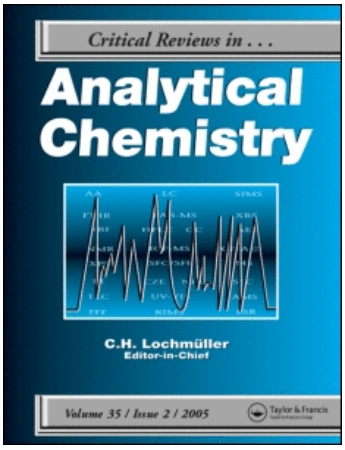

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Direct Processing and Analysis of Solid and Other Complex Samples with Automatic Flow Injection Systems

Zheng-Liang Zhi[ab]; Angel Rios[a]; Miguel Valcarcel[ac]
[a] Department of Analytical Chemistry, Faculty of Sciences, University of Cordoba, Cordoba, Spain [b] Department of Environmental Science and Engineering, Nanjing University, Nanjing, China [c] Dept. of Analytical Chemistry, Faculty of Sciences, C/O San Alberto Magno s/n, Cordoba, Spain

To cite this Article Zhi, Zheng-Liang , Rios, Angel and Valcarcel, Miguel(1996) 'Direct Processing and Analysis of Solid and Other Complex Samples with Automatic Flow Injection Systems', Critical Reviews in Analytical Chemistry, 26: 4, 239 — 260
To link to this Article: DOI: 10.1080/10408349608050576
URL: http://dx.doi.org/10.1080/10408349608050576

Critical Reviews in Analytical Chemistry, 26(4):239–260 (1996)

Direct Processing and Analysis of Solid and Other Complex Samples with Automatic Flow Injection Systems

Zheng-liang Zhi, Angel Rios, and Miguel Valcarcel***

Department of Analytical Chemistry, Faculty of Sciences, University of Cordoba, Cordoba-14004, Spain

* Present address: Department of Environmental Science and Engineering, Nanjing University, Nanjing-210008, China

** Author for correspondence: Dept. of Analytical Chemistry, Faculty of Sciences, C/.San Alberto Magno s/n; E-14004-Cordoba, Spain Tel.: +34 57 218616; Fax: +34 57 218606

ABSTRACT: The use of a suitable sampling or sample-processing unit connected to a flow injection system can significantly expand the scope of application of the flow injection technique by enabling the direct introduction and treatment of solid and other complex samples in a single automated system. In recent years various sample pretreatment techniques, including electrolytic dissolution, on-line leaching, continuous microwave digestion, on-line sterilizable membrane dialysis/filtration, pervaporation, dynamic gas-extraction, and flow-reversal liquid-liquid extraction, among others, have proven useful for solving a variety of analytical problems in conjunction with flow injection systems. The principles behind these techniques and their applications in fast assay and on-line process monitoring are reviewed and discussed. Selected major advances in this research field are outlined.

KEY WORDS: automatic sample treatment, flow injection technique, process monitoring, complex (solid) samples.

I. INTRODUCTION

The complexity of the matrices of the real samples typically encountered in environmental, industrial processes, and biotechnological studies hampers the direct analysis of key components by use of a specific detection technique. Special attention thus must be devoted to the development of effective methods for sample pretreatment, which is often the bottleneck of the overall analytical process. Most sample pretreatment procedures are still performed manually. They tend to be highly variable in nature, complex, labor-intensive, difficult to control systematically, and the source of bias error and contamination problems, particularly when larger number of samples are involved. Automation of the sample preparation step (usually referred to as preliminary operations) of the analytical process for this type of sample is a complex task that can rarely be accomplished satisfactorily in a simple way,[1] even though it is critical for enhancing sensitivity and selectivity, improving the accuracy and precision of routine laboratory assays or R&D

1040-8347/96/$.50

strategies, and ensuring rapid analyses or obtaining reliable (near) real time profiles for chemical fluxes involved in industrial applications (process monitoring or quality control testing). The development of on-line sample treatment procedures and the implementation of a variety of continuous separation and/or preconcentration techniques in the analytical systems is a key approach to improving analytical features.[1] In this respect, flow systems in general and the flow injection (FI) technique in particular have proven useful for automating not only serial assays,[2] a fairly unexplored use, but also for handling contaminant-free complex samples and the direct pretreatment of solid and liquid samples with harsh chemical matrices such as solid, particle and colloidal suspensions, as well as the macromolecular-containing samples.[3] This type of sample and FI systems are essentially incompatible; concilling the two would probably rely largely on the development of an effective sampling interface between the real sample and the detector in order to avoid matrix effects. Much research in recent years has been aimed in this direction. Some innovations suitable for processing this type of sample in a continuous way in an on-line association with a special detector in a flow injection system have been developed in order to automate (or at least simplify) analytical procedures and solving various specific analytical problems.

Some well-documented, comprehensively reviewed continuous separation approaches (coupled to FI systems) to the analysis of relatively complex liquid samples are not discussed here because most only allow the analysis of liquid (aqueous) samples, with no provision for the treatment of solid and related complex samples.

Sampling interfaces of use in direct processing and handling of solid or complex matrix samples by the continuous flow technique can be classified into three main categories according to the way the analyte is transferred into the analytical flow system, namely, (1) by partial or complete dissolution of the sample with the aid of external energy (e.g., electronic, ultrasonic, microwave) supplied by some physical means; (2) by using a continuous sterilizable membrane filtration/dialysis unit to remove interfering particles and macromolecules present in real samples; and (3) by isolating the analyte from the complex sample matrix using other nonchromatographic continuous techniques such as gas extraction, pervaporation, or, occasionally, liquid-liquid or liquid-solid extraction.

The state of the art of three above-mentioned types of techniques is discussed and reviewed separately below, with an emphasis on the applications in routine laboratory analyses and on-line industrial process stream monitoring. The real assets and potential shortcomings of each particular assembly are also commented on.

II. ON-LINE ELECTROLYTIC DISSOLUTION

Electrolytic dissolution is an efficient way of accomplishing fast continuous dissolution of solid metal samples for chemical analysis. It is a suitable sample preparation technique that can be coupled on-line to various determination procedures in order to circumvent the time-consuming step involved in sample preparation. In this technique, the metal sample is used as the anode of a Teflon electrochemical dissolution chamber that also includes a cathode. When an accurate d.c. pulse is applied between the electrodes over a short, precise interval of time, a portion of the sample is dissolved into a continuously flowing electrolyte that is transported by the electrolyte solution toward the detection system.

Electrolytic dissolution with flow injection analysis was first proposed for monitoring the soluble aluminium content in steel by

Bergamin and co-workers.[4] The dissolution system involved an electrolysis cell for sample dissolution, a debubbler for removing the gases evolved during electrolysis, a filter unit for removing solid particles and a determination unit based on a post-column reactor for photometric reaction with Eriochrome Cyanine R. The potential problem is that the electric current added cannot be increased indefinitely (beyond 600 mA) because of the formation of solids that clog the filter in a very short time, while a long electrolysis time results in the amount of the analyte dissolved per time unit approaching constancy, thereby giving rise to a stationary signal. Thus, aluminium in steel can only be determined over the range 0.01 to 0.13% (w/w).

An improved flow system was proposed by the same research group for the spectrophotomic determination of molybdenum in alloys that surpasses the previous system in various respects.[5] Thus, the debubbling unit and filtering device were removed from the electrolytic dissolution unit and placed in the determination flow manifold, which led to a more stable system and more precise results. A commutator was included, which permitted the injection of standard solution into the sample zone, thereby simplifying the application of the standard addition method. Solution leakage was observed in sample changeovers because the dissolution chamber was a discrete unit, where the start and stop of the electrolyte flow was controlled by the commutator.

On-line electrolysis was used for sample preparation in the determination of copper in aluminium alloys.[6] The coupled electrolytic dissolution-FAAS approach was demonstrated to be technically much simpler because when using the FAAS detector with the debubbling device no post-column reactor is necessary.

The electrolytical flowing attack method combined on-line with ICP emission spectrometer and was first tested by Ohls and Flock for rapid simultaneous determination of alloy elements (Cr, Ni, Mn, and acid-soluble Al) in steel and other iron alloys containing less than 0.3% carbon.[7] The multielement capabilities of the detector allow the accurate determination of the quantitative composition of steel samples. On-line electrodissolution coupled with ICP-AES was also adopted by Bergamin an co-workers for the simultaneous determination of Cr, Ni, Mn, Si, and Fe in stainless steel.[8] The method is applicable to samples containing elements such as carbon, sulfur, and phosphorus at levels not exceeding 2% (w/w). Yuan et al. reported a method for the direct simultaneous multielement analysis of solid metal samples (aluminum alloys) by the same technique. Metals such as Zn, Si, Fe, Mn, Cr, Mg, and Cu can thus be determined simultaneously over the range from 10 to 100 g/g.[9]

The principal advantages of the flow electrodissolution approach are saving time in sample preparation, reduced manipulation, and acceptable repeatability. The rapid analyzer for controlling aluminium in steel based on a special electrolysis cell for the rapid dissolution of solid steel samples and a computer-controlled flow injection spectrophotometer reported by Coutinho et al.[10] is now commercially available. However, the performance of this technique may seemingly be improved somewhat by introducing the following modifications in the manifold: (1) an 8-port injection valve involving two flowthrough electrolytic cells in the closed loop system operating in an alternate fashion should shorten the start-up time, thus increasing sample throughput and facilitating sample changeover; and (2) introducing a back-flushing program for the in-line filter removal of the solid produced during operation would allow the system to work continuously over longer periods. Electrolytic dissolution also has some disadvantages, such as a limited scope of applications. In fact, it is only applicable to metals in alloys or steel;

other potential drawbacks are that it needs appropriate solid standards for calibration and a that slight compositional difference between the standard and the sample may dramatically alter the amount analyte that is dissolved by a given amount of electricity.

