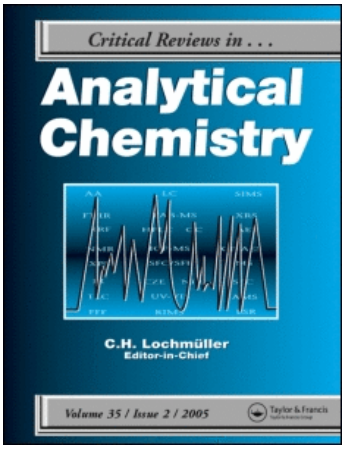

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Simultaneous Determination of Several Components by Flow Injection Analysis

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Simultaneous Determination of Several Components by Flow Injection Analysis

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ABSTRACT: Simultaneous determination of multiple components by the flow injection technique (MC FIA) of various configurations with a single detector (single or multichannel), or with several detectors in series or in parallel, is described. Exclusive instrumental modules, i.e., multifunctional valves, special detectors, and their combinations are also given. Special techniques employing liquid-liquid extraction, membrane separation, packed microcolumn reactors, immobilized enzyme reactors, etc. are discussed. The applicability of MC FIA in simultaneous determination and speciation of metal ions, inorganic anions, and organic compounds in diverse samples is also presented.

KEY WORDS: multicomponent analysis, flow injection analysis, speciation, simultaneous determination, sequential determination, inorganic analytes, organic substances, metal ions.

I. INTRODUCTION

With ever-increasing demands being placed on the speed and complexity of analyses, the simultaneous determination of several components in a single sample has been gaining in importance. Classical separation techniques, such as chromatography, electromigration, extraction, etc. are among the most frequently used conventional methods for sample treatment prior to measuring the concentrations of the analytes. The techniques solve important problems related to selectivity improvement in a large number of determinations, especially in connection with the growing complexity of the sample matrix. Chromatographic and electromigration techniques allow highly efficient separation of a large number of compounds of diverse character in a single run, but they are relatively time consuming, with the typical sample frequency not exceeding several samples per hour. Instrumentation is rather expensive and, in addition, it produces an excessive amount of information that is usually not required for practical use.

The development of different instrumental methods for the simultaneous determination of several components in the same sample without previous separation has occurred recently. This is partly because modern analytical instrumentation offers an excessive amount of information, only a small part of which is usually employed for quantitative purposes. This is a major alternative to sequential determination and, in addition, facilitates the automation of the procedure involved by avoiding the customary separation or masking agents formerly used.

The application of continuous flow analyzers, mainly flow injection analyzers (FIA), for the simultaneous determination of several components in very small sample volumes (typically tens or hundreds of microliters) has been challenging for many years because of the simplicity of adaptation and the great variability of flow injection manifolds. The rapid development of

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multicomponent flow injection analysis (MC FIA) is perhaps due to great improvements in sampling frequency, reduction of sample and chemical consumption, and variable selectivity and sensitivity of flow-through detection systems.

MC FIA manifolds in different configurations have been described by many workers, most of whom used relatively complex flow systems with single or multiple detectors, either in series or parallel. A large number of analytical procedures, including those employing different separation and/or preconcentration techniques, have been devised in combination with all common detection systems. Their combinations as part of the FIA technique are easily adaptable for the simultaneous determination of two or more parameters in the same sample.

The concept of MC FIA is defined as the determination of several species in the same sample injected into a single FIA manifold.[1-3] In contrast, one definition of *speciation* is a method for determination of the various individual physicochemical forms of the same element.[1-3] The advantages and difficulties of these very progressive methods, with some analytical applications, were discussed in several monographs[1-4] and comprehensive reviews.[5-9] Method feasibility has been demonstrated for different kinds of analytes.

II. INSTRUMENTATION

In classical MC FIA adaptation (Figure 1), aqueous sample solutions are usually introduced continuously or in definite volumes into a continuous aqueous stream, which serves as both the reagent and carrier stream in the simplest single-line version. The aqueous sample solution can also be merged and mixed with another separate aqueous stream containing an organic analytical reagent (OAR), spectral buffer, etc. Appropriate chemical reactions, as well as physical processes of mass transport in the solution, take place in a reaction and mixing coil before entering the detection system. An analytical signal is treated in the usual manner, with the analyte concentrations being calculated from the peak heights, the peak areas, or the peak widths, or using different mathematical algorithms for data evaluation.

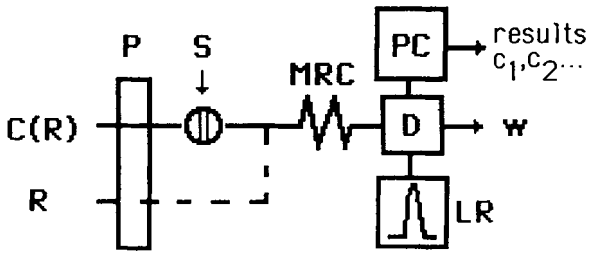

FIGURE 1. A single-line FIA manifold. C = carrier stream; R = reagent stream; P = peristaltic pump; S = sample; MRC = mixing and reaction coil; D = detector; LR = line recorder; W = waste.

Several fundamental problems must be considered when constructing an MC FIA system, especially in connection with sample introduction, data treatment, and kinetic aspects of the processes in solution. Most components of MC FIA systems are commonly used in classical FIA. Comprehensive reviews of the basic components of FIA systems have been published. Component design and selection is based on the same principles used for the classical FIA systems. Therefore, only the components that are unique to MC FIA systems are described here. Some special modifications of the detection systems are also mentioned.

A highly stable pumping system is usually required in MC FIA because more rigorous control of the total flow rate and its long- and short-term stability are of importance, especially for fast-reading/scanning multichannel detectors. Rigorous control of the flow rate is not always possible with peristaltic pumps. The biggest drawback of these pumps is the presence of pulses in the stream generated (even in the presence of pulse dampers). The frequency of pulsation is comparable to the reading frequency of fast reading/scanning detectors; thus, measurement precision is negatively influenced. Flow rate also changes significantly during the starting period of the peristaltic pump and ultimately with the aging of the pumping tubes. Therefore, conditioning of pump tubing is required in order to assure constant flow rates.

High-performance liquid chromatographic (HPLC) pumps with pulse dampers and pressure regulators are an expensive solution to the problem, especially when several independent streams

are required. Linear plunger or syringe pumps are preferred for MC FIA with fast-scanning multichannel detectors because the reproducibility of the results is significantly influenced (RSD from fractions to units of percentages for linear plungers and peristaltic pumps, respectively) by the type of pump used.[10] HPLC pumps cannot ordinarily be used with a high salt content in the flowing stream. A suitable circulating pump with a small dead volume is not commercially available. Displacement techniques, involving either a constant gas overpressure with the aid of inert gas from cylinders or pumping air into a closed container (usually a thick-walled bottle), or aqueous streams forced by gravitation are widely used, inexpensive alternatives.

Several somewhat complex designs of injection devices for dedicated use in MC FIA manifolds have been presented in the literature. Six-port rotary loop injectors are the most frequently used designs. The use of a coupled multiple injection system in which valves are internally coupled in series or in parallel is recommended for creating double peaks and/or for simultaneous or sequential sample and reagent introduction as plugs into a single or multichannel MC FIA manifold.

A configuration of internally coupled valves with a packed microcolumn located in the loop of the secondary valve[11] was proposed for speciation of several elements. Depending on the column characteristics, a washing stream flowing in the opposite direction to the sample may be required. Washing steps can be performed conveniently using the proposed configuration.

A simple and versatile pneumatically operated two-layer rotary valve was described for simultaneous introduction of samples and diversion of analysis streams in MC FIA.[12,13] Applications include valve configurations with time-controlled sample volumes and with loop-controlled sample volumes in one or two loops. Both configurations are useful in routine analyses of samples containing highly varying analyte concentrations. The usefulness of the valve for ion-exchange preconcentration procedures was also demonstrated.

A single eight-port valve or the parallel configuration of two six-port valves is very suitable for creating a merging zone or zone penetration modes, but it can also be used for creating the "sandwich-zone" technique (Figure 2). A complex 16-port injection valve (Figure 2) with two identical sample loops, one loop filled with a discriminating (masking) agent and one loop filled with a separation liquid (carrier solution typically) enclosed inside the particular loop, has been used for instantaneous introduction of two plugs of the sample separated from each other by the long segment of the separation liquid.[14] A simple modification allows creation of the "sandwiched-zone" technique.

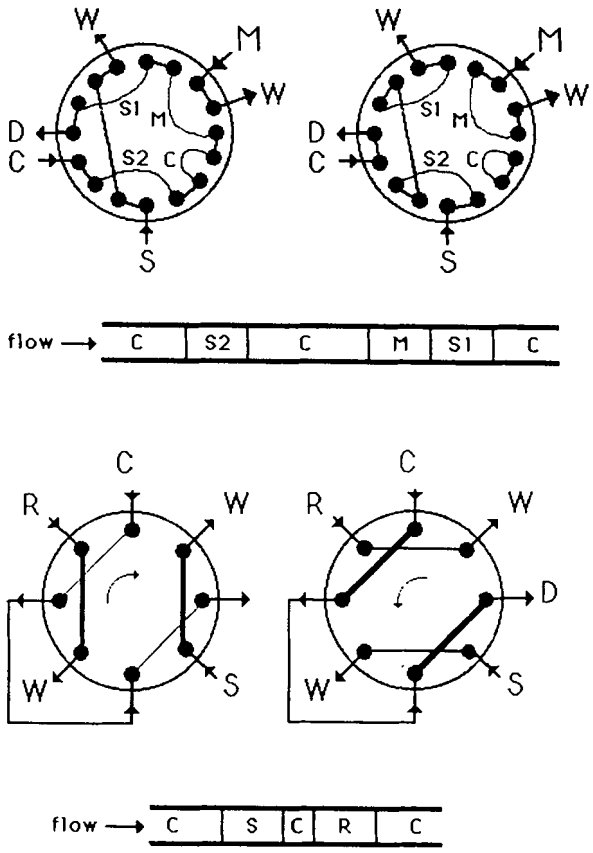

FIGURE 2. Schematic diagrams of 16- and 8-port injection valves (top and bottom, respectively) with a sequence of reaction components. M = Masking (discriminating) agent and its plug; S1, S2 = sample loops and sample zones; other symbols, see Figure 1.

The selection of suitable detection systems is limited by the requirement of variable selectivity. Electrochemical and optical detection systems are generally preferred. Simple and less expensive potentiometric (ion-sensitive electrodes [ISE], glass pH electrode, chemically [ion] sensitive field effect transistor [CSFET, ISFET] sen-

sors, etc.), as well as amperometric and other electrochemical sensors are frequently used. A single common reference electrode situated at the exit of the measuring cell is frequently used in MC FIA configurations that employ several detectors (Figure 3).

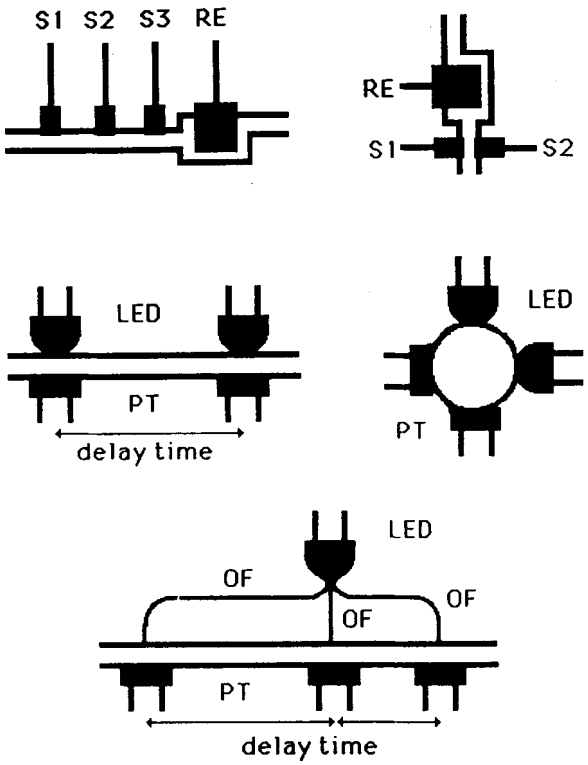

FIGURE 3. Schematic diagrams of in-series configurations of electrochemical (top) and LED photometric "on-tube" detectors, with or without optical fibers (bottom and middle, respectively). S1, S2, S3 = Electrochemical sensors; RE = reference electrode; LED = light emitting diode; PT = phototransistor; OF = optical fiber. Time delay is determined by the mutual position of the sensors and flow rate.

The use of expensive optical detectors is limited in this context. They are most frequently used in kinetic studies at constant time, which is defined by the mutual position of the detectors and injection device in the MC FIA system. They are also employed in different MC FIA configurations for some special analytical problems or in combination with other detectors for speciation. They can also be used for the simultaneous determination of several components using a single OAR or a mixture of several OARs when the components to be determined differ significantly in their optical parameters.

Solid-state, flow-through photometric detectors incorporating light-emitting diodes (LEDs) as the sources visible, ultraviolet (UV), and NIR radiation, and photodiodes or phototransistors as detectors, provide simple, reliable, and low-cost alternatives to commercially available spectrophotometers. In the simplest design, the LED and phototransistor are glued directly into a nontransparent detector body and the flowing stream comes into direct contact with the plastic surfaces of the solid-state components. Alternatively, both demountable active components are located behind small glass focusing lenses. An "on-tube" design has the advantage of negligible sample dispersion within the flow cell, which is realized by the perpendicularly oriented (semi)transparent capillary tube.

Several LEDs can be situated along the tube to form an in-series configuration (Figure 3), or a single multicolor LED can be operated by the computer to establish appropriate conditions to select a suitable diode with the required optical parameters. A flow photometer with a multicolor LED light source, using microcomputer control of photometric measurements and data processing, was applied to the simultaneous determination of several analytes.[15,93] Single or multichannel optical detectors, using a monochromator or a single or multiple source of monochromatic light, with optical fibers as light guides, can also be used. They are our inexpensive alternative to the in-series or in-parallel configurations of single or multiple photometric detectors.

