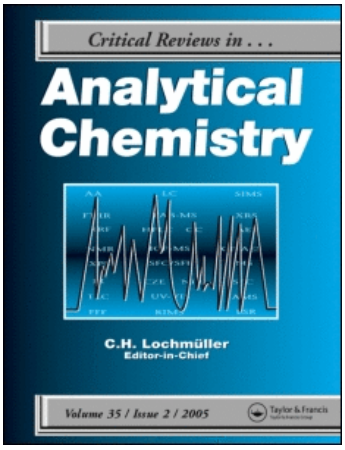

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Liquid-Liquid Extraction Flow Injection Analysis

Vlastimil Kubá[a]
[a] Department of Analytical Chemistry, Masaryk University, Brno, Czechoslovakia

To cite this Article Kubá, Vlastimil(1991) 'Liquid-Liquid Extraction Flow Injection Analysis', Critical Reviews in Analytical Chemistry, 22: 6, 477 — 557
To link to this Article: DOI: 10.1080/10408349108051643
URL: http://dx.doi.org/10.1080/10408349108051643

Critical Reviews in Analytical Chemistry, 22(6):477–557 (1991)

Liquid-Liquid Extraction Flow Injection Analysis

Vlastimil Kubáň

Department of Analytical Chemistry, Masaryk University, CS-61137 Brno, Czechoslovakia

KEY WORDS: liquid-liquid extraction, flow injection analysis, continuous flow analysis, instrumentation, theory, application in chemical analysis.

I. INTRODUCTION

In spite of the great improvement in selectivity and sensitivity of modern analytical instrumentation, the classical preconcentration and separation techniques, such as liquid-liquid extraction, ion-exchange chromatography, precipitation, etc., are still widely used to improve analytical measurements, especially in connection with trace and ultratrace analysis. Liquid-liquid extraction is among the most frequently used conventional methods for sample pretreatment preceding measurements of the analyte concentration. It offers a potential for selectivity and sensitivity improvement in a large number of determinations.

By use of partitioning between an organic and an aqueous phase, an analyte can be separated from an interfering matrix, or interfering matrix components can be removed from the sample in order to increase the selectivity of the determination of the analyte. Furthermore, an analyte can be concentrated by extracting it from a large volume of an aqueous sample into a small volume of an organic phase, thus improving the detection limits.

Manual liquid-liquid extraction procedures are usually very tedious, involving a large consumption of solvents and chemicals, and are subject to potential contaminants from the atmosphere and chemical glassware. In addition, the conventional liquid-liquid extraction process requires manipulation with significant volumes of hazardous and/or toxic organic solvents. The handling of a large number of samples and the introduction of bias and errors associated with the various requisite manipulations must be also taken into account.

The culmination of these factors is that sample preparation, particularly for a very complex matrix, is often the time-limiting step for the determination. Hence, there is an increasing need for mechanized or automated sample preparation by either direct or indirect coupling of a liquid-liquid extraction system to the analytical instrument since this seems to be the best way to overcome the aforementioned disadvantages.

The rapid development of automated liquid-liquid extraction is perhaps due to the broad use and importance of the liquid-liquid extraction process. One of the most effective ways to shorten the duration of this process has been the construction of dynamic "on-line" liquid-liquid extraction systems applying the principles of continuous flow analysis. A centrifugal and air-segmented flow analyzer first incorporated liquid-liquid extraction concepts to automate the separation technique as a logical step. Subsequently, equipment for flow injection analysis (FIA) also has been used to modify this separa-

1040-8347/91/$.50

tion technique since liquid-liquid extraction in FIA is rapid, with little interference from emulsions, and since the absence of air bubbles in FIA facilitates separation of the two immiscible phases used in the extraction process.

Liquid-liquid extraction has been semiautomated or automated by several workers, principally using air-segmented flow systems. The earlier studies of the utility of liquid-liquid extraction in FIA were simultaneously carried out by Karlberg and Bergamin and their co-workers[1,3] in 1978. Since then, a large number of analytical procedures have been devised, in combination with many common detection systems.[3–147] Currently, approximately 30% of all preconcentration methods in FIA employ liquid-liquid extraction procedures. The advantages and difficulties of this very progressive methodology, including some analytical applications, have been discussed in several monographs[94,113,125,126] and a number of comprehensive reviews.[7,9,14,21,26,38,59,61,63,68–71,76,82,87,95,98–100,118,141–143] Hence, the feasibility of the methodology has been demonstrated many times.

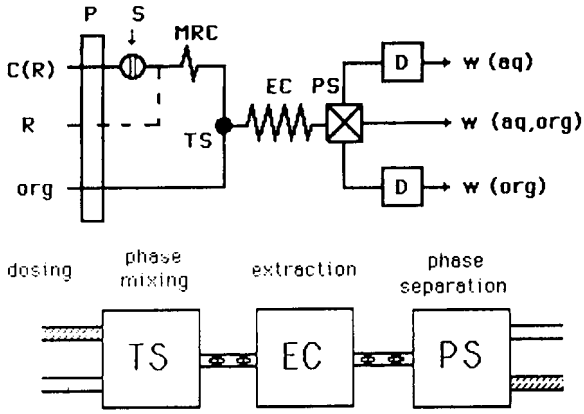

FIGURE 1. Liquid-liquid extraction FIA manifold. C: carrier stream, R: reagent stream, org: organic phase, aq: aqueous phase, P: peristaltic pump, S: sample solution, V: injection valve, MRC: mixing and reaction coil, TS: phase segmenter, EC: extraction coil, PS: phase separator, RC: restriction coil, D: flow through detector, DB: displacement bottle, w (aq), w (org), and w (aq,org): waste for aqueous, organic, and nonseparated segmented flow with possible emulsion.

II. PRINCIPLES OF LIQUID-LIQUID EXTRACTION FIA

Regardless of the way in which the liquid-liquid extraction step is performed — via a manual batch procedure or by use of some kind of mechanized or automated system — three basic operations are usually necessary: (1) the organic and aqueous immiscible phases must be dispersed in defined volumes; (2) the phases must be brought into intensive contact with each other for the extraction to take place; and (3) the phases must be physically separated from each other after the extraction event in order to make the chemical separation meaningful.

In classical FIA adaptation (see Figure 1), aqueous sample solutions are usually introduced continuously or in definitive volumes (<100 to 200 μl) into a continuous aqueous stream. This stream serves as both a reagent and a carrier stream in the simplest single-line FIA version. The aqueous sample solution can also be merged and mixed with another, separate aqueous stream containing an organic analytical reagent, spectral buffer, etc. In this way, appropriate chemical reactions as well as solution homogenization take place in a reaction-and-mixing coil before entering a phase segmenter.

The resultant aqueous stream (ideally pulseless) of an extractable component is then segmented with an organic immiscible solvent stream at the segmenter mixing point, where more or less reproducible droplets of one phase in the other are formed. The size of the droplets is regulated by a combination of gravity, density, and interfacial and hydrodynamic forces. The geometry of the inner capillary system of the segmenter and the quality of the surface also play a role.

The droplets move into the outflow channel after having been formed and tend to minimize their interfacial area with the other phase and to maximize the contact surface area with the wall material of the outflow tubing, thereby wetting it. This process results in the formation of independent, more or less regular segments of both phases in a single moving stream which then enters the extraction coil.

Depending on the outflow channel material and on the material of the extraction capillary coil, the solvent that has the greater affinity for

the tubing material coats the tubing walls with a very thin film, representing a relatively stationary phase. The film of one solvent surrounds the deformed spherical, ellipsoidal, or tubular segments of the other solvent. A film of the organic phase surrounds the aqueous segments in the PTFE capillaries, while the segments of organic phase are surrounded by the aqueous phase film in the glass or metal tubing. The former case is more frequently used in liquid-liquid extraction FIA; the latter plays an important role in the reextraction step of two-step liquid-liquid extraction FIA.

The extraction process occurs principally in the extraction coil, and to a lesser extent in the segmenter and the phase separator. During extraction the extractable sample components are transported from a relatively homogeneous aqueous solution of higher analyte concentration into segments of the immiscible organic phase through the segment interface. The interfacial area available for the extraction consists of menisci between the two phases and a film surrounding the segments. An analyte diffuses to the interface between the two phases, and extraction equilibrium is reached, the attainment of which depends on a number of factors which are discussed later.

Naturally, the degree of extraction is a function of the residence time of the analyte in the extraction coil, which is affected by the coil length and the flow rate. Extraction efficiency in the extraction coil is usually high and often virtually complete in several seconds.

The influence of the flow rate and system manifold parameters on the extraction efficiency depends on the kinetics of the extraction process and the mass transport processes involved. The extraction rate increases with decreasing segment size and decreasing inner diameter of the extraction tube; hence, the use of a narrower extraction tube and small/short segments enhances extraction. The choice of the material used for the extraction coil indirectly influences the extraction efficiency; the efficiency is high for stripping an analyte from the aqueous to the organic phase for PTFE coils, while it is very low for glass coils. In contrast, the efficiency of analyte stripping from organic to aqueous phase is higher for glass coils than for PTFE coils.

The segments of the aqueous and organic phases are subsequently separated in a phase separator into individual streams. The extractable analyte in the receiving phase is determined using a flow-through detection system. The analytical signals are treated in a conventional manner, with the analyte concentration calculated from the peak height, the peak width, or the peak area. In principle, the other separated phase is directed to waste through a restriction coil (which also controls the separation efficiency of the phase separator).

The principal operations of liquid-liquid extraction FIA also characterize the three basic components of liquid-liquid extraction FIA systems (see Figure 1): (1) a segmenter (or confluence point) for mixing the organic and aqueous phase streams, providing alternate, regular segments of both immiscible solvents in a uniform segmented flow in a single channel; (2) an extraction coil, in which the solute is transferred from one phase to the other; and (3) a phase separator, the mission of which is to continuously and quantitatively separate the segmented outlet stream of the phases.

A wide variety of principles applicable to liquid-liquid extraction exist, such as that performed in liquid-liquid extraction FIA. Consequently, various types of system manifold arrangements of different complexity are described in the literature. These arrangements or operational modes include:

1. *Without phase separation:* this is the simplest mode, the aqueous solution of the sample being injected into a single-channel manifold together with the organic phase stream (or an extractable substance in the organic phase injected into the organic or aqueous phase stream)
2. *Single extraction:* the segmentation system is located prior to or following the injection valve
3. *Multiple extraction:* in which the separation process is repeated several times by using the same or a different extractant in successive steps
4. *Back extraction:* a multistage extraction mode in which the aqueous sample is first

extracted into an organic medium and then back-extracted into another aqueous phase, where measurements are performed

5. *Special techniques:* such as closed-loop systems, systems with nonsegmented stream, systems without a phase separation unit, or systems with liquid separation membranes

Manifolds of different complexity have been described for common or special liquid-liquid extraction FIA methods, most of which are depicted in Figures 2 to 11. The simplest manifold type is the single-line manifold without phase separation, in which the sample solution, present in an aliquot of the aqueous or organic phase, is injected into the continuous flow of the organic phase.[4,16,49] An aqueous phase carrier stream is seldom used for transport of the sample in the organic phase. Usually, no chemical reaction occurs (Figure 2A), or it can occur only in the aqueous segment (Figure 2B), or it can occur in a separate step.[48] Flame atomic absorption spectrometry (AAS), fluorimetry, or fast scanning "on-tube" spectrophotometry are generally used as the most suitable detection systems.