III. ON-LINE LEACHING IN A CLOSED-LOOP RECIRCULATING SYSTEM

Leaching is a liquid-solid extraction process that uses a liquid solution to selectively extract one or more desired analyte from a complex solid matrix. Direct leaching of a solid sample can be accomplished by using a closed-loop recirculating system, including a sample cell or cartridge with a special filter for retaining the sample. The leaching stream is allowed to circulate through the solid sample for a preset time. Ultrasonic irradiation and heating (by immersing the sample cell in a thermostated water bath) are sometimes used as a physical means for accelerating the partial dissolution of the sample when the leaching kinetics is rather slow. This approach is potentially applicable to any pulverizable sample (e.g., plant material, soil, coal, filtration sorbents, or liquid samples with precipitation problems) under contamination-free conditions.

The applicability of the flow injection on-line leaching approach has been demonstrated by directly treating solid samples (soil, ground plant materials) using acid as the leachate and ultrasonic irradiation and heat. Total boron in soils[11] and total iron in plant materials[12] were determined by these approaches. The leaching time was greatly reduced by applying ultrasonic irradiation and heating when the leaching solution was passed through the solid. The on-line leaching/flow injection approach was also used for the determination of acid-soluble calcium in charcoal with an AAS detector;[13] no physical energy was applied during the leaching step in this approach, but the cycling time was rather long (i.e., 10 min).

A flow injection system coupled to an open/closed loop recirculating system sample pretreatment unit for automatic multistep leaching was used for the determination of the cation-exchange capacity (CEC) of soils.[14] The manifold used for CEC determination consisted of two distinct blocks, namely, the open/closed loop reciculating system for sample pretreatment and the flow injection analysis system for residual magnesium determination. The open/closed recirculating system (Figure 1) included a filter cartridge for retaining solid soil particles, a peristaltic pump channel for propelling the solution, and one switching valve for delivering both the waste and the sample. Cations were first displaced from the exchange sites on soil colloids by passing dissolved barium(II) through the unit. Soil was then bound barium exchanged with magnesium(II) by adding a magnesium sulfate solution that was successively circulated through the system, which aided to further remove barium as a sulfate precipitate. By activating the switching valve, the clear liquid filtrate obtained was introduced into the determination manifold, where the concentration of residual magnesium (II) was determined and from it the sample CEC was calculated. Because the entire pretreatment was done in a filter cartridge included in a closed flow system, manipulation of the soil sample was greatly reduced. Moreover, the method is more simple, rapid, and reliable than its manual countpart.

IV. ON-LINE MICROWAVE SAMPLE TREATMENT

One of the most frequently tested approaches for on-line solid sample preparation is microwave-assisted acid digestion, which can be directly or indirectly coupled with different detection techniques such as

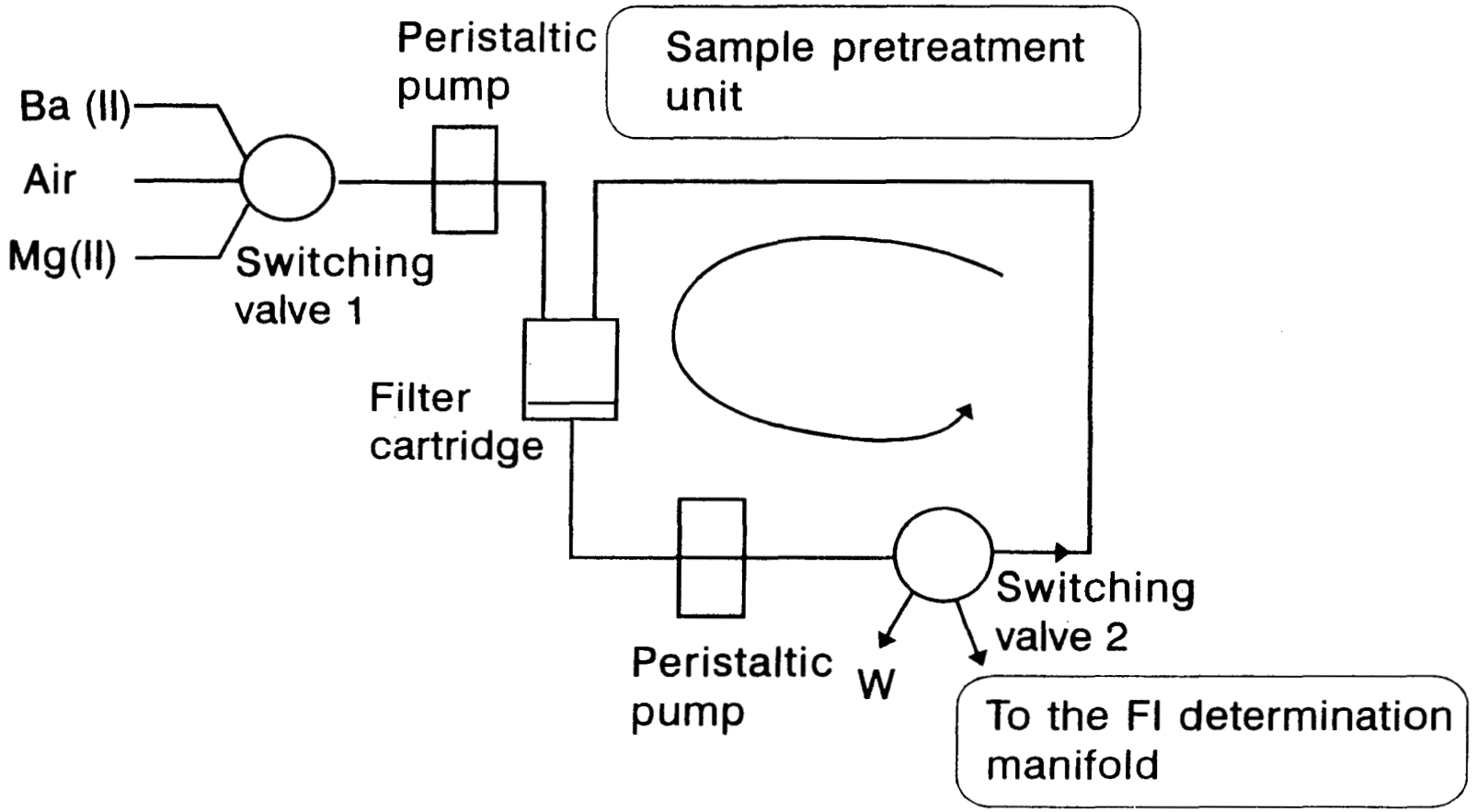

FIGURE 1. Open/closed loop reciculating flow manifold used as a sample pretreatment unit for the determination of the cation-exchange capacity of soils.

amperometry and photometry, ICP atomic emission, and atomic absorption spectrometry.[15] The incorporation of a microwave oven into a shorter FI manifold offers many advantages over off-line methods, including digestion times, digestion of troublesome matrices, and dissolution in a closed environment (which reduces volatile analyte losses and atmospheric contamination and increases personal safety through reduced exposure to hazardous acid fumes and minimized reagent handling).

Effective manifolds for on-line microwave-assisted acid attack can be classified into two categories according to the location of the digestion unit in the manifold: before and after the sample injection valve (Figure 2). In the former arrangement, the continuous-flow circuit (digestion at atmospheric pressure) and the stopped-flow procedure (at high pressure) are the two most frequently employed modes. After microwave digestion, the slurred sample is passed through the loop of the injection valve to effect discrete sample delivery to the detector. In the second type of arrangement, the digestion unit is placed after the injection valve. The injected sample (normally a slurry) is digested when flowing through the microwave digestion unit, subsequently being cooled in an ice-bath to reduce the amount of water vapor, and degassed whenever necessary prior to being continuously transferred to the detection system.