A detector with a dual flow cell positioned in a single light path (Figure 4) was used to simplify the FIA manifold and to reduce its cost. A delay coil, a packed microcolumn reactor, or similar differentiating elements can be positioned between cells to provide for various experimental conditions. A photometric system with reference and sample cells was introduced as an alternative to the configuration with two detectors in parallel.[16] The organic and aqueous phases passed through the sample and reference cells of a double-beam spectrophotometer, respectively, after their separation by a dual membrane phase separator at different residence times (Figure 8). The

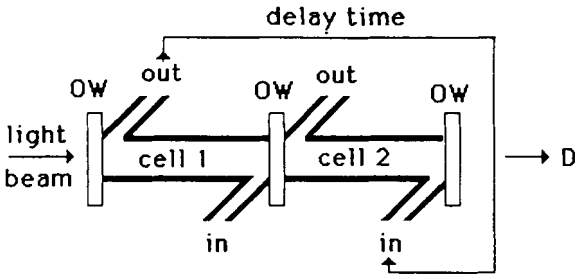

FIGURE 4. Schematic diagram of the dual flow cell photometric detector. OW = Optical window. The time delay is determined by the actual distance between the cells (reaction coil can be placed between the cells) and the flow rate.

detector was modified to allow electronic switching of the sample/reference designation of both flow cells by setting a double-pole, double-throw relay. The relay, used to establish either normal or reversed flow cell operation, was controlled with an electronic timer after a preselected time delay to measure both phases. The zones produce positive and negative peaks corresponding to the extractable and nonextractable species. A similar system was used in a single-phase MC FIA for the simultaneous monitoring of the two species in a two-line configuration with a single reductor column.[17]

Optosensing[18] allows real-time monitoring of signal changes (dA/dT), so that the rate of color development during the reaction step of the analysis can be measured. The system uses an inexpensive spectrophotometer with a net path length through the silica-based C-18 sorbent. The system was applied to illustrate the extension of the use of hydrophobic sorbents in FIA for preconcentration of anions on a packed column reactor and for on-column detection. A kinetic-based optosensing method was developed in which differences in the reduction rate for heteropoly complexes allow the simultaneous determination of phosphate and silicate. Partial least-squares (PLS) analysis was used to evaluate the data. Predicted errors were obtained for both components. A reversed-flow elution method using refractive index detection was explored as an alternative to optosensing.

The splitting and confluence points are most frequently realized by a single W-, Y-, or T-component. The purpose of the splitting point is to divide, continuously and reproducibly, a single carrier stream containing a sample zone into two separate flow streams with a preselected ratio. They are then directed to the particular channels of the manifold. At the confluence point, these streams are mixed into one uniform stream entering a single detector. Both devices have to maintain the concentration profile of the analyte, prevent deterioration of the original concentration gradient, and prevent or minimize any further dispersion and dilution of the zone over the wide range of experimental conditions.

III. CLASSIFICATION OF MC FIA MANIFOLDS

A wide variety of principles are applicable to the simultaneous determination of several components performed in narrow-tube systems such as FIA. Consequently, different types of connecting manifolds of varying complexity, are described in the literature for common or unique MC FIA methods. The complexity and wide range of applications cause difficulties in manifold classification. The most commonly used classification systems are based on the relationship between the number of species to be determined, the number of detectors used, or the injection principle.

A single or multichannel detector can be used to assay several species, or the FIA system may consist of several single-channel detectors, each for a particular species to be determined. This subgroup is defined as multidetection systems. The mutual position of the detectors in the MC FIA manifold and their characteristics (principle, selectivity, sensitivity, etc.) can be used as a secondary criterion. In the former case, a distinction can be made according to whether the detectors are arranged in series or in parallel.

Another principal criterion is the injection principle. Systems with single and multiple (simultaneous or sequential) injection devices can be selected as another subgroup — multidetermination. Injection devices of different complexity have been described in the literature for common or special applications. Table 1 gives a

TABLE 1
Characterization of MC FIA Systems

No. of Detectors	Configuration	Characterization
Several	In series	Single injection, selectivity principle identical
		Multiple injection
	Parallel	Multiple injection
		Single injection
		Zone sampling
Single		Zone splitting
		Fast reading/scanning
		Gradient technique

survey of the basic classification of MC FIA systems. Other schemes, i.e., in time and in space detection classification, etc., are also used.

A. Systems with Several Detectors

Two different classes of MC FIA configurations with several detectors can be distinguished, depending on the injection and transportation technique applied (series or parallel). The configurations are usually simpler in relation to the data handling and to operate but are more expensive MC FIA manifold designs, in comparison to single-detector systems. They are less sensitive to interferences and probably also to interionic interactions among the components, particularly when detectors of different principle are used.

Particularly promising techniques for simultaneous determination of several species and/or speciation are those that employ two or more different detection modes. Several detectors of variable selectivity and sensitivity in different configurations are most frequently applied for the simultaneous determination of the species of diverse nature, while detectors in series operated at the same experimental parameters are preferred for differential kinetics-based determinations.

Combinations of detectors of different types and selectivities (destructive and nondestructive, selective and nonselective) can be adapted for the determination of different forms of the analyte in solution (speciation). An analytical signal from nondestructive sensors, such as ISE, ISFET, etc., corresponds to the concentration of the ionic form of the species, while the analytical signal from destructive sensors (atomic absorption spectrometry [AAS], atomic emission spectrometry [AES], etc.) corresponds to the total concentration of the species in solution.

Separation techniques, e.g., membrane separation and packed columns, can also be used for sample pretreatment and subsequent determination of several components (hydrogen sulfide, carbon dioxide, and sulfur dioxide, etc.) by an in-series configuration with nonselective (potentiometric, conductimetric) and highly selective spectrophotometric detectors. These techniques, in combination with various discrimination techniques, which are based on differences in acid/base, redox, and other properties of the species, can also be used for sample pretreatment. The subsequent speciation of dissolved gases and total content of the anions evolving the gases (e.g., hydrogen sulfide and sulfide concentrations, respectively) can be detected by in-series or in-series/in-parallel configurations with potentiometric, conductimetric, and spectrophotometric detectors.

The serious drawback of systems with flame atomic absorption spectrometry (FAAS), inductively coupled plasma optical emission spectrometry (ICP OES), and flame (FOES) detectors is that they require an additional water line to accommodate higher nebulizer uptake (Figure 5, center). The flow rate is usually adjusted between 3 and 5 ml/min. Flow rates for FIA systems and other detectors are generally lower.

The systems are relatively expensive due to the presence of several detectors. Simplified systems with a double pass sample zone, in a single

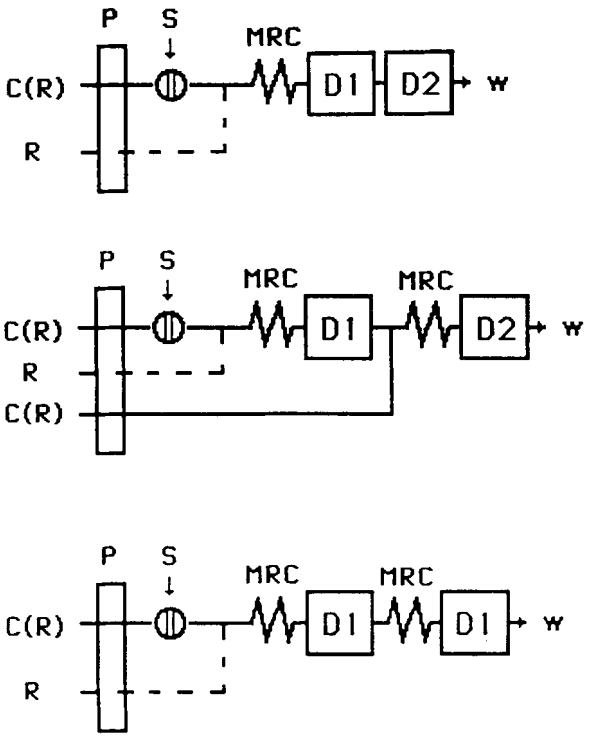

FIGURE 5. MC FIA manifolds with detectors in in-series arrangement. Two detectors of different selectivity (top), different principle (center), and identical operational parameters (bottom).

measuring cell in a closed loop[19] or with iterative reversal systems, have been described. Detection systems with two separated flow cells positioned either in a single or double light beam and also different LEDs-based detector configurations have also been used.

1. Detectors in Series

Three different combinations can be used in this particular configuration (Figure 5) involving multiple detectors. These are

- Different selectivity (different nature of analytes or reaction products)
- Different principles (destructive and nondestructive, selective and nonselective) for speciation or determination of the actual concentration of the ionic form and total concentration of the analyte
- Different operational parameters (kinetic measurements, conversion techniques for speciation, ligand exchange reactions)

Single injection of a sample is most frequently used.

In this particular system, the single sample zone is introduced into a single-line MC FIA manifold. The sample zone or the zone of the reaction products sequentially passes each individual selective detector. The analytical signal obtained by each detector corresponds to the concentration of the particular species. Complex mathematical treatment of experimental data can be used for (1) elimination of interionic interferences of other components of interest; (2) minimization of matrix effects; and (3) correction of differences in zone dispersion.

a. Detectors of Different Selectivity

In-series arrangements employing multiple electrochemical detectors[20–26] positioned along the capillary tube, with a reference electrode situated at the exit of the measuring cell, are popular (Figure 3). Miniature ISE electrodes or ISFET sensors can also be positioned[24] perpendicular to the main axis of a single measuring flow cell (Figure 3, right top). Such systems can also be used in single MC FIA configurations. The latter configuration eliminates the influence of sample zone dispersion, because measurements are made at the same position in the FIA manifold. The same configuration can be realized employing[27–29] several photometric detectors (Figure 3, center). The configuration is suitable for measurement of two different species based on differential kinetics of metal ion catalysis. A composite manifold has been designed that consists of a flow cell containing four ISEs and two spectrophotometric transducer flow cells.[21]

b. Detectors Based on Different Principles

Promising techniques for speciation and/or MC FIA are those that employ two or more dif-

ferent detection principles in series[30] because the configuration is generally less sensitive to interferences. Only a few papers describe manifolds using two different detectors for speciation of inorganic compounds.[30–36] Most utilized spectrophotometric and AAS detectors for speciation of different metal ions. Combinations of amperometric, or ISE detectors with FAAS, or molecular spectrophotometry[21,34–36] are frequently used. Combinations of detectors that operate at the same parameters are seldom employed in this context.

Analytical signals of nondestructive sensors, such as ISE and ISFET, correspond to the concentration of the ionic form of the species, while the analytical signal of destructive sensors (AAS, AES, etc.) correspond to the total concentration of the species in solution.[30] In addition, the introduction of a discrimination element or masking agents between both detection systems improves separation of the complexed and uncomplexed ions, thus permitting the determination of free *and* total analyte concentration.[37]

Combinations of potentiometric, conductimetric, and photometric detectors in series can be used for speciation or for the determination of volatile species. The method involves preseparation of volatile analytes with a flow-through gas-diffusion unit. Nonselective electrochemical detectors allow the determination of total concentration of ions that evolve gaseous species due to changes in acidity or conductivity of a suitable acceptor stream flowing through a membrane diffusion unit. The response of a selective spectrophotometric method corresponds to the content of a particular species (sulfur dioxide, carbon dioxide, hydrogen cyanide, etc.). The discrimination of the mass transport through the membrane, based on acid/base and/or redox properties of the species, can also be successively used to improve selectivity.

c. Detectors Operated at Identical Parameters

This particular system is frequently used for kinetic measurements and for techniques based on ligand exchange reactions, redox-differentiated reactions, etc.[38,39] A sensitive catalytic method using FIA has also been presented for the simultaneous determination of trace amounts of diverse ions.[40–46] The cation-exchange separation of analytes from matrix metal ions, which also serves for mutual separation of those metals, can be directly coupled on-line with a catalytic photometric detector in a continuous-flow system.[44] Catalytic methods are based on measurement of the increase in the absorbance, at a particular wavelength, resulting from the metal ion-catalyzed oxidation of a suitable OAR. The method of analysis is simple, rapid, and accurate, and can be achieved in a continuous and nearly closed system.

The use of detectors operating in series using identical parameters can be modified by using a single detector with two independent flow cells situated in a single light path (Figure 4) or a photometric detector employing an optical fiber system transmitting a monochromatic light from a common source (e.g., monochromator or LED source).

2. Parallel Configuration of Detectors

Multichannel FIA systems, with multiple detectors in parallel, can be divided according to the injection principle or the transport mode into several subsystems. These include a single injection (splitting zone), simultaneous multiple injection, and zone sampling (Figure 6). Detectors that exhibit different selectivity, sensitivity, and operational principles are frequently combined. Detectors working under identical conditions are seldom used in parallel configurations. The complexity of valve system injection devices, as well as of the FIA manifold, seem to limit the practical value of the methods.

a. Splitting Zone Technique

A single sample plug is injected by a single-loop injector into a carrier stream. The plug may be mixed with the reagent solution or spectral buffer in this simple, but efficient, technique. After mixing, the initial injected volume is divided into two or more secondary parts in a defined and highly reproducible and precise manner, preferably without deterioration of the original sample concentration gradient. Each par-

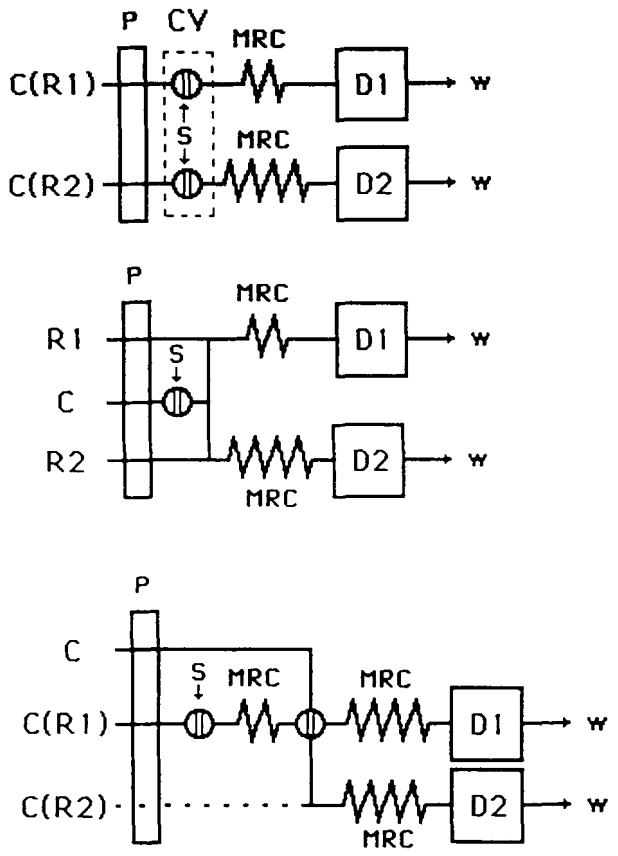

FIGURE 6. MC FIA manifolds showing a parallel arrangement of two detectors and simultaneous (top) and single injection with a splitting zone (center) and zone sampling techniques (bottom). CV = Internally coupled injection valves.

ticular subplug, which maintains the concentration profile of the original plug of the sample at the splitting point, is transported through the separate channel of the FIA system.[21,47–51]

The secondary plugs of the same sample may be optionally mixed with suitable OARs to form the reaction products of different physicochemical properties. The same effect can also be produced by packed microcolumn reactors (enzymatic, reductor, etc.), which transform the analyte species into a single form (lower or higher oxidation state, simple ion, etc.).

