valve is rapidly turned to the inject position. The sample is thereby inserted into the carrier stream.

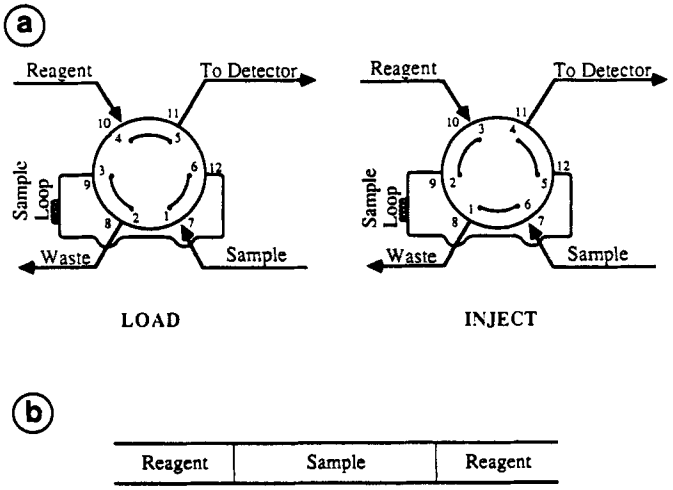

FIGURE 4. (a) Six-port injection valve. Port numbers 1 to 6 refer to those on the rotor, while port numbers 7 to 12 designate those on the stator. The valve configurations are shown in the LOAD and INJECT positions, respectively. (b) The sample zone injection as a result of a configuration like (a). The sample is juxtaposed on both sides by reagent.

b. SLIDER VALVES

A volume-based injection technique pioneered by Bergamin F° et al.[22] uses commutators (slider valves). The commutator consists of two fixed external plates and a central, movable plate. This type of valve is illustrated in Figure 5. In the load position, the sample fills a cavity or external loop of the central plate and the carrier stream flows through the valve via a bypass channel. The central plate is then moved to the inject position in which the sample is inserted into the carrier stream. Com-

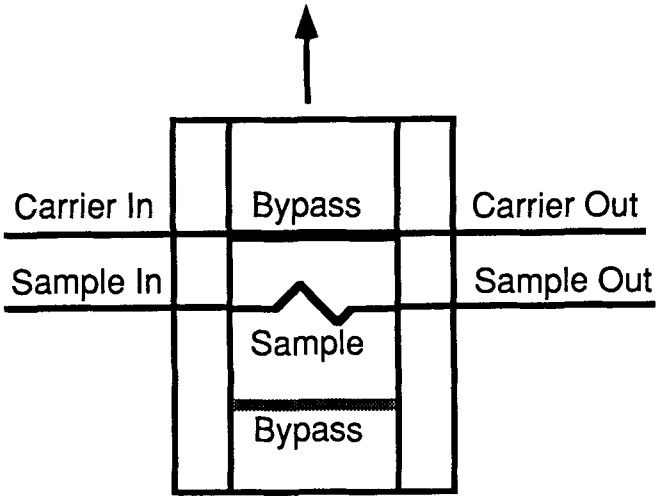

FIGURE 5. Slider valve (commutator). In the load position, the sample loop is in line with the sample channel. The carrier is flowing through a bypass channel. The sample is then injected when the sliding portion of the valve slides down such that the sample loop is now in line with the carrier.

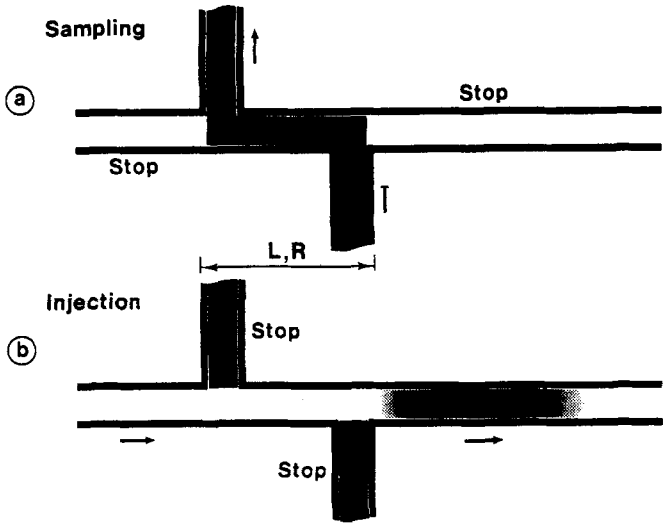

FIGURE 6. Hydrodynamic injection technique. (a) A fixed volume of sample is metered into the conduit defined by length, L, of tubing of radius R. During the load position, the carrier pump is stopped. (b) The carrier pump is started and the sample pump is stopped, propelling the injected sample into the device and toward the detector.

mercially available slider valves from Altex Corporation closely approximate the commutator design.

c. HYDRODYNAMIC INJECTION

Hydrodynamic injection is a volume-based method which does not require moving parts.[23] The valve is replaced by two tees, connected by a channel of known volume. This volume defines the volume of injection. The carrier stream is stopped, and the sampling stream is simultaneously turned on to fill the injection volume. The sampling stream is then turned off and the carrier stream is turned on to complete the injection cycle. The principle of such a valve is shown in Figure 6. The precision of this injection technique is much worse than rotary valve injection [2.5% relative standard deviation (RSD) compared with 0.3% RSD, for peak height in a series of ten injections]. The major drawback to this method is the difficulty in balancing hydrodynamic forces. Zagatto et al. have described a hydrodynamic injection procedure in which a commutation device is used.[24] Inclusion of a valve, however, defeats the purpose for which hydrodynamic injection was developed, i.e., to eliminate moving parts and concomitant wear.

3. Pretreatment by Injection

In addition to being used as a method for the introduction of a sample into the carrier stream, "injection" valves have been used to perform other pretreatment procedures. The technique of zone sampling has been used for dilution (see subsection B following[40]), and zone trapping has been used for incubation of the sample and reagent in slow reactions.[25] In zone trapping, the sample is first injected into a reagent carrier stream. When the sample zone fills a second injection valve, the valve is closed, thus trapping the sample and reagent mixture. Following a well-defined incubation period, the sample is reinjected into the carrier to be detected downstream. This method is proposed as an alternative to stopped flow FIA as a means to accommodate slow reactions. The chief advantage is cited as increasing throughput and sensitivity by truncating the sample to the most concentrated portion of the zone.

a. MERGING ZONES

Sample and reagent are simultaneously injected into two carrier streams and merge at a confluence point in the merging zones technique. The Brazilian group has written a series of papers describing the merging zones technique and some interesting applications.[26,27] Simultaneous injection of sample and reagent can be achieved through the use of an automated two-valve system or one double injection valve.[22,26] Such a system is shown in Figure 7. The merging zones method was described using the double proportional commutator which allowed simultaneous injection of both sample and reagent with a single device.[26] Although the merging zones method was developed as a means to reduce reagent consumption, the technique has also been used for addition of unstable reagents, pH adjustment, and a way to avoid baseline drift.[28] Although widely used, this method is difficult to implement in practice due to the precise timing required. The principle of merging reagent and analyte zones is best achieved by the zone penetration technique.

b. ZONE PENETRATION

Zone penetration is similar to the merging zones method except that the sample and reagent slugs are injected into a single carrier stream. Under the laminar flow conditions in

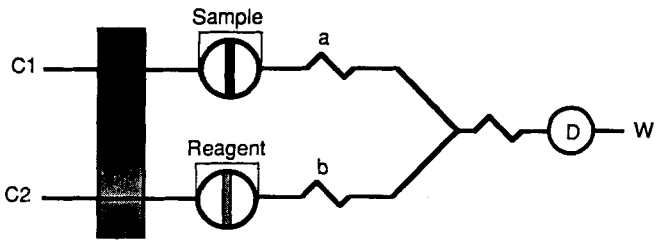

FIGURE 7. Merging zones manifold. The apparatus consists of two injection valves and two separate carrier streams (C1 and C2), each with a particular flow rate. After injection (usually synchronous), the injected volumes flow through separate reactors. The samples (usually one sample and one reagent) merge at a confluence point. The lengths of the reactors (a and b), the timing of injection, and flow rates are set for the desired amount of overlap of the sample and reagent boluses.

FIA, the flow rate in the center of the tube is two times faster than the average flow rate. This enables one zone to penetrate the other, as shown in Figure 8. The split loop method introduced by Ruzicka and Hansen is one way to achieve zone penetration using a single valve.[29] Yerian et al. used the split loop injection technique for the pretreatment of serum samples with urease to liberate ammonia for detection by optosensing flow injection analysis.[30] Other double injection rotary valves and commutators may be used for zone penetration with an added advantage: the volume separating the sample and reagent zones may be varied to give the exact degree of mixing desired. A single-line "dilution" system may be envisioned where there is only minimal mixing of reagent and sample at the edges of the concentration gradients, where dilution is large.