On-line sample pretreatment is of particular interest to the determination of mercury and other volatile elements such as arsenic, selenium, and lead because of the well-known problems associated with the volatilization of these elements, the risk of contamination, adsorption losses, etc. during the extensive sample pretreatment procedure. On-line microwave oven digestion can accelerate the analyte conversion from its chemical forms bonded to the organic matrix and thus the transformation of the analyte species into a well-defined, unbound oxidation state such as Hg(II), Se(IV), Te(IV), As(III), Sb(III), Bi(III), and Pb(IV). Subsequently, the analytes can be determined by the cold vapor and hydride generation atomic absorption techniques.[16] Thus, mercury in whole blood,[17] slurred solid samples,[18] and water and urine[19] were determined by FI

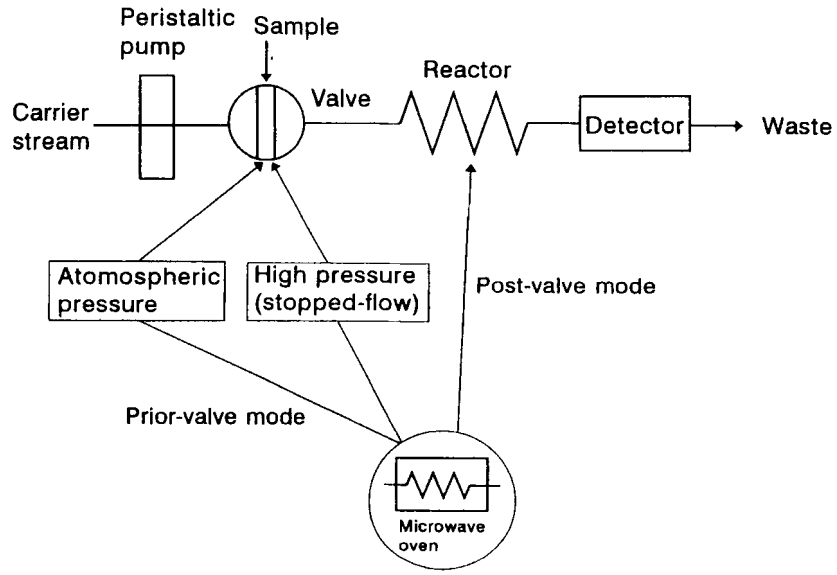

FIGURE 2. Schematic diagram showing the two positions where a microwave oven unit can be incorporated into a flow injection system.

cold vapor atomic absorption spectrometry after post-valve microwave on-line treatment. Cabrera et al. used on-line microwave oven digestion for the determination of Pb in beer, juice, and slurred fruits. They found on-line microwave digestion to increase the efficiency of Pb hydride generation by approximately 50% and to significantly increase of the selectivity.[20]

High-performance liquid chromatography (HPLC) with post-column on-line microwave-assisted digestion and hydride generation atomic absorption detection has also been adopted by Lopez-Gonzalvez et al. to determine arsenite, arsenate, dimethylarsinate, monomethylarsonate, arsenobetaine, and arsenocholine in drinking water, sewage, and harbor seawater, synthetic fish extract, and sediment extract.[21] The efficiency of microwave oven decomposition for organoarsenicals was found to be close to 100%, and detection limits between 0.3 and 0.9 ng were achieved for all the species tested.

On-line microwave digestion is also considered a suitable approach for the preparation of samples for FAAS analysis. An early approach for on-line treatment of liquid samples (whole blood) was incorporated into FAAS by using a double injection valve to introduce the sample and reagent in parallel into a flow system. The mixture was subsequently passed through a Pyrex coiled decomposition tube located inside the microwave oven in order to mineralize the blood and the digest was finally carried to the AAS nebulizer.[22] This simple approach, however, has some disadvantages, such as the short residence time of the sample/reagent mixture in the microwave oven (less than 25 s in order to minimize the production of acid fumes during digestion), so it is only suitable for liquid samples requiring little digestion.

Haswell and Barclay tested a continuous flow injection system with post-valve microwave digestion with the sample as a slurry[23] coupled on-line to an FAAS for total element determination. Bubble formation during digestion was controlled by post-digestion cooling and pressure regulation. The results for reference materials examined for Ca, Fe, Mg, and Zn determinations were in good agreement with certified values. Spiked elemental recoveries were typically in the range 94 to 107%, and the sample throughput was 30 to 60 samples per hour.

The early prior-valve microwave digestion approach developed by Burguera and Burguera[24] using a microwave-assisted digestion procedure combined with FI and FAAS for the determination of metals in biological tissues such as liver and kidney is not a truly an on-line approach. Carbonell et al. developed an approach allowing homogenized acid slurry to be continuously passed through a PTFE coil located in a microwave oven for digestion, the digested sample being subsequently cooled and degassed and then passed through a rotary valve to fill the loop. After the sample had been completely digested, the loop of the closed system was injected into the carrier stream and transported to the AAS flame. Lead in sewage sludge was determined at a sampling frequency of 12 h^{-1}, which was limited mainly by the digestion time.[25] A similar approach was used for the determination of copper and manganese in various matrices, including artichokes, diet foods, sewage sludge, and different reference materials.[26]

Stopped-flow microwave-assisted digestion at high pressure can occasionally be used to increase the digestion efficiency. Such is the case with the approach reported by Karanassion et al., where a coiled PTFE tube serves as both a sample container and a digestion vessel that is filled with a mixture of acid and water slurry.[27] Sample flow is stopped and the coil tube sealed while microwave power is applied for a preset time. This methodology was developed for sample preparation in the ICP-AES analysis of powdered botanical and biological reference materials, where Al, Ba, Ca, Cu, Fe, Mg,

and Zn were determined simultaneously. Gluodenis et al. developed a stopped flow microwave heating reactor for sample preparation in AAS analysis.[28] Slurred samples were digested in a glass reactor mounted inside a microwave oven where the pressure inside was maintained at 459 lb in^{-2} and continuously monitored for 5 min during digestion. After cooling, the reactor was vented and the contents were flushed out into a calibrated flask and diluted to volume. This procedure can be only considered a semiautomatic approach because there is no direct connection to the detector; however, it can effect total dissolution with a minimum amount of residual dissolved carbon, so it is suitable for materials containing large amounts of organic matrix.

On-line FI microwave oven digestion can be applied to the automatic solid sample preparation for graphite furnace atomic absorption spectrometry (GFAAS) despite the discrete, non-flow-through nature of the technique. Burguera et al.[29] developed an approach allowing mineralization of sample slurry to be accomplished in a PTFE coil located inside a microwave oven downstream of the injection valve. After passing a degasification unit, a precise volume of the mineralized sample that was collected in a capillary of a sampling arm assembly was introduced into the graphite furnace through a solenoid. Lead in biological materials was thus determined at concentrations down to 70 g l^{-1}. A similar manifold was also been developed by the same group for the determination of iron and zinc in adipose tissue.[30] A small modification of the manifold allowed the introduction of carbon tetrachloride plugs in order to restrict sample dispersion for the determination of titanium as titanium dioxide over the linear range from 0 to 0.7 g ml^{-1} in soap samples.[31]

Other successful applications involving FI with an on-line microwave oven digestion unit include the determination of total dissolved phosphorus (TDP)[32,33] and total phosphorous (TDP plus particulate phosphorus except inorganic polyphosphates)[34] in waste waters; chemical oxygen demand (COD) in well, river, and canal water, sewage and food industry waste;[35] and urea, as ammonia, in natural waters.[36]

V. ON-LINE STERILIZABLE MEMBRANE DIALYSIS/FILTRATION SAMPLING DEVICES

The continuous on-line determination of key components in the medium during fermentation is a prerequisite for the effective process monitoring and control of bioreactors. The demand for the continuous monitoring device is becoming even more important when a continuous biotechnical process is to be developed. Direct measurement in the bioreactor with some type of biosensor is one plausible solution; however, until recently, truly *in situ* sterilizable sensors for measuring key substrates and metabolite concentration were not yet available. Hence, much effort has been expended in developing on-line sampling devices that may be fully automated and integrated between the process and detection system. This has promoted rapid advances in on-line analyses of biotechnological processes with special emphasis on sampling and coupling to flow injection and column liquid chromatography.

Early on-line monitoring systems relied mainly on the use of a continuous-flow, air-segmented system; however, uses in biotechnology were restricted by cell growth in the nutrient-rich permeating flow in the system after a few days of continuous operation. As an alternative, flow injection is becoming the most frequently applied technique for the on-line monitoring of chemical variables. The advantages of this technique are a much lower likelihood of cell growth in the system, a short response time, long-term stability, low reagent consumption, and modest

maintence requirements. However, it is difficult to determine several compounds simultaneously in a single run; also, accurate quantification requires selective enzymatic or chemical reactions. On the other hand, chromatography can be used successfully in some cases for multideterminations in fermentation broths.

On-line measurements usually requires direct sample introduction into the analytical system, which normally entails using a continuous, sterilizable filtration interface (stream sterilized at 121°C) to remove interferring broth components and particles or colloidal substances while keeping contaminating microbes out of the fermenter. A major problem encountered in the use of a pressure-driven membrane filtration process is that the filtration rate is often seen to decline with time. A decline in the filtration rate can be caused by several factors, including concentration polarization and fouling.[37] This may be caused by adsorption of sample constituents onto the membrane, precipitation of salts, pore blocking by particulate matter, etc. Ideally, the interface between the bioreactor and the analytical system used to solve or minimize these problems should meet the following criteria:

1. It should not disturb the fermentation process and, in particular, cause no infections. This in turn requires the use of materials that can be easily stream sterilized *in situ* or in an autoclave.
2. The filtration unit should be compact with low hold-up or dead volume, especially on the filtrate side, in order to ensure that the sample closely represents what is happening in the fermenter during a rapidly changing process.
3. The system must be technically simple to maintain. The use of standardized commercially available filters and connections of different sizes is desirable.
4. The sampling devices should have reasonable long-term reliability (membrane fouling should remained minimal for many hundreds of hours of fermentation).
5. Calibration of the determination system together with a sampling device should be possible.