Each subplug of the sample or reaction product reaches the individual detection system situated in the particular channel of the FIA manifold. For example, analytes can be sensed by a dual-channel flame photometer in one channel and a dual-channel FAAS spectrometer in another channel for the simultaneous determination of Na and K, and Ca and Mg, respectively.[51]

b. Zone Sampling

An unusual, but rather interesting, principle is based on the zone-sampling concept where different parts of the partially dispersed single-sample zone can be separated and introduced into two or more individual channels of the FIA system. Two detection systems of different selectivity and/or a significant difference in sensitivity (several orders of magnitude) and a multichannel injector device or two injectors in series in the FIA manifold are essential for this technique.[52,53]

An electronically time-operated valve injection system can be used to select a suitable part of the zone to pass through the secondary sample loop for delivery to a particular channel. The central part of the zone, which is less dispersed, can be separated and transported to the less sensitive detection system. The combined parts of the more dispersed zone can be sensed by a more sensitive detector. A precisely defined volume of a strongly dispersed trailing part of the zone can alternatively be separated for a particular detection system while the rest of the zone continues to its own detection system.

One or both secondary zones can be merged with suitable OARs to produce reaction products of different physicochemical parameters. In this particular case, it is possible to determine two or more components with very low consumption of the sample and reagents. A combination of AAS and spectrophotometry or electrochemical detection seems to be preferable, in that simpler chemistry treatment is required.

c. Multiinjection

A complex injection device can also be used for the simultaneous introduction of precisely defined volumes of several zones of a single sample into the separated individual channels of the FIA manifold.[54–57] The zones are mixed with the suitable OARs and sensed by a particular detection system. The number of sample loops of the in-

jector device and the number of independent channels of the FIA manifold have to be the same as the number of the species to be determined. A single multichannel loop injector (from 8 to 16 ports) or several simple pneumatically or electronically operated injectors in parallel can be used for introduction of the sample plugs. The advantages of the complex MC FIA manifold are disputable when compared to two single-line manifolds. The system can be useful when very slow kinetic reactions are in use and when excessive delay time between particular zones of the same sample drastically decreases the sampling frequency.

B. Systems with a Single Detector

These systems are more frequently used than the previous group because they are generally less expensive due to the presence of a single detector. Appropriate MC FIA manifolds are, of course, more flexible. Two main groups can be distinguished: (1) sequential detection (double peak, time resolved), with a single or multiple injection (simultaneous or sequential); and (2) simultaneous detection with a single injection (Figures 7, 8, and 9). In the former case, a single-channel detector is used in connection with a differentiation element (delay coil, packed reactor, etc.) and a splitting-zone technique or with a dual injection system in which the valves are coupled in series or in parallel. A confluence point is situated in front of the detector. A multichannel fast scanning detector with the appropriate software for the mathematical treatment of the experimental data is used either for a single scan, or for multiple scanning at a preselected frequency for each sample, in the latter case.

MC FIA with a single multichannel fast-scanning detector with adjustable selectivity and sensitivity and appropriate computer programs for mathematical "separation" of the components is one of the most promising techniques for the future. This is due to the simplicity of the manifold, the need for minimum chemistry, and the increase in speed of the "separation" of the components. The main disadvantage of these detectors is their cost, but a decrease in the price of the computers and data acquisition units, as well as the instrumentation, can be expected in the near future. Much cheaper LED-based multichannel instrumentation, fiber optic-based detectors, or simple dual-wavelength detectors can

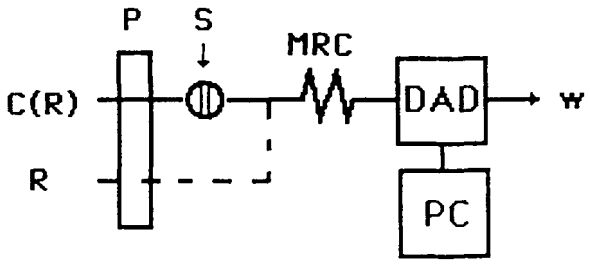

FIGURE 7. MC FIA manifold with a multichannel fast-scanning detector (diode array UV-VIS, ICP OES, etc.) operated by a computer (PC).

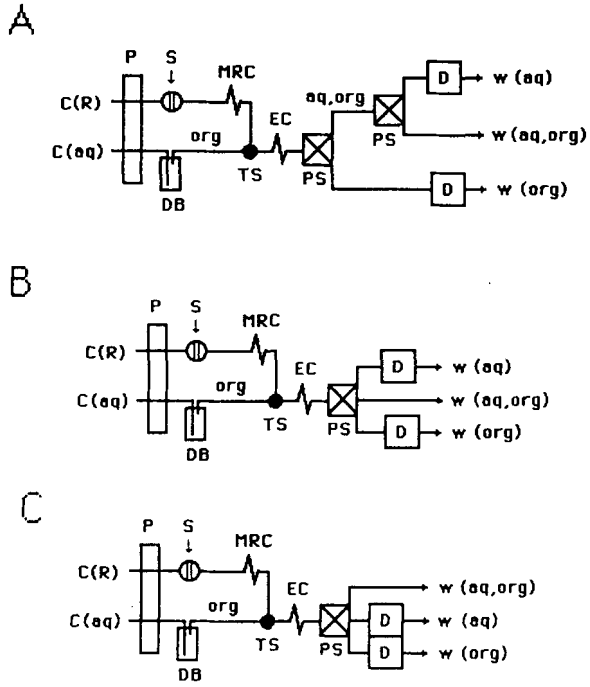

FIGURE 8. Schematic diagrams of MC FIA manifold for liquid-liquid extraction with two phase separators in series (top); a dual-phase separator and two independent detectors (center); or single double-beam photometric detector with sample and reference flow cells for aqueous and organic phases, respectively (bottom). DB = Displacement bottle; aq = aqueous phase; org = organic phase; TS = phase segmenter; EC = extraction coil; PS = phase separator; w(aq), w(org), and w(aq,org) = waste for separated aqueous, separated organic, and unseparated aqueous and organic phases, respectively.

be more convenient for less complicated applications at present.

A single optical detector can replace the two detector systems when two flow cells are in a common light path (modification of the FIA manifold depicted in Figures 5, 6, and 8) when sample and reference cells are used (Figures 9 and 10), or when two independent flow cells for sensitization of two different reaction products are used.

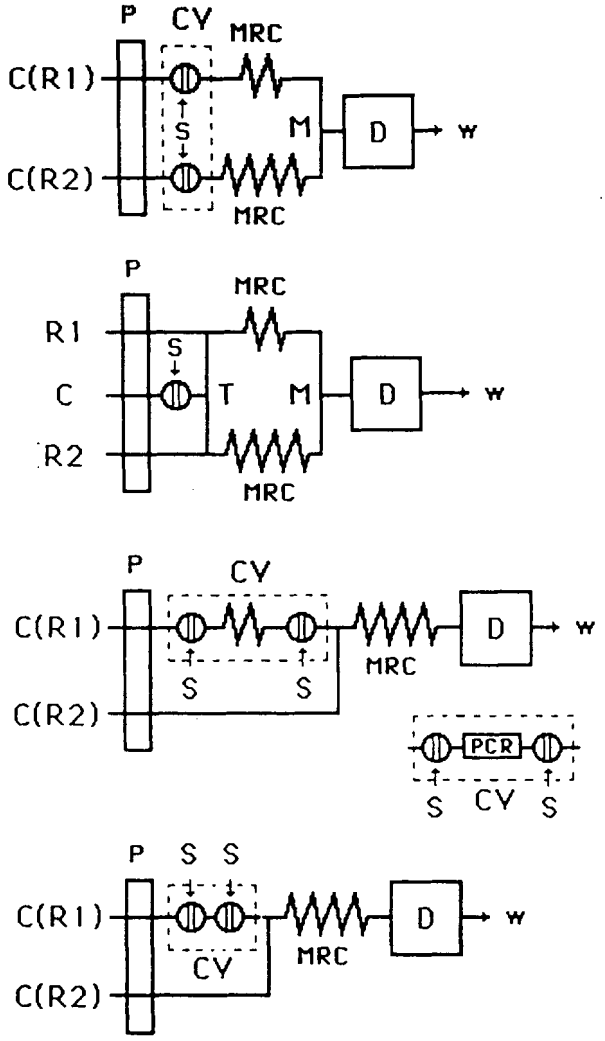

FIGURE 9. Schematic diagrams of MC FIA manifolds with a single detector with a simultaneous injection (top), single injection with zone splitting (second from top) and subsequent merging of the streams, or with a simultaneous injection of two sample plugs into a single-line MC FIA manifold (dual-peak and zone penetrating techniques). T = Splitting point; M = merging point.

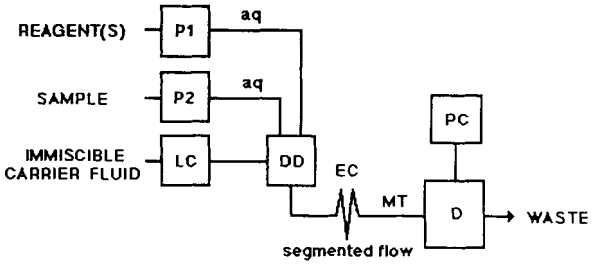

FIGURE 10. Schematic diagram of MC FIA manifold for liquid-liquid extraction FIA without phase separation. P1, P2 = Peristaltic pumps; LC = liquid chromatographic pump; DD = dual-channel coaxial phase segmenter; MT = measuring FEP transparent tubing; D = fast reading on-tube photometric detector with millisecond time resolution; PC = PC/AT personal computer.

A reaction/retention coil or a discriminating element (enzymatic, ion-exchange, or reductor reactor) can be situated between the splitting and the confluence point in front of the two cells and/or between the cells. The coil allows complete temporal separation of the two sample zones. Thus, two independent peaks are obtained, each corresponding to the concentration of the particular components.

1. Simultaneous Detection

Great potential exists in the use of multichannel optical detectors, which permit measuring the analytical signal over a wide spectral region. Some examples of these detectors include UV-VIS diode array detectors,[10,58–71] FOES and ICP spectrometric detectors,[53,72–73] and molecular emission cavity analysis (MECA)[74] detectors (Figure 7). Less attention has been paid to the application of fast-scanning electrochemical detectors.[75,76]

Diode array detectors (DAD) or fast-scanning UV-VIS detectors with vibrating or rotating optical elements interfaced to a suitable computer allow spectral information to be acquired over rather short intervals (tens to hundreds of milliseconds). The temporal scanning of an absorption or emission spectrum is performed at the maxima of the analytical signal, or during other time-

dependent characteristic points of the analytical signal (e.g., inflection point, etc.). Information can be integrated as successive reaction zones pass through the optical flow cell at a constant, preselected, sampling frequency (tens to hundreds of milliseconds). This method might enhance the determination of more complex kinetic information of the processes.

Such information in turn allows quantitative results for the sample components to be obtained with a degree of accuracy that depends to a significant extent on the calibration procedure used. The consecutive data treatment allows a number of parameters, such as kinetic or equilibrium constants of the reaction or the instantaneous concentrations of all components, to be evaluated. A number of components can be effectively determined on the basis of their own characteristic absorption or emission. The characteristic absorption or emission of the reaction products formed by the individual components of the analyzed mixture with a suitable nonselective OAR or a mixture of highly selective OARs can also be used effectively.

Direct MC FIA methods based on the measurement of characteristic UV or VIS absorption or secondary emission by all the components investigated, without the addition of any reagent, can give reliable results at major and minor concentration levels (RSD <1 and 10%, respectively), provided their optical characteristics are sufficiently well separated and the spectral interferences are minimized.[58,65,68] The accuracy and precision of the results of indirect MC FIA methods using a single, nonselective OAR exhibiting low selectivity and reacting with all components in the sample to be determined are often poorer due to the small differences between the spectral parameters of the reaction products, mutually as well as with respect to the reagent.[50,60–62,64–66]

It is reasonable to expect the accuracy and precision to improve with the use of several highly selective OARs,[10,63,70] each of which reacts with a single component of the solution with a comparatively high value of the selectivity coefficient (multiligand MC FIA). Compared to the conventional MC FIA employing a single, nonselective OAR, the multiligand MC FIA affords more accuracy and precision. This is due to the greater differences between the spectral characteristics of the reaction products, whereas in conventional single-ligand MC FIA, these are determined basically by the properties of the analyte.

Of intrinsic factors, inter-ionic interactions affect the basic spectral parameters of the compounds, thereby causing variations in their values with changing concentration ratios. This effect can be partly eliminated by using the values of spectral parameters obtained in conditions closely approaching the expected composition of the actual sample mixtures to be analyzed, by multivariate calibration, or by employing mathematical treatments that take these interactions into account as an additional linear or nonlinear term.

No significant differences are generally obtained between the results for overdetermined systems (number of wavelengths, NL, is greater than number of components, NC) and for classical systems (NL equals NC); precision and accuracy are not influenced by NL. The measurements at the absorption maxima of the particular chelates with a single dual-wavelength detector are usually sufficient for obtaining precise results in MC FIA.