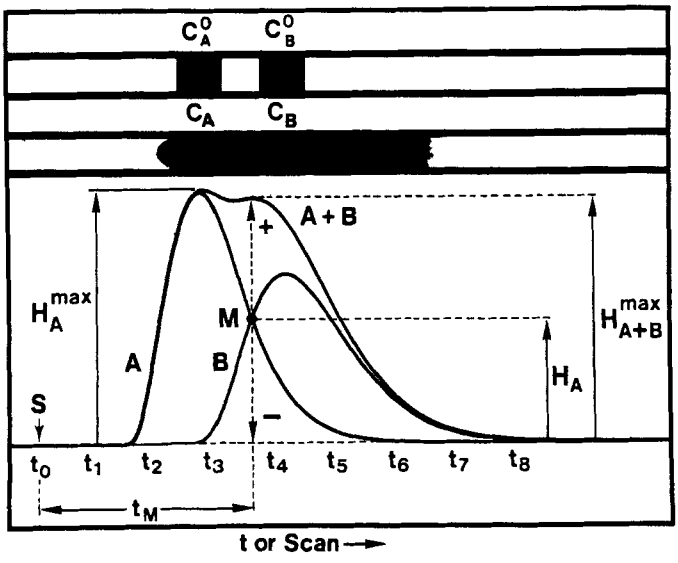

FIGURE 8. Zone penetration technique. Top: separately injected zones C_A^0 and C_B^0 mutually penetrate each other forming a composite zone, the degree of penetration governed by the initial distance between the zones. Bottom: separate (A,B) zones and composite (A+B) response curve.

c. NESTED LOOP INJECTION

The external loop in a commutator or rotary valve may be used to accommodate any of a number of other pretreatment devices such as gas diffusion, dialysis, or microcolumn modules. Figure 9 shows typical nested loop injection techniques. Nested loop injection provides a means to perform pretreatment of the sample prior to injection into the FIA system. Such pretreatment units which may be used include dialyzers, gas diffusion units, solvent extraction units, or filters. The most simple nested loop injection device incorporates a second sample loop, as shown in Figure 9b. A variant of this technique, the split loop injection, is illustrated in Figure 9c. The injection technique became more sophisticated by including a microcolumn (Figure 9d), a dialysis or gas diffusion membrane (Figure 9e), or even a detector (Figure 9f). See references for further examples of nested loop techniques.[18,31,65]

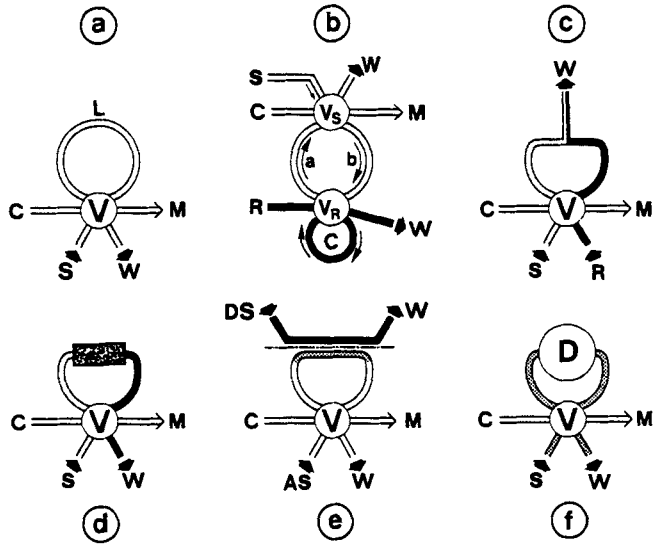

FIGURE 9. A conventional sample loop (a) may be augmented (b) by the addition of a second (nested) loop, or (c) may be split to allow injection of two zones. A microcolumn placed in the loop (d) for preconcentration or matrix removal concomitant with injection. Similarly, sample cleanup may be achieved by placing a dialysis or gas diffusion unit in the loop (e). A further alternative is the placement of a detector in the sample loop (f).

B. Dilution Techniques

Before beginning discussion of the available techniques for dilution of a sample, the extent of dilution must be defined. The dispersion coefficient, with roots in the chemical engineering literature,[11,12] has been used as a measure of dilution. The dispersion coefficient is

$$D = C^0/C$$

where C^0 is the concentration of the injected species prior to dispersion, as measured by the steady-state absorbance signal.

C is the concentration of the species at the time for which D is calculated. This principle is shown in Figure 10. The concentrations can be calculated using a calibration curve obtained with the detector. Empirically, D^{max} is determined as the ratio of the steady-state response to the response at peak maximum which has a concentration, C^{max}:

$$D^{max} = C^0/C^{max}$$

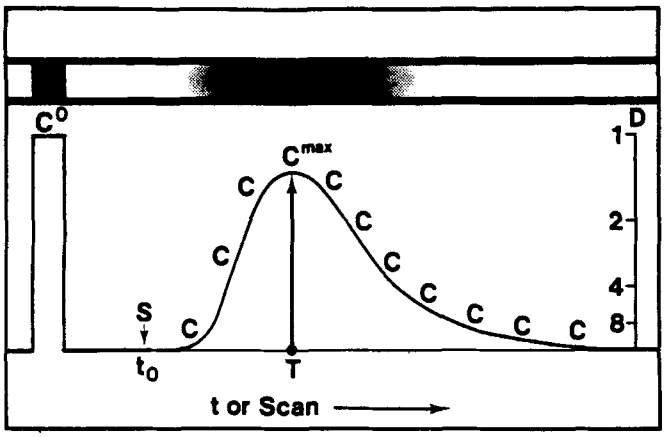

FIGURE 10. An originally homogeneous sample zone (top left) disperses during its movement through the FIA conduit. The zone undergoes a change from initial plug shape (bottom left), with concentration C^0, to an exponentially modified Guassian shape (bottom right) with a maximum concentration in the zone of C^{max}. However, a gradient of concentrations is present from 0 concentration to the zone of C^{max}.

For convenience, the superscript "max" will be dropped, assuming concentration measurements will be made at the peak maximum, unless stated otherwise. Dispersion coefficients are defined as limited (D = 1 to 3), medium (D = 3 to 10), and large (D > 10).[11] Dilution, as defined by the attenuation of the detector response to the sample's concentration, has occurred when dispersion coefficient values are greater than 1.

Another measure of dilution, the inverse mole fraction, X^{-1}, has been suggested by Whitman and Christian[44] as

$$X^{-1} = n_{inj}/n_{det}$$

where n_{inj} is the number of moles injected and n_{det} is the number of moles detected. As X^{-1} is increased, the dilution is increased. This dilution measurement is only valid for systems where the number of moles of analyte is decreased prior to measurement. Diluting a sample k-fold and measuring the area without using the dilution system yields A^0, which is used for calibration. If the undiluted sample is then subjected to the dilution system, and its area, A_{det}, is measured, an expression for the X^{-1} may be written:

$$X^{-1} = k A^0/A_{det}$$

This definition is useful when multiline FIA systems, such as dialysis and zone sampling, are employed.

1. Gradient Dilution

Perhaps the simplest and most often overlooked method of attenuation of the sample's response is gradient dilution.[32] Through reproducible evolution of the sample concentration profile, each element of the profile yields useful information which is often redundant. If the peak maximum does not lie in the calibration range, then measurement of the zone can be made at a time on the profile which yields a decreased response. By selection of measurement times longer than the time at peak maximum (T in Figure 10), an element with decreased concentration (C in Figure 10) is measured, yielding a proportionately decreased absorbance. This measurement is reproducible from sample to sample, and can be calibrated, provided that care is taken to measure the response at the same time after injection. A further attractive feature of this technique is that it requires no modification of the apparatus.

2. Gradient Chamber

Gradient devices were developed to mix a sample through mechanical means, providing significant dilution and pretreatment of samples with difficult matrices (e.g., viscous samples). The dispersion coefficient of a sample after passing through a gradient chamber is much higher than the dispersion coefficient of a similar sample which has passed through a capillary. This is shown in Figure 11. The dispersion coefficient, D, is proportional to the ratio of the volume of the mixing chamber, V_m, to the injected volume, S_v. Additionally, the gradient device has been used for FIA titrations. One drawback of the chamber approach is that the chamber has an exponentially decreasing concentration profile in time which broadens with increasing V_m/S_v ratios. Consequently, the cycle time t_{cyc}, which is the minimum time required between injections, will increase. Furthermore, the amount of dilution provided by the system is fixed, which does not allow adjustment of the response when a sample measurement is out of range (either low or high).

Pioneering work on a gradient chamber-based voltammetric system was made by Nagy and co-workers.[33] Ruzicka and Hansen[34] made initial FIA developments of the technique and discussed its theoretical and practical aspects and applications. Stewart and Rosenfeld[35] demonstrated the usefulness of a gradient chamber for dilution and scale expansion in FIA. They cited the need to increase the dynamic range of the method to higher concentrations. Three articles by Pardue and co-workers[36-38] discussed the mechanism of peak width broadening as a result of using the gradient chamber and demonstrated its ability to increase the dynamic range of FIA measurements.