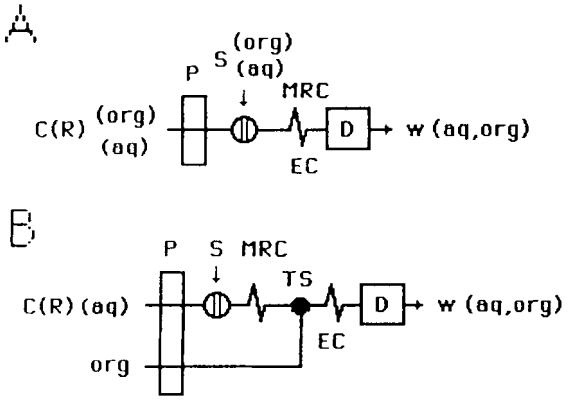

FIGURE 2. FIE manifold with injection of the sample in aqueous or organic phase into the organic (aqueous) phase carrier stream without phase separation and without (A) or with the phase segmenter and chemical reaction (B).

A simple version of an unsegmented system (Figure 3A) is one in which a liquid membrane separator or "dialysis module" allows mass transfer through a hydrophobic, porous membrane from

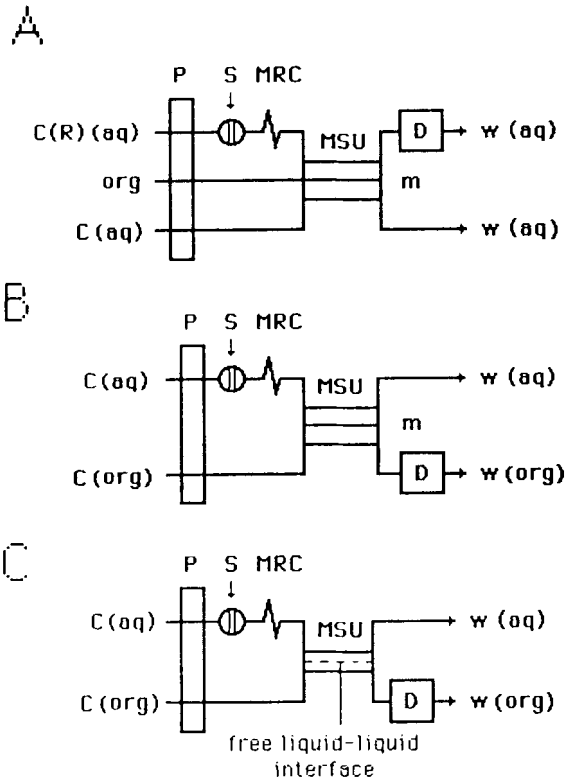

FIGURE 3. FIE manifolds with unsegmented streams and the "dialysis-form" membrane separation units (MSU) with a continuously wetted liquid (A) membrane (m), a microporous or nonporous membrane (B), and with an open liquid/liquid interface (C).

aqueous sample solution, continuously passing the module, into the organic (or aqueous) recipient phase (stagnant or flowing) stream.[65,74,77] The membrane can be untreated or pretreated with an organic solvent or with an organic analytical reagent dissolved in the suitable organic phase (Figures 3A and 3B). A silicone rubber nonporous membrane has also been used for the transfer of organic analytes into a suitable organic solvent (e.g., hexane, methanol).[158]

In the original manifold of Karlberg[1] and Bergamin[3] (Figure 4A), a defined volume of the sample is injected into a carrier or reagent stream which is then segmented with an immiscible solvent. Analyte extraction occurs in an extraction coil of narrow-bore PTFE tubing. After separation, the organic (or aqueous) phase passes through a detector (usually photometric) system. A manifold with the segmentation system prior to the injection valve (Figure 4B) minimizes sec-

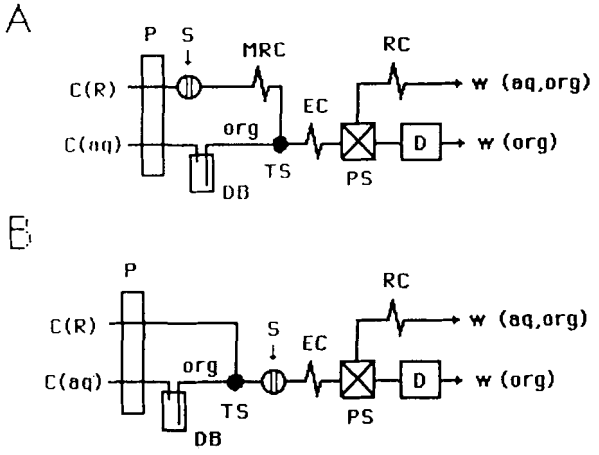

FIGURE 4. Classical FIE manifolds with an injection prior to (A) or after the phase segmenter (B).

ondary sample dispersion during its transport through the reaction and mixing coil and through the segmenter.[106] This system could be used with or without phase separation.

An off-line system (Figure 5) also is often used in which the extractable component is collected in plastic containers or in a graduated vessel and subsequently diluted to a known volume. An aliquot of the diluted (or undiluted) sample is then injected into another system (e.g., a liquid chromatograph, an AAS [electrothermal or with flame], etc.). The sample collection procedure is believed to average any fluctuation in concentration.[40]

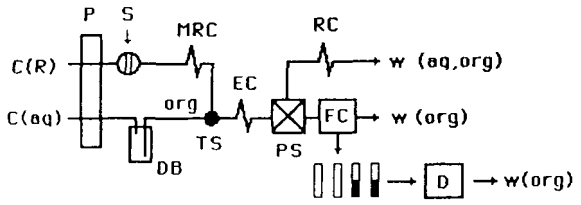

FIGURE 5. A FIE manifold with the collection of the sample zones by a fraction collector (FC) and with the subsequent off-line determination by AAS, GC, HPLC, etc.

The manifold with two phase separators in series has been used in which one phase separator directs the separated organic phase into the detector flow-through cell, while the aqueous phase with the traces of the organic phase passes the second phase separator (having a hydrophilic membrane). The separated aqueous stream flows through the second detector flow cell (Figure 6A) to detect water-soluble analyte species. Single or double detector systems can be used for the simultaneous monitoring of both phases.[22,31]

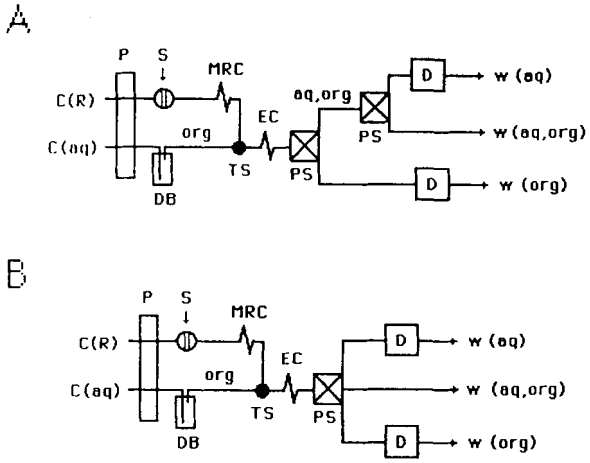

FIGURE 6. FIE manifolds for the simultaneous monitoring of both phases separated by two membrane phase separators in series (A) or a single dual-phase separator with lipophilic and hydrophilic membranes (B) and two independent optical detectors. aq, org: partly separated segmented stream.

A similar manifold utilizes a dual membrane separator[51] that allows partitioning of the segmented flow into three individual channels, two of which are sensed. Simultaneous detection of extractable and nonextractable species in the aqueous and organic phases is performed using a single dual-wavelength detector or two parallel detectors, respectively (Figure 6B).

The use of two autonomous systems (Figure 7) is very popular with flame AAS, where widely different flow rates are required for extraction and nebulizing. The liquid-liquid extraction FIA system is operated at normal flow rates, and the organic phase is separated and a portion is periodically injected into an independent carrier stream which is continuously pumped into the spectrometer acting as the detector. Advantages of this system are that it is closed, so that atmospheric contamination is excluded during most

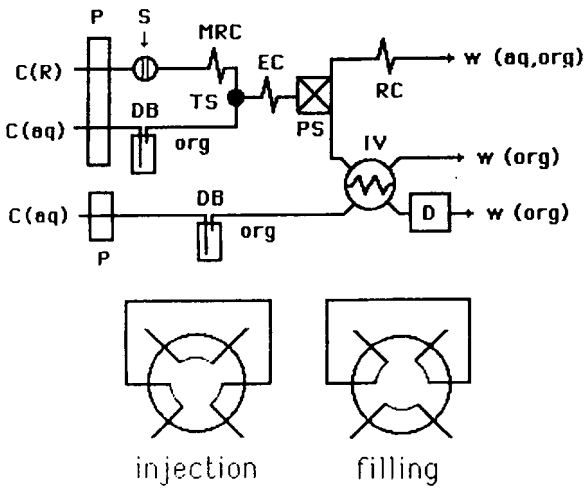

FIGURE 7. An autonomous FIE and a measuring system with common injection valve (IV) after the phase separator; filling and injecting positions of the injection valve (IV) are depicted in the bottom.

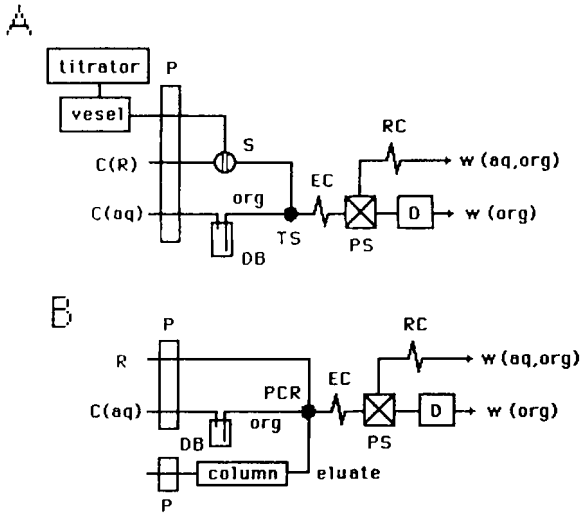

FIGURE 8. FIE manifolds for continuous monitoring of the chemical equilibria or processes (A) and a postcolumn segmented reactor (B) for HPLC or IC with simultaneous addition of the derivatizing reagent (R) and organic phase for segmentation of an effluent from a chromatographic column or from a packed-reaction column (extraction can or cannot take place).

of the procedure, fewer surfaces need cleaning, and the method can be automated.[28]

Currently, the phase separator can be placed in either its original position, or prior to the second injection system, in such a manner that the separation process results in a continuous stream of the organic phase containing the analyte, which fills the loop of the injection valve.