Another important factor in MC FIA can be the mutually competitive equlibria of analytes and other components which can affect the completeness of the formation of analytically usable reaction products, thus giving rise to apparent deviations. In this respect, the multiligand MC FIA approach employing two or more highly selective OARs, each reacting predominantly with one of the components to be determined, is more convenient. The reaction products formed offer, by a suitable choice of OARs, a higher variability than is possible in conventional MC FIA. In this manner, a higher selectivity of the simultaneous determination and improved accuracy and precision of the data can be achieved by a shift of the reaction equilibria in favor of the analytically usable reaction products. The experimental conditions, such as concentration ratios among all the components, acidity of the reaction mixture, etc., have to be more precisely established.

The absorption of the free OAR in the nearby spectral range, particularly for organic dyes with extensive π-electron systems, also plays an important role. Because the reagent must be used in an excess concentration with respect to the

total concentration of the analytes, the absorption of the OAR in conventional MC FIA is often commensurate with, or even higher than, the total absorption of the reaction components. This, together with nonselective absorbance, has a serious negative effect on the accuracy and precision of the determination.

Several factors, including the volume of sample introduced, exposure time, and ICP operating conditions, that influence the determination of various metal ions in biological and clinical samples were investigated by using the FIA-ICP-OES technique. The results obtained show that the technique is superior to the method of continuous nebulization ICP-OES in analysis efficiency, precision, matrix effect, and sample introduction.[53,72,73,77]

The possibilities for using fast-scanning voltammetric detectors with a cyclic voltammetric unit connected to a three-electrode cell (carbon-paste working electrode, Ag/AgCl reference electrode, and glassy-carbon auxiliary electrode) for potential scans were explored for individual and multi component determination of phenolic compounds in water.[78] The resolution, and hence the selectivity, obtainable from the electrode processes can be improved by derivatizing the current/potential recordings. Individual and simultaneous determination of three species can be achieved by judicious selection of measurement potentials, and, in the worst case, by use of simultaneous equations.[78]

The above-mentioned procedures give sufficiently accurate results rather rapidly for 2 to 5 or, occasionally, for a higher number of components. Thus, little experience has been gained so far with mathematical handling of experimental data from MC under dynamic conditions of FIA. It can be concluded that the characteristic feature of measurements in MC FIA in flow conditions gives poorer analytical signal reproducibility, compared to the stationary conditions. This results in poorer analytical accuracy and precision.

Among the primary parameters affecting reproducibility are the rapidity and reproducibility of sample injection into the carrier stream; pressure pulses occurring while the injector valve is switching between injection and filling positions; and constancy of the flow rate of the carrier stream; the latter can be achieved by employing a pulseless plunger pump.

In the absence of matrix effects, the standard spectral parameters used for the chemometric resolution of the components of interest, are obtained from standard solutions of pure analytes by so-called "univariate calibration". The lack of additivity of the spectral parameters of pure components in mixtures has so far been overcome by obtaining their standard spectral parameters from standard mixed solutions by "multivariate calibration". The calibration models using PLS regression are superior to the results obtained by traditional univariate calibration. A novel standardization method using gradient scanning technique was also proposed to achieve multicomponent standard addition at different sample/standard ratios in a single injection.[79]

In summary, the MC FIA methods, which are rapid and show great promise for use in analytical practice, give accurate and precise results for systems with negligible intra-ionic interactions. Complex information can alternatively be obtained from continuous scanning of the analytical signal at different parts of the sample zone than by using any other MC FIA technique.

2. Sequential Detection (Dual-Peak Techniques)

The amount of information provided by conventional flow-injection systems can readily be enhanced by simple modifications. The different possibilities available for obtaining several peaks per injected sample were reviewed.[7] Among these topics are simultaneous and sequential multidetection, multicomponent determinations (including speciation), reaction-rate methods, and automatic amplification of conventional analyte concentration ranges. There are numerous principles applicable for sequential detection, most of which are based on dual-peak measurement. Classification of the techniques is very confusing.

3. Splitting Zone Technique

This system is similar to that with two detectors in parallel. However, physicochemical

parameters of the reaction products that are to be sensed by a single detector must be similar or identical. Sample throughput is lower due to the sequential detection after differentiation in time by in-line discriminating elements.

A single sample plug is injected into a carrier stream and split into two or more streams.[80-83] Each stream may or may not then be merged with a suitable OAR solution and mixed in reaction coils of different geometries. Different discrimination, transformation, and separation techniques are widely used for formation of a single detectable product or several products of similar or identical physicochemical parameters. The streams are merged into one stream at a confluence point prior to or just after the reaction is completed and the product(s) is (are) sensed by a suitable detector. The plugs of the reaction products are temporally and spatially separated due to the difference between the geometries of the reaction coils or other discriminating devices; thus, two separate peaks are obtained.

The sample can also be split into two flows, one of which is treated directly with a suitable OAR and sent to the sample flow cell of a double-beam spectrophotometer. This gives positive absorbance values and allows direct determination of one particular species in the sample. The other flow goes through a packed reduction (oxidation) microcolumn, where the other species is transferred to a common reaction product.[84,85] The analyte can also be converted into a single species by a ligand-exchange reaction, a masking agent, or a discrimination element. The sample is then treated with the same OAR, and the overall mixture is sent to the reference cell of the same double-beam spectrophotometer. This process gives negative peaks corresponding to the sum of the absorbances of the products.[86]

The procedure was automated using a digital potentiometer, interfaced with the register output of a spectrophotometer. A specific program was developed, allowing data storage of the successive expectations, data treatment for calculation of the calibration curves, and automatic reports of the content of the particular species in the samples.

Single or multiple (subsequent or simultaneous) splitting of a single-sample zone can also be used in connection with several different packed column (enzyme, ion-exchange, reductor, etc.) reactors. The main role of the reactors is to transform species of a different nature to a common product (usually hydrogen peroxide, NADH, a lower or higher oxidation state of elements, etc.) to be sequentially sensed by a single detector (see below).

A nested-loop valve system makes simultaneous determination possible by splitting each injected sample into two sections, one of which undergoes a differential reduction process. This allows two different species to be detected as two sequential peaks.[87]

4. Gradient Techniques

Applying the acidity or concentration gradient technique is effective for cases in which the existence of the analytically usable reaction products of the species to be determined with a single nonselective or mixture of several highly selective OAR depends strongly on acidity or concentration of the components in solution.[88-92] Masking agents can also be used.

A larger volume of the sample is recommended in this particular technique. The volume is injected into the continuous stream of the OAR with experimental conditions (acidity, concentration) which differ by several orders of magnitude. The appropriate acidity and/or concentration gradients are formed at the interfaces between the sample zone and the OAR solution and in the center of the zone due to the diffusion and dispersion of the solutions. The sample penetrates into the OAR zone and vice versa. The components to be determined react with the reagent, depending on the local experimental conditions. Thus, two or more peaks can occur for a single injection.

5. Different Injection Techniques[29,47,52,57]

Several techniques can be used for creating double peaks in single- or multiple-line MC FIA manifolds. The injection of a large volume of the sample (1 to 1.5 ml) into a continuous flow of a carrier stream or into a single-line FIA manifold is used most frequently. The central part of the

plug remains undispersed and can be used for sensing self-absorbing species. The leading and/or trailing parts are controllably dispersed by the mass transport of the reaction components, thus forming two reaction zones.[94-96] The particular parts correspond to the differences in kinetic parameters or differences in acidity or concentration conditions. Two peaks, somewhat separated from each other, are formed. Thus, two or three different species can be determined simultaneously in a single injection.[96] These parts can be used for detection of the species of interest after their selective reaction with suitable reagent(s). These temporally separated peaks can also be used for speciation or for elimination of interferences when the reaction rate of the analyte and interferents differ by more then one order of magnitude.

FIA manifolds that have an adapted injection device, which works in several positions depending on the number of the species to be determined, can be used for creation of the sample and suitable reagent(s) solutions sequence. In each position, the appropriate pair of sample and reagent loops is filled by the sample and reagent solutions while the other pair is washed by the carrier stream into the capillary system of the FIA analyzer. Each pair corresponds to the particular OAR and the same sample solution. The determination is done by the single detector in several sample zones produced by the injection device by simply changing the position during the passage through the detector cell.

The manifold with parallel injection valves allows the simultaneous injection of several sample plugs into individual channels.[33,94,95,97-99] The time delay between the arrival of the plugs at the detector is determined by the placement of a packed reactor and/or reaction coil (Figure 9).

In a manifold with serial injection valves, two sample aliquots are injected into a single line. The valves (and also the plugs) are separated from each other by the packed reactor and/or reaction coil.[100] Two peaks per injection are obtained with a single detector in both cases; the first yields the unconverted species and the second corresponds to the sum of converted and unconverted species. This system was used for the fluorimetric determination of urea and ammonia.[97]

Another system uses a single detector with two flow cells aligned with the same optical path (Figure 4). This system yields two peaks corresponding to two individual zones of sample solution injected simultaneously into separated carrier streams of reagent in a two-line system. Taking advantage of the different residence times of the samples in the manifold lines, the resulting color formation is measured by a single optical detector with two separated flow cells aligned within the same optical path.[101]

Determination of two components in a single sample by a single injection and using a single detector was based on the synchronized injection of a large volume of sample and a small volume of reagent into separated streams. These were then merged at a confluence point and mixed downstream with a chromogenic reagent stream for detection. This system was used for Fe(II)/Fe(III) speciation,[102] determination of Ni(II) aquoion, and Fe(III) ion after its reaction with thiocyanate.

6. Sandwich Zone Technique

This technique is very similar to the one described previously. In this technique, a sample zone is sandwiched between two plugs of two different reagents, a reagent and a masking agent, or a reagent and an inert carrier. The sample zone is injected into the system by using a complex, internally coupled injection device. The 16- or 8-port multifunction valve is an easy and convenient way to intersperse calibration standards and sample determinations by the sandwich zone technique.[103-105] The sample penetrates through both interfaces into the reagent(s) plugs, forming two different reaction systems. The yields of the reactions in both reaction systems depend on the local experimental conditions. The sample can additionally be merged with another OAR before entering the detector. Both interfacial parts of the sample zone react with two different OAR or at two different experimental conditions, thus two different species can be determined. The advantages of using derivative over normal recordings were also considered.

This method was used for Fe(II)/Fe(III) speciation[104,105] when the sample was sandwiched between a zone of water and a zone of ascorbic

acid. It was subsequently merged with 1,10-phenanthroline. The analytical signal of the central part of the zone corresponds to Fe(II), while the signal of the trailing part corresponds to the total amount of Fe.

The second approach is based on the use of a single-line flow-injection system, using the formation of a double peak as a result of injecting a large sample zone sandwiched between reagent zones of appropriate composition. In this manner, two time-resolved signals for the kinetically governed processes can be obtained and used for quantification of the individual species.

The complex 16-port injection valve (Figure 2, top) with two identical sample loops, one loop filled with a masking, an oxidizing, or a reducing agent and one loop filled with a separation liquid, has been used for the instantaneous introduction of two plugs of the sample separated from each other by the long segment of the separation liquid. The system exploits the simultaneous intercalation of a modifying reagent plug and two small sample plugs into the same carrier stream by making effective use of the 16-way valve.[14] One plug with the masking agent and the other untreated plug are merged downstream with a suitable OAR to form reaction products of the binary system. Two well-separated peaks are obtained using a single detector, the first one corresponds to the concentration of one component, which did not react with the masking agent, while the second one corresponds to the sum of both species. The concentration of the second species of interest can be calculated from the difference. The system has been used for the simultaneous determination of Ca(II) and Mg(II) in water[8] and speciation of Fe(II)/Fe(III) ions.[14]

7. Zone Penetration

Zone penetration and similar double-injection techniques are based on the simultaneous injection of sample and reagent zones into a single carrier stream. In the former case, the zones are separated by a definite volume of the carrier liquid, while in the latter case, the zones are in direct contact. The zones penetrate into each other during the transport through the FIA manifold. A reaction and mixing coil facilitates radial mixing.[58]

Three distinct regions can be identified on the dispersion profile due to mass transport. The trailing and leading parts contain the reagent and the sample only. The central part contains both components; the concentration ratio, and eventually the acidity gradient, changes. A single or a double peak thus can be obtained, depending on the volume of the carrier liquid intercalated between the reagent and sample zones and/or on the residence time. By choosing an appropriate time interval after injection, various concentration ratios and/or experimental conditions can be reproducibly selected. More complex information can also be obtained by a repetitive scan on different parts of the plug.

The systems are sensitive to any alteration to the FIA manifold (sample and reagent volumes, concentrations and acidities of the solutions, flow rate, inner diameter of the tubes, reaction coil length, etc.), which results in distortion of the concentration profile of the plug.

C. Special Techniques of MC FIA

1. Packed Reactor Systems

The presence of enzymatic reactors and ion-exchange, oxidative, or reductor packed column reactors for the conversion of several species into a common reaction product dramatically reduces the cost per analysis and the complexity of the configuration. Thus, their use in routine practice is very convenient.[69,106–120] A single column, or several columns in series or in parallel, are commonly used systems. A single sample zone is injected into a carrier stream and split into two or more[114,118] independent streams. Each of these streams passes through a specific reactor, or one of them is directed to the confluence point through an empty column (Figures 11 and 12).