Gisin et al.[39] examined the problem theoretically and demonstrated that the precision of peaks obtained by the use of a gradient device surpassed the precision offered by the more conventional and common capillary FIA system. They reported

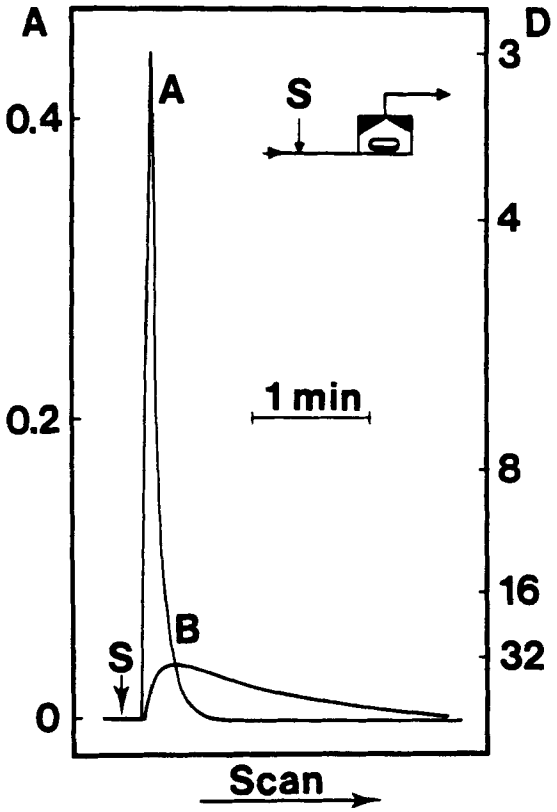

FIGURE 11. Gradient chamber-based FIA dilution system. By including a gradient chamber of volume V_m (here 1.9 ml), the dispersion may be made much higher than for a system in which a gradient chamber is not used. The attenuation is proportional to the volume of the chamber. "A" is the recorded peak without the mixing chamber and "B" is the peak with the chamber.

dispersion coefficients on the order of 1000, with a RSD of less than 1%. As in the next example, a piston pump and automated injection valve were used, which increased the possible precision. In fact, Gisin et al.[39] recommended the use of a piston pump for increasing precision over that of the peristaltic pump for FIA applications where precise, pulse free measurements are required.

3. Zone Sampling

It is possible to remove a small portion of the developed sample zone and inject this aliquot into a second flowing stream. Such a process is shown in Figure 12. This provides two advantages: (1) an aliquot is taken which is then further diluted, reminiscent of classical volumetric dilution, and (2) the injection into a second stream may allow further chemistry to occur. Reis et al.[40] used the commutator device[23] to provide sampling of the zone and injection into a second stream. A rotary injection valve may also be used in place of the commutator used in this example. Reis et al.[40] reported a dispersion coefficient of 100.

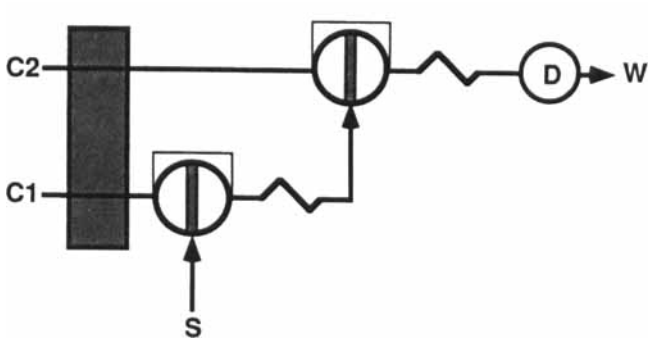

FIGURE 12. Zone-sampling dilution system. Through use of a second injection valve, a small (ca. 25 μl) portion of the dispersed zone can be captured and injected into a second carrier stream, providing increased dilution.

It is possible to combine dilution techniques, and the resultant method often retains the advantages offered by the components. For example, Toei improved the range and precision of dilution attainable by zone sampling, employing a gradient chamber as the dispersion unit in the FIA manifold.[41] The sample was injected into a gradient chamber FIA system. The effluent from the chamber was injected by a second valve into a second carrier and detected.

Garn and co-workers used a similar approach to achieve dilutions on the order of 1 million-fold.[42] They used two gradient chambers in series, with a zone sampling configuration, as shown in Figure 13. A sample was injected into a stream leading into the first chamber, then a small portion of the trailing edge of the concentration profile exiting the gradient chamber was injected into a second chamber, the eluent of which passed through a flow cell. Even with dispersion coefficients on the order of 10^6, they were able to obtain RSDs better than 2% while reducing any physical effects that the matrix may contribute. This method has been quite successfully applied to the on-line process analysis of industrial dyes.

4. Split Zone

The split zone dilution technique is conceptually similar to the zone sampling technique.[43] Here, however, the entire portion of the FIA concentration profile from some preset time (the delay time) to the long time limit of the zone is cleaved from the rest of the profile and analyzed. The manifold design for such a system is shown in Figure 14. Essentially, if the total area of the FIA peaks is the integral of the profile from $t = 0$ to infinity, then the area of the peak detected when using the split zone technique is the integral of the concentration profile from the delay time to infinity. By increasing the delay time, the area decreases. In this fashion, dispersion coefficients on the order of 15,000 were reported. A gradient chamber was used in another variant of the system, the use of which increased the precision of the dilution and removed any effects that viscosity may have on the dispersion. Furthermore, Clark et al.[43] derived an equation which would allow dynamic ad-

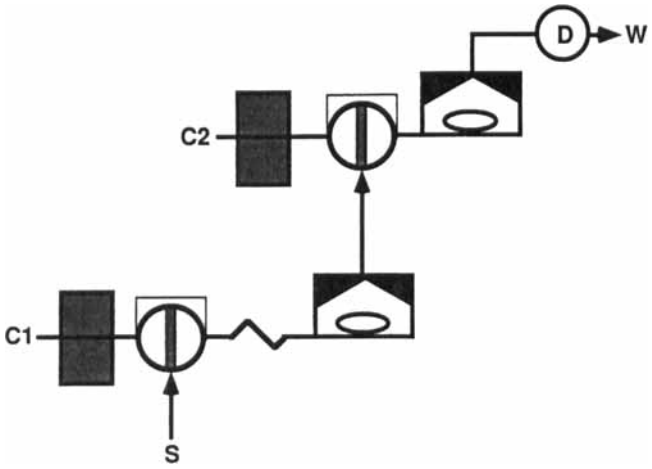

FIGURE 13. Gradient chamber-based, zone-sampling dilution system in which a gradient device is used for the initial dispersion. A second injection valve is used to sample the highly dispersed zone and inject this sample into a second carrier, C2, which undergoes further dilution by the second gradient chamber.

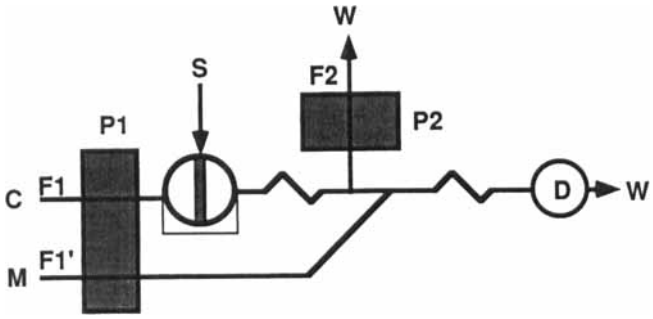

FIGURE 14. Split-zone dilution system in which a sample is injected into a carrier (C) which is flowing through the injection valve at flow rate F1. A second pump is aspirating at flow rate F2, which is greater than F1, such that all carrier and sample are propelled toward pump 2. The difference between F1 and F2 is provided by a second carrier (M) propelled by pump 1. At the time at which the desired amount of sample is between the tee piece and the injector, pump 2 is stopped and the remaining portion of the sample is propelled through the detector and analyzed. By increasing the delay time, increasing dilutions may be achieved.

justment of the dilution, as required when a sample to be analyzed is found to be out of range of the detector and must be further diluted. The equation allows one to back calculate the original concentration of the sample.

5. Cascade Dilution

Whitman and Christian described a simple, rapid, and reproducible sample dilution system, applying sample volume reduction through stream splitting using differential pumping coupled with a true dilution by merging with a subsequent carrier stream.[44] The system is shown in Figure 15. One can achieve a 500-fold dilution without long residence times. Whitman and Christian[44] derived an equation which allows one to

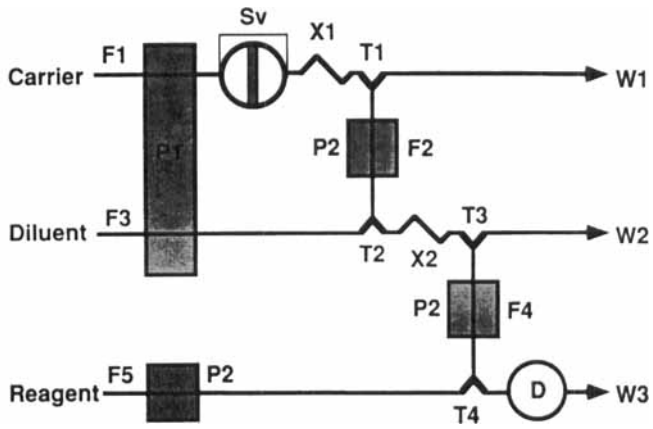

FIGURE 15. Schematic of the cascade dilution system manifold. P1 and P2 are peristaltic pumps, X1 and X2 are mixing coils, F1 to F5 are flow rates, D is the detector, and T1 to T4 are confluent mixing tees.

set the dilution factor simply by setting the flow rates of the various streams. Furthermore, this technique can be run strictly independently of computer control or pump timing. The method was applied to dilute a 1.7 *M* chloride sample prior to analysis. It should be noted that the reproducibility of this technique and the split zone technique depend on maintaining reproducible flow rates.

C. Membrane Techniques

Membrane techniques are often used in flow injection analysis for two reasons. They are most often employed to separate the analyte of interest from the bulk matrix, which often contains interfering species or particulates, as shown in Figure 16. The second use is purely as a method for dilution. The injected sample is carried to the membrane by a donor stream. The donor stream may or may not react with the analyte. The analyte then selectively passes through the membrane to an acceptor stream. The acceptor stream normally contains a reagent with which the analyte forms a detectable species. The reader is referred to theoretical discussions of mass transport for dialysis[45] and gas diffusion.[46] Ion selective electrodes also employ membranes in flow injection analysis.