The system for the study of chemical equilibria[12] in two phase systems and of the experimental conditions for analytical procedure using a continual titration technique is useful when a great deal of information on the experimental condition is needed (Figure 8A).

Solvent segmentation was also introduced into liquid chromatography to prevent dispersion in postcolumn derivatization, with or without extraction involved (see Figure 8B), or the same manifold can be used for preseparation or preconcentration of ionic species on the ion exchange column. The reaction time between the separated constituents in the sample and the added reagents could then be increased without significant band broadening if the effluent stream was segmented with an organic solvent.

The classical air-segmented extraction system approach, using glass as the material for the extraction coil, is shown in Figure 9. If a narrow PTFE tube is used, a counter pressure is easily built up because of the compressibility of the air

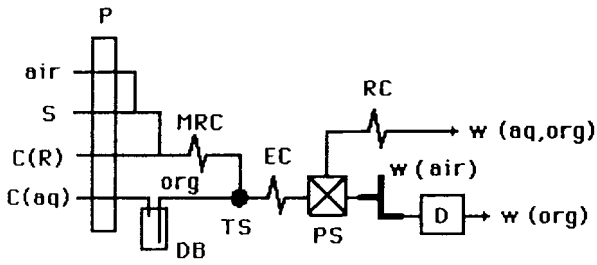

FIGURE 9. An air-segmented FIE manifold with a debubbler prior to the flow-through detector. w(air): an outflow channel from the debubbler.

segments. The secondary reagent addition after phase separation is also possible.[148–150]

A double-step extraction (back-extraction) is preferable for the work with ETA AAS because the final extract is an aqueous solution (Figure 10A). A high enrichment factor can be achieved because of the low solubility of organic solvents in water.[36,40] The first extraction takes place in a Teflon tube wound in a coil with a diameter of several centimeters.

The organic phase containing metal chelates is separated in the phase separator and continues to the second segmenter where it is interspaced

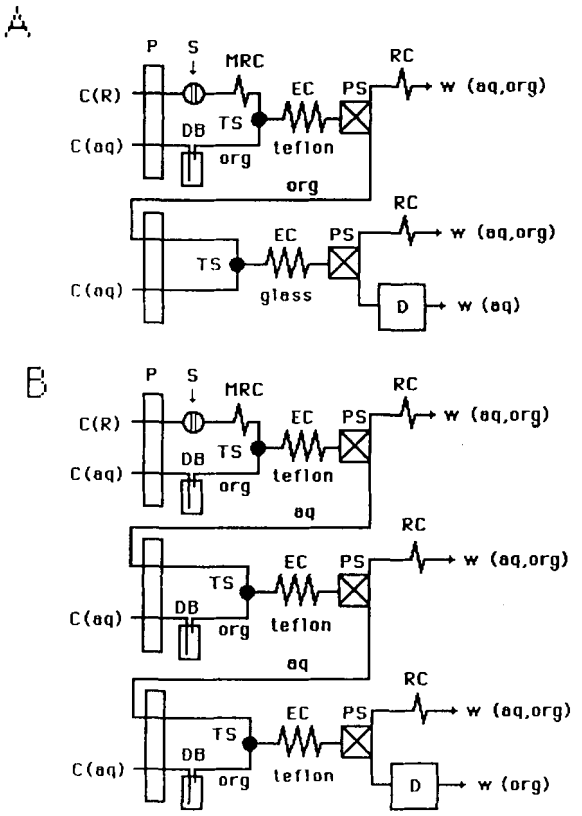

FIGURE 10. FIE manifolds for liquid-liquid extraction and back-extraction (A) from aqueous into organic phase (in a Teflon extraction coil) and vice versa (in a glass capillary, aqueous phase is used for sensing) and for multiple extraction (B) with the same cleanup procedure (first step) and subsequent extraction with the same or different solvents and resampling the aqueous phase (second step) and organic phase used for sensing.

with an aqueous solution containing striping reagent (forming stronger complexes with chelating reagent and displacing analyte from chelates). The back-extraction takes place in the second extraction coil (usually a glass or metal capillary) but now goes into the aqueous phase. The organic phase is discarded while the aqueous is collected in plastic cups for subsequent measurement in ETA AAS. The steady-state method of the continuous pumping of the analyte into the system is more convenient and reliable and it is actually faster than the injection technique because of the added dispersion in the sample loop with the latter technique. A system similar to this can also be used for sequential multistep separation process[20,23] with identical or different solvents (Figure 10B).

The sample collection procedure averages any fluctuation in concentration if an off-line configuration is used. Tailing occurs mainly when high concentrations of analyte are present due to the adsorption of the species on the tube walls. The second extraction step adds little to the enrichment but simplifies the ETA AAS measurements significantly.[40,126]

The extraction FIA system with closed loop (Figure 11) is based on the continuous circulation of the organic phase in the closed loop connected to the fresh aqueous stream, which continuously enters the extraction coil by a four-way segmenter and exits to the waste stream after separation in the membrane separator. A higher enrichment factor depends on the residence time of the organic solvent in the closed loop.[92,103]

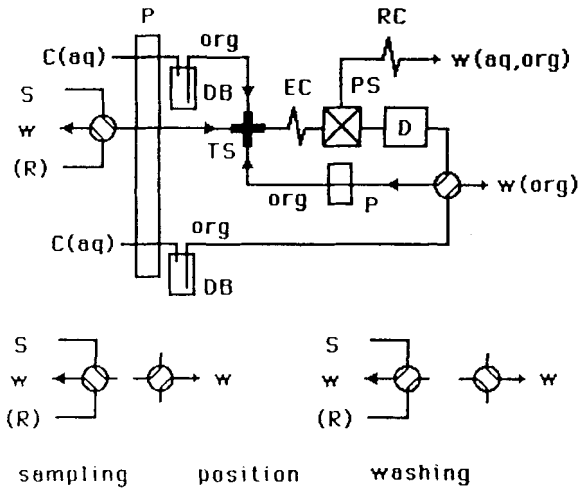

FIGURE 11. A closed-loop FIE system with a four-channel segmenter for continuous introducing a makeup flow of the organic phase (top line) and continuous introduction of the fresh sample solution. An on-loop photometric detector is used for continuous monitoring of the analyte concentration after the phase separator. Sampling and washing positions of the two valves are depicted in the bottom.

III. INSTRUMENTATION

Several fundamental considerations must be taken into account when designing a liquid-liquid extraction FIA system. These are connected to reproducible segmentation of the two immiscible phases; optimization of the geometry of the complete liquid-liquid extraction system; the selec-

tion of proper construction materials for all individual system components; and a highly efficient and fast separation of the phases after reaching the separation equilibria.

All of the individual steps are important for obtaining high sensitivity in sample analysis with the lowest possible sample zone broadening. Separation and segmentation of the two immiscible phases are the most important steps, but also are the most difficult procedures of this technique; consequently, they are also the most frequently discussed in the literature.

Most components of liquid-liquid extraction FIA systems are commonly used in a classical one-phase FIA. The exceptions are the segmenter, the extraction coil, the phase separator, and several special components (e.g., the transport unit, the restriction coil, etc.). Comprehensive reviews of the basic components of the one-phase FIA system are reported elsewhere;[94,113,125,126] therefore, only the components exclusive to liquid-liquid extraction systems are described herein. Special modifications of detection systems and appropriate interfaces are also mentioned.

All components used in liquid-liquid extraction FIA should be selected with particular consideration to the aggressive properties of the organic solvents used in the extraction process since some solvents may attack materials ordinarily used in FIA. Therefore, special components, all connectors, and all tubing should be composed of chemically inert materials, such as fluoroplastics, glass, platinum, titanium, or stainless steel. Other components, such as the injector and the reaction and mixing coils, can be the same as those used in classical one-phase FIA systems[94,113,125,126] since their function is exactly the same as in one-phase manifolds.

The selection of suitable detection systems is limited by the presence of trace concentrations of one of the solvent phases in the other, as the solubility of solvents is usually quite low (but measurable). Optical detector systems are preferred to electrochemical detectors, with the influence of the organic phase being less important for the former.

A. Transport Units

An aqueous stream can be created using a standard peristaltic pump with ordinary PVC tubing, or by several other techniques.[94,113,125,126] In contrast to this, a flow of the organic solvent is usually created by use of:

1. A peristaltic pump with pumping tubes comprised of inert material (such as modified PVC [Tygon], silicone rubber, flexible polyurethane [Tygotane], or fluoroplastics [Acidflex, Viton, etc.]) since the flexible tubes commonly used in FIA are useless
2. A liquid chromatographic pump (piston or syringe) with pulse dampers and pressure indicators
3. A displacement technique, involving pumping an aqueous stream into a closed container (usually a thick-walled bottle) by using a peristaltic pump with ordinary pumping tubes; the container is completely filled with an organic solvent, which is replaced at a constant aqueous flow rate and forced into the FIA system (see Figure 12)
4. A constant gas overpressure (using pressurized inert gas), forcing the organic phase through the FIA system from a closed container

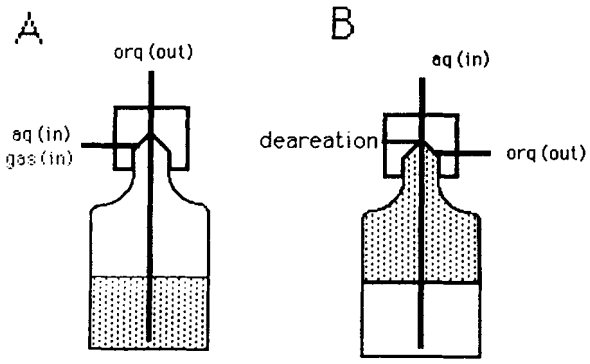

FIGURE 12. Two possible applications of the liquid/liquid displacement technique for an organic solvent being heavier (A) or lighter (B) than water and for the constant air pressure displacement technique (A). Deaereation channel can or cannot be used in a bottle cup (A, B).

A stable pumping system is usually required in liquid-liquid extraction FIA. Rigorous control of the flow rate is not always possible with peristaltic pumps since nonpolar solvents attack normal, inexpensive pump tubing material; and even some more expensive commercial solvent-resistant tubings (Viton, Acidflex) tend to deteriorate with time.[63] Small particles flaking from the inside wall of the tubes tend to clog the porous membrane of the phase separator, further aggravating the problem of flow rate maintenance.