Samples injected into the system can be split into two particular streams with an appropriate ratio. Each stream separately passes through one of the two separated columns (Lichrosorb NH_2, activated with glutaraldehyde) containing immobilized cholesterol oxidase and cholesterol es-

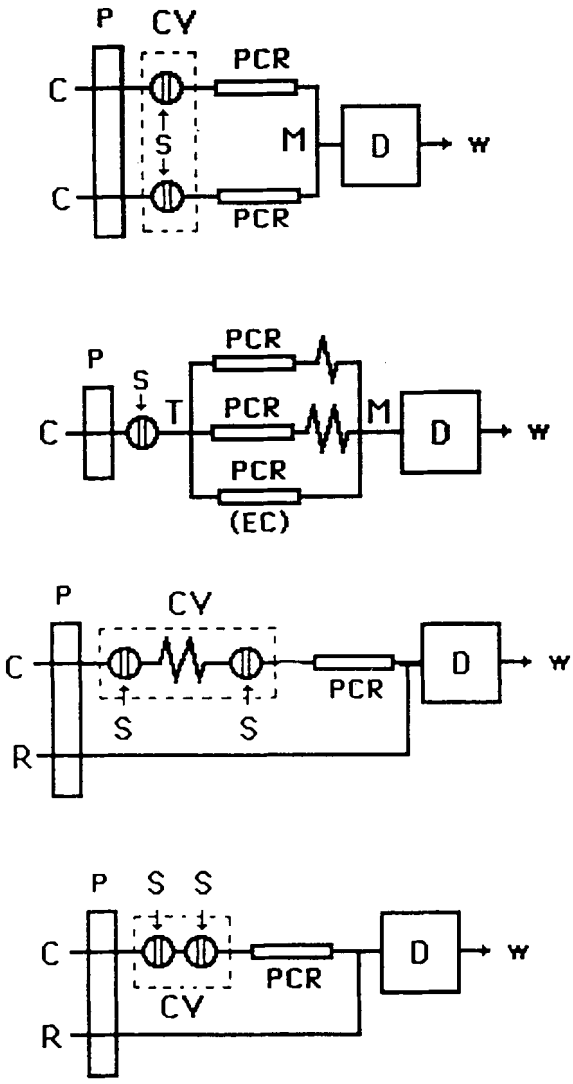

FIGURE 11. Schematic diagram of MC FIA manifold with packed column reactors. P = Peristaltic pump; C = carrier stream; R = reagent; CV = internally coupled injection valve; S = sample; PCR = packed column reactor (enzyme, reductor, etc.); M = mixing point; D = detector; EC = empty column; w = waste.

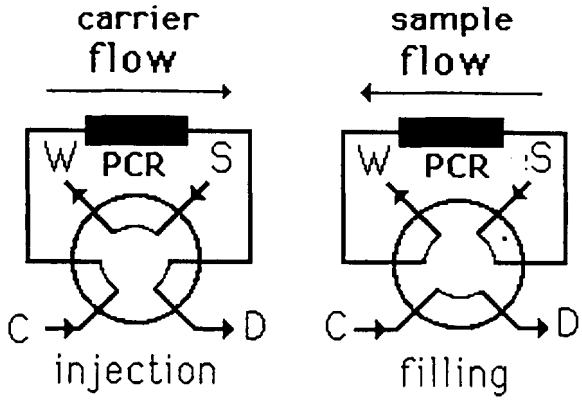

FIGURE 12. Filling and injection positions of the valve with packed column reactor in sample loop position for reversal MC FIA manifold.

terase, and glucose oxidase, respectively. The course for glucose determination is longer than the other so that hydrogen peroxide of different origins can be measured separately with an amperometric flow-through peroxidase electrode in the form of two separated peaks.[107]

The simultaneous assay of two enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), using enzyme-immobilized open-tubular reactors in parallel has an additional channel for evaluating and correcting for the error resulting from pyruvate in the sample sera. The recognition of the signal pattern from three channels of the FIA system makes possible the primary screening of diseases.[108]

A blood serum sample is split so that part passes through an immobilized cholesterol esterase column, before passing through an immobilized cholesterol oxidase column, and the other portion only passes through the latter column. Separated peaks are obtained for free and total cholesterol, with measurements being made of the hydrogen peroxide generated from the oxidase reaction by a hexacyanoferrate (II)-mediated peroxidase electrode. A single-column manifold was used for the simultaneous determination of urea and ammonia in which an enzymatic reactor converted urea into ammonia, which was determined by the same reaction as the ammonia itself.

An ion-exchanger of silica gel microcolumn placed in one channel of a dual channel FIA manifold can also be used for separation of an interferent(s) or a particular component of a reaction mixture[121–127] Two plugs of the same sample are sequentially introduced by a multifunctional injection valve into a reagent stream. Subsequently, the first plug passes through the IC column to adsorb the interferent or analyte and the second plug passes through the empty column. Two peaks are produced. The first peak corresponds to the analyte and the second one to the sum of the analyte(s) and interferent. The interferent or the analyte concentration can be calculated from the difference. The system was

used for individual and sequential determination of V(V) and Ti(IV) with hydrogen peroxide.[33]

Two different species can be determined by splitting the injected sample. A portion of it passes through the reductor column and a delay coil before both streams are recombined. The unreduced portion precedes the remainder of the sample in mixing with the reagent for spectrophotometric detection. Two peaks are produced for each sample.[128]

The sample can also be injected simultaneously by an internally coupled injection device at two inlet points that are separated by a reductor column. The two plugs are then allowed to meet the OAR solution. The colored plugs reach the detector cell at different times, giving double peaks, and are monitored at the same wavelength. The first peak affords the concentration of Fe(III) plus Ti(IV) and the second the concentration of Ti(IV).

2. Liquid-Liquid Extraction

Liquid-liquid flow injection extractions can be easily adapted for the simultaneous determination of several components. The species can be nonselectively extracted into an organic phase (Figures 8 and 10), which is detected by a multichannel detector. Alternatively, the partition of the extractable and nonextractable species between both immiscible phases is detected with two detectors, which are either in series or in parallel, or by a single, fast-reading "on-tube" detector.[130]

The system with two[129] independent detectors was used for the simultaneous monitoring of both immiscible phases. These were first separated using two membrane separators (lipophilic and hydrophilic) connected in series or with the aid of a dual-membrane separator in which the organic phase passes through a Teflon membrane and the aqueous phase passes through a filter paper membrane (Figure 8, top). Simultaneously, the phases are directed to the detectors, and analytical signals are fed into digital integrators. This system was used for the simultaneous determination of diphenhydramine and 8-chlorotheophilline in a pharmaceutical preparation[131] after their separation into cyclohexane and water at pH 10, or for the simultaneous determination of acidity constants.[132]

The combination of two spectrophotometric detectors was used for direct automated extraction ratio measurements. The organic and aqueous phases were detected by two independent computer-controlled diode-array detectors. The full data set was treated, and the composition (2:2:1) and rate constant of the system of U(VI) with (4-benzoyl-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-thione)-trioctylphosphine were determined.[133]

To decrease the complexity of the system, both phases were monitored by a single detector (Figure 8, bottom) in which the organic phase flowed through the sample flow cell, and the aqueous phase flowed through the reference flow cell.[132]

A fast-reading, computer-controlled, "on-tube" photometric detection system (millisecond time resolution)[134–139] is very suitable for some applications in MC FIA without phase separation (Figure 10). A detection system with a transparent fluoroplastic thin-walled capillary or with a special glass capillary flow cell with optical fibers oriented perpendicularly to the main axis of the flow cell was used for computer "phase separation" in MC FIA. A sophisticated "sorting" computer program allows separation of analytical signals measured directly on the aqueous and organic segments and the subsequent determination of extractable or nonextractable species.

The systems were used for the simultaneous determination of pesticides in wastewaters,[134] for the simultaneous determination of both extractable and nonextractable species[137], and determination of water/1-octanol partition coefficients.[135,136]

In-line spectrophotometric detectors situated on a closed-loop system were used for the continuous monitoring of an analytical signal within or out of the system.[138,139] In the first arrangement, the buildup of the extracted species is monitored directly, and a derivative can be formed inside of the loop by addition of a suitable reagent. The other arrangement involves measurement of the analyte after a preselected time delay, which results in a transient signal typical of FIA.

3. Closed-Loop Systems with an On-Loop Detector

A configuration for unsegmented MC FIA was suggested in which the entrapment of the sample plug into a closed system allows its repetitive passage through a single detector (thus providing transient signals) until the sample is completely dispersed into the carrier. The proposed system features the iterative pass of the reacting plug through the detector several times in one (closed-loop system[19,122,130,137,140]) or alternating (iterative-reversal system[141]) directions. The number of peaks obtained per sample is a function of its volume, flow rate, and reactor length. The envelope of the maxima (or minima) of these peaks defines a kinetic curve that allows the application of conventional kinetic methods of determination (initial rate, fixed and variable time). It also provides for calculation of the partial reaction orders and rate constants and application of dilution and amplification methods for concentrated or diluted samples, respectively (Figure 13).

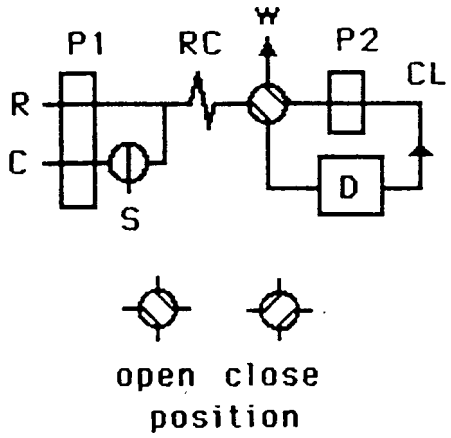

FIGURE 13. Schematic diagram of MC FIA manifold with closed-loop system.

The information obtained can also be used in the simultaneous determination of species by kinetic, catalytic, and ligand-exchange methods. The analysis information obtained has been used for Cr(III)/Cr(VI) speciation, the simultaneous determination of Fe(III) and Co(II) via the EGTA/4-(2-pyridylazo) resorcinol (PAR) ligand displacement reaction, and for the simultaneous determination of Fe(II) and Fe(III) with 1,10-phenanthroline, which selectively reacts with Fe(II).

The cyclic flow-injection configuration allows the repeated passage of the reacting plug through the detector, which results in multiple-peak recordings.[142] From the data obtained, which correspond to a typical kinetic curve, the required sensitivity can be selected by using procedures based on fixed-time measurements (peak maxima or minima) or reaction-rate measurements (signal increment between two successive maxima or minima).

4. Membrane Separation Techniques

A system with three double-tube separation units, each with an inner tube of microporous PTFE and an outer tube of PTFE, has been proposed for the continuous, simultaneous determination of halogenated amines and free chlorine in water. The quantitative reaction between chloramines and iodide was used in order to quantify conveniently the chlorinated species as available chlorine. Mono- and dichloramines and chlorine concentrations can be simultaneously determined by this method, as can inorganic chloramines and chloroamino acids.[143] A similar system with a PTFE membrane was used for the simultaneous determination of cyanide and thiocyanate[144] or nitrate and amonia.[145]

IV. APPLICATION OF MC FIA IN CHEMICAL ANALYSIS

A. Inorganic Cations

1. Metal Ions (Table 2)

Alkali and alkali-earth ions (K^+, Ca^{2+}) and other inorganic ions (NH_4^+, Cl^-, NO_3^-, and PO_4^{3-}) in plant nutrient solutions[21] were determined with four ISEs and two spectrophotometric detectors. Simultaneous detection of Ca ions and the pH with ISFETs has been demonstrated as a model system for *in vivo* measurements.

Potassium, Ca, and pH (over the range 1 to 13) were determined with two pH-sensitive neutral carrier/PVC electrodes (tridodecylamine and

TABLE 2
Determination of Inorganic Cations, pH, and Other Parameters by MC FIA

Analyte	Note	Ref.
pH	PVC, pot	20
pH, alkalinity, ionic concentration	pot, MAS	36
pH, Cl^-	pot	22
pH, Ca(II)	pot	157
Na, K	MAS	161
Na, K, Cl^-	pot	26
Li, Na, K, Ca	FF	79
Na, K, Ca, Mg	FF, AAS	51
Na, K, Ca, Cl	pot	23
NH_4^+, No_3^-, Cl^-	pot	54
K, NH_4^+, Ca, Cl^-, NO_3^-, PO_4^{3-}	ISE, MAS	21
Li, Na, K, Ca, Mg, Cu, Fe, Zn	ICP	72
Na, Ca, Mg, Al, Fe, Cu, Cr, Mn, Zn	ICP	77
Ca, Mg	AAS	156
Ca, Mg	MAS	165
Ca + Mg, Ca	MAS, XO	149
Ca, Mg	MAS CPA3	88
Ca + Mg, Ca	MAS	152
Ca, Mg	MAS, DAD, AA3	62
Ca, Mg	MAS, kin.	41, 43
Mg, Sr	MAS, kin.	40
Ca, Mg, Sr	MAS, kin.	42
Ca, pH	pot	157
Ca, N, P	MAS	155
Ca, La	MAS	27
Fe(III), Al(III), Zn(II)	MAS, LED, XO	15, 93
Fe(II), Cr(VI)	MAS	103
Fe(III), Ti(IV)	MAS, Tiron	95
Fe(III), Co(II)	MAS, PAR/EGTA	140
Fe(III), Ni(II)	MAS	7
Fe, Ni	MAS	58
Fe(II), Fe(III), Ti(IV)	MAS, Tiron	102
Fe(III), Ni(II)	MAS	96
Fe(III), V(V)	MAS	164
Fe(III), Ni(II)	MAS, DAD	172
Fe(III), H^+	MAS, DAD	61
Co, Mn	MAS	44
Cu, Pb, Cd, Zn	amp.	75
Cu, Pb, Tl, Sn	pot.	76
Cu, Al, Fe	MAS	47
Cu, Hg	Fl.	46
Cu, Ni, Co, U	MAS, DAD	65
Cu, Ni, Co(III)	MAS, DAD, 1PAN4S	64
Cu, Fe	MAS, DAD, 1PAN7S	66
Cu, Fe	MAS, DAD, NC/Ph	70
Cu, Fe	MAS, DAD, BC/BP/FZ	10, 63
Cu, Ni, Pd	MAS	71
Co, Ni	MAS, PAR	38
Co(II), Mn(II), Ni(II), Cu(II)	MAS, PAR	91
Co(II), Ni(II)	MAS	171
Zn, Cd	MAS, DAD	60
Zn(II), Cd(II)	MAS	121

TABLE 2 (continued)
Determination of Inorganic Cations, pH, and Other Parameters by MC FIA

Analyte	Note	Ref.
Al(III), Fe	MAS/AAS	53
Pr, Sm, Nd	MAS, DAD	65
U, Th	MAS	87
V(V), Pb(II)	MAS, PAR	92
V(V), Ti(IV)	MAS	33
Bi(III), Pb(II)	MAS, AA3	89

Note: MAS = Spectrophotometry; pot = potentiometry; Fl. = fluorometry; AAS = atomic absorption spectrometry; amp. = amperometry; FF = flame photometry; kin. = kinetic method; DAD = diode array detector; PAR = 4-(2-pyridylazo)resorcin; Ph = 1,10-phenanthroline; 1PAN4S = 1-(2-pyridylazo)-1-naphthol-4-sulfonic acid; NC = neocuproine; BC = bathocuproinedisulfonate; BP = bathophenanthrolinedisulfonate; FZ = Ferrozine; XO = Xylenol Orange; LED = light emitting diode detection; AA3 = Arsenazo III; CPA3 = chlorophosphonazo III; ICP = inductively coupled plasma atomic emission spectrometry.

octadecyl isonicotinate, respectively) in a low-dispersion miniature potentiometric flow cell designed specifically for use in conjunction with a multichannel data acquisition system.[20] Alkalinity, pH, and total ionic concentration in drinking water (bottled, urban, and natural) was based[36] on pH measurements by a glass-calomel microelectrode, while the alkalinity and total ionic concentration were determined by FIA titration, acid-base reactions, and spectrophotometric detection.