The various reported automatic continuous sampling interfaces that meet the abovementioned criteria are of two different types: those where the sampling unit is mounted inside the fermenter and those where it is placed outside the fermenter (in a recycle loop connected to it). Figure 3 shows a typical schematic diagram for the two types of sampling interface.

A. *In Situ* Sampling Modules

This type of sampling unit often consists of a stainless steel carrier serving as a support for a porous membrane. It is placed at a well-stirred position in the bioreactor to avoid or reduce membrane clogging. The filtrate is then withdrawn by means of a peristaltic pump and transported further for analysis. The main features of this type of analyzer and selected applications are presented in Table 1. A further list of early sampling and sample pretreatment devices used in connection with flow systems has been reported.[37] In an *in situ* module, a true picture of the conditions in the bioreactor is obtained, but some obstacles still remain. Thus, the bioreactor tends to be clogged very quickly, must have a relatively high short response time (20 to 40 min), allows no membrane changeover during fermentation, and poses calibration problems.

Worth special mention is a disk-shaped probe-type *in situ* membrane module recently marketed by Advanced Biotechnology Corporation (Germany) and named ABC module.[40] The membrane module can be inserted through a standard 19- or 25-mm port in the top plate of a bioreactor, or, alternatively,

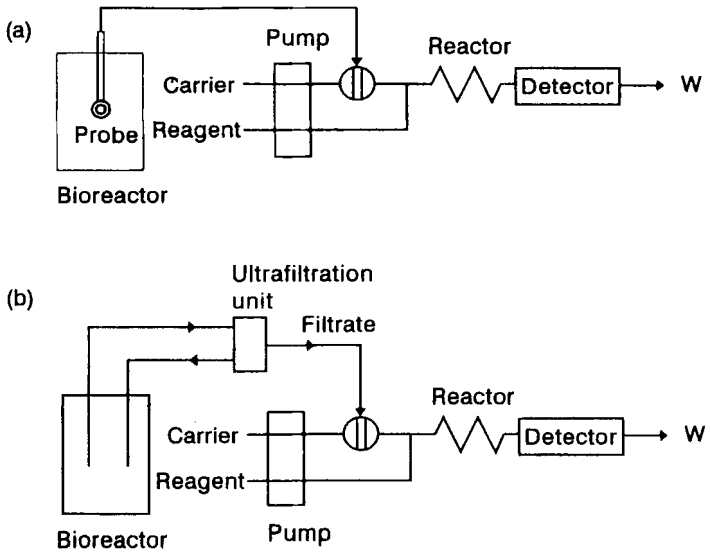

FIGURE 3. Schematic illustration of the two types of sampling units for fermentation monitoring: (a) probe-type sampling unit mounted inside the fermenter; (b) sampling unit located in a recycle loop outside the fermenter.

Table 1. Applications of probe-type ultrafiltration/dialysis devices in fermentation monitoring

Fermenter	Analyte	Analyser	Sampling device and membrane	Sampling time	Typical monitoring time	Ref.
Fermentation of glucose by *Saccharomyces cerevisiae*	ammonium, phosphate, glucose, volatiles and dissolved gases	automatic analyser and mass spectrometer	0.2-μm Nylon membrane, flat microfiltration unit	13 min	20 h	38
Penicillin fed-batch	penicillin V and by products, degradation products and their precursors	HPLC	porous-plate microfiltration with membrane 10,000 dalton cut-off	35 min	300 h	39
Penicillin V fed-batch	reducing sugars, dissolved organics, ammonium, urea, sulphate, phosphate, penicillin V	air-segmented automatic flow analyser, HPLC	disk-shaped sterilizable ultrafiltration probe with polycarbonate membrane	20-40 min	1000 h	40
Penicillin fed-batch	glucose, ammonium	semi-on-line FIA	polypropylene, polycarbonate, ceramic material	2.5 min	200 h	41
Recombinant *Escherichia coli* culture	cell metabolites, intracellular enzyme etc.	FIA, HPLC	ABC tubular filtration module	-	24 h	42
Fed-batch Penicillin V fermentations	penicillin V	FIA	ABC tubular filtration module with 0.22-μm pore size membrane	-	200 h	43
Fermentation broth, milk, waste water	glucose	HPLC, FIA	microdialysis probe with membrane cut-off at 30,000 dalton	10 min	1-2 weeks	44
Fermentation Module for *Saccharomyces cerevisiae*	ethanol, glucose	autoanalyser	dialysis probe	-	48 h	45
Penicillin and ethanol	carbohydrates	HPLC	dialysis probe	-	48 h	46

through a 25-mm port below the liquid surface. When inserted from the top of the bioreactor, one or more extension tubes are needed to place the membrane below the liquid surface. Liquid passing the membrane is controlled in 16 grooves, and these permeate streams are joined in four openings spaced evenly along the membrane holding unit. Through these four openings, the permeate enters a central channel and a continuous sample stream leaves the unit from the top of the probe to the analyzers. Christensen et al. characterized this type of device and found that a constant flow rate of 1 ml min^{-1} may be obtained through the 200-h fermentation by restricting the permeate flow with a peristaltic pump at the beginning of fermentation in order to avoid early membrane fouling. The lag time of the ABC module is approximately 2.5 min and is mainly dependent on the membrane thickness. The ABC tubular filtration module coupled on line with FI or HPLC has been used for monitoring different components of bioreactors.[40–43]

(Micro)dialysis probes have also proven successful as sampling devices in continuous monitoring of biotechnical and miscellaneous applications.[44–46] It allows continuous withdraw of very small, representative samples that do not affect the volume of the test solution (organ or fermentation), so it is especially suitable for small-scale bioreactor monitoring.

B. Sampling Devices Placed Outside the Bioreactor

In this case, the membrane module is placed in a loop outside the bioreactor and the fermentation broth is pumped out of the bioreactor into the sampling unit and, after filtration, back again. It is possible to change the membrane during fermentation, but there may well be an effect on the performance of microbial cultures owing to circulation in the loop. It is therefore important to minimize both the residence time in the loop and the ratio of loop volume to working volume of the bioreactor. This type of sampling unit exists in different designs and a number of them are now commercially available, as reflected in the following examples.

1. BIOPEM Module

The first stream sterilizable (cross-flow filtration unit) was designed by Kroner and Kula,[47] which was commercially marketed and called BIOPEM. This dynamic filtration device can provide a continuous sampling stream, separating enzymes from the cell mass and particulate broth components continuously, prior to the determination system. The BIOPEM module contains a magnetic stirrer placed above the membrane to create a tangential flow and is connected to the bioreactor via a bypass (Figure 4). The broth is pumped continuously through a filter by a peristaltic pump with a moderate feed rate and across a microporous or ultrafiltration membrane through which a continuous filtrate stream is produced. This stream is directly connected to the analyzing system (flow injection analyzer) or a fraction collector. The system allows continuous operation under aseptic conditions. The BIOPEM module coupled with a continuous flow analysis system was used for the analysis of alkaline protease and pullulanase during fermentation of *Bacillus licheniformis* and *Klebsiella* pneumonia on a 30- and 70-l pilot scale. It was also used to monitor the concentration of the inducer L-phenylalanine in the fermentation of *Rhodococcus sa.* M4 over a period of at least 87 h.[48] The same device was used monitor of the disintegration of microbial cells[49] and for protein detection.[50]

Although the BIOPEM module has been used successfully for bacterial and yeast fermentations in the pilot plant, the major drawback is its relatively high response time (approximately 2 min with a permeate flow of

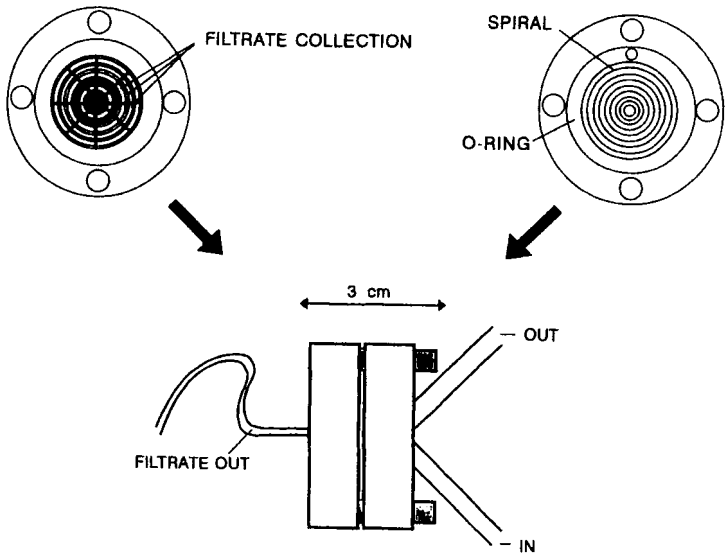

FIGURE 4. Schematic diagram of the tangential flow filtration module accepting any 47-mm-diameter membranes. (Adapted from Reference 52.)