In principle, the flow should start and stop instantaneously for precise control of stream movement, especially for intermittent pumping functions. A small inner holdup volume is desirable to permit rapid startup and short washout periods. The biggest drawback of reciprocal pumps is the generation of pulses in the stream. The flow rate changes rather significantly during the starting period of the peristaltic pump, so that some conditioning of the pump tubing is required in order to obtain a constant flow.[94,113,125,126]

A reasonably constant flow rate of the organic phase can be easily and effectively delivered by a peristaltic pump using Tygotane tubes (a flexible polyurethane). This is the only material capable of tolerating MIBK and methanolic solutions during long operating periods (10 h); the only limitations are that small-diameter Tygotane tubing is not commercially available and it increases the dead volume of the unsegmented part of the extraction FIA system.[103]

HPLC pumps are an expensive but effective solution to the problem, especially when several independent solvent flows are to be used; however, they cannot be used with a low-pressure system flowing stream without introducing an additional column to produce a necessary overpressure for their proper function. Syringe pumps are very expensive. However, a well-defined segmentation pattern can be created through the use of alternately operated stepper-motor-driven microsyringe pumps.[130] Chemically inert circulating pump with a small dead volume is not as yet commercially available.

Displacement techniques, using either aqueous phase pumping or constant gas pressure,[22] can in many cases offer less expensive and better alternatives. Membranes (made of chemically inert materials or a thin layer of an insoluble substance) can be used to prevent or minimize the solubility of the gas or water in the organic solvent. It is sometimes difficult to obtain a constant flow rate through an extraction FIA system using constant gas pressure since small irregularities in the flow rate may result in tube clogging or pressure variations in one of the containers.

B. Segmenters

Phase segmentation involves dividing the continuous flows of the organic and the aqueous phase into one uniform stream with alternating segments. The immiscible phases are brought together in a narrow tube in a controlled manner so that defined segments of each phase are formed. Segmentation and separation of the two immiscible phases are of crucial importance to the quality of the results obtained. Detailed studies of factors controlling segment size and the reproducibility of segmentation have focused on segmenters of the gravity/density and hydrodynamic types.[85,114,115,132,133]

There are two major variables to consider in connection with segmentation: segmentation reproducibility and segment size. While segment size may not affect the extraction efficiency of a fast extraction process or when large sample volumes are introduced into the system, it could theoretically affect the efficiency of slower systems.[23,85] The maximum segment size is determined by the interfacial tension of the organic and aqueous phases, both between each other and between a phase and the tubing material, such that the maximum segment size decreases with decreasing values of interfacial tension $\gamma_{o/a}$. The choice of tubing material, tubing dimensions, and geometry of the mixing chamber also is critically important. The possibilities to vary segment length, however, are limited with most available segmenters.

Accurate performance of the segmenter is key to the successful development of continuous liquid-liquid extraction. To be sure, the overall segmentation process can negatively influence sample dispersion, extraction rate, and phase separation.[23,85] Reproducibility of segmentation is often not satisfactory, and the operation of seg-

menters at the high flow rates and phase flow rate ratios often sought in connection with sample workup is limited. Reproducible segmentation can improve the precision of signal measurements, and may frequently simplify signal evaluation. Finally, it may even be possible to eliminate phase separation altogether if the segmentation reproducibility is sufficient to allow precise timing of the measurement intervals.

Several segmenter types of varying efficiency have been described in the literature. The most common segmenter types are T-piece segmenters made of glass,[6,15] fluoroplastics,[13,22,78] stainless steel[97] or glass-lined T-pieces of stainless steel, and combinations of hydrophobic and hydrophilic materials.[50,64] Improved glass A8-T and A10-T fittings[1,10,15,65,103,107] and T-pieces made of fluoroplastics with inserts of fluoropolymer tubing[46,47,54,78] or with an enlarged inner diameter of the outflow channel[74,81,85] have found wide use. Different configurations of Y-[40,51,57,99,118] or W-pieces[24,25,58,108,118] made of glass or fluoroplastics and four-way fittings[92,103] have also been recommended. More recently, a coaxial (falling drop) segmenter has been introduced[129,133,144–147] in an attempt to overcome some of the disadvantages of the other segmenter types.

The geometry of the inner capillary system of the T-shape segmenter[6,93,132] and the geometry of the confluence chamber of the coaxial segmenters have also been carefully investigated.[133] Flow systems without phase segmentation[4,16,18,48,65,74,77] and the use of sample introduction downstream from the segmenter have been suggested[106] since extraction efficiency, extraction rate, and sample dispersion (peak broadening) are all influenced by the segmentation pattern. Furthermore, a multichannel dropping segmenter has been used for homogenization and the instantaneous introduction of sample and reagent aqueous solutions into the continuous flow of the other immiscible solvent[143–145] directly inside the segmenter. Other studies showed that conventional loop injectors operated by a cycling motor with adjustable filling/draining times and a brief intermittent period can be used at low flow rates or for introducing very long segments[124,143] of an immiscible solvent into the continuous flow of the other phase.

Existing segmenters can thus be divided into two general groups, depending on their principle of segmentation (postsegmenter introduction of the sample being a special case):

- Continuous flow segmenters (including those which operate on the basis of differences in gravity or density)
- Mechanical types of segmenters

Comparison of several parameters of the various types of segmenters indicates that the best segmentation pattern and the best segmentation reproducibility are produced by the coaxial segmenter, the modified A8-T fitting, and the conventional loop injector (see Table 1).

1. Continuous Flow (Gravity/Density; Hydrodynamic) Segmenters

The segmentation process is based on the principle that droplets or small plugs of one immiscible phase are formed in the continuous flow of the other solvent in a small mixing chamber of the segmenter. Another principle leading to segment formation is the "ripple"-forming process connected with the destruction of the thick layer of one solvent in the other one formed on the inner tube walls. The former principle operates in a hydrophilic compartment of the segmenter (glass or metallic tee piece, or modified Technicon A8-fitting), while the latter occurs in segmenters made from a lipophilic material (such as fluoroplastic) or in cases where the walls of the compartment are contaminated by lipophilic impurities. The latter principle also controls segmentation at very high flow rates, flow rate ratios (Q_a/Q_0), or when a very high overpressure is applied.[144]

The continuous (ideally pulseless) streams of two immiscible solvents meet at the segmenter mixing point. More or less reproducible droplets or plugs of one phase are obtained. The size of the droplets is governed by the equivalence of the gravity, density, interfacial tension, and mainly hydrodynamic forces as the droplet grows in size, until the interfacial force is greater than the sum of the gravity, density, and hydrodynamic forces. The droplet size also depends on the material of the inner wall of the segmenter

TABLE 1
Comparison of the Basic Parameters of Different Types of Segmenters[143]

Parameters	A8-T glass	T-glass	T-PVDF	Coaxial	Loop magn inj. valve	
Q_a(ml min^{-1})	<4	<4	<3	<8–10	>8	2.5
Q_o(ml min^{-1})	<1.5	<1.6	<1.5	<0.8, 1.5[a]	3	2
Q_a/Q_o	<15	<15	<3–5	<40	—	2
L_s(org)	<38	<45	<8	<50	>>	>1
$s_r(5)$[b]	2, 12[c]	9, 12[c]	12	4, 4[d]	—	6[e]
$s_r(10)$[b]	7, 25[c]	33[c]	—	7, 12[d]	—	—

[a] Freon-113; see text for other solvents, 1.2 ml min^{-1} for Freon-113.[126,129]
[b] Relative standard deviation at Q_o = 0.5 ml min^{-1} and at Q_a/Q_o 5 and 10, respectively.
[c] "Ripple" forming principle.
[d] 0.35 and 0.25 mm inlet glass tubing.
[e] At $Q_a = Q_o$ = 0.5 ml min^{-1}, 19% at Q_o/Q_a = 0.5/1.5 ml min^{-1}.

and, to a great extent, on the geometry of the inner capillary system of the segmenter.

Space-oriented forces affect the skewing process of the droplets in continuous segmenters, the degree of influence of each depending primarily on the flow rate of the carrier stream, the mass of the droplet, and the orientation of the capillary system in space or its orientation among the capillaries.

The droplets subsequently move on into the outflow channel and tend to minimize their outer surface area toward the other phase and/or to maximize the contact surface area with the wall material by wetting it. A more or less regular segmented flow with independent segments of both phases is obtained.

Depending on the material of the outflow channel and the extraction coil, the solvent having the greater affinity toward the tubing walls covers them with a thin film that surrounds the deformed spherical, tubular, or ellipsoidal segments of the other phase. In PTFE tubing, the film of the organic phase will surround the aqueous segments, whereas in glass or metal tubing the segments of the organic phase will be surrounded by a film of aqueous phase.

The size of the contact area between the phases is influenced mainly by the segment length in the extraction coil. Hence, the segmentation pattern must be controllable and constant during the entire analytical procedure. It is necessary during manifold optimization to consider kinetic efficiency, total extraction yield, and peak (sample zone) broadening.

a. Noncoaxial Segmenters: Classical T-, Y-, and W-Pieces

The classical T-, Y-, and W-pieces made of homogeneous materials (e.g., fluoroplastics, stainless steel or glass, glass-lined T-pieces made of stainless steel, or metal capillaries with a fluoropolymer coating [in T-shaped segmenters]) are the most widely used segmenter types. Capillary tubes made of glass, stainless steel, and other materials in various geometrical configurations and combinations of materials have also been used for phase segmentation. The segmenters are commonly used with the aqueous phase entering through a horizontal inlet capillary which is axially oriented to the main segmenter axis, and the organic entering through the upper or lower capillary (depending on the density difference between the two solvents). However, other orientations also are often used (see Figure 13).

The T-shaped glass segmenters function by the wetting and skewing principle at low or mod-

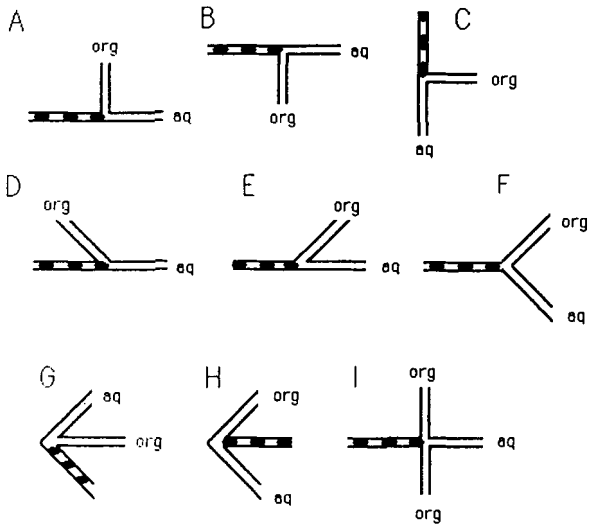

FIGURE 13. Classical T-pieces (A, B, C), Y-pieces (D, E, F), W-pieces (G, H), and four-way (I) segmenters with the most often used orientation of the aqueous, organic, and segmented flows, but also other orientations are possible.