1. Dialysis

Dialysis is used for several purposes in flow injection analysis. Dialysis is usually used for separating an analyte from a difficult matrix; it can also be used for dilutions and to increase the overall selectivity of the analytical procedure. Cellulose acetate membranes are most often used in flow injection analysis. These membranes act as size exclusion devices. The size of the molecule that may pass through the membrane is determined by the membrane's pore size. A simple dialysis unit is constructed by milling flow channels for the acceptor and donor streams in two plates mirroring each other. The dialysis membrane is pressed between these plates. The Bran-Lubbe Corporation sells dialysis modules with premounted dialysis

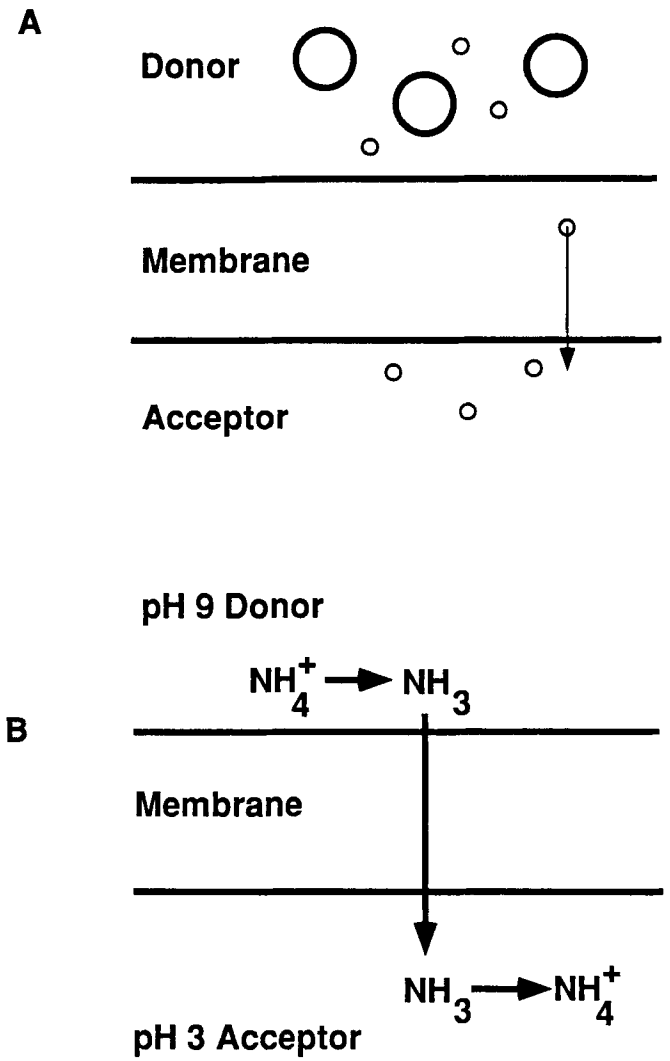

FIGURE 16. Typical membrane techniques include (A) dialysis where small molecules are allowed to cross the membrane into an acceptor stream and larger molecules are excluded, and (B) gas diffusion membrane where only gaseous molecules are allowed to cross into an appropriate acceptor.

membranes for use with their AutoAnalyzer instruments that are suitable for use in flow injection apparatuses.

Typically, dialysis allows a low molecular weight analyte to be separated from larger molecules or particulates in the sample matrix. For example, van Staden has described a FIA-dialysis system in which chloride is determined in whole cow's milk.[47] Casein, a relatively major component of milk, interferes with the determination of chloride using a chloride-selective electrode. The interference was easily removed by the addition of the dialysis module. Xie and Christian used a dialysis module in conjunction with a lithium ion-selective electrode.[48] The dialysis membrane removed matrix effects often present in blood serum.

Another use of dialysis in flow injection analysis is to perform simple dilution. When a sample bolus flows past a dialysis membrane, ions and molecules will diffuse into the acceptor stream. Because diffusion is a random process, only some of these will diffuse into the acceptor stream. Typically, 3 to 30% of a solute will pass through the membrane, the fraction being constant over a moderate concentration range, determined by the individual system.[49] Conditions of both the donor and acceptor streams may be manipulated to vary the degree of dilution obtained. One of the first FIA papers by Ruzicka and Hansen[50] describes the use of dialysis as a means to dilute chloride and phosphate in blood serum. In this method, dialysis is used to perform two pretreatment procedures: separation of the analytes from a difficult matrix and dilution of the sample prior to reaction.

2. Gas Diffusion

Gas diffusion is a powerful method to separate an analyte from the bulk interfering sample matrix. A sample is injected into a reagent/carrier that converts the analyte of interest into a gaseous form. Similar to dialysis, the donor stream flows past a membrane (Teflon, polypropylene, or silicone rubber), behind which an acceptor stream is flowing that can reabsorb the gas. For example, by injecting a sample containing ammonium ions into a basic carrier, ammonia is formed. The ammonia will diffuse across a hydrophobic membrane into an acceptor stream. The acid acceptor converts the ammonia back to ammonium ion, thereby increasing the pH of the acceptor stream containing an indicator which is detected downstream.[51] The sample may contain particulates or other potentially interfering species, which will not traverse the membrane.

Marstorp et al. have used a gas diffusion membrane for the separation of acetoacetate from whole milk following conversion to acetone.[52] It is important in the dairy industry to monitor for subclinical ketosis in cows to optimize their dietary energy balance. The flow injection method allows high accuracy and throughput.

D. Solvent Extraction Techniques

Solvent extraction fulfills several different pretreatment tasks: sample cleanup, removal of interferences, elimination of matrix effects such as viscosity, and preconcentration. When an analytical determination is sensitive and rapid, but the analyst is required to perform a manual solvent extraction, the technique's usefulness is potentially compromised by two problems: (1) manual extraction is very time intensive and (2) the sample is, by necessity, open to atmospheric contamination which may contribute interferences. Solvent extraction by FIA (SE-FIA) alleviates these problems by providing a rapid, automated extraction in a closed system. A generic solvent extraction device is shown in Figure 17. Typical segmenters and phase separators are shown in Figure 18. In addition, solvent waste problems are minimized since the volume requirements are reduced dramatically. Only liquid-liquid solvent extraction is discussed in this section. Commonly used extraction solvents

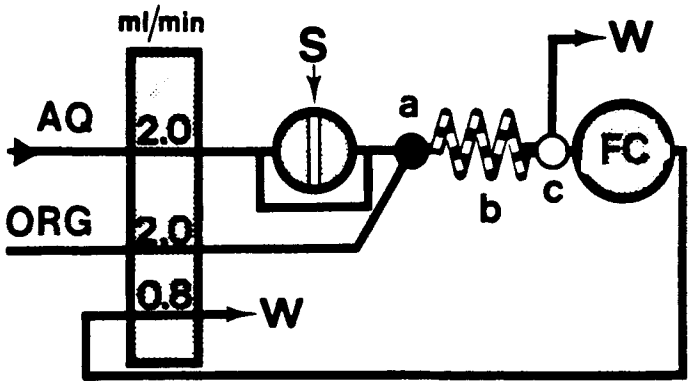

FIGURE 17. Solvent extraction manifold. A sample (S) is injected into an aqueous carrier (AQ) which contains a reagent forming a complex or ion pair with the analyte present in the sample, thereby increasing its partition coefficient for the selected organic phase. The aqueous phase merges with an organic phase (ORG) at the segmentor (a) and extraction occurs in the segmented stream (b). Finally, the phases are separated via a phase separator (c) and the organic phase is analyzed.

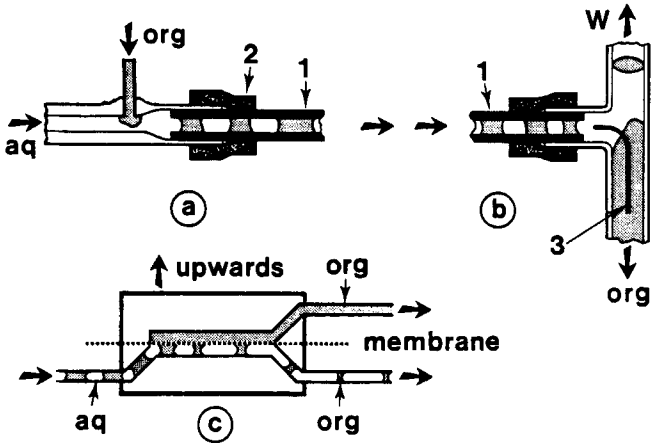

FIGURE 18. Segmenter and separator units used for solvent extraction. In the segmenter (a), the aqueous stream is merged with an organic stream, causing segmentation. (b) A simple phase separator for organic solvents with greater density than water is shown. The Teflon string (3) assists the separation. (c) A membrane phase separator with a hydrophobic membrane such as Teflon, which allows passage of the organic phase but not water is shown.

and reagents which are abbreviated in the text are listed in Table 1.