0.5 ml min^{-1} and a circulation flow of 33 l h^{-1}) as the chief result of the large dead volume of the stirred chamber in the module. Moreover, Christensen et al. found that the module is very poor for sampling during fermentation with filamentous fungi, which they ascribed to insufficiency of the stirrer to prevent material build up.[41]

2. Tangential Flow Filtration Modules

The use of a cross-flow, tangential flow filtration (TFF) module has received considerable attention in recent years. This technique was first proposed by Henry and Allred a few years ago for cell harvesting and enzyme recovery,[51] but its wide use as a sampling unit for FI and HPLC analysis started only in recent years. This type of sampling unit exists in several different designs and two of them, namely, FAM (Waters Filtration/Acquisition module) and A-SEP filter module (Applikon), are now commercially available.

An assembled filtration sampling device (FAM) is shown in Figure 4. Its two parts are made of stainless steel a 47-mm-diameter hydrophilic membrane filter disc is sandwiched between the shells, each with helix-shaped grooves machined into them. The larger channel on one shell drives the process flow; the smaller channel on the other shell drives the filtrate outside. The bioreactor solution is driven to flow tangentially along the filter and thus separated into two streams (viz. the particle retentate and the particle-free filtrate). This movement of fluid across the surface of the filter prevents sedimentation of materials on the membrane. After filtration, the retentate is returned to the stirred fermenter and the filtrate is transferred to a switching valve placed close to the sampling device. This valve allows alternate passage of either the filtrate or standards into the preparation system, where degasification and dilution take place. The diluted unsegmented sample is sent directly to the determination system to be introduced by filling the loop of a injection valve. The filtrate flow rates can be maintained at 0.15 to 0.30 ml min^{-1}, provided a high fluid recycle rate (e.g., 50 ml min^{-1}) is adopted. The module has a very low response time (10 and 25 s for the FAM module and A-SEP module, respectively) and lower residence times for the broth in the circulation loop. Thus, the effects of anaerobic conditions and substrate depletion in the loop are minimal,

but the circulation itself may affect the morphology of the microorganisms owing to the high linear velocities obtained in the tubings.

Buttler et al. investigated the filtration characteristics of a tangential flow filtration module (FAM) that served as a sampling unit in conjunction with column liquid chromatography. They found that the Waters FAM sampling unit could be used for analyses of carbohydrates in complex media involved in small-scale fermentations, and that they yielded recoveries close to 100% for some small sugars. However, amino acids and other organic acids were found to exhibit a more complex membrane transport behavior in complex matrices.

Fully or partially automated on-line systems have been developed and adopted for monitoring bioprocesses by the use of this technique coupled with FIA or liquid chromatography. Part of these applications are summarized in Table 2, which shows information on the components determined, type of fermenter, sampling unit, and membrane used, as well as the reported typical monitoring time. From this table, the following conclusions can be drawn: (1) relatively few compounds have been determined; (2) flow injection manifolds involving a selective enzymatic or chemical reaction are the most choice for on-line monitoring of chemical variables (whenever multicomponents are to be determined several different manifolds, each dedicated to one individual component are usually employed); (3) commercially available ultrafiltration sampling units, such

Table 2. Applications of the tangetial flow filtration unit in fermentation monitoring

Fermenter	Analyte	Sampling device and membrane	Anal. system	Monitoring time	Ref.
E. Coli, *S.Cerevisiae*, and *S. Pilosus* fed-batch	glucose, ethanol, phosphate, ammonium	FAM, 0.2-μm Durapore	FIA	180 h	52
Saccharomyces cervisiae model fed-batch	glucose, ammonium, total protein	FAM, 0.1-μm Millipore membrane	FIA	82 h	53
penicillin fermentation	ammonium, glucose	FAM, polysulphone membrane	FIA	200 h	41
mouse-mouse hybridoma and *Clostridium thermosulfurogenes* fermentations	monoclonal antibodies and pullulanase isoenzymes	TFU and BIOPEM, 0.22-μm Durapore membrane	FIA	240-450 h	54
E. coli fermentation	acetate and phosphate	A-SEP, 0.2-μm Durapore membrane	FIA	30-35 h	55
Candida rugosa fermentation	glucose	FAM, 0.45-μm membrane	FIA	30	56
Saccharomyces cerevisiae fermentation	five carbohydrates and ethanol	FAM, 0.22-μm Durapore membrane	HPLC	24 h	57,58
Bacillus polymyxa and *Klebsiella oxytoca* fermentations	major products and volatile metabolites	FAM, 0.2-μm Durapore membrane	FIA-MS	8-15 h	59

as FAM or A-SEP, furnished with a standard disk-shaped 0.2- or 0.45-m pore size membrane, can be satisfactorily used to produce particle-free samples from rather different fermentation processes involving bacterial, yeast culture, and filamentous fungial broth; and (4) the sampling device can be used for long-term (typically days to weeks) fermentation monitoring.

In addition to fermentation monitoring, TFF was used by Pedersen et al. for on-line analysis of waste waters.[60] They developed a system allowing continuous monitoring of the concentrations of phosphate, ammonia, and nitrate in different parts of a wastewater treatment plant over a period of at least 24 h. The membrane must be changed every 3 weeks. Bacterial growth in the filtrate system was prevented by manually flushing the system with 0.5 *M* HCl on a daily basis.

3. Cross-Flow, Hollow-Fiber Ultrafiltration Modules

One other type of cross-flow filtration unit is the hollow-fiber ultrafiltration module. On-line monitoring of components during the production of cephalosporin C was developed by Bayer et al. by using a cross-flow, hollow-fiber filtration module and air-segmented flow systems for which quantifying glucose, ammonia, phosphate, sulfate, methionine, and caphalosporin.[61] The hollow-fiber filtration module with a 0.2-m pore size polypropylene membrane supplied a continuous, sterile filtrate of cell- and solid-free broth from the high-protein containing medium. No change in the concentration of small molecules was found to occur during permeation, but an appreciable amount of proteins also passed through. A dialysis cell for removing proteins was therefore incorporated into the automatic analyzer. The response time for the complete analysis was approximately 37 min owing to the relatively long transfer distance between the fermenter, filtration module, and analyzer system and the small sampling rate (approximately 0.3 ml min^{-1}).

Van de Merbel et al. reported an automated on-line system for monitoring low-molecular-weight compounds during fermentation based on the same ultrafiltration device coupled on-line with HPLC.[62] Filtration was performed by continuously pumping the fermentation broth through the module at a flow rate of 100 ml min^{-1} and applying a pressure of 0.3 to 0.4 bar. The retentate was pumped back to the fermenter and the filtrate transferred on-line to a loop mounted on a six-port switching valve connected to the LC system. The residence time of the fermentation broth outside the fermenter was only 12 s; however, the actual sampling times were 12 and 20 min for gluconic acid and anaerobic beer fermentation, respectively. The delay time between sampling and data presentation was found to be influenced by membrane parameters such as the membrane-area-to-dead-volume ratio and membrane thickness, as well as by the composition of the fermentation broth. Membrane fouling can be minimized by closing the filtrate outlet between two sampling periods, thus canceling the pressure difference across the filter. The system was used to determine sugars, alcohols, and organic acids during gluconic acid and beer fermentation for at least 250 h with complex fermentation broth under both aerobic and anaerobic conditions. More than 300 analyses were performed during this period. A similar system was adopted by the same research group for on-line monitoring of low-molecular-weight components such as lactose, glucose, and fructose in an *E. coli* batch fermentation broth.[63]

4. On-Line Analysis with Self-Cleaning Filtering System

Saltzman et al. reported an on-line analysis system for monitoring low levels of hydrogen sulfide in lean alkanolamine solution using a highly sensitive analyzer based on

the measurement of a UV-absorbing chromophore formed by the amine-HS_2 and a special self-cleaning filtering system.[64] The filtering system consisted of two filter elements aligned in series with the bypass flow and filtrate outputs feeding a common manifold. While approximately 50 ml min^{-1} of the sample flowed through one filter element, the other filter element was backflushed with filtrate displaced in a piston cavity by an air-actuated piston. Every 30 s the operation of each filter element reversed its operating mode so that one element was washed while the other was used to pass the sample. This filter system was found to operate in a reliable, accurate way for over 10 months without a problem.