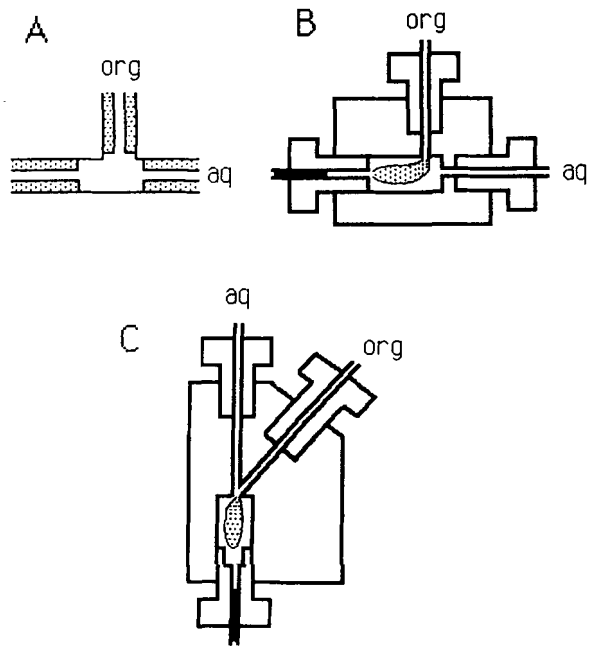

FIGURE 14. An improved glass T-piece with fluoropolymer tubing inserts (A), a fluoropolymer T-shape segmenter with an enlarged outflow channel (B), and an improved Y-piece with an outflow channel made of a fluoropolymer screw (C).

erate flow rates. The segment volume is controlled by the position and orientation of the input and output channels, and by the inner diameter of the input channel (and to a great extent, that of the output channel). The resulting force vector (controlling the segment size) is influenced by the angle between the two inlet tubes and by the orientation of the outlet tube. The maximum droplet size is limited by the resulting force vector and by the volume of the compartment at the junction of the T-piece.

An improved glass T-piece segmenter may be realized by inserting one or more concentric fluoropolymer tubes into the outlet (and sometimes also into the inlet) tubing of a conventional glass T-piece (see Figure 14A). The inner diameter of the fluoropolymer tubes as well as their distance from the position of the inlet tubing positively influence[132] the reproducibility of the segmentation process. The maximum droplet size is limited by the volume of the compartment formed by introducing fluoropolymer tube inserts into all branches of the glass T-piece. The volume of the droplets is, in this case, influenced by the wetting ability of the organic solvent at the moment when the surface of the distorted droplet contacts the end of the fluoropolymer tubing.

This type of segmenter yields reproducible segmentation over a wide range of flow rates at a constant organic phase flow rate. The length of the organic segments is reduced with increasing flow rate of the aqueous phase and reaches a limiting value at higher aqueous phase flow rates. The aqueous segment length increases at low Q_a and at high total flow rates. The organic segment length increases nearly linearly with increasing organic phase flow rate. Both decrease with increasing total flow rate at a constant flow rate ratio.[132]

The organic segment length shows a nearly linear dependence on the distance of the end of the fluoropolymer tubing from the joint of the T-piece (from 3 to 15 mm). At shorter distances, the segment lengths of both phases, and segmentation reproducibility, are drastically or markedly decreased, respectively, as a result of ripple segmentation. Segmentation reproducibility is satisfactory at moderate flow rates, and decreases at higher flow rates due to the formation of very small droplets at the end of the fluoropolymer tubing insert.

The T-shaped segmenters made of fluoro-

plastics are in many cases able to produce small and reproducible segments, assuming optimum geometry of the inner capillary system. They work best at low flow rates and at phase flow rate ratios Q_a/Q_0 close to unity. At higher flow rates and flow rate ratios, the reproducibility is not satisfactory.

An ordinary T-piece gives segments of a reproducible size.[13,110] The mutual positions of the inlet and outlet tubes can influence the efficiency of the segmentation process. One type (Type B) (see Figure 13B) was found to be best,[93] but its benefits were not marked and could not be confirmed at all at higher flow rates.[65,132] Other combinations of organic and aqueous phase inlet orientations gave an uneven flow. The relative segment length changes only negligibly with the angle between the vertical axis and the axis of the organic phase inlet channel, and the reproducibility of segment formation decreases when the angle is increased.

With Q_a/Q_0 near unity, the length of the aqueous and organic segments are approximately equal when segmentation is regular. The segment lengths depend on the total flow rate Q_t (within a limited range), and decrease with increasing Q_t. Above some limiting value, $Q_{t(lim)}$, the segmentation pattern is usually regular at the segmenter but irregular in the extraction coil as a result of ripple segmentation or combination of segments during passage through the extraction coil.[132,133] At a given Q_t, longer segments are obtained, depending on the interfacial tension $\gamma_{o/a}$ and the viscosity ratio η_0/η_a of both solvents.

Increasing the inner diameter of the outflow channel (0.3 to 0.7 mm ID for inlet and 0.5 to 2 mm ID for outlet tubes; Figure 14B) results in longer segments with this kind of segmenter, a prerequisite being that the organic phase forms a very thick film on the walls of the compartment or that it can fill the whole diameter of the channel before the organic segment is skewed off by the aqueous flow. At high phase flow rate ratios, this phenomenon cannot occur and the segments are actually formed in the extraction coil. A significant decrease in the flow rate in this part of the system also occurs.

The length of the segments is determined by the inner diameter of the outlet tubing so that the T-piece in its original shape gives shorter segments; however, the segment length is about four times longer when the outlet is twice enlarged.[65] Refined versions of this design are commercially available (e.g., the Tecator design), where the outlet channel volume can be varied by exchanging fittings. In this way, segment lengths between 2 and 15 mm can be obtained. Neither of these segmenter types use materials displaying different wetting characteristics. Thus, segment size control is limited in comparison with the A8-T fitting.

The relative segment length changes with the angle between the vertical axis and the axis of the organic phase inlet channel and reaches a maximum between 135° and 180°. The reproducibility of segment formation decreases when the angle is increased. The maximum relative segment length is usually achieved when the aqueous phase flows through the T-segmenter from the bottom to the top along the longer axis while the organic phase flows horizontally. The minimum segment length was achieved in the reverse position. The difference in segment length between the vertical organic flow stream inlets (see segmenters A and B, respectively, in Figure 13) was less evident with the aqueous phase flowing horizontally along the main segmenter axis.

Irregular segmentation due to breakup of some of the segments, as a result of irregularities in the walls of the drilled channels, has also been observed. In fact, smoothness of the walls is as important for obtaining even segments as is avoidance of impurities or precipitates causing deposits. The geometry of the segmentation point was found to be critical; both the size of the mixing cavity and its proximity to turbulent points (i.e., points at which the flow direction changes abruptly) must be optimized so that segments of uniform size result.[65]

In the absence of fluoropolymer tubing inserts, an irregular segmentation pattern can develop in a Kel-F tee-piece (commercially available), usually displaying both long and short segments. This was observed to be due to creeping of the organic phase along the walls into the aqueous inlet capillary of the T-piece, where it formed an adherent droplet located approximately 1 or 2 mm into the branch.[78] The droplet grew in size until it formed a constriction large enough to occasionally cause breakage of the

aqueous stream at the junction of the branches where it normally occurs. Installing short, flared pieces of fluoropolymer tubing into all three capillary branches of the narrow bore cylindrical chamber eliminated the problem over a wide range of Q_t.

The ability of the organic solvent to wet the wall material of the segmenter is important in segmenters made of fluoroplastics, where the ripple segmentation mechanism predominates.[143] The segments are formed in the extraction coil after the segmenter, and segmentation is facilitated by a somewhat pulsating flow. The pulsating flow might cause the segmentation pattern to be in phase with pulsation. However, excessive pulsation or irregular flow gives rise to uneven segmentation for both classical and modified T-shape segmenters.

The optimum design for stainless steel phase segmenters[151] was found to consist of three 0.18 mm ID capillaries joined as close as possible in a stainless steel block (0.25 mm ID bore) at 30/30° angles among them, with the aqueous phase entering through the upper capillary, the organic through the center capillary, and the segmented flow exiting through the lower capillary (Figure 13G). Here, the smallest segment possible is approximately 20 μl. Using larger capillaries (0.25 and 0.30 mm ID), other geometries (45°, 90°, and 120°), and a larger bore (0.7 mm ID) in the steel block, produced two- to threefold increases in segment size.

The W-piece segmenters made of Teflon or PVDF (polyvinyldifluoride) with 45° angles between the inlet and outlet channels[23,24,108] and Y-type segmenters have also been used with very good results[4,40,96] and give reproducible segmentation (Figure 13D to H). In the modified Y-piece,[40] the organic segments form on the hydrophobic surface of a Teflon screw which is screwed into a Perspex block containing connectors for the aqueous and organic solvent lines (see Figure 14C). The segmentation is regular at the high ratios of aqueous to organic phase flows which are needed in some applications, and is not changed when the segmenter is rotated by 180°. Adsorbed solids may, however, cause irregularities in the segment formation and make the segmentation position dependent.

A model having a continuously adjustable length of forming chamber has also been used, and at certain settings it produced segments of widely different sizes. When the flow rate was increased at such a setting, the forming chamber tended to switch to modes yielding segments of one half or one third of the previous size. A large hysteresis was observed when the flow rate was decreased due to the change in the segmentation mode.[134,142–147] The dimensions of the forming chamber may be critical, and the optimum may be different for different solvents.

The major advantages of the fluoropolymer segmenters are that they are inexpensive and readily available. These segmenters offer relatively consistent segmentation, but the segments are fixed in size. The size is large compared to the smallest segments obtainable with an A8-T fitting. Also, a disadvantage of these segmenters is that they are not good at directing the flow, i.e., too high an overpressure applied to the outlet of the segmenter may result in the direction of the flow being reversed in one of the solvent delivery channels. The choice of channel material is most important (particularly important are the wetting properties of the organic solvents used in liquid-liquid extraction FIA). Teflon is not ideal because changes in the segmentation mode often cause irregularities in the segmentation.[132]

b. Noncoaxial Segmenters: The A8-T Fitting

A standard A8-T glass Technicon connector, with glass and platinum inlet capillaries and glass outlet tubing with two concentric pieces of fluoropolymer tubing inserted into the outflow channel, has often been used as a segmenter. The working principle is based on the formation of a droplet of the organic phase in the aqueous phase stream when the organic stream is led into the platinum capillary at right angles to the aqueous stream (which enters the glass capillary) (Figure 15).