1. Common Methodologies and Uses

a. ION PAIR FORMATION

Karlberg and Thelander presented the first use of SE-FIA.[53] They extracted caffeine, present in acetylsalicylic acid preparations, from a basic aqueous phase into a chloroform organic phase. The chloroform solution contained 1% tetrapropyl ammonium bromide which forms an ion pair with sodium lauryl sulfate present in the acetylasalicylic acid preparations. The sodium lauryl sulfate would otherwise interfere with the extraction. They demonstrated excellent reproducibility (0.80% RSD) at caffeine concentrations of 0.274 m*M*. They were able to analyze 100 samples per hour. Karlberg and Thelander[53] demonstrated a rapid, nonlinear rise in the extraction efficiency with respect to length of coil (for short coils), and this increase in efficiency leveled off with long reaction coils.

Kawase and co-workers reported on the determination of anionic[54] and cationic[55] surfactants using SE-FIA based on ion-pair formation. Methylene blue was used to form an ion pair with the five anionic surfactants, including sodium dodecyl sulfate. Cationic surfactants, such as alkyl(C_{10}–C_{18})-pyridinium chlorides, were used as test analytes. A sample was reacted with Orange II reagent to form an ion pair. The ion pair was then extracted into chloroform in a long (3.25 to 5.0 m) extraction coil. The phases were separated using a dual phase separator arrangement. The first phase separator was similar to the one described by Karlberg et al.,[53] the second separator was based on a gradient chamber with a porous PTFE membrane. The purpose of the chamber was to reduce the baseline noise resulting from inflow of the aqueous stream into the separated organic stream. The working range reported was 15 μ*M* to 1.25 m*M* of the anionic surfactant, and 0.1 to 1.0 m*M* of the cationic surfactant, each with a sampling frequency of 80 per hour. A linear response was reported over a range of concentrations, with a reproducibility of 1.5%.

The ion-pair extraction technique was used by Silva et al.,[56] who developed a method allowing the sequential analysis of nitrate and nitrite in food products. They extracted a nitrate-bis(neocuproine)copper(I) ion pair into methylisobutyl ketone (MIBK), followed by AAS determination of the copper. Nitrite

Table 1
Commonly Used Extraction Solvents and Reagents

Abbreviation	Common name	IUPAC name
MIBK	Methyl isobutyl ketone	4-Methyl-2-pentanone
APDC	Ammonium pyrrolid-inedithiocarbamate	—
DEDC	Diethyldithiocarbamate	—
DDDC	Diethylammonium -*N*,*N*-diethyldithiocarbamate	—
PTFE	Poly(tetrafluoroethylene)	—
	Lumogallion	[4-Chloro-6-(2,4-dihydroxyphenylazo)-1- hydroxybenzene-2-sulfonic acid]
SDS	Sodium dodecyl sulfate	—
6-Me-PAA	6-Methylpicolinealdehyde azine	—
	Teroladine	N-t-Butyl-1-methyl-3,3-diphenyl-propylamine
PAN	1-(2-Pyridylazo)-2-napthol	
PAR	4-(2-Pyridylazo)resorcinol	
DDC	Diethyldithiocarbamate	
DTC	Bis(carboxymethyl) dithiocarbamate	

is oxidized to nitrate with cerium(IV) sulfate in the neocuproine/copper carrier. Both ions are thereby determined. In a subsequent run, sulfamic acid is present in the carrier, which reduces nitrite to nitrogen, and nitrate is determined alone. Nitrite is obtained by difference. Each ion could be determined in the presence of the other up to a tenfold excess. The detection and quantification limits were found to be 0.04 and 0.13 μg/ml nitrate and nitrite, respectively. They found chloride to be the most serious interference. However, by addition of chromotropic acid, the chloride could be measured, thereby allowing quantification of the nitrite and nitrate.

b. METAL-LIGAND EXTRACTIONS

Before metals can be extracted into an organic phase, they are usually complexed with an organic ligand. This classical extraction technique can be automated by FIA. Klinghoffer et al.[57] used a dithizone solution in carbon tetrachloride to extract lead and cadmium from aqueous solutions. Samples were prepared which contained lead, cadmium, and zinc. During the cadmium determination, the lead present in the sample was prevented from forming a complex with dithizone through adjustment of the pH using disodium hydrogen phosphate. Excellent reproducibility was reported for cadmium concentrations as low as 0.1 ppm. Alternatively, the cadmium was masked by complexation during the lead determinations using diammonium hydrogen citrate. The lead exhibited good reproducibility in the 0.2 to 1.0 ppm range. In either case, the zinc was masked with $K_4[Fe(CN_6]$. The method of "FIA scanning" was discussed, in which a single concentration of lead was injected into a FIA system. The pH of the carrier, originally being acidic, was slowly made alkaline. The pH was monitored and plotted simultaneously with the FIA peaks at a very slow chart speed, obtaining peak height trends. This method allowed the selection of the optimum pH for determination of the lead. A clear maximum in absorbance was apparent at pH 4.8, which was determined to be the optimum pH for lead determination.

c. SAMPLE WORKUP

Nord et al.[58] used SE-FIA as an automated sample workup tool to extract terodiline from blood serum prior to analysis by gas chromatography (GC). Terodiline is known to adsorb on PTFE and polypropylene. Accordingly, the system was constructed using stainless steel and copper tubing with glass connectors. The phase separator was constructed using a PTFE membrane sandwiched between two pieces of Perspex. The injected serum sample was merged with dilute NaOH and subsequently segmented with an organic phase consisting of n-heptane with 2% (v/v) pentanol and 200 ng/ml PR-185 hydrochloride internal standard. A switching valve was used to collect a fraction at the appropriate time. The time was empirically set in order to collect the terodiline-rich extract. Then the collected fraction was manually injected into a gas chromatograph. Extraction yields were 60% when a 4-m extraction coil was used. Exact detection limits were not listed by the authors, but peak height ratios between the terodiline and the internal standard appeared reasonably linear down to 25 ng/ml.

Audunsson[59] discussed a method which coupled SE-FIA directly with a gas chromatograph. A sample containing amines with mol wt < 200 is injected into an alkaline carrier. The sample is then brought in contact with a Teflon membrane on which n-undecane is immobilized. The neutral amines are extracted into the membrane, followed by back extraction into a stagnant acidic acceptor stream on the opposite side of the membrane. When sufficient time is allotted for the extraction/back extraction to occur, the acceptor stream is moved to a second valve where a heartcut of the zone is injected onto a GC column. This technique is illustrated in Figure 19. RSDs less than 40% were reported for amines in urine down to 1 ppb.

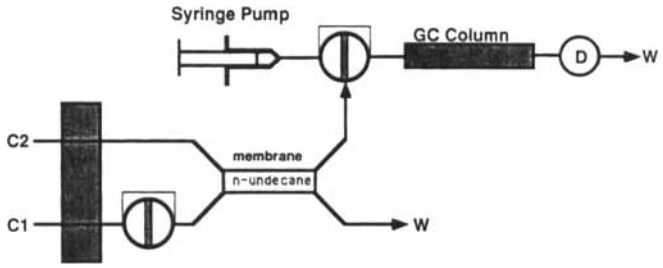

FIGURE 19. An SE-FIA system directly coupled to a gas chromatograph. C1 is an aqueous carrier and C2 is an organic carrier. Details are found in the text.

d. ELIMINATION OF INTERFERENCES

One of the major objectives of all extraction methods is to separate the analyte from interferences. Ogata et al.[60] employed the reaction of silicate with ammonium molybdate which forms a colored silicomolybdate complex for the determination of silicate in the 0.01 to 1.0 μg ml^{-1} range. Phosphate is also known to form a complex with molybdate, which leads to interference in the silicate determination. Urine samples containing silicate and phosphate are injected into a 0.2 *N* HCl carrier stream. The injected samples pass through a Dowex column with molybdate adsorbed to the resin. The phosphate in the samples is adsorbed to the column whereas the silicate is not. The samples are then merged with a stream containing ammonium molybdate. MIBK is then added by confluence to segment to aqueous stream. The silicomolybdate complex is extracted into the MIBK segments. Next, the segments are separated and the silicomolybdate complex is measured spectrophotometrically. The RSD of the measurement of silicate was 2.5%.

Another example of SE-FIA to assist in interference elimination is given by Sweileh and Cantwell[61] in which they extracted Zn(II) from a matrix which contains Fe(III). Fe(III) is reduced to Fe(II) with ascorbic acid. KSCN is then added to the Fe(II)-Zn(II) mixture by confluence. This mixture is then segmented by MIBK and the $Zn(SCN)_2$ is extracted into the

MIBK segments. Excellent results were reported. When a 2% Fe solution containing 0.5 ppm Zn was analyzed with direct aspiration, i.e., without FIA solvent extraction, the zinc concentration was found to be 0.92 ppm based on the calibration curve. The same solution when extracted using the SE-FIA gave a zinc concentration of 0.47 ppm. The authors suggested that SE-FIA could be routinely used for elimination of many kinds of matrix effects and spectral interferences.

2. Unique Phase Segmentation and Separation

The devices shown in Figure 18 are used to provide segmentation and separation of the phases once extraction is complete. However, these devices sometimes are not quite robust enough, and therefore alternative segments and separators have been developed.