A gradient-scanning technique was applied to the simultaneous flame photometric determination of alkali and alkali-earth ions (Li^+, Na^+, K^+, Ca^{2+}) in soil extracts and tap water, using a fast-scanning monochromator and a storage oscilloscope to obtain spectra on different sections of the injected sample zone.[79] A combination of flame photometry and AAS makes it possible to analyze Na, K, Ca, and Mg in surface, ground, and domestic water.[51]

The same ions (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}) and heavy metals (Cu, Fe, and Zn) were determined in blood serum by ICP OES using aqueous synthetic multielement solutions for calibration. The matrix interference of undiluted human and bovine serum was minimized or eliminated at low injection volumes and/or by using relatively high radiofrequency power.[72] An ICP OES method for Na, Ca, Mg, Al, Fe, Cu, Cr, Mn, and Zn determination in biological and clinical samples is superior to continuous-nebulization ICP-OES in analysis efficiency, precision, matrix effect, and introducing the amount of sample.[77]

Calcium and Mg were determined in microliter samples of standard, control, and fresh blood sera by AAS using the merging zone method.[156] The total amount of Ca and Mg was determined in waters by micellar solubilization spectrophotometry with Xylenol Orange and cetyltrimethylammonium bromide in ammonia buffer (pH 10.5) containing triethanolamine. Calcium alone was determined in an NH_3-NH_4Cl-sodium citrate buffer solution.[149] The same determination in natural waters was performed with chlorophosphonazo-III in triethanolamine/HCl buffer (pH 7.0) and 1.6 m*M* HCl (pH 2.2), respectively.[88] Determination of Ca and Mg (hardness) in natural, drinking, urban, and bottled water was also performed by MC FIA titration with spectrophotometric detection.[152] A method using

Arsenazo III, based on the use of a DAD and merging zones, is applicable to the resolution of mixtures in which the chromogenic reagent has a high absorbance. The spectrum strongly overlaps those of its complexes (therefore the excess reagent is considered as another component).[62] A differential two-point kinetic assay analysis for Mg and Ca ions with phthalein complexon as the indicator is based on the dissociation of their cryptand (2.2.1.) complexes using Na ion as the scavenger. A similar method for Mg and Sr ions uses an acid dissociation of the *trans*-1,2-diaminocyclohexanetetraacetate complexes of Mg and Sr using Cu(II) ions as the scavenger.[40,41] The limiting factor of the methods is that a rather pronounced difference must exist between the rates of reaction of the metal complexes.

Protein (as NH_3 by the indophenol method at 660 nm), P (as molybdophosphoric blue), and Ca (as cresolphthalein complexes at 580 nm) were determined in a wide range of animal feeds with a single digestion.[155] Lanthanum and the total quantities of La and Ca ions in synthetic mixtures and bioinorganic samples were determined with two spectrophotometers by using 2-(2-arsenophenylazo)-7-(2,4,6-trichloro phenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid and 2-(2-phosphono-4-chlorophenylazo)-7-(4-fluorophenylazo)-1,8-dihydroxy-3,6-disulfonaphthalene, respectively.[27]

A flow photometer with a multi-LED detector[93] was applied to the simultaneous determination of Al and Zn in alloys with Xylenol Orange[15] in acetate buffer for Al and Zn ratios, which vary from 0.01 to 100. Total amounts of Fe and Cr(VI) were determined with a single spectrophotometric detector by the sandwich technique.[103] A nested-loop valve system makes the simultaneous determination of Th and U possible by detecting two sequential peaks. For this analysis, each injected sample was split into two sections, one of which underwent a differential reduction process.[87]

Iron(III) and Ti(IV) were injected simultaneously at two inlet points separated by a silver reductor column. The two plugs were allowed to meet the Tiron solution, which was used as a color-developing reagent, giving double peaks (430 nm). The first peak yields the concentration of Fe(III) plus Ti(IV) and the second peak gives the concentration of Ti(IV) in standard reference rock samples, respectively.[95] Individual and sequential determinations of V(V) and Ti(IV) as their peroxo complexes were performed using a modified injection valve that introduces two samples sequentially into the reagent stream. A cation-exchanger minicolumn incorporated on-line into the V manifold removed the Ti complex and allowed the V to be determined selectively while the other peak corresponded to the sum of both elements.[33]

The determination of Ni(II) in the presence of Fe(II) is based on the formation of double peaks in a single-line system, with multiple vertical (absorbance) measurements at 395 nm of the peak profile while the center of the sample zone remains unmixed. Nickel(II) is determined by direct spectrophotometry of the Ni(II) ion at the center of the sample zone. Iron(II) is first oxidized on-line by peroxydisulfate to Fe(III), which complexes with thiocyanate to form the intensely red complex.[7]

Trace amounts of Co and Mn in high-purity Al were determined[140] after cation-exchange separation of Co and Mn from matrix metals. A catalytic photometric detector, which is based on the Co and Mn-catalyzed oxidation of protocatechuic acid by hydrogen peroxide in alkaline medium was used. Metals such as Fe, Si, Cu, Mg, Zn, Cr, Ti, Zr, V, and Ca do not interfere with this determination.[140] Bismuth(III) and Pb(II) were determined with Arsenazo III by merging two different solutions, one of them at a constant flow rate and the other at a variable flow rate. This produced a carrier that was pH adjusted to the desired pH value.[83] A closed flow system with a single spectrophotometric detector allows determination of Fe(III) and Co(II) via EGTA/PAR ligand displacement kinetic methods.[122]

Two or more components (Cu, Pb, Cd, and Zn) were determined in a single sample with a system including a square-wave amperometric detector, a microprocessor-based potentiostat, and a microcomputer.[75] Potentiometric stripping analysis of Cd, Pb, Tl, and Sn in various supporting electrolytes was outlined with regard to the application of the matrix-exchange technique. Complexing agents, such as citrate, tartrate, and

ammonia, led to a drastic potential shift for bivalent ions, thus removing the limitations for the simultaneous determination.[76]

Several valves with different functions along the reversed-FIA manifold allow photometric determination of pollutants such as Cu, Fe, and Al in wastewaters using a single configuration.[47] A catalytic-fluorometric method for determination of Cu(II) and Hg(II) is based on their catalytic effects on the oxidation of dipyridyldiketone phenylhydrazone and 2,2′-dipyridylketone hydrazone, respectively, using the FIA stopped-flow mode.[46] The different rates of the dissociation of the citrate complexes of Co(II) and Ni(II) at pH 9.25 are used for the two-point kinetic assay. The absorbance of their complexes with 4-(2-pyridylazo)resorcinol formed in a subsequent rapid reaction[38] are measured. Ethylene diaminetetraacetate (EDTA) prevents interference from Cu(II), Mn(II), Zn(II), Cr(III), Pb(II), and V(V) at 10 ppm.

The creation of known and reproducible pH gradients allows the simple and accurate determination of Co(II) and Mn(II), and of Ni(II) and Cu(II), in the presence of similar amounts of Co(II) with PAR in binary mixtures. In the latter example, Co is kinetically masked by virtue of the relatively slow dissociation of the $Co(HPAR)^+$ complex.[91] The temporal changes in the peak heights due to the V-PAR complex, formed at pH 2, and the Pb-PAR complex, formed at pH 9, were used for the determination of Pb in the presence of varying amounts of V. An aqueous HCl sample containing Pb(II) and V(V) at pH 2 was injected into a stream in 10 m*M* PAR buffered in ammonia at pH 9.9. Vanadium could be determined with only limited precision in the presence of a constant amount of Pb.[92]

A configuration involving zone sampling and merging zones allowed the simultaneous spectrophotometric determination of Al with Eriochrome Cyanine R as the reagent and of Fe by AAS in plant digests. The zone-sampling approach permits an easier pH control in the Al determinations so that interferences caused by variations in sample acidity are avoided without the need for very concentrated buffers. The merging zones configuration greatly reduces the consumption of reagents.[53]

A multichannel UV-visible DAD enables several components with sufficiently separated absorption bands to be determined simultaneously.[65] Binary and ternary mixtures of Cu(II), Ni(II), Co(II), and U(VI) were analyzed for their major and minor components. The method was applied to a simultaneous determination of Pr(III), Sm(III), and Nd(III) in oxidic concentrates of rare earths at different concentration ratios,[65] and of free acid and metal concentrations in hydrolyzable metal ion solutions (Fe was chosen as an example).[61]

Calcium and Mg were determined with Arsenazo III in mixtures in which the chromogenic reagent had a high absorbance. The spectrum strongly overlaps those of its complexes, therefore, the excess reagent is considered as another component.[62] Recently, 2-(2-pyridylazo)-1-naphthol-4-sulfonic acid (1PAN4S) and its 1PAN7S analog were used for determination of Cu(II), Ni(II), and Co(III) by reverse-flow injection analysis (r-FIA)[64] in 1PAN4S 0.12 m*M* and sodium iodate 1.2 m*M* in 0.2 *M* acetate buffer medium at pH 5.00 and for determination of Cu(II) and Fe(II) ions, respectively.[66] The absorbance at 550 nm is related to the 1PAN7S chelates of Fe(II) and Cu(II) and that at 764 nm to the Fe(II) chelate alone. Interference from Zn is avoided by the addition of nitrilotriacetic acid; however, Ni interferes.[66]

MC FIA using several highly selective reagents, permits improved selectivity, accuracy, and precision of determination compared to methods employing a single nonselective reagent.[10] A 1:10 mixture of phenanthroline/neocuproine was used for the determination of Cu(II) and Fe(III) based on the sum of the absorbances at several wavelengths and on monitoring absorbances at wavelengths away from the absorption maximum, respectively. The determination of nitrite via the Griess reaction is used as another example.[70] Mixtures of 0.6 m*M* disodium bathocuproinedisulfonate (BC) with 0.2 m*M* disodium bathophenanthrolinedisulfonate (BP) or 2 m*M* BC with 0.2 m*M* ferrospectral in a medium of formate buffer pH 3.5 and ascorbic acid was used for the determination of Fe and Cu in water samples.[10] These reagents were also used for the determination of Fe and Cu in deproteinated blood serum in the presence of 0.1 *M* formate buffer

(pH 3.5), 10 m*M* ascorbic acid, and 0.3 *M* trichloroacetic acid. The concentrations of both elements calculated for overdetermined systems (10 or 11 wavelengths) at the absorption maxima for the individual chelates or (at 2 or 3 wavelengths) gave satisfactory results.[63] Determination of binary and ternary mixtures of Cu, Ni, and Pd involves the sequential measurement of the absorbances of metal-EDTA complexes at 700, 590, 380, and 340 nm at pH 3.2 (phthalate buffer).[71]

2. Speciation of Metal Ions (Table 3)

Determination of mixed oxidation states of Fe(III) and Fe(II) in mineral process liquors[55] employs parallel FIA manifolds with simultaneous sample introduction into parallel streams by coupled injection valves. A closed-loop system with multidetection based on the iterative pass of the reacting plug through a single on-loop detector n times[19] was also employed for speciation of Fe. The determination of Fe(II) and Fe(III) was based on the reaction with 1,10-phenanthroline, which selectively reacts with Fe(II). The determination of Fe(III) was based on its prior reduction with hydroxylamine.[19]

The system, which uses a single injection and a single detector, was based on the synchronized injection of a large volume of sample and a small volume of ascorbic acid into separated streams. The streams were then merged at a confluence point and mixed downstream with a chromogenic reagent detection stream. It was used for Fe(II) and Fe(III) speciation employing detection with 1,10-phenathroline.[99] The same system[14] exploited the simultaneous intercalation of a modifying reagent plug and two small sample plugs into the same carrier stream by making effective use of a 16-way valve.

Iron(II) and Fe(III) were determined by relying on their different kinetic-catalytic behavior in the redox reaction between leuco Malachite Green and peroxodisulfate, with and without the presence of the activator 1,10-phenanthroline. This analysis[94] is based on the fact that one of the chemical reactions is very rapid, whereas the other is comparatively slower. In the first system, two individual zones of a sample solution are injected simultaneously into separated carrier streams of reagent in a two-line system. Taking advantage of the different residence times of the samples in the manifold lines, the resulting color formation is measured by a single optical detector

TABLE 3
Metal Ion Speciation by MC FIA

Analyte	Note	Ref.	Analyte	Note	Ref.
Fe(II)/Fe(III)	MAS, Ph	55	Cr(III)/Cr(VI)	MAS	127
Fe(II)/Fe(III)	MAS, Ph	19	Cr(III)/Cr(VI)	MAS	153
Fe(II)/Fe(III)	MAS, Ph	14	Cr(III)/Cr(VI)	MAS	81
Fe(II)/Fe(III)	MAS, Ph	99	Cr(III)/Cr(VI)	MAS	82
Fe(II)/Fe(III)	MAS	94	Ce(III)/Ce(IV)/Ce	FI	84
Fe(II)/Fe(III)	MAS, Ph	17	Cu(II)/Cu (compl.)	AAS, ie	37
Fe(II)/Fe(III)	MAS, Ph	104	Ca(II)/Ca	AAS/MAS	146
Fe(II)/Fe	MAS, Ph	105	Ca(II)/Ca	pot	24
Fe(II)/Fe	MAS, Ph	85	Metals	AAS/MAS	32
Fe(III)/Fe	MAS, T	101	Metals	MAS	83
Fe(II)/Fe	AAS	31	Fe(II)/Fe(III)/Ti	MAS, T	102
Fe(II)/Fe(III)[a]	pot/AAS	30	Metals	MAS	141

Note: ASC = Ascorbic acid; ie = ion-exchange packed column reactor; T = Tiron.