VI. PERVAPORATION

Pervaporation can be defined as a combined separation technique that integrates an evaporation and a gas-diffusion process in a single step. It is based on the evaporation of volatile substances contained in a heated donor stream through a porous hydrophobic membrane for their separation from complex matrices. Pervaporation can be used for analytical purposes in order to protect a biosensor or other detector from suspended solids and macromolecular compounds in the fermentation broth when applied to the on-line monitoring of a biotechnological process. Prinzing and co-workers have designed a laboratory-built continuous pervaporation module consisting of an upper and a lower compartment separated by a horizontal semipermeable membrane.[65] The carrier stream enters the lower compartment but is separated from the membrane by an air gap. The carrier stream is then heated, which results in a vapor pressure difference across the membrane that provides the driving force for the separation process. Thus, volatile analytes diffuse across the air gap and pass through the membrane to an acceptor stream that drives them to the biosensor. The approach was applied to the determination of ethanol and biacethyl in simulated brewing mixtures. Recently, an improved pervaporation module integrated in a flow injection enzymatic manifold was developed by the same research group for the determination of ethanol in both beer and baker's yeast cultures.[66]

Pervaporation can also be coupled to flow spectrophotometry,[67] electrochemical detection[68] and even mass spectrometry or gas chromatography-mass spectrometry[69] for detecting the volatile analytes. The absence of between the sample matrix and membrane, and the features of pervaporation, are especially suited to the monitoring of complex samples (e.g., biological fluids, fermentation, and other industrial process samples).

VII. GAS-EXTRACTION APPROACH

Gas extraction or dynamic head space methodology provides an indirect method for the analysis of samples containing organic or inorganic volatile species. Because only the gas phase in contact with the sample (and not the sample matrix itself) is taken for analysis, gas extraction can be used for the determination of trace concentrations of volatile substances in dirty, viscous, and nonhomogeneous samples, which are difficult to handle directly by conventional flow or chromatographic systems. The fact that no membrane is necessary makes this approach suitable for use whenever a complex sample matrix may hinder mass transfer by clogging the membrane.

The dynamic gas extraction approach was first used in combination with a flow injection manifold by our group for the direct determination of triethylamine in fish samples.[70] In the manifold, Figure 5, a laboratory-made sampling device based on dynamic gas-extraction, was used to continuously supply the gaseous sample from the alkalized sample. A flow-reversal injection mode using no debubbling unit was used to

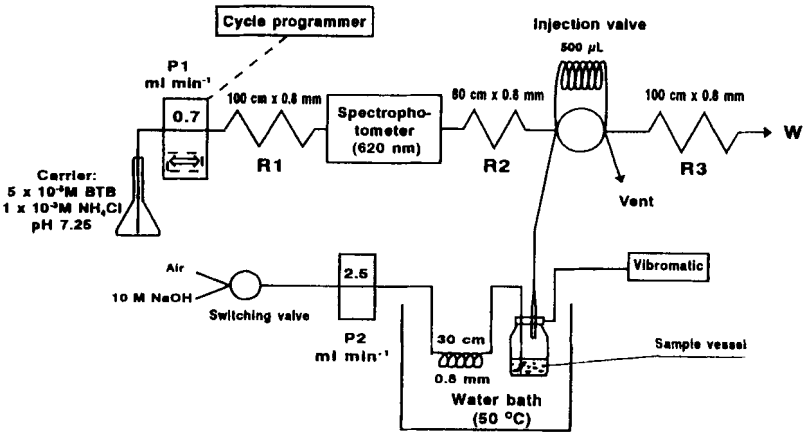

FIGURE 5. Flow-reversal FI/gas extraction approach for the direct determination of TMA in fish.

directly treat and process the gaseous sample introduced via an injection valve. A pH indicator (Bromothymol Blue) aqueous solution of pH 7.25 served as a carrier and to detect the amine by a gas-liquid dissolution reaction that is monitored *in situ* with a spectrophotometer. Trimethylamine was determined over the range 0.1 to 10 g ml^{-1} at a rate of about 10 samples per hour. The chief advantages of this method are that all measurements are made in aqueous solutions and that it requires no prior separation of sample matrix; also, it features great instrumental simplicity because the evolved analyte is determined by integrating gas dissolution, color development, and detection in a single, dynamic step.

By using a similar device, we recently developed a method for sulfur dioxide determination in solid and liquid food samples.[71] The method allows the quantitation of 0.75 to 75 ppm of analyte in real samples. Should higher sensitivity be required, an alternative approach also based on the gas-extraction sampling device but involving *in situ* concentration and continuous monitoring of the reaction product can be used (Figure 6). The kinetic features of the absorbance-time profiles obtained in real time are exploited for quantitation (by the fixed-time or tangent method). Application of the method is recommended for samples containing less than 3 g ml^{-1} SO_2.[71] The manifold based on the gas-extraction and the flow system can be readily applied to the determination of other volatile substances (determinations providing a suitable derivatizing reaction for detection are available). Quantitative analyses, however, require that the standard solution be prepared in a well-matched sample matrix or the standard-addition method be used for calibration.

VIII. FLOW-REVERSAL INJECTION LIQUID-LIQUID EXTRACTION FOR OIL SAMPLE ANALYSIS

The conventional method for the chemical analysis of oils and fats is slow and tedious because it usually includes a number of time-consuming steps such as weighing, dilution, mixing, and, in most cases, liquid-liquid or liquid-solid phase extraction. So far, there are only a few FIA references for the development of automatic methods for the analysis of oils,[72–75] because of the difficulty involved in dealing with this kind of sample. The complex sample matrices involved lead to early determination of the membrane in traditional flow injection liquid-liquid extraction manifolds, including a membrane separator. The flow-reversal injection liquid-liquid extraction approach,

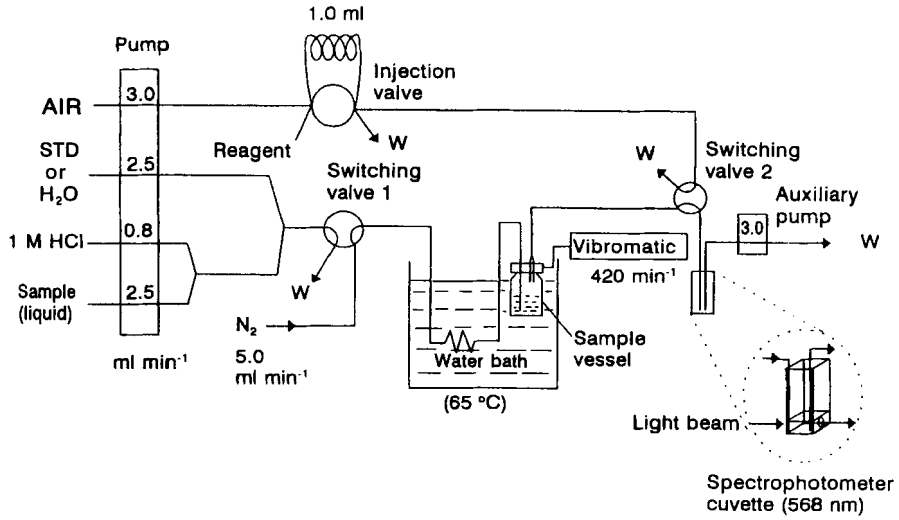

FIGURE 6. FI manifold involving *in situ* concentration and continuous monitoring/gas extraction for sulfur dioxide determination in solid and liquid samples.

which requires none of the typical units for this continuous separation technique, is more versatile usable with a wide range of complex samples.[76] This technique has been exploited by Mesa et al. for the direct treatment of oil samples by using a mode of reversed liquid-liquid extraction (the analyte is extracted into the aqueous phase) for the determination of polyphenols over the range of 100 to 900 g ml^{-1} in olive oils after reaction with Folin-Ciocalteau reagent in the flow manifold.[77]

Recently, we developed an automatic method for free fatty acid determinations in olive oils based on the flow-reversal injection liquid-liquid extraction approach.[78] The manifold used, shown in Figure 7, is based on the extraction of the reaction product

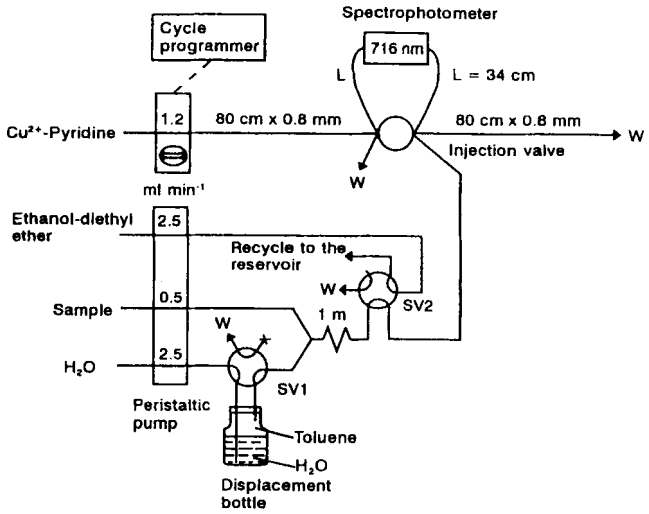

FIGURE 7. Schematic diagram of the flow reversal injection system for liquid-liquid extraction in the determination of total free fatty acids.

formed (copper soap) into the organic sample phase that fill the loop of an injection valve that accommodates the flow-cell (detector). A cycle programmer controls the iterative reversal of the flow direction as many times as required in order to facilitate the reaction between the Cu^{2+}-pyridine and FFA at the organic-aqueous interface in the reaction coil. The accumulation and dispersion of the Cu^{2+}-FFA complex in the organic phase located upstream the detector is continuously monitored by a photometer, which provides a multipeak recording for the process. The preset duration of each cycle prevents the aqueous phase from reaching the flow-cell, thus avoiding parasitic signals during measurements. In this way, total FFA was determined over the range 0.06 to 9% in olive oils at a sampling frequency of 12 h^{-1}.