Bäckstrom et al.[62] discussed a phase separator based on a fluoropore filter supported by a Teflon-coated filter screen. A CU-diethyldithiocarbamate (DEDC) solution in chloroform was used as a test analyte. A NaCl aqueous solution was added by confluence to the organic stream to partition the organic stream and increase the pressure drop across the dialysis membrane. This was done in order to test the separator stability. A 1.5 m length of 0.5 mm internal diameter (I.D.) tubing was used as a pressure restrictor which increased the pressure drop to 1 atm above ambient pressure. When the pressure drop across the membrane was ambient pressure, 18% of the organic phase was lost. Increasing the pressure drop to 0.3 to 1 atm above ambient pressure decreased the loss of organic phase to 0.1 to 0.5%. However, when increasing the pressure drop further to 2 atm, significant amounts of sodium were found in the organic phase. Pressure drops of 0.2 to 1 atm were recommended for optimal separation and recovery as well as to decrease the penetration of the aqueous phase through the membrane. The separation efficiency was also measured for various aqueous-organic phase volume ratios. The range of phase volume ratios (Q_w/Q_o) was 32 to 0.053. The loss of organic phase in any case was less than 0.8% when using CCl_4. The loss of organic phase under similar conditions when using freon was less than 0.4%.

An unsegmented extraction system developed by Sahlestrom and Karlberg[63] avoided the problem of separation of the two immiscible phases following extraction. They devised a technique in which the aqueous sample is injected into an aqueous carrier which contacts a PTFE membrane, held rigid by two phase separator plates and a membrane support. A thin film is formed by a chloroform organic phase present on the opposite side of the membrane. The organic acceptor stream, similar to that shown in Figure 18c but being supplied from a reservoir, is stopped during the time which the sample is diffusing across the membrane in order to form the thin film on the membrane. This thin film leads to increased surface area for extraction and acts as the acceptor (extracting) stream. They achieved extraction efficiencies typically between 8 and 18%. While this is far less than routine SE-FIA efficiencies of 60 to 96%, they suggested that the system is useful for "coarse" extractions.

Imasaka et al.[64] used SE-FIA coupled with fluorometric detection to determine gallium in the 4 to 80 μM concentration range. They described a simple phase separator which was designed around a PTFE membrane. Lumogallion was used to extract the gallium from an aqueous stream into isoamyl alcohol. Extraction efficiency coupled with the fluorometric determination was best when the extraction coil length was 35 cm and the tubing inner diameter was 1 mm. This configuration yielded a linear calibration curve to 80 μM. Longer coils or tubing of narrower diameter yielded a sigmoidal curve due to the collection stream being over enriched with the gallium-lumogallion pair. They employed a pH scanning method similar to that described earlier[57] to determine the optimum pH range for extraction and detection of the gallium.

Canete et al.[65] employed a novel extraction method which did not rely on phase separation. Instead, they designed a system in which a Hellma flow cell with a volume of 18 µl was placed in a 490 µl injection loop. This is a variant of the nested loop design described earlier and illustrated in Figure 9f. The injection loop-flow cell moiety was filled initially with the organic phase (chloroform). Next, the injection loop was placed in the inject position (like a typical FIA experiment) and the carrier, which contained the analyte, began to flow through the injection loop. It is important to note that at this stage two aqueous-organic interfaces were present, one at the inlet and one at the outlet of the injection loop. Additionally, the analyte concentration near the interfaces was initially homogeneous. The carrier was then subjected to repetitive flow reversals, which gradually enriched the analyte in the organic phase. The volume of the injection loop containing the organic phase and the duration of the flow reversals were adjusted such that the organic phase-aqueous phase interfaces never reached the flow cell. The analyte used in this study was the anionic surfactant SDS. An ion pair is formed between the SDS and methylene blue, which is added by confluence prior to injection of the organic phase (chloroform) similar to the extraction reported by Kawase et al.[53] described earlier. The ion pair is extracted into the chloroform. A gradual increase in signal due to the ion pair extracted into the organic phase was demonstrated, with an increasing number of flow reversal cycles.

3. Multiple Extraction

Manual extractions often include several stages to enhance separation. This principle has been investigated for use in FIA. For example, Shelly et al.[66] reported on a three-stage extraction system, in which polynuclear aromatic compounds (PNA) were extracted from a burned oil extract. The system is shown in Figure 20. Three extractions were carried out sequentially. A sample is injected into pentane which merges with DMSO. The DMSO phase (after phase separation), containing the PNAs and other polar compounds, is then mixed with water. The

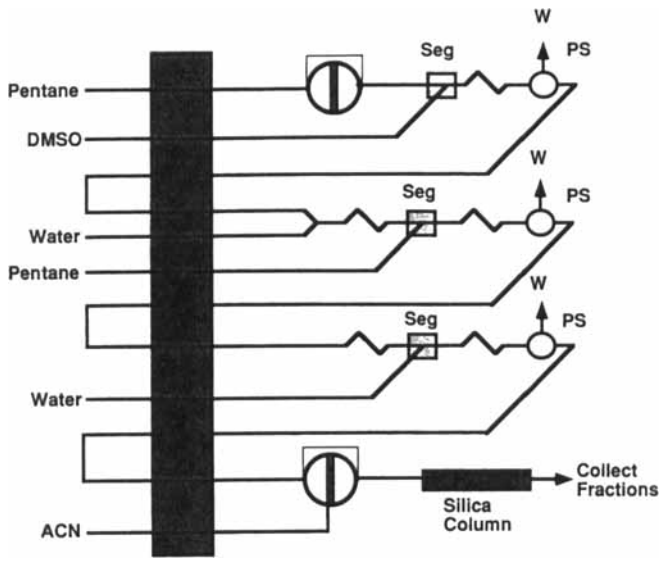

FIGURE 20. A multistage SE-FIA system. Seg are segmentors and PS are phase separators. A sample is injected which is subjected to a series of three extractions. The analyte is finally adsorbed on a silica column and subsequently eluted by injection of acetonitrile. The appropriate fraction is collected and injected onto a HPLC column.

DMSO-water mixture is segmented with pentane, which back extracts the PNAs. The pentane phase is washed with water to remove any remaining hydrophilic materials. The pentane phase is passed through a silica column. The adsorbed sample is then eluted with acetonitrile and collected. The collected material is finally analyzed by reversed-phase HPLC. The automated sample cleanup procedure just described was compared with a similar manual extraction. Excellent similarity was seen between the two, although the magnitude of the peaks on the chromatogram demonstrated that the recovery efficiency of the automated method was not quite as good as the manual method. The authors cited the lack of vigorous conditions in the FIA method as a reason for the decreased efficiency.

Bengtsson and Johansson[67] performed a back extraction which further purified the sample, in addition to the purification achieved by the first extraction step. Cd, Cu, Pb, Co, and Ni were complexed using ammonium pyrrolidinedithiocarbomate (APCD) and diethylammonium-N,N-diethyldithiocarbamate (DDDC) and were extracted into freon segments. The freon segments passed through the first of two Teflon extraction membranes. The resulting organic phase which contained the metal complexes entered a second segmentor. An aqueous solution containing an excess of mercury(II) formed segments in the organic carrier. The mercury ions formed strong carbamate complexes and displaced the APDC and DDDC from the metals in the organic phase. The liberated metal ions were then back extracted into the aqueous phase, which were separated, and then were measured by atomic absorption. The authors reported enrichment factors of 15 to 20, preconcentrating samples from the submicrogram per liter range to their detectable range of 20 to 50 $\mu g\ l^{-1}$. This technique can be operated in a continuous mode of extraction. Instead of using a finite sample volume which typifies FIA, the sample can be extracted continuously, and the measured response attains a steady-state value.

4. Continuous Extraction

Certain applications may dictate the use of a continuous solvent extraction system. For example, a continuous flow system might allow continuous preconcentration of a dilute analyte until the appropriate concentration of analyte is reached. Bergamin F° et al.[68] described a solvent extraction method for the determination of molybdenum in plant ash solutions down to concentrations of 0.05 ppm. They used tubing with an I.D. of 0.85 mm, a 0.11 ml phase separator, and an 80 µl flow cell. They injected a sample volume of 8.75 ml which essentially was the same as a single, continuous plug of sample, which reduces dispersion. The reagent (thiocyanate) and the extractant (isoamyl alcohol) are added in sequence by confluence. Extraction occurs, and the organic phase is separated and the molybdenum measured by spectrophotometry.

Nord and Karlberg[69] reported on the use of a continuous flow SE system. A sample, containing Cu, Zn, Pb, and Ni, is pumped into the extraction system via a two-way valve which selects between the sample and a water rinse stream. A contiguous, nondispersed zone of sample reaches the confluence point where the reagent APDC is added and mixed. The aqueous phase which contains the metal-APDC complexes is then partitioned with MIBK. Following extraction of the metal-APDC complexes into the MIBK, the organic segments are separated from the aqueous segments. At the appropriate time, a second switching valve directs the resultant organic stream into the AA spectrometer for detection. Selectivity is achieved by using the AAS inherent specificity.

Gallego and Valcarcel[70] used SE-FIA to preconcentrate and indirectly measure perchlorate. The method is based on the extraction of copper(I) into 6-Me-PAA in MIBK in the presence of anions such as perchlorate. The amount extracted is proportional to the concentration of anion present. The perchlorate sample was pumped, and a solution of Cu(I) in the presence of ascorbic acid and sodium acetate was continuously added by confluence. The sample/carrier merged with the 6-Me-PAA/MIBK solution, and the Cu was extracted into the organic phase. Following separation using a T-separator, the copper-6-Me-PAA complex was determined using AAS. The analytical range was given as 0.1 to 5.0 $\mu g\ ml^{-1}$. The relative standard deviations for the 2 $\mu g\ ml^{-1}$ perchlorate standard was 0.70%. The detection limit was given as 70 ng/ml. Optimum conditions were also listed, as well as interferences from other anions. SCN^- was listed as the most serious interference.