The droplet grows in size until it comes into contact with the fluoropolymer tubes or until it reaches its maximal volume (V_{max}), i.e., when the distance between the platinum and fluoropolymer capillaries is sufficiently long to prevent any contact with fluoropolymer. The droplet is

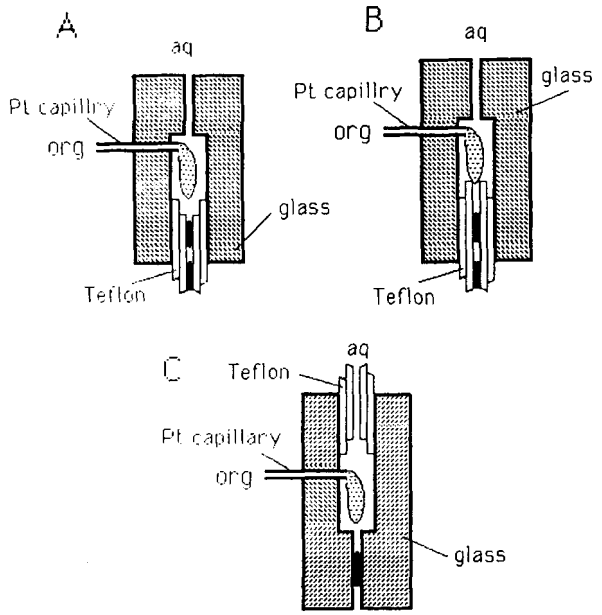

FIGURE 15. Improved A8-T fittings with the glass and platinum inlet capillaries and two fluoropolymer tubing inserts in an outflow channel (with two possible positions A, B) and the A8-T fitting in reverse position (C).

then dislodged to form a segment since it does not wet the glass surface. The volume of the droplet (and the segment length) are therefore determined by:

- The volume of the glass compartment between the platinum capillary and the edges of the two concentric fluoropolymer tubes
- The inner diameter of the platinum capillary
- The flow rate ratio
- The viscosities of the fluids
- The interfacial tension, $\gamma_{o/a}$
- The gravity force
- The hydrodynamic force of the flow of aqueous phase

In such a case, the segment size can easily be adjusted by changing the position of the edge of the inner tube when using a particular solvent system. Appropriate positioning is necessary in order to obtain a regular segmentation pattern. Adjusting the mutual positions of both tubes will also change the segment length.[6,132] The maximum size of the segments is determined by the interfacial tension between the organic and aqueous phases. Regular segment lengths of up to 50 mm, from 10 to 20 mm, and about 2 to 3 mm can be produced using an A8-T fitting in the chloroform/water, the pentanol/water, and *n*-butanol/water system, depending on $\gamma_{o/a}$ value.

Increasing the flow rate Q_a of the aqueous stream at constant flow rate Q_0 of the organic phase using a very long distance between the platinum and fluoropolymer capillaries results in a decrease of the organic segment length (the distance preventing any influence of the wetting properties of the lipophilic capillaries on the segment formation process).[132] In contrast, the length of the aqueous segments increases rapidly and varies exponentially, or nearly linearly, with the flow rate of the aqueous phase at low and high total flow rates, respectively. In addition, the sum of the lengths of the organic and aqueous segments increases at higher aqueous flow rates.

The segment length in the organic phase is practically not influenced by the flow rate Q_0 of the organic phase. The length of the aqueous segments decreases with increasing organic flow rate Q_0, and reaches a limiting value at a very high Q_0 (which depends principally on the glass compartment size). The influence of the total flow rate is usually negligible over a wide range. The reproducibility of segmentation is satisfactory over a relatively wide range of the flow rate Q_a of the aqueous phase and flow rate ratio Q_a/Q_0; however, it decreases at very low and very high flow rates and flow rate ratios. The best reproducibility is achieved in the flow rate ratio interval, $Q_a/Q_0 = 1 - 5$.

Small changes in spatial orientation do not influence the segment length or the reproducibility of segmentation, especially not at a high flow rate Q_a of the aqueous phase. Turning the axis of the inlet/outlet glass capillary around the horizontal axis of the platinum capillary increases the segment length. The segment length reaches its maximum value after 180° orientation due to the decreased influence of the gravity/density force.

Lipophilic impurities on the glass wall surface, other changes of the hydrophilic properties of the walls, a short (<2 mm) distance between the platinum and fluoropolymer capillary ends, or use of very high total flow rates or flow rate ratios cause a change from regular droplet formation segmentation to "ripple" segmenta-

tion.[143] In such cases, the glass surface is covered by organic phase or a small droplet adheres to the end of the platinum capillary, and a short bridge of a hydrophobic interface results. Also, relatively large droplets of organic phase can cling to the entrance and cause undue dispersion.[40] The stream of organic phase then flows continuously into the fluoropolymer capillary without regular droplet formation inside the glass compartment.

Segmentation takes place inside the extraction coil at some distance from the edge of the capillary, which depends on the orientation of the main segmenter axis, the coiling of the outflow capillary, its position, etc. The segment length is decreased drastically, the segmentation pattern becomes irregular, and the segmentation reproducibility decreases to 20 to 50%. The segment lengths of the organic and aqueous phases remain unchanged or decrease with increasing total flow rate. This is in contrast to the regular segmentation mode, where the segment lengths remain constant or increase with increasing total flow rate.

Any changes in the laminar character of the flow due to changes in the inner diameter of the tube, fluctuations in the flow rate, insertion of sharp edges, etc. destroy the organic layer formed on the fluoropolymer tube walls in a laminar flow of both phases. Instead, small droplets or plugs are formed. This "ripple" process of droplet formation is more sensitive to any irregularities in the experimental conditions than the classical droplet formation process and results in impaired segment formation reproducibility. The "ripple" process can be partially overcome by insertion of a second fluoropolymer tube.

This phenomenon rarely occurs at low aqueous flow rates, but frequently occurs at very high Q_a/Q_0 or at very high back pressures. The perpendicular flow of aqueous phase will then deform the droplet shape and slide it along the surface of the walls of the glass compartment. The stream of organic phase can finally bridge over this hydrophilic surface, forming a continuous film of organic phase that changes the mode of segmentation.

The spreading of the organic droplet along the walls of the glass compartment is very important at higher Q_a. The droplet bends somewhat with the stream and becomes distorted to some extent at all flow rates. At very high flow rates this, together with the gravity force, will control the droplet size. This phenomenon becomes expressed more for a fluoropolymer compartment in the common T-segmenter than for the glass compartment of the A8-T segmenter.[132]

The modified A8-T segmenter cannot be used with standard tubing connectors of the low-pressure HPLC type, which is a drawback in routine work. Serious leakage often occurs at high total flow rates or at high overpressure (due to the phase separator or the restrictor coil) since this type of segmenter was designed for low-pressure segmented continuous flow analyzer systems. It can be readily used with a T-shape phase separator or in systems without phase separation, but difficulties are encountered in systems employing phase separators of the membrane type.

The segment size is influenced by surface-active substances, such as anionic surfactants.[6] The segment length is constant with no surfactant present; however, with increasing surfactant concentration, the segment length decreases significantly, and peak height decreases more rapidly for shorter segments than for longer ones. This phenomenon depends on the amount of surfactant at the interface between the phases, changing the interfacial tension. The segment length increases with increasing methanol content, and peak tailing is reduced as a result of increases in extraction efficiency and rate, via a reduction of the lipophilic nature of the fluoropolymer tubing walls causing adsorption of ion associates from the aqueous stream. The reproducibility also decreases with increasing methanol content in the reagent stream.

The modified A8-T fitting has the advantage of using variable segment lengths over a wide range and of being very good at maintaining the direction of flow. This type of fitting can tolerate even high back pressures if it is tightly fitted to the extraction system; however, the separation between segments is sometimes not consistent.[23]

The dead volume at the confluence point is too large for organic flow rates that are much smaller than the aqueous flow rate. When segmentation is controlled by droplet formation, segment length can be easily varied by the Q_a/Q_0 flow rate ratio, or (better) by the distance of the fluoropolymer tubes from the platinum capillary

end. Segmentation was found to become irregular at higher phase flow rate ratios, and, even with careful adjustment, occasional droplets of larger size can be dislodged.

Fluctuations in solvent flow rate or solvent flow rate ratio caused by segmentation has the adverse effect of forcing some of the phase intended for resampling out to waste at the phase separator. Hence, uneven segmentation leads to a decrease in sample recovery.[23] Segmentation in the back-extraction step is not as critical since the organic and aqueous phase segments are often about the same size.[40,128,129,132] However, irregular segmentation increases pulsation.

c. Noncoaxial Segmenters: The Four-Way Fitting[92,103]

A four-way fitting has also been used as a segmenter, allowing a small "make-up" stream of the organic phase to be continuously introduced into the segmented stream as a constant fraction of the total flow (Figures 9 and 13I). This additional stream is introduced to compensate for the finite loss of MIBK through the phase separator and for the solubility of the organic phase in the aqueous phase.[103] However, to prevent dilution, only a very low flow rate of the "make-up" stream is used.

d. Coaxial (Gravity/Density) Segmenters[128,132,133,142–147]

The coaxial segmenter (falling drop) consists of two basic components (see Figure 16): a PVDF screw with a single or multiple capillary inlet channel, and a segmenter body with inlet and outlet capillary channels for delivery of aqueous (organic) phase and drainage of the segmented flow stream, respectively. A glass capillary inlet tube is pressed into a PVDF screw (Figure 16A) for introduction of organic phase,[128,133] while a single[146] or multiple[144,147] capillary channel system is drilled inside the PVDF screw (Figure 16B and C) for introduction of the aqueous phase.[144] When assembled, the inlet capillary system ends in the conical housing of the compact segmenter body, made of Perspex or PVDF (Figure 16A).

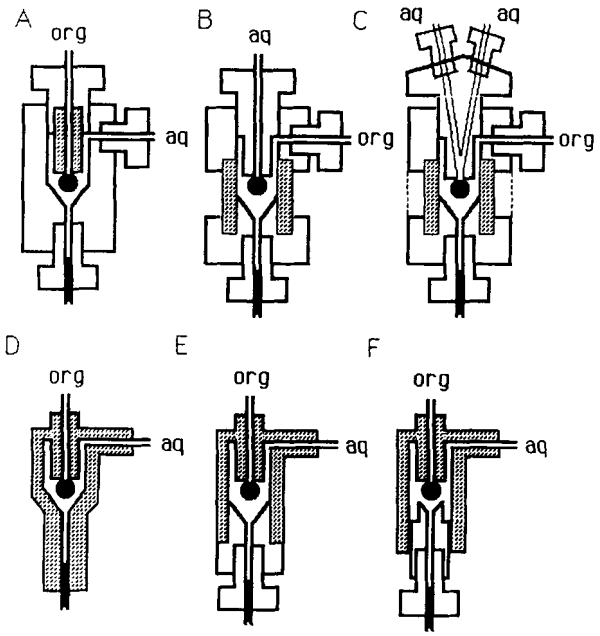

FIGURE 16. Coaxial segmenters of different geometries made of PVDF or Perspex (A), PVDF body with a thick wall glass confluence chamber and PVDF conical compartment with reverse-oriented phase flows and a PVDF screw with a single- (B) or a multiple-channel inlet system (C) for continuous introduction of aqueous stream(s), an all-glass segmenter (D), and a glass segmenter with the two different geometries of the PVDF conical compartment (E, F).