IX. OTHER APPROACHES FOR THE ANALYSIS OF SAMPLES WITH COMPLEX MATRICES

Solid-phase extraction (SPE) is a simple, selective alternative to liquid-liquid extraction and related techniques. Complex sample matrices can be eliminated (cleaned-up), and the use of organic solvents is avoided or reduced. Also, SPE is fast and can be automated easily. It is applicable to both metal ions and organic species in liquid, gaseous, and "dirty" samples.

The general mechanism of solid-phase (liquid-solid) extraction involves the stationary phase immobilized on a solid support. There are many sorbent materials available for SPE; silica gels bonded with a variety of functional groups (alkyl-, phenyl-, cyano-, and diol-) are commonly employed to provide specific interaction with the analytes. With an appropriate sorbent and by judicious choice of pH and eluting solvents, selective retention of the compounds of interest is feasible with minimal retention of interfering substances.

SPE has been widely adopted as a sample clean-up technique for preparing samples for HPLC analysis. SPE and FI have been used jointly mainly for the on-line preconcentration of trace elements and differentiations of species.[79] Nevertheless, SPE has also proven applicable to organic species. Thus, a method for automatic determination of bitterness in olive oils using a FI-sorbent (C_{18} bonded silica) extraction system was developed by Garcia-Mesa et al.[80] Recently, FI-SPE was applied to the selective determination of phenols at subnanogram-per-milliliter levels in waters and soil leachates.[81] The SPE procedure included in the manifold effectively replaced the traditional distillation or extraction step usually required to remove aromatic amines and related compounds in the standard method for total phenols. SPE can also be implemented in FIA by placing the solid support in the flow-cell of a nondestructive optical detector and integrating retention and detection of an analyte, with or without a prior or *in situ* derivatization reaction (flow-through sensors).[82]

Supercritical fluid extraction is a very efficient technique for the extraction of organic chemicals from different matrices such as solid food, soil, and sediment samples. The SFE method decreases solvent use and extraction time compared with the traditional extraction method. It is believed that SFE will be an effective option or even replace many current extraction procedures.[83] The combination of SFE and continuous flow techniques for the analysis of solid foodstuffs was recently demonstrated.[84] However, SFE does not offer an immediate solution to all sample preparation problems. Moreover, the high purchase and maintenance costs of SFE are major hindrinces to its adoption as routine laboratory equipment.

Until recently, most standard methods for the determination of volatile analytes such as ammonia, hydrogen cyanide, phenol, hydrogen sulfide, sulphur dioxide, etc. still called for predigestion and predistillation of the sample to recover complex forms of an analyte and then to remove the analyte from a pontentially interferring matrix. Flow in-

jection analyzers employing a flow stream digestion and distillation device in which the sample to be analyzed is digested and distilled continuously are a front-end extension of the method. However, the several shortcomings of this approach (e.g., long start-up and line-out periods, variable recoveries resulting from the short residence times required, large volumes, and carry-over problems) have led continuous distillation procedures to decline. In fact, a few new approaches have been reported in recent years, prominent among which are the determination of ammonium and nitrate nitrogen in a variety of digest extracts and soils,[85] and that of phenols in waste waters.[86]

The incorporation of a robotic station or an intelligent autosampler has proven an excellent approach to automating basic operations such as weighing, grinding, sieving, homogenization, sample transport, etc. These tasks are almost inaccessible to other types of autoanalyzers. On the other hand, these approaches may be very slow in performing such simple operations as reagent addition, matrix separation and preconcentration, derivatization, etc. Moreover, the complexity of an FI manifold can be dramatically reduced if the sample to be introduced is pretreated. Thus, the incorporation of a robotic station in an FI manifold appears to be a promising way of performing difficult tasks that evade other automatic approaches. The applicability of robots coupled to FI systems has been demonstrated with the automated determination of total vitamin C in foods,[87] wear metals in used lubricating oils,[88] and total polyphenols in virgin olive oils.[89] The greatest assets here are enhanced throughput and simplicity and decreased costs (some sophiscated modules of robotic stations can be replaced with straightforward FI equipment).[90]

CONCLUSION

Direct processing and analysis of solid and other troublesome samples by use of automatic flow systems and sampling interfaces has resulted in significantly improved analytical performance, particularly with respect to the overall analysis time, traditionally considered critical in process monitoring and other fast assays. However, available reliable sampling interfaces for this type of sample are scant and limited in performance; also, their applications span a few areas. The development of a variety of flexible sampling interfaces for use in monitoring in various long-term industrial processes is therefore a desirable goal.

ACKNOWLEDGMENT

Financial support provided by the CICyT (PB95–0977) is acknowledged.

REFERENCES

1. Valcarcel, M., Luque de Castro, M. D., and Tena, M. T., *Anal. Proc.*, **1993,** *30,* 276.
2. Clark, G. D., Whitman, D. A., Christian, G. D., and Ruzicka, J., *Crit. Rev. Anal. Chem.*, **1990,** *21,* 357.
3. Valcarcel, M. and Luque de Castro, M. D., *Fresenius J. Anal. Chem.*, **1990,** *337,* 662.
4. Bergamin, H. F., Krug, F. J., Zagatto, E. G. A., Arruda, E. C., and Coutinho, C. A., *Anal. Chim. Acta,* **1986,** *190,* 177.
5. Bergamin, H. F., Krug, F. J., Reis, B. F., Nobrega, J. A., Mesquita, M., and Souza, J. G., *Anal. Chim. Acta,* **1988,** *214,* 397.
6. Yuan, D., Wang, X., Yang, P., and Hung, B., *Anal. Chim. Acta,* **1991,** *243,* 65.
7. Flock, J. and Ohls, K., *Fresenius' Z. Anal. Chem.*, **1988,** *331,* 408.
8. Bergamin, H. F., Krug, F. J., Nobrega, J. A., Oliveira, P. V., Reis, B. F., and Gine, M. F., *Anal. Chim. Acta,* **1991,** *245,* 211.
9. Yuan, D., Wang, X., Yang, P., and Hang, B., *Anal. Chim. Acta,* **1991,** *251,* 187.
10. Coutinho, C. A., Arruda, E. C., Mundim, M. J., and Pacheco de Araujo, W., *Steel Res.*, **1990,** *61,* 201.

11. Chen, D., Lazaro, F., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chim. Acta,* **1989,** *226,* 221.

12. Lazaro, F., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chim. Acta,* **1991,** *242,* 283.

13. Tyson, J. F., Bysouth, S. R., Graszcyk, E. A., and Debrah, E., *Anal. Chim. Acta,* **1992,** *261,* 75.

14. Zhi, Z., Rios, A., and Valcarcel, M., *Anal. Chem. Acta,* **1994,** *298,* 287.

15. Lofty, D., *Lab. Equip. Dig.,* **1992,** *30,* 13.

16. Tsalev, D. L., Sperling, M., and Welz, B., *Analyst,* **1992,** *117,* 1729 and 1735.

17. Guo, T. and Baasner, J., *Talanta,* **1993,** *40,* 1927.

18. Morales-Rubio, A., Mena, M. L., and Mclead, C. W., *Anal. Chim. Acta,* **1995,** *308,* 364.

19. Welz, B., Tsalev, D. T., and Sperling, M., *Anal. Chim, Acta,* **1992,** *262,* 91.

20. Cabrera, C., Madrid, Y., and Camara, C., *J. Anal. Atom. Spectrom.,* **1994,** *9,* 1423.

21. Lopez-Gonzalvez, M. A., Gomez, M. M., Camara, C., and Palacios, M. A., *J. Anal. Atom. Spectrom.,* **1994,** *9,* 291.

22. Burguera, M. and Burguera, J. L., *Anal. Chim. Acta,* **1986,** *179,* 352.

23. Haswell, S. J. and Barclay, D., *Analyst,* **1992,** *117,* 117.

24. Burguera, M. and Burguera, J. L., *Anal. Chim. Acta,* **1988,** *214,* 421.

25. Carbonell, V., de la Guardia, M., Salvador, A., Burguera, J. L., and Burguera, M., *Anal. Chim. Acta,* **1990,** *238,* 417.

26. Carbonell, V., Morales-Rubio, A., Salvador, A., and de la Guardia, M., *J. Anal. Atom. Spectrom.,* **1992,** *17,* 1085.