Alternatively, the continuous monitoring of a process stream may be made using a continuous solvent extraction system. Atallah et al.[71] continuously extracted a sample containing the analyte by merging an aqueous stream which contained the sample with a slower flowing organic phase stream (Figure

21). After segmentation and extraction, the organic phase is separated from the aqueous phase. The aqueous phase is discarded, and the organic phase is redirected back to extract fresh sample in a closed loop fashion. Thus, the concentration of the analyte is steadily increased in the organic phase. This system can be used for analysis as well as a preparative technique.

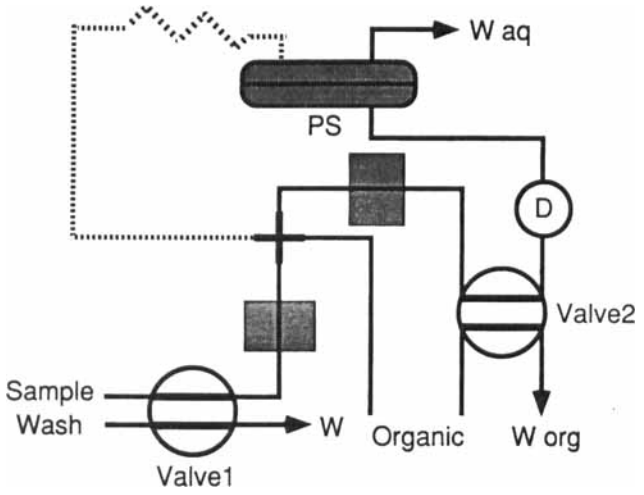

FIGURE 21. Continuous loop solvent extraction system where a sample stream is continuously merged via valve 1 with an organic stream and segmented. Extraction occurs in an extraction coil and the phases are separated by a phase separator (PS). The organic stream passes through a flow through detector (D) and then may be recirculated via a switching valve, valve 2, and further enriched in the analyte. Alternatively, the organic stream may be sent to waste in preparation for another analysis.

5. Extraction Theory References

There have been a number of articles which treat the extraction process theoretically. While the scope of this review is to discuss the current techniques of sample handling and pretreatment by FIA, certain theoretical articles have practical importance. Bäckstrom et al.[72] discussed factors which affect dispersion in phase separators. Sweileh and Cantwell[73] discussed the use of peak height measurements in solvent extraction FIA. Johansson et al.[74] examined the determination of extraction coefficients using SE-FIA. In an article by Lucy and Cantwell,[75] the kinetics of extraction were reported. Specifically, they examined the contributions of segment length, interfacial area, the segment aspect ratio, and the coil tightness of the extraction coil on the extraction rate.

E. Preconcentration Techniques

There are many cases where the analyst must determine the concentrations of elements using detection methods whose detection limits are greater than the concentration range of the sample. There are methods by which one can perform preconcentrations manually, such as solvent extraction or affinity chromatography. However, many of these techniques are quite time consuming.

There have been FIA methods developed which perform preconcentration steps automatically. An excellent review of preconcentration methods[76] and a recent work on further development and synergism with liquid chromatography[77] demonstrate that preconcentration using microcolumns in FIA has many advantages over the traditional, manual techniques.

Most of the FIA preconcentration methods are based on the use of a microcolumn to significantly reduce the volume of the analyte, from a large injected volume (about several hundred microliters) to a small detected volume (about several microliters). Essentially, the sample is adsorbed on the solid support in the column. This is accomplished via ion exchange or formation of a chelate — either on the column or prior to adsorption. The rest of the sample's matrix is washed away by the carrier. An abrupt change of carrier then elutes the adsorbed analyte into as small a volume as possible. The volume reduction can be as significant as several milliliters into several microliters, thereby preconcentrating (locally) more than several hundred-fold.

Ion-exchange methods of preconcentration are used in which elution is achieved by replacement of cationic analyte molecules with hydrogen ions or anionic analyte molecules by hydroxide ions. Similarly, the metal ions may adsorb onto a ligand bound to a suitable solid support. Typical ion-exchange elution then follows. Sorbent extraction/preconcentration methods use adsorption of metal chelates or analytes with appropriate organic functionality onto the column support, followed by elution through abrupt changes in polarity of the carrier. Such columns can be incorporated into a manifold as shown in Figure 22. Here, a nested loop approach is used, where in the load position the sample is loaded on a timed basis onto the column, and in the inject mode the eluent elutes the sample. The research in this field has been directed toward finding sorbents and eluents which yield the greatest preconcentrating ability.

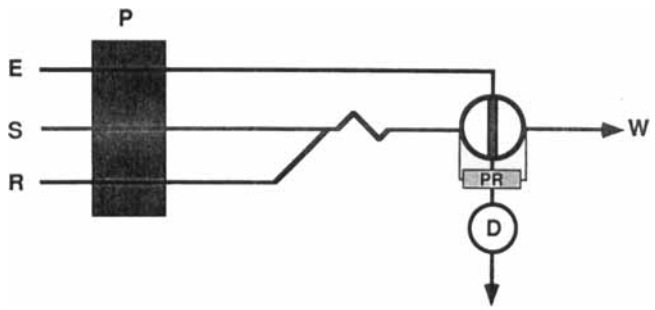

FIGURE 22. Manifold incorporating a column for sample preconcentration. The sample, S, is merged with a reagent, R, if necessary, and loaded onto a packed reactor. As discussed in the text, the reactor can be an ion exchange column or an affinity column. In either case, after an appropriate load period, the valve is switched to the inject mode and the eluent, E, elutes the adsorbed sample. The metal ions are then detected using a suitable detector.

1. Ion Exchange

Bergamin F° et al.[28] first reported the use of an ion exchange reactor coupled with FIA to preconcentrate samples containing micrograms per liter of ammonium ion. A reactor containing a cation exchange resin was embedded in the sample loop. The sample was loaded on the reactor on a timed basis. In the inject mode, a NaOH eluent stream eluted the sample off the reactor. The eluted ammonium ion formed a colored complex with the Nesslar's reagent and was detected spectrophotometrically.

A frequently cited reference in the FIA preconcentration literature is that of Olsen et al.,[78] who used a Chelex-100 reactor to preconcentrate seawater samples prior to analysis by AAS. In a comprehensive study, the authors described a method to preconcentrate heavy metals such as Pb and Cd in the seawater samples more than tenfold. They loaded the 5-cm reactor with sample (2 ml) and subsequently eluted the analyte with HNO_3 and detected it by AAS. The limit of quantification of Pb using AAS was reduced from 227 to 15 ppb.

In two papers, Hartenstein et al.[79,80] demonstrated the efficacy of sample preconcentration prior to ICP-AES. They used a chelex ion-exchange reactor onto which they loaded the sample, using a load time to suit the analytical requirements, but typically in the 40 to 190 s range. Elution was achieved using 2 *M* HNO_3 in an ICP nebulizer, allowing simultaneous preconcentration and detection of Ba, Be, Cd, Co, Cu, Mn, Ni, and Pb. Peak height enhancements of 18- to 74-fold were achieved. Recoveries were in most cases greater than 95%, with low parts per billion detection limits of these elements.

Cox and McLeod[81] employed an activated alumina reactor for the preconcentration of Cr(III) from human urine matrix. A 0.2 to 10 ml sample containing the Cr(III) was injected into the FIA manifold, where the Cr was absorbed onto the reactor. A 200 μl portion of 2 *M* HNO_3 was used to elute the Cr(III). Recoveries of 93% were reported. The method is improved when a large sample volume is used. For example, using a 200 μl sample, the limit of detection (LOD) was 0.92 $\mu g\ l^{-1}$ and the RSD for $\mu g\ l^{-1}$ was 12%. However, when a 10 ml sample was injected, the LOD decreased to 50 $ng\ l^{-1}$, and the RSD for the 10 $\mu g\ l^{-1}$ sample was 2.4%. This method was reported to reduce matrix effects from Na and K, while interference from Ca and Mg was still noted.

Malamas et al.[82] reported on a procedure in which metal ions, including Cu(II), Co(II), Cd(II), Pb(II), and Zn(II), were preconcentrated using 8-quinolinol immobilized on porous glass. The sample is loaded onto the reactor in a continuous fashion for a desired period of time. The volume of the sample is then determined based on the sample flow rate. A 400 μl portion of 0.5 to 1.0 *M* HNO_3 was used to elute the sample. Using a sampling time of 25 min, a 500-fold preconcentration could be achieved. A standard deviation of less than 1% was reported for 0.6 ppm Cu using AAS determination. Recoveries were good for these metals in samples with pH of 6.5. Marshall and Mottola[83] have discussed the effects of pH, reactor dimensions, temperature, and flow rate on the breakthrough capacities of the reactor. The breakthrough capacity is a measure of the capacity of the reactor to retain the analyte. They have also studied the selectivity of sorbent extraction for transition and alkaline earth metals [Cu(II), Fe(III), Ni(II), Co(II), Ca(II)] onto the reactor.

Wei et al.[84] discussed the need of robust extraction methods which would not be easily compromised by conditions of on-line industrial analysis. The possibility of corruption of the Teflon extraction membrane, for example, precludes the use of this type of solvent phase separation device in industrial situations. They discussed a novel technique in which a sample of gasoline which contained mercaptans was loaded onto an anion exchange reactor. The mercaptans were adsorbed by the hydroxyl groups on the reactor. An aqueous, alkaline plug eluted the sample from the reactor; however, now the aqueous plug is juxtaposed by organic solvents on both sides. A second valve was used to perform a "heart-cut" of this aqueous zone and inject it into a second, aqueous carrier. The mercaptan was derivatized with aqueous DTNB and detected optically. The system, reminiscent of zone sampling, is illustrated in Figure 23. The system performed well for sample cleanup. An additional benefit of this approach was that the zone was transferred into the easier-to-handle aqueous phase.