The confluence chamber, made of a thick wall glass tube and a conical PVDF insert, and an outlet channel of fluoropolymer capillary at the bottom of the conical chamber also has been used (Figure 16B and C). An all-glass coaxial segmenter having an integral conical confluence chamber (Figure 16D), and a glass segmenter having a PVDF conical insert in the straight glass tube (Figure 16E and F), have been compared with the above-mentioned segmenters.

The working principle of the single or multichannel segmenters is the formation of small droplets (usually in the order of units to tens of microliters) of one solvent in a continuous flow of another fluid, immiscible with the first. Each component in the aqueous reaction mixture is transported through one of the capillary channels in the screw, converging at the common confluence point at the junction of the inlet tubing capillary system when using the multichannel segmentor (see Figure 16C). The organic (or aqueous)

phase flows through the glass (fluoroplastics) inlet capillary when organic (or aqueous) phase is used as a droplet-forming phase in a single-channel segmenter.

Droplet size depends predominantly on gravity/density forces since the influence of hydrodynamic forces is negligible. The droplets, of defined volume, either descend or ascend (depending on the density difference $\Delta\rho_{o/a}$), through a small conical chamber filled with flowing phase which does not form droplets. Its flow has a negligible influence on droplet size due to the formation of a whirl, depending on the inner/outer diameter of the end of the screw.

The droplet is then transported through the outlet channel, where the segment develops. For a reaction system, droplets of the homogeneous reaction mixture are then transported through the fluoropolymer capillary system of the analyzer in the form of separate segments of aqueous phase (forming a closed reaction system) which are completely isolated from each other by a film of organic phase that prevents analyte carryover.

The segmenters[133] function effectively over a wide range of flow rates of both phases, and over a range of flow rate ratios from 2 to 35–40. The segment length of the droplet-forming phase increases linearly, with the inner diameter of the inlet capillary at constant flow rates of both phases. It is not changed with the flow rate of the droplet-forming phase at a constant flow rate of the other phase, up to the limiting value at which the jet effect appears, depending on the inner capillary diameter and character of the solvent. Segment length is only slightly influenced over the whole range of its own flow rate. Optimum segmentation reproducibility is achieved for inlet capillaries with ID from 0.1 to 0.3 mm for all tested organic solvents and water, and it decreases in the case of other IDs.

The length of the segments of the phase *not* forming droplets increases nearly linearly with the flow rate of the other phase and with the total flow rate (i.e., slopes of segment length vs. flow rate are close to unity), and decreases rapidly with increasing flow rate of the other phase, according to changes in the dropping frequency of the droplet-forming phase.

Segmentation reproducibility for organic phases decreases in the order Freon-113 > chloroform > MIBK > carbon tetrachloride. Uneven segmentation has rarely been noted with MIBK, and only exceptionally with carbon tetrachloride at low organic flow rates where small droplets of the organic solvent were cut off at the end of the segments. Values for appropriate flow rates and the flow rate ratios must be established for each solvent at a given inlet capillary ID and defined experimental conditions. Improved segmentation reproducibility is usually achieved at a medium total flow rate and a flow rate ratio between 5 and 15, when the flow rate of the droplet-forming phase is in its optimal range. The best diameter of the organic phase inlet tubing varies between 0.10 and 0.35 mm ID, depending on the character of the solvent and its flow rate, but generally 0.25 ± 0.05 mm ID can be used for most organic solvents.

For all tested solvents, an uneven segmentation pattern is obtained at a very low flow rate of the droplet-forming phase and a constant moderate flow rate of the other phase. The segmentation reproducibility decreases from units up to tenths of a percent. At higher organic phase flow rates, a jet effect appears, depending on the solvent character, and the segmentation reproducibility decreases sharply. Segmentation breaks down completely as a result of this effect.

Changing the orientation of the main axis of the coaxial segmenter from the horizontal position has a negligible effect on the segment length, but over 10°, the segment length decreases by between 25 and 50% for both phases. No significant difference between the lengths of the organic segments in straight and coiled fluoropolymer tubing (30 mm coil diameter) was found at moderate flow rates, but the segmentation reproducibility was better for coiled than for straight tubing.

Up to a 5% content of inorganic salt (such as NaCl) in the aqueous phase increases the length of the organic segments by 4% due to the increase in density of the aqueous phase. In this case, the segmentation reproducibility remains constant or increases only slightly. Using a methanol content of up to 15%, or content of a nonionic surfactant of up to 1%, causes a decrease in segment length by <10%. The segmentation pattern and segmentation reproducibility are strongly impaired by a higher content of either of these additives,

and segmentation breaks down completely at a methanol concentration above 30% or a surfactant concentration above 1.2% due to the change in surface tension.

Larger segments are produced by the coaxial segmenter than by classical segmenters. This segmenter works better than those at high phase flow rates and phase flow rate ratios. At very high flow rates and phase flow rate ratios, there is a tendency for small organic segments to decrease in size and for larger organic segments to grow as they pass through a fluoropolymer extraction coil propelled by a peristaltic pump. In order for the segments to survive, it is necessary that they have a certain minimum volume, which may be attained using a 0.25 to 0.50 mm ID glass capillary.[38] To prevent the influence of aqueous flow stream pulsation and formation of a whirl, the inner glass capillary can be screened by thin wall tubing.

A coaxial segmenter seems to be very convenient in many applications, generally offering comparable segmentation reproducibility to the loop injector over a wide range of flow rates of the two solvents. The use of coaxial segmenters does not introduce any additional moving parts into the liquid-liquid extraction FIA system. The results thus far indicate that a considerable improvement in performance can be obtained with this new segmenter design. Apart from providing a two- to threefold increase in segmentation reproducibility, a coaxial segmenter can be operated over a wide range of flow rates and phase flow rate ratios than previous segmenter designs. An additional advantage is that this segmenter behaves according to a simple and well-established theory, making it possible to calculate, *a priori,* the parameters of the segmentation pattern.

In this case, the segment lengths of the two immiscible phases can be varied over a relatively wide range, from units to tens of millimeters (from 3 to 50 mm for the organic phase, and from 10 to 300 mm for aqueous phase), simply by changing the inner diameter of the inlet capillary and by changing the flow rates of the two solvents. The segment length of the droplet-forming phase can easily be predicted and is independent of the majority of factors influencing segmentation in other segmenter types (e.g., flow rates of the two solvents, the flow rate ratio, etc.).[133]

The segmentation reproducibility depends on the cleanliness of the walls of the mixing compartment and the flat end of the inlet capillary. The segmentation pattern can be changed by the accumulation of lipophilic substances on the walls of the confluence compartment. Periodically, the walls of the conical housing become coated with a thin film of organic phase due to irregularities in segmentation by the small droplet formation at the end of the regular segment or to variability in the volume of the organic solvent clinging to the surface of the conical portion of the outlet channel. Segmentation reproducibility may be decreased by as much as several tenths of a percent. Careful washing of these surfaces with ethanol and periodic grinding of the capillary end can enhance segmentation reproducibility, especially after long, dry storage of the system. Air bubbles must also be removed from the segmenter, especially in the space between the glass capillary and the mixing compartment walls. However, this is less critical for coaxial segmenters than for most other types of segmenters.

The relatively large dead volume of the conical chamber (ca. 0.1 ml) tends to increase sample and reagent consumption and presents the risk of contamination of the samples by sample/reagent carryover. These factors are less serious at high aqueous flow rates and high flow rate ratios used in certain applications (such as with AAS, and when high enrichment factors are desirable).[128,129] The dead volume can be minimized by miniaturization of the confluence compartment. Furthermore, the distance of the inlet glass capillary from the conical housing and the inner and outer cross-sectional diameters of the confluence chamber must be optimized for the desired droplet size since they also greatly influence the cutoff process. The nominal volume of the conical chamber must be greater than the actual droplet volume to avoid contaminating the walls of the confluence compartment with organic phase.

The compact PVDF body segmenter has the disadvantage of being opaque, and consequently, visual checking of the segmentation process is not possible. Clearly, visual checks are important

for detecting when the walls of the confluence chamber become coated by lipophilic substances. Perspex, however, is attacked by most common organic solvents, and the conical compartment thereby is difficult to machine to the desirable wall smoothness. Compact body segmenters and those with a confluence chamber made of a thick wall glass tube tightly pressed into a compact FP4 fluoropolymer body (Figure 16C) are less sensitive to leakage at higher back overpressure and thus are more suitable for systems with membrane phase separators.

Coaxial segmenters generally function better at moderate or high, than at low flow rates since the phase droplets are often destroyed on the walls of the conical chamber, resulting in a decreased segmentation reproducibility. A hydrophilic construction material (e.g., glass) is recommended for use in the confluence chamber (particularly in the conical part of the chamber).

The geometry of the confluence chamber (and especially of the conical part) should be optimized to decrease the dead volume of the segmenter and to increase the segmentation reproducibility. It should be noted that the use of a narrow angle in the conical part of the confluence chamber increases the risk that droplets may split off small particles that can adhere to the walls of the compartment or the conical housing. This phenomenon occurs more frequently in conical chambers made of Perspex, probably because they are difficult to machine to a desired wall smoothness and because the wetting ability of water is less on Perspex than on glass. The phenomenon was rarely observed in the PVDF conical housing and has not been seen in integral conical compartments made of glass.

The analytical signal obtained using a multichannel dropping segmenter is comparable to that obtained by steady-state measurements in which aqueous solutions of extractable reaction product are introduced directly into the flow of organic phase. Instantaneous extraction of a reaction product speeds up the reaction rate when an extractable product is formed from nonextractable reaction components. No significant lengthening of the extraction/reaction coil is needed when a dropping segmenter is used for instantaneous preparation of a reaction mixture or for the direct introduction of a pre-prepared extractable product solution into the system.

Direct "in-segmenter" introduction of the sample and organic analytical reagent using a multichannel segmenter can eliminate the reaction/mixing coil, and thus most problems connected with its use. The use of a multichannel dropping segmenter in liquid-liquid extraction FIA can also eliminate the need for a phase separator and a sample injector, thus significantly simplifying the manifold.