27. Karanassios, V., Ci, F. H., and Salin, E. D., *J. Anal. Atom. Spectrom.,* **1991,** *6,* 457.

28. Gluodenis, Jr., T. J. and Tyson, J. F., *J. Anal. Atom. Spectrom.,* **1993,** *8,* 697.

29. Burguera, J. L. and Burguera, M., *J. Anal. Atom. Spectrom.,* **1993,** *8,* 235.

30. Burguera, J. L., Burguera, M., Carrero, P., Rivas, C., Gallignani, M., and Brunetto, M. R., *Anal. Chim. Acta,* **1995,** *308,* 349.

31. Burguera, M. and Burguera, J. L., *Lab. Robot. Autom.,* **1993,** *5,* 277.

32. Hinkamp, S. and Schwedt, G., *Anal. Chim. Acta,* **1990,** *236,* 345.

33. Williams, K. E., Haswell, S. J., Barclay, D. A., and Preston, G., *Analyst,* **1993,** *118,* 245.

34. Benson, R. L., McKelvie, I. D., Hart, B. T., and Hamilton, I. C., *Anal. Chim. Acta,* **1994,** *291,* 233.

35. Balconi, M. L. and Borgarello, M., *Anal. Chim. Acta,* **1992,** *261,* 295.

36. Schmitt, A., Buttle, L., Uglow, R., Williams, K., and Haswell, S., *Anal. Chim. Acta,* **1993,** *284,* 249.

37. Ogbomo, I., Prinzing, V., and Schmidt, H. L., *J. Biotechnol.,* **1990,** *14,* 63.

38. Schmidt, W. J., Meyer, H. D., Schhgerl, K., Kuhlmann, W., and Bellgardt, K. H., *Anal. Chim. Acta,* **1984,** *163,* 101.

39. Moller, J., Hiddessen, R., Niehoff, J., and Schhgerl, K., *Anal. Chim. Acta,* **1986,** *190,* 195.

40. Neihoff, J., Moller, J., Hiddessen, R., and Schhgerl, K., *Anal. Chim. Acta,* **1986,** *190,* 205.

41. Christensen, C. H., Nielsen, J., and Villadsen, J., *Anal. Chim. Acta,* **1991,** *249,* 123.

42. Schhgerl, K., Brandes, L., Wu, X., Bode, J., Ree, J. I., Brandt, J., and Hitzmann, B., *Anal. Chim. Acta,* **1993,** *279,* 3.

43. Carlsen, M., Johansen, C., Min, R. W., Nielsen, J., Meier, H., and Lantreibecq, F., *Anal. Chim. Acta,* **1993,** *279,* 51.

44. Mandenius, C. F., Danielsson, B., and Mattason, B., *Anal. Chim. Acta,* **1984,** *163,* 135.

45. Gibson, T. D. and Woodward, J. R., *Anal. Chim. Acta,* **1988,** *213,* 61.

46. Marko-Varga, G., Buttler, J., Gorton, L., and Gronsterwall, C., *Chromatographia,* **1993,** *35,* 285.

47. Kroner, K. H. and Kula, M. R., *Anal. Chim. Acta,* **1984,** *163,* 3.

48. Nalbach, U., Schiemenz, H., Stamm, W. W., Hummel, W., and Kula, M. R., *Anal. Chim. Acta,* **1988,** *213,* 55.

49. Recktenwald, A., Kroner, K. H., and Kula, M. R., *Enzyme Microb. Technol.,* **1985,** *164,* 607.

50. Hustedt, H., Kroner, K. H., and Kula, M. R., *Biotechnol. Forum.,* **1985,** *2,* Section 2.

51. Henry, J. D., Jr. and Allred, R. C., *Dev. Ind. Microbiol.,* **1972,** *13,* 177.

52. Garn, M., Gisin, M., Thommmen, C., and Cevey, P., *Biotechnol. Bioeng.,* **1989,** *34,* 423.

53. Chung, S., Wen, X., Viholm, K., de Beng, M., Christian, G., and Ruzicka, J., *Anal. Chim. Acta,* **1991,** *249,* 77.

54. Freitag, R., Fenge, L., Scheper, T., Schhgerl, K., Spreinat, A., Antranikian, G., and Rraune, E., *Anal. Chim. Acta,* **1991,** *249,* 113.

55. Forman, L. W., Thomas, B. D., and Jacobson, F. S., *Anal. Chim. Acta,* **1991,** *249,* 101.

56. Valero, F., Lafuente, J., Poch, M., Sola, C., Araujo, A. N., and Lima, J. L. C., *Biotechnol. Bioeng.,* **1990,** *36,* 647.

57. Buttler, T., Gorton, L., and Merko-Varga, G., *Anal. Chim. Acta,* **1993,** *279,* 27.

58. Buttler, T. A., Johansson, K. A. J., Gorton, L. G. O., and Marko-Varga, G. A., *Anal. Chem.,* **1993,** *65,* 2628.

59. Hayward, M. J., Kotiaho, T., Lister, A. K., Cooks, R. G., Austin, G. D., Narayan, R., and Tsao, G. T., *Anal. Chem.,* **1990,** *62,* 1798.

60. Pedeersen, K. M., Kummel, M., and Soeberg, H., *Anal. Chim. Acta,* **1990,** *238,* 191.

61. Bayer, Th., Herold, Th., Hiddessen, R., and Schhgerl, K., *Anal. Chim. Acta,* **1986,** *190,* 213.

62. van de Merbel, N. C., Lingeman, H., and Brinkman, U. A. Th., *Anal. Chim. Acta,* **1993,** *279,* 39.

63. van de Merbel, N. C., Kool, I. M., Lingeman, H., Brinkman, U. A. Th., Kolhorn, A., and Rijke, L. C., *Chromatographia,* **1992,** *33,* 525.

64. Saltzman, R. S., *Anal. Chim. Acta,* **1986,** *190,* 227.

65. Prinzing, V., Ogbomo, I., Lehn, C., and Schmidt, H. L., *Sen. Actuators, B,* **1990,** *B1,* 542.

66. Ogbomo, I., Steffl, A., Schumann, W., Prinzing, U., and Schmidt, H. L., *J. Biotechnol.,* **1993,** *31,* 317.

67. Mattos, I. L. and Luque de Castro, M.D., Talanta, 1995,

68. Papaefstathiou, I., Tena, M. D., and Luque de Castro, M. D., *Anal. Chim. Acta,* **1995,** *308,* 246.

69. Usthche, H. and Histi, G., *J. Membr. Sci.,* **1981,** *8,* 105.

70. Zhi, Z., Rios, A., and Valcarcel, M., *Anal. Chem.,* **1995,** *67,* 871.

71. Zhi, Z., Rios, A., and Valcarcel, M., *Analyst,* **1995,** *120* (in press).

72. GarcRa-Mesa, J. A., Luque de Castro, M. D., and Valcarcel, M., *Analyst,* **1993,** *118,* 891.

73. Garcia-Mesa, J. A., Luque de Castro, M. D., and Valcarcel, M., *Lab. Robot. Autom.,* **1993,** *5,* 29.

74. Canham, J. S. and Pacey, G. E., *J. Am. Oil Chem. Soc.,* **1987,** *64,* 1004.

75. Linares, P., Luque de Castro, M. D., and Valcacel, M., *Anal. Chim. Acta,* **1989,** *225,* 431.

76. CaZete, F., Rios, A., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chem.,* **1988,** *60,* 2354.

77. Mesa, J. A. G., Linares, P., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chim. Acta,* **1990,** *235,* 441.

78. Zhi, Z., Rios, A., and Valcarcel, M., *Anal. Chim. Acta* (in press).

79. Welz, B., *Microchem. J.,* **1992,** *45,* 163.

80. Mesa, J. A. G., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chim. Acta,* **1992,** *261,* 367.

81. Zhi, Z., Rios, A., and Valcarcel, M., *Analyst* (in press).

82. Valcarcel, M. and Luque de Castro, M. D., *Analyst,* **1993,** *118,* 593.

83. Hedrick, J. L., Mulcahey, L. J., and Taylor, L. T., *Microchim. Acta,* **1992,** *108,* 115.

84. Tena, M. T., Luque de Castro, M. D., and Valcacel, M., *Anal. Chem.,* **1995,** *67,* 1178.

85. McLeod, S., *Anal. Chim. Acta,* **1992,** *266,* 107; and 113.

86. Kwade, C., Voigtlander, R., and Cammann, K., *Fresenius J. Anal. Chem.,* **1992,** *342,* 426.

87. Vanderslice, J. T. and Higgs, D. T., *J. Micronutr. Anal.,* **1989,** *6,* 109.

88. Granchi, M. P., Biggerstaff, J. A., Hillard, L. J., and Grey, P., *Spectrochim. Acta,* **1987,** *42B,* 69.

89. Garcia-Mesa, J. A., Luque de Castro, M. D., and Valcarcel, M., *Anal. Chem.,* **1993,** *65,* 3540.

90. Garcia-Mesa, J. A., Luque de Castro, M. D., and Valcarcel, M., *J. Flow Inject. Anal.,* **1990,** *10,* 262.