[a] $[Fe(CN)]^{3-}/[Fe(CN)]^{4-}$

with two separate flow cells aligned within the same optical path.

The second approach is based on the use of a single-line flow-injection system, using the formation of a double peak as a result of the injection of a large sample zone, sandwiched between reagent zones of appropriate compounds. In this manner, two time-resolved signals for the kinetically governed processes can be obtained and thus used for quantification of the individual species.[94] An internal coupling of valves allowed the calibration standards and the sample to be interspersed when samples (1500 μl) were inserted between zones of water and ascorbic acid solution with the subsequent addition of 1,10-phenanthroline at pH 5.0. The signal provides a plateau region corresponding to Fe(II) followed by a peak corresponding to the total Fe.[104,105]

Application of a reducing column allows speciation of Fe(III) and determination of Fe(II) and total Fe[85] after passage through a Jones reductor minicolumn before spectrophotometric detection with 1,10-phenanthroline in citrate buffer of pH 5.0. Iron(II) and total Fe are determined by splitting the injected sample so that a portion passes through the reductor column and a delay coil before both streams are recombined. The unreduced portion precedes the remainder of the sample to mix with the reagent for spectrophotometric detection. Two peaks are produced for each sample, the first being a measure of Fe(II), the second of total Fe.[85] Iron(III) and total Fe were determined with Tiron as the color developing reagent using a single detector with two flow cells aligned with the same optical path to yield two peaks corresponding to Fe(III) and total Fe in the sample or using simpler instrumentation with a multidetection system.[101]

Speciation of Fe(II) and Fe(III) cyano complexes, $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$, utilizing electrochemical and atomic absorption detectors in series[30] is based on amperometric detection of one species at a Pt electrode. The required potential is applied and the resulting reduction or oxidation current of the appropriate Fe cyanide complex is measured. Total Fe in both species is determined by an AAS detector.

Speciation of Cr(VI) and Cr(III) is based on the separation of the two valences of Cr on an ion-exchange column packed with either a chelating resin with salicylic acid functional groups or with 8-quinolinol immobilized on porous glass, and FAAS determination of the separated species.[127] Several different configurations for the simultaneous and sequential spectrophotometric speciation of Cr(VI) and Cr(III) at the milligram per liter level in natural waters and wastewaters are based on r-FIA and completely continuous modes.[153] A closed FIA system with multidetection by a single detector was extended to the simultaneous determinations for Cr speciation, with injection of the reagent(s) into the sample solution (which acts as the carrier).[153]

Cerium(III) was determined by injection into a carrier stream of HCl, $HClO_4$, or H_2SO_4 and monitoring of its native fluorescence.[84] Cerium(IV) was similarly determined by incorporating a Zn reductor minicolumn into the system. Splitting the injected sample so that only part passes through the reductor and the remainder bypasses it allows total Ce and Ce(III) to be detected from the two sequential fluorescence peaks obtained.[84]

Free and EDTA-complexed Cu^{2+} ions were speciated by FAAS with an ion-exchange flow-injection system.[37] Total Ca and free Ca(II) in milk were determined by AAS (422.7 nm) and by spectrophotometry (580 nm), respectively. Interference in the determination of free Ca was eliminated by using a dialyzer, which also separated the total and free Ca.[146] Interference from phosphates in the determination of total Ca by AAS was overcome by using a dinitrogen oxide-acetylene flame with the necessary suppression with K^+.

B. Inorganic Anions (Table 4)

A review of methods for speciation of nitrogen as nitrite/nitrate by flow-injection spectrophotometry was presented, with distinction being made between sequential and simultaneous determinations. A new simultaneous method for this speciation was proposed, making use of an inner coupling valve system that clearly improved on earlier designs.[6]

Nitrate was determined by displacing thiocyanate from an anion-exchange minicolumn and detected spectrophotometrically after reaction with Fe(III).[123] The efficiency of Pb- and Au-

TABLE 4
Simultaneous Determination of Inorganic Anions

Analyte	Note	Ref.	Analyte	Note	Ref.
NO_3^-/NO_2^-	MAS	6	As/Sb/Se	ICP, HG	73
NO_3^-/NO_2^-	MAS	123	CN^-/SCN^-	MAS	39
NO_3^-/NO_2^-	MAS	86	CN^-/SCN^-	MAS	144
NO_3^-/NO_2^-	MAS	100	CN^-/I^-	MAS	45
NO_3^-/NO_2^-	MAS	128	$CN^-/NO_3^-/HS^-$	MAS	29
NO_3^-/NO_2^-	MAS	154	HSO_3^-	MAS	67
NO_3^-/NO_2^-	MAS	11	HSO_3^-/Asc.	MAS	151
NO_3^-/NO_2^-	MAS	49	HCO_3^-/HSO_3^-	pot/MAS	50
NO_3^-/NO_2^-	MAS	57	Cl^-/Cu(II)	amp	150
NO_3^-/NO_2^-	AAS	159	Cl^-/PH	pot	22
NO_3^-/NO_2^-	AAS	160	Cl^-/SO_4^{2-}	MAS	35
NO_3^-/NH_3		145	Cl^-/Na/K	pot	26
NO_3^-/NH_4^+, Cl^-	pot, MAS	54	Cl^-/Na/K/Ca	pot	23
Total P/N	MAS	147	diff. S		74
Total P/N	MAS	52	diff. S	MAS	166
Total P/N	MAS	80	P(III)/P(V)		24, 48
P/N/Ca	MAS	155	Si/P		124
P/Si/As		125	P/Si		170
P/Si/As		126	P/Si		142
P/Si		18			

Note: HG = Hydride generation technique; diff. S = different anionic forms of sulfur; P/Si/As = phosphate, silicate, arsenate; Si/P = silicone and phosphorus; P/Si = phosphate and silicate; P/N/Ca = protein nitrogen, phosphorus, and calcium.

loaded ion-exchange columns in removing anionic interferences was investigated. It was necessary to incorporate a copperized Cd reductor to reduce Pb or Au ions released by these columns, which otherwise depress the nitrate response. Incorporation of a Pb/Au ion-exchange column allows the determination of nitrate in tap water. The water sample containing nitrates and nitrites is split into two flows, one is directly treated with the azo dye reagent and sent to the sample flow cell of a double beam spectrophotometer. This gives positive absorbance values, allowing direct determination of nitrite in the sample. The other flow goes through a Cd reduction microcolumn, where nitrates are reduced to nitrites. The sample is then treated with the azo dye reagent and the overall mixture is sent to the reference cell of the same spectrophotometer. This gives negative peaks, corresponding to the sum of the absorbances of both ions.[86] Configuration with internally coupled valves and a reductant column located in the loop of the secondary valve overcomes shortcomings in the simultaneous determination of nitrite and nitrate.[100]

Nitrites were determined in feed, food, water, and soil extracts by photometry of the red dye formed by reaction with sulfanilamide and *N*-(1-naphthyl)ethylene diamine in acid solution. Nitrates were determined in the same samples after reduction[128] by Cd or at 520 nm using 1-naphthylamine-7-sulfonic acid and sulfanilamide[154] after the samples passed through a reduction column filled with Cd-coated Zn granules, which converts nitrates to nitrites.

The in-valve reduction technique of nitrate on a copperized Cd column situated in one loop of a two-position sampling valve[11] prior to the sampling system was also used. Nitrite from the samples, as well as that formed in the reduction procedure, is sampled by a second valve and introduced into the flow system. Both valves are

synchronized in such a way that there are two peaks. One corresponds to the sum of nitrate and nitrite, and the other corresponds to the nitrite only. The merging zones approach can be used to minimize reagent consumption, when nitrite is diazotized and coupled with *N*-(1-naphthyl)ethylene diammonium dichloride.[49,57]

Ammonia and nitrate in river water[145] were fluorometrically determined with *o*-phthalaldehyde reagent after the nitrate was reduced to ammonia in an alkaline medium by $TiCl_3$. The ammonia generated in alkaline solution is separated with a tubular microporous PTFE membrane. Nitrite stoichiometrically interferes with the method but can be masked by sulfanilic acid.

Total P and total N in wastewater samples[147] were first digested with peroxodisulfate in a heated capillary tube containing a Pt wire. Subsequently, independent determinations of phosphate and nitrate were made using molybdate and Malachite Green. The merging zones approach, which uses a multiple proportional injector for introducing samples and reagents into water carrier streams in such a way that there is only one analysis path with one detection unit, reduces consumption of reagents to the microliter-per-determination range. For both Berthelot and molybdophosphate reactions,[52] catalysis is employed. A dual-channel manifold with a single or with two spectrophotometers[80] was also used.

Protein (N), P, and Ca were determined in a wide range of animal feeds after a single digestion[155] by the indophenol method, the molybdophosphate reaction, and Ca-cresolphthalein complexes, respectively. The phosphomolybdenum blue reaction for the determination of phosphate was also used as a model to illustrate the extension of the use of hydrophobic sorbents for the preconcentration of an anion and for on-column detection, i.e., optosensing.[18] The synergistic relationship between the rates of formation of the phosphate and silicate heteropoly complexes allows the simultaneous determination of phosphate, in the parts-per-billion range, and silicate, in the parts-per-million range.

Phosphate, silicate, and arsenate have been determined by adapting an anion-exchange column[125] with a mixture of 0.1 *M* KCl, 10 m*M* NH_4OH, and 1 m*M* EDTA solution. The order of elution is silicate, phosphate, and arsenate. Determination is based on the absorption at 610 nm of the heteropoly blue formed with ascorbic acid as the reducing agent. Arsenate was determined by the formation of arsenomolybdate blue, in which the sample and Mo(VI) solution were mixed to form the heteropoly acid, then the acid was reduced with ascorbic acid to the blue form.[126]

Phosphate and phosphonate were determined with two detectors located in series. An Mo(V)-Mo(VI) reagent was used to detect P(V) at the first detector and P(III) at the second detector, following oxidation by a sulfite solution.[28] This in-series detection system, linked to a liquid chromatograph, was useful for both the separation and the identification of various oxo acids with P(V) and P(III) units.[28] A similar system with a single detection system[48] was useful as a postcolumn reaction detector for HPLC of various oxo acids of P(III) and P(V).

Silicon and P in biological standard materials (bovine liver [NBS], chlorella, and pepperbush [NIES])[124] were determined after the materials were ashed, fused with a Li carbonate boric acid mixture, and dissolved in a hydrochloric acid solution. Interfering cations were removed by a simple cation-exchange column filtration. The dry acid effluents were fused with a small amount of sodium carbonate for depolymerization, taken up in diluted EDTA solution, separated on TSK-gel SAX, with a mixture of 5 m*M* NaCl/10 m*M* NH_3/1 m*M* EDTA as an eluent, and analyzed for silica and phosphorus.[142]

Arsenic, Sb, and Se, by hydride generation and ICP OES with a simultaneous three-channel system, give[73] peak signals three times lower than steady-state signals for a continuous introduction system. By contrast, the precision was better due to reduced pump pulsations, the reduced sample size needed for the analysis, and the potential for a greatly increased rate of sample throughput in the FIA system.[73] The cyanide and thiocyanate method involved a two-step procedure in which the total concentration of both species is first determined (using sodium isonicotinate/sodium barbiturate reagents). Next, the cyanide is complexed with Ni(II) and thiocyanate is quantified in synthetic sample solutions.[39] The cyanide concentration is calculated by difference. Both ions were also determined by the pyridine/barbituric acid method after diffusion through a micropo-

rous tubular PTFE membrane module from the phosphoric acid donor stream to a phosphate or carbonate buffer acceptor stream.[144] Thiocyanate reacts slowly with chloramine-T at pH 8.1, so that cyanide can be determined without interference from thiocyanate. Total cyanide and thiocyanate are determined at pH 6.0. Bromine interferes with the determination of cyanide and thiocyanate at both pH 6.0 and 8.1. Hexacyanoferrate(II) and hexacyanoferrate(III) interfere at pH 8.1, but not at pH 6.0. Cyanate, oxaloacetate, oxalate, tartrate, albumin, globulin, and lysozyme do not interfere.[144]

Catalytic determination of thiocyanate and iodide with a double-injection technique was applied to the redox reaction between Ce(IV) and As(III).[45] The catalytic activity of thiocyanate was suppressed selectively by pretreatment with Ce(IV).

The appropriate in-series configuration r-FIA with two injection valves allowed the insertion of the specific reagents for the photometric determination of each species at different points in the water and wastewater streams and monitoring of two different combinations of anionic pollutants (sulfide, cyanide, and nitrite).[29]

Amplification and dilution multidetection techniques were considered for the manipulation of analysis sensitivity. These techniques were also considered for broadening the determination range of an analyte with maximum accuracy while using a conventional spectrophotometric detector in linear or cyclic flow systems and a DAD for monitoring several wavelengths simultaneously. The formaldehyde/pararosaniline/sulfite system was used for studying these techniques.[67] Ascorbic acid and sulfite determination in orangeade and lemonade is based on the reaction of the species with chloramine-T with the use of a dual injection valve.[151]

Chloride and sulfate were determined in natural waters.[50] Residual chlorine and Cu(II) in water samples taken from swimming pools[150] were determined by amperometry with two polarized Pt electrodes based on the oxidation of iodide. Interferences of Fe(III), Cu(II), nitrite, and atmospheric oxygen are eliminated in the proposed procedure.[150] Chloride and pH were determined with an in-series ISE arrangement after sample injection into a carrier buffer solution.[22]

Carbon dioxide and sulfur dioxide in complex matrices in fruity wine[35] were sensed by two detectors in series, a potentiometric detector responsive to both analytes, and a photometric detector for sulfur dioxide only (*p*-rosaniline formaldehyde method) after preseparation of the analytes with a flow-through gas-diffusion unit.[35]

C. Organic Substances (Table 5)

Ammonia and hydrazine react with *o*-phthalaldehyde and mercaptoethanol to form fluorescent derivatives at different pH values. This is due to formation of zones of different pH with a novel mode of forming pH gradients.[90] A stop flow method increases the ranges that can be quantified in samples containing hydrazine and ammonia in ratios between 0.3 and 70. Ammonia and urea can be determined in water samples using the same principle in a dual injection system with the valves coupled in series or in parallel.[97] An enzymatic reactor converts urea into ammonia, which is determined by the same reaction. These methods are tolerant to foreign species commonly found in water.[97]

An asymmetric merging zone FIA configuration allows determination of urea and ammonia[98] using a reagent-injection configuration that includes a dual injection valve (for insertion of Nessler's reagent and for accommodating the enzyme reactor). The resolution of the two peaks obtained on each injection allows the determination of both analytes in mixtures.[98]

A fast-scanning voltammetric detector allows individual and multicomponent determination of phenolic compounds (phenol, guaiacol, and 2,4-dichlorophenol) in water by normal and derivative flow-injection/cyclic voltammetry.[78] A cyclic voltammetric unit with a three-electrode cell (carbon-paste working electrode, Ag/AgCl reference electrode, and glassy-carbon auxiliary electrode) improves the resolution and selectivity obtainable from the electrode processes by derivatizing the current/potential recordings.