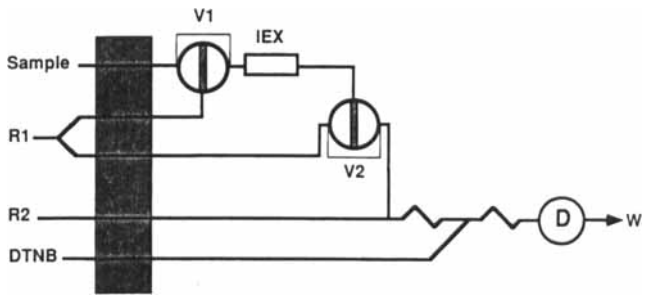

FIGURE 23. Manifold for sorbent extraction and elution. A gasoline sample containing mercaptans is continuously loaded onto an anion exchange column via the first injection valve (V1). A 60 μl portion of an alkaline reagent, R1, is then injected to elute the sample from the column. The center of the aqueous bolus containing the mercaptan analyte is removed by the second injection valve (V2) with a sample loop volume of 15 μl and injected into R1 and merges with a buffering reagent R2. DTNB is added by confluence, forming a colored product which is subsequently analyzed.

2. Sorbent Extraction

Ruzicka and Arndal[85] described the first use of sorbent extraction in FIA. They pointed out that chelates such as immobilized 8-quinolinol are successful for pretreatment purposes, yet are limited in use to just a few elements. A more flexible technique would be to absorb a metal-chelate complex in one step and elute it in another. The metal chelate would be formed prior to being absorbed on the reactor. 8-Quinolinol, 4-(2-pyridylazo)resorcinol (PAR), 1(2-pyridylazo)-2-napthol (PAN),

and diethyldithiocarbamate (DDC) were used in this study. The absorbed complex is then eluted by reversing the polarity of the carrier. They used methanol to elute the complex. They used C-18 bonded silica as the sorbent material. It is clear that, although C-18 was used in this study, other appropriate sorbents such as C-2 or C-8 could be used depending on the conditions required. In addition, with recent advances in solid supports, other supports such as polymeric-based bonded phases have been found useful in conditions with extreme pH. Using a 1 ml sample, preconcentration factors of 60-fold were obtained in less than 2 min. This is not as large as the 500-fold preconcentration factor achieved above using the chelating reactor, but the time between samples is dramatically decreased. The implications of this work are that, with few foreseen exceptions, most analyte-chelate combinations which have been developed for use in solvent extraction could be used in sorbent extraction FIA. Sorbent extraction preconcentration techniques add several advantages to preconcentration, including mechanical simplicity, no organic waste, and flexibility in application to different analytes.

An example of preconcentration of anionic species by sorbent extraction FIA is the work of Lacy et al.[86] The phosphate in the sample was reacted with ammonium heptamolybdate, forming phosphomolybdate. This species is then adsorbed to a C-18 column. The molybdate is then reduced with ascorbic acid. Measurement is made directly on the column which allows a 40-fold increase in the sensitivity over conventional sorbent extraction followed by elution.

A similar technique was discussed[87] in which a sample containing metals such as V, Cr, Ni, etc. were injected into a system which then forms a chelate with DTC. The chelate is then adsorbed onto a polystyrene-divinylbenzene column. Interferences from the matrix pass through the column unretained. The carboxyl groups on the metal complexes are then ionized with a basic carrier, eluting the metals. The metals are then analyzed with an ICP-MS. They used this technique with biological and environmental samples, with little matrix contributions for either. Limits of detection for these metals were in the 8 to 80 ng/L range.

Since sorbent extraction has been primarily developed as a means of sample pretreatment prior to introduction to a HPLC column, a wealth of information exists in the chromatography literature on this topic. An example of precolumn preconcentration technique which is pertinent here is the work of Trippel-Schulte et al.[88] Trippel-Schulte et al. used a reactor packed with a macroreticular styrene divinylbenzene copolymer, onto which they injected a sample containing a carcinogenic aromatic amine analyte. They eluted the analyte with methanol following this preconcentration and sample cleanup step. Such references will undoubtedly serve as a starting point for future development of sorbent extraction FIA methods.

3. Flow Reversal

Canete et al. demonstrated that gaseous samples can both be determined and preconcentrated using flow reversal FIA.[89] A gaseous sample is loaded into an injection loop yielding two gas-liquid interfaces. Repeated flow reversal cycles mix the sample and reagent, forming a detectable product. Preconcentration is achieved by repeated filling of the sample loop, allowing more product to be formed. This technique is very similar to the extraction solvent extraction technique described by Canete et al.[65]

III. CONCLUSION

It is clear that FIA offers many unique advantages for the handling and pretreatment of samples. Industrial, clinical, and research laboratories could and do currently benefit by using such techniques. Obviously, the design of the FIA apparatus and technique will have to fit the need of the analysis at hand. None of the techniques listed above are expected to be entirely appropriate for the analysis at hand. However, the authors have chosen one or two methods for each technique which promises good utility and flexibility, and which the authors recommend as a beginning for the reader's pretreatment technique. These techniques are shown in Table 2.

Table 2
Preferred Methods of Sample Pretreatment

Pretreatment	Appropriate FIA technique	Ref.
Dilution	Gradient chamber zone sampling	42
Preconcentration	Sorbent extraction	86, 87
Extraction	Liquid-liquid extraction	53
	Continuous loop extraction	69, 71
Dialysis	Size exclusion dialysis and simple dilution	49
Gas diffusion		50, 51

FIA is now recognized as a viable and appropriate method of performing analyses of industrially important analytes. The ultimate goal, of course, is the direct coupling of FIA to the process where it can analyze the process and directly contribute to its control. However, the analytes of interest are seldom present in suitable matrices or concentration ranges. Indeed, the methodology for manual analysis of industrial analytes (and others) often include several pretreatment steps. As shown above, there are several methods each for preconcentration or dilution or for sample/matrix removal, including solvent extraction or filtration. The very conditions which prohibit analyses or which would foul or destroy the detector can be systematically and automatically removed using FIA. Furthermore, such industrial samples may be toxic or radioactive. Instead of allowing the possibility of accidental exposure of the analyst, the use of FIA would dramatically reduce these risks. Pretreatment by FIA offers increased throughput while simultaneously decreasing reagent consumption and man-hour involvement.

Our industrial colleagues share a consensus that FIA will be a method of choice for process analysis as robust components become commercially available. Flow injection systems are now being offered which, like process chromatographic systems, employ robust, commercially available injection valves and manifolds which are manufactured in Teflon or stainless steel. Detectors are being manufactured that employ rugged flow-through cells. Furthermore, pumps are available which are designed to propel aqueous and nonaqueous solvents.

A FIA apparatus can easily be built using commercially available components or complete systems can be purchased which vary in design. While the authors do not endorse one model over others, one commercially available system designed specifically for process analysis can be obtained from FIAtron Inc. (Oconomowoc, WS), which is based on a pressurized solvent system to propel the carrier. This apparatus is designed to reside at the process line and can accomodate a high pressure sample line.

This group is focusing on efforts to develop portable flow injection analyzers. The entire system can be transported in a "suitcase" and can be battery operated. It is controlled by a laptop computer and was designed for automatic analysis. It is our belief that such instruments which can be easily put in service on the process line would allow great flexibility in the diagnostics of the process.

At the other end of the scale are laboratory applications where pretreatment using FIA enhances the performance of a wide range of instruments — photometric, atomic, or electrochemical. Automated pretreatment serves two primary purposes: enhancement of the analytical range and matrix removal. Governmental, academic, and private analytical laboratories are using FIA to increase their efficiency and precision.

Clinical laboratories have equally challenging sample pretreatment problems. Most clinical laboratories must analyze hundreds of samples daily, many of which contain human pathogens. FIA has been shown to be well suited for enzymatic analysis and for performing serial assays.[10,11] It only makes sense that the sample be pretreated with the same technique by which the measurement is being made. Conversely, as shown earlier, FIA is remarkably flexible in its use with other techniques. Many clinically important compounds are analyzed using a gas chromatograph. However, as is often the case, derivatization, filtration, separation, dilution, or preconcentration is often required prior to injection onto the column. FIA offers a simple means for such precolumn sample pretreatment.

Regardless of the application, when repetitive analyses need to be made which require sample pretreatment, FIA is a viable means to perform the pretreatment. Discarding for a moment the barriers between techniques which terminology often erects, using the technique which works and provides the correct answer is the final goal for most analysts. Of course, an equally important problem is constructing the analytical system so that it conforms to the other constraints of the analytical problem. Whether FIA is used to pretreat a sample prior to injection on a chromatographic column or a chromatographic step is used prior to injection into a flow injection analyzer, the bottom line has to be the correct analysis of the sample. The authors believe that hyphenated techniques will continue to be developed which employ FIA. One may envision the development of continuous, bedside patient monitors or robust industrial analyzers, both being capable of direct, on-line sampling, pretreatment, and analysis. In any case, the vast reduction of the turnaround time required for analysis will allow both critical medical and industrial processes to be made on a more timely basis.

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