Monochloramine, dichloramine, and free chlorine in a water purification plant[143] can be determined with a system with three double-tube separation units, each with an inner tube of microporous PTFE and an outer tube of compact

TABLE 5
Determination of Organic Substances by MC FIA

Analytes	Note	Refs.
Hydrazine, ammonia	Fl.	90
Urea, ammonia	Fl., MAS	97, 98
Phenols	volt	78
Chlorine, chloramines	MAS	143
Ascorbic acid, HSO_3^-	MAS	151
Ethanol, acetaldehyde	MAS	117, 69
Methanol, ethanol	MAS, en	115
o-, *m*-, *p*-Cresol, naphthols	MAS, DAD	68
ATP, Glucose-6-phosphate	en, pot	106
LDH	MAS, en	148
AST, ALT	en	108
Glucose, cholesterol	pot, en	107, 110
Glucose, sucrose	en, pot	113, 120
Glucose, fructose, sucrose	amp, en	114
Glucose, glycerol		167
Glucose, urea	pot	25
Choline, acetylcholine	en, pot, amp	109, 112
Lovastatin, butylated hydroxianisole	UV, el., amp	34
Cholesterol total/free	en	111
Triglycides, β-D-galactose	en, Fl.	116
Glycine, cysteine	Fl, en	56
Cystine, cysteine	en	163
L-Lactate, β-D-glucose, glycerol	en	118
L(+)- and L(−)-lactic acid	en, pot	119
Pyridoxal, pyridoxal 5-phosphate	Fl	12
Phenylephrine HCl, pheniramine	MAS, Ext.	16
Diphenhydramine, 8-chlorotheophyline	MAS, Ext.	129
Procycline HCl	MAS, Ext.	131
Pesticide	MAS, Ext.	134
Lactate dehydrogenase	MAS, serum	148
Steroids, Bili acid	Fl, CL	158
Chloropromazine, promethazine	MAS	162
Propene oxide, propene 1,2-diole	en	168
Diquat, Paraquat	MAS	169
Teniposide	MAS, DAD, plasma	59

Note: ATP = Adenosine-5-triphosphate; AST = asparate aminotransferase; ALT = alanine aminotransferase; Ext. = liquid-liquid extraction; LDH = lactate dehydrogenase; volt = cyclic voltammetry; CL = chemiluminescence; en = enzymatic packed column reactor; UV = MAS in ultraviolet range; el. = electrochemical detector.

PTFE. The reaction between chloramines and iodine is used in order to quantify the species as available chlorine. Inorganic chloramines (ammonia derived) and chloroamino acids (amino acid derived) can be distinguished. Ascorbic acid and sulfite in orangeade and lemonade can be determined with chloramine-T, by use of a dual injection valve.[151]

Individual and simultaneous determination of ethanol and acetaldehyde in wines by use of immobilized alcohol and aldehyde dehydrogenases are carried out on a suitable enzyme reactor. The sample is injected with a dual injection valve into two channels of different length, each containing an enzyme reactor. One peak per analyte is obtained[117] when a configuration with a single-beam spectrophotometer or with a diode-array detector and dual reagent injections[69] is employed.

Hydroxylated aromatic isomers, such as of 1- and 2-naphthol in binary mixture, and binary

or ternary mixtures of *o*-, *m*-, and *p*- cresol, were determined by the same indicator reaction (coupling of a diazonium salt to the hydroxylated compound) with a diode array photodetector.[68]

Adenosine-5′-triphosphate (ATP) and glucose 6-phosphate were effected with immobilized enzymes[106] in two paths, i.e., a hexokinase-immobilized reactor and an ATP-dehydrogenase reactor, and fed into a flow-through glassy-carbon electrode using a carrier solution of Tris/HCl buffer.[106]

A cyclic configuration allows kinetic determination of lactate dehydrogenase (LDH) in blood serum by repeated passage of the reacting plug through an on-loop detector. Multiple-peak recordings correspond to a typical kinetic curve. The required sensitivity can be selected by using procedures based on fixed-time measurements (peak maximum or minimum) or reaction-rate measurements (signal increment between two successive maxima or minima).[148]

Glucose and cholesterol were determined as separated peaks in blood serum by the combined use of immobilized glucose oxidase and cholesterol oxidase enzyme reactors in parallel and a peroxidase electrode.[110] Free and total cholesterol in blood serum were determined by using immobilized enzymes[111] after the sample was split so that part passed through an immobilized cholesterol esterase column, before passing through an immobilized cholesterol oxidase column, and the other portion only passed through the latter column. The separated peaks correspond to free and total cholesterol, with measurements being made of the H_2O_2 generated from the oxidase reaction by a hexacyanoferrate cholesterol-mediated peroxidase electrode.[111]

Glucose and total cholesterol in blood serum were determined with enzymes[107] immobilized on Lichrosorb NH_2 columns, activated with glutaraldehyde. Samples split into two streams passed through the columns. One column contained immobilized cholesterol oxidase and cholesterol esterase, and the other contained glucose oxidase and a delay coil about 6 m long. Two separate peaks of H_2O_2 of different origins were measured with an amperometric flow-through peroxidase electrode.[107]

Asparate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using enzyme-immobilized open-tubular reactors in parallel. An additional channel allows evaluation and correction of the error resulting from pyruvate coexisting in sample sera. The results corresponded well with those obtained by a conventional UV method. The recognition of the signal pattern from the three channels makes possible the primary screening of diseases.[108]

Choline and acetylcholine, and lipids in clinical and biochemical samples are quantified by using acetylcholinesterase and choline oxidase enzyme-immobilized on columns. An amperometric detector measures the hydrogen peroxide produced enzymatically. An on-line ion-exchange column is used to remove interferences at the amperometric detector during analysis of blood and brain samples. A combination of immobilized lipid enzymes, phospholipase-D, lipase, and glycerol-3-phosphate oxidase, is used for the determination of total phospholipids.[109]

Acetylcholine and choline, using immobilized choline-esterase and acetylcholin-oxidase enzyme reactors, respectively, were detected with an enzyme electrode.[112] The dual paths were joined, mixed with a flow of 0.3 m*M* potassium hexacyanoferrate(II) solution, passed through a mixing coil, and analyzed on peroxidase detectors.[112]

Lovastatin and butylated hydroxyanisole (BHA, an antioxidant) in tablets[34] were determined with in-series UV chromophore and oxidative amperometric detection. The UV detection is specific for the drug in the presence of potential autoxidation products as well as BHA and its oxidation products. Electrochemical detection in series is specific for low levels of BHA in the presence of its oxidation products, as well as the drug and its potential autoxidation products.[34]

Glucose and sucrose were determined using a manifold comprising columns of invertase/mutarotase and glucose (immobilized on controlled-pore glass) in sequence with incorporated controlled bypass around the invertase column. This allowed one sample to traverse both columns (giving a response from glucose and sucrose) and the next to pass through only the glucose oxidase column (giving a glucose response only). The amperometric detection of the hydrogen peroxide produced[120] was measured. Further modification allows a single sample to be split between the

two flow paths, so that two peaks are obtained in sequence (sucrose/glucose and glucose).[120]

Glucose and sucrose were determined in soft drinks utilizing immobilized invertase-mutarotase-glucose oxidase enzyme bioreactors.[113] Glucose, fructose, and sucrose in mixtures were determined by using parallel configurations of enzyme-immobilized reactors. The hydrogen peroxide produced (for glucose and sucrose) and hexacyanoferrate(II) (for fructose) were monitored amperometrically.[114] Sucrose determination was performed with a glucose-eliminating reactor, which was placed just before the sucrose reactor. Interference of ascorbic acid was completely eliminated by using an ascorbate-eliminating reactor, which was placed before the sample injection valve.

Triglycerides of clinical importance and β-D-glucose were determined using immobilized enzyme whisker-walled open tubular reactors.[116] Lipoprotein lipase and glycerol dehydrogenase were immobilized on the inner walls of the reactors. Triglycerides were measured by monitoring fluorometrically the resulting NADH. The completion of the hydrolysis of triglycerides required more than 10 min, so the stopped-flow procedure was adopted at this stage. Before the peak corresponding to triglycerides appears, a glucose dehydrogenase-immobilized reactor was placed as the second channel in a splitting-confluence FIA system to determine another species of clinical importance, β-D-glucose. Triglycerides and β-D-glucose in the concentration ranges of clinical significance could be determined simultaneously with satisfactory reproducibility.[116]

Glycine and cysteine (based on their reaction with phthaldialdehyde) were determined[56] using a dual-channel FIA configuration with a selecting valve. L-Lactate, β-D-glucose, and glycerol were determined by the fluorometric detection of the resulting common product, NADH, using enzyme-immobilized open-tubular reactors in parallel.[118] Despite a somewhat complex splitting-confluence flow system (the optimal geometry was carried out based on the Taylor-Aris equation), reproducible results were obtained. Assignment of the enzyme reactors to the channels should be made by taking into account the concentration ranges of the analytes to be measured and the differences in the rates of individual enzyme reactions.[118] L(+)- and D(−)-lactate dehydrogenase reactors in parallel with a diaphorase electrode give separated peaks for L(+)- and D(−)-lactate acid isomers.[119]

A methanol and ethanol differential-kinetic enzymatic method is based on the use of a reactor containing alcohol oxidase immobilized on controlled-pore glass.[115] This method also involves the aldehyde/*p*-rosaniline/sulfite-coupled reaction and a twofold halting of the flow (in the enzymatic reactor to favor the oxidation reaction and in the detector flow cell to perform kinetic measurements) per sample assayed.[115]

Pyridoxal (Py) and pyridoxal 5-phosphate (Py5P) in human serum were determined based on the oxidation reaction of these compounds in the presence of cyanide, yielding fluorescent substances.[12] The sequential method involves the use of a diverting valve to provide the suitable carrier for the determination of each of these compounds. The other methods use the native fluorescence of Py and that of the oxidation product of Py5P (simultaneous method) and the fluorescence of both oxidation products (differential-kinetic method), respectively.

Phenylephrine hydrochloride and pheniramine maleate in nasal spray were determined by solvent extraction-FIA using two porous-membrane phase separators (Teflon and paper membranes) and one photometric detector.[16] Interfering species, thimerosal maleate and benzalkonium, are removed from the injected sample using miniature on-line ion-exchange columns. At pH 13, pheniramine is quantitatively extracted into the chloroform phase and phenylephrine remains in the aqueous phase. Dramamine tablets were analyzed for both diphenhydramine and 8-chlorotheophylline in the same way. At pH 10, diphenhydramine is quantitatively extracted into the cyclohexane phase and 8-chlorotheophyllineremains in the buffer phase. This pH was chosen after measuring the sigmoid, extraction-pH profiles of the drug components. Assays are performed at the rate of two per minute and with a precision and accuracy of 1%.[129]

V. CONCLUSION

The MC FIA procedure in continuous mode differs from that in the manual batch analysis because equilibrium is seldom achieved due to

the limited residence time and kinetics of the process. It also differs whenever a masking of interferents is necessary. In the manual procedure, the masking agents are added to the sample solution well in advance of detection; therefore, the complexation has sufficient time to occur. In flow systems, all of the reactions take place nearly simultaneously, in no less than several seconds; therefore the kinetics of the reactions plays an important role.

Kinetic aspects, on the other hand, can improve selectivity by discrimination of some chemical reactions. However, the equilibrium data can serve as a good basis for selection of the experimental conditions. The final choice has to be made from experiments performed under exactly the same dynamic conditions as those used for the MC FIA analytical procedures. The typical reproducibility and precision of most MC FIA analytical procedures are comparable to those obtained with the manual procedures, despite the several additional factors influencing the measurement and data treatment procedures.

Application of fast multichannel flow detectors with high scan speed and reading of the analytical signal allows significant improvements in the experimental data obtained. It also allows an increase of about one order of magnitude of the number of results obtained during the time period. Due to the decrease in cost of hardware and software of all parts of the instrumentation, and the implementation of computers into the instrumentation, MC FIA should play a very important role in many branches of analytical chemistry in the near future.

MC FIA with either optical or electrochemical detection is being used with success for the simultaneous determination of several components in a single injected volume of several microliters. Most MC FIA procedures, especially those using metal chelates with OARs, belong to the nonselective procedures. Thus, they are often combined with selective detection systems. AAS, AES, ICP OES, fluorometry, and, especially, spectrophotometry belong to the most popular of these detection systems.

Despite the relatively small number of papers dealing with the simultaneous determination of several components by FIA, this very effective technique has increasing importance for routine practice and research. MC FIA has many advantages over the other automatic methods, even though there are several negative aspects. It is mainly done because of the simplicity of the instrumentation, the high sampling frequency and information, precision and reproducibility, and, last but not least, because of the low cost per analysis. The merits of MC FIA can be expressed in terms of a decrease in the sample and chemical consumption, the possibility of measuring samples containing different concentrations, the use of new calibration techniques, as well as an indisputable increase in the sampling frequency and its information value.

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