Thus, several separate inlet capillaries meeting at a common confluence point of very small diameter[147] can be used for such applications as dilution (changing Q_1/Q_2 for sample and water), reagent introduction, titration (changing Q_1/Q_2 for sample and titrand), calibration (changing concentration or Q_1/Q_2), gradient formation (two pumps for gradient or gradient pump), and kinetic measurements (different Q_t or L_{EC}).

The high degree of segmentation reproducibility attainable with this kind of segmenter can improve the precision of signal measurement and can often simplify signal treatment. Finally, it may even be possible to eliminate the phase separation process altogether since the segmentation reproducibility is sufficient to allow precise timing of measurement periods.

2. Mechanical Segmenters

a. Loop Injector-Type Segmenters

All the above-mentioned segmenters work relatively well over a wide range of organic phase flow rates and phase flow rate ratios, but the total volume of the droplets and the phase segment length are strongly influenced by the aqueous phase flow rate or by surface active substances, such as surfactants.

In some studies (especially when it is preferable not to use a phase separator), very precise and reproducible segments are needed. In such cases, it is crucially important that the segment length be unaffected by experimental factors.[145] Segmentation can then be implemented using a pneumatic or motor-driven loop injector (Figure 17). Very precise and reproducible volumes of

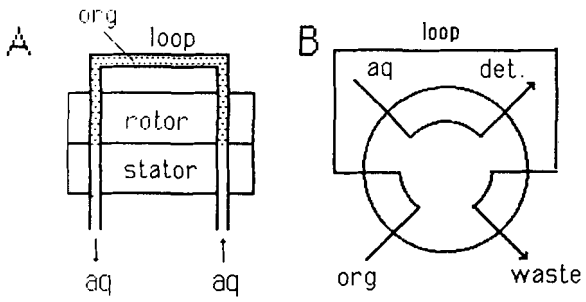

FIGURE 17. A rotary loop injector (A) and a six-port rotary valve (B).

one phase can be introduced into a continuous flow of the other phase, with the segment length being controlled only by the preselected volume of the injector loop.[145]

Segmentation reproducibility decreases with increasing length of the extraction capillary (L_{EC}) and with increasing flow rate (Q_t). Inadequate segmentation reproducibility most often occurs at very low flow rates. Small droplets of organic or aqueous phase are, in these cases, formed at either end of the segments as a result of incomplete "washing out" of the sample loop at low flow rates. The same phenomenon can be observed at high flow rates, when the narrow (relative to their length) aqueous segments split off small droplets. Alternatively, the thick film of organic solvent formed on the fluoropolymer tubing wall at high flow rates may split off droplets by the "ripple" process.

Segmentation, however, is quite regular over a wide range and total length of the extraction coil. A mechanical segmenter, allowing precise timing of alternating segments of organic and aqueous segments, may make it possible to eliminate phase separation altogether. Conventional loop injectors operated by a cycling motor operated with adjustable fill/drain times and a brief intermittent period can be used at low flow rate ratios, or for producing long segments. Phase separation may be eliminated by the use of a specially designed mechanical segmenter, making possible precise timing of the segmented stream passing through the detector cell.

b. Intermittent Pumping

The introduction of reproducible volumes of one phase into a continuous flow of the other can also be realized by the intermittent pumping principle.[94,113] In this case, two microcomputer-controlled peristaltic pumps equipped with stepper motors can produce a reproducible segmentation with a variable segment volume ratio, provided that large segment volumes are acceptable. Unlike classical liquid-liquid extraction FIA in which the immiscible phases are segmented by continuous segmenters leading to the formation of segments with random size, a well-defined segmentation pattern can be generated by the use of alternately operated stepper-motor driven microsyringe pumps.[130] A resulting uniform segmented flow (with approximately 14-μl volume segments) allowed subsequent computer-controlled digital "phase separation".

c. Magnetic Valves

A magnetic valve system (Figure 18) controlled by a cycle timer can also be used to segment two immiscible solvents. The segment length is controlled by the flow rate and by the cycling frequency, and it can be easily varied over a wide range (from millimeters to tens of millimeters) by changing these parameters.[143,145]

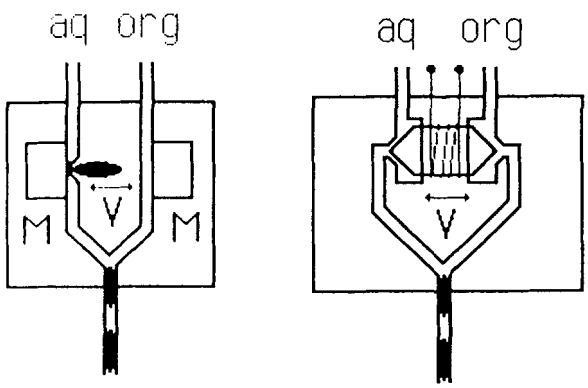

FIGURE 18. Three-port magnetic valves with electromagnet(s) (M) and a magnetic valve (V).

Such systems work reproducibly at moderate flow rates and in a narrow interval of flow rate ratios close to unity. The best reproducibility is usually achieved at a cycling frequency ranging from units to tens of cycles per minute. At higher flow rates or higher flow rate ratios (especially when solvents of different viscosity and interfacial tension are used), serious pulsation occurs, and the segmentation reproducibility decreases rapidly (up to tens of percent, compared to the original reproducibility of units of percent found at $Q_a/Q_0 = 1$). Serious pulsation can also occur if the magnetic valve is operated at a slow cycling frequency.

3. Postsegmenter Sample Injection[106]

A relatively large dispersion occurs in all conventional liquid-liquid extraction FIA systems in which samples are introduced into an aqueous phase carrier stream. The dispersion is lower when samples are introduced into a segmented (rather than unsegmented) flow stream (see Figure 4A and B) since dispersion of the sample during its transport through the reaction tube and phase segmenter is eliminated. Analytically, the sensitivity, calibration graph linearity, and sampling frequency are higher than that obtained using conventional techniques due to the sharper and higher peaks. The peak shapes are also more symmetrical, illustrating that dispersions of sample components, both before reaching and within the segmenter, represent major contributions to peak broadening.

C. Extraction Coils

There are two principal considerations to be made when choosing an extraction coil: the material the coil is made of and the coil dimensions. With respect to the former, the question is whether to use hydrophilic (glass or metal capillary) or lipophilic (fluoroplastics) material. The choice of which coil material to use depends on whether the sample will be extracted from the aqueous into the organic phase or vice versa. The former case is mainly used in classical liquid-liquid extraction systems, whereas the latter is typically used in two-stage extraction systems; however, both types of materials have been used. The choice of coil material can indirectly influence the extraction efficiency and the sample dispersion, changing the film thickness.

The second consideration, concerning the coil dimensions, can affect sample dispersion and extraction efficiency, as related to kinetic efficiency, total extraction yield, and peak broadening. The liquid-liquid extraction process requires that the segmented phases remain in contact while the analyte approaches a state of thermodynamic equilibrium in partitioning between the two phases. The extraction coil can be made sufficiently long that transfer kinetics do not represent a limiting factor in the extraction efficiency. Any increase in coil length beyond this point results in increased sample dispersion without any concomitant increase in sample recovery, and is therefore undesirable.

The extraction coil is usually a relatively narrow bore Teflon tube (0.2 to 1.5 mm), several decimeters to several meters long. The coil geometry, the inner wall surface quality, and the spatial orientation influence the extraction rate, the extraction efficiency, and the yield of the extraction process. To the extent possible, the coil should be free of any sharp changes of inner diameter and sharp bends and edges. Its inner surface should be smooth and completely free of deposits that can change the hydrophobic character of the tube material.

Extraction coils have been used in various configurations of flexible Teflon tubing, but the simplest and the most frequently used coils have a symmetric or helical shape, with a coil diameter ranging from units to tens of centimeters. The horizontal position of the main axis of the coil forces the mixing process, and introduces a secondary flow rate perpendicularly oriented to the axial, which increases mass transport.

The secondary flow patterns are established in response to centrifugal force in coiled extraction capillary. They bisect the capillary profile, reducing the diffusion distance by one half. The radial secondary flow causes effective mass transport, interchanging material in a slower moving streamline with material in a faster one.[113] Hence, mass transport is forced by intrasegmental movement due to the viscous drag of the two

immiscible fluids. Curved and coiled extraction coils should, therefore, give better internal radial mixing in the segments and result in a more effective extraction.

The secondary flow in a coiled tube is disrupted when its coiling radius or its position are changed, and as a consequence, the mechanical forces acting on sample dispersion become altered. It should be noted that a coil diameter of up to 60 or 80 mm does not adversely affect the analytical signal.[73,103] The intense secondary flow can sometimes negatively influence the extraction efficiency.

Coils containing small diameter glass or Teflon beds or glass wool plugs, or which are subjected to high frequency agitation have been shown to promote contact between the two phases in flow analysis systems. Auxiliary methods (e.g., immersion of the coil in a thermostatic bath maintained at an elevated temperature, or subjecting the coil to an ultrasonic field) have also been used to improve mass transfer efficiency. The use of knitted (or even knotted) open tubular waves (which effectively reduce axial dispersion in homogeneous systems) have been found to reduce band broadening for some single phase FIA applications.[94,113] Convoluted coil configurations with small turn radii (as in a serpentine reactor) severely disrupt laminar flow and can be potentially more efficient than a simple coil.[94,113] However, liquid-liquid extraction in a coiled tube is as effective in reducing band broadening as a knitted open tube of the same internal diameter since average peak area variances show no statistical difference.

At very short (or zero) residence times, the relative peak area was always less in a straight tube than in coiled or knitted tubes since some finite time is necessary for equilibrium to be established between the two phases. There was no obvious difference between coiled and knitted tubes, suggesting no real difference in extraction efficiency. Furthermore, there was no reduction of band broadening noted. The coiled tube is simpler to use and has a lower back pressure than other, more complicated configurations. A straight tubing also is the simplest and most frequently used configuration when a very short residence time is preferred. In some cases, segmentation reproducibility and analytical signal parameters are better for straight tubing than for coils.[144]

The viscosity difference between the two immiscible solvents manifests itself in the extraction process as an inhibitor of diffusion. With increasing viscosity, the rate of mass transfer decreases, thereby decreasing radial mixing and increasing the residence time of the sample component(s).

Segment length of the extraction coil is the principal parameter controlling the magnitude of the contact area between the two phases. An irregular segmentation pattern results in losses of the solvent by the wetting process because of a varying interfacial film thickness. Serious coalescence of segments of different sizes is due to differences in the linear velocity of segments having different geometries because of the differences in the viscous drag of the wetting phase. This implies that the segmentation pattern must be under control and constant during the entire analytical procedure.

The enrichment factor (the ratio between the flows of aqueous phase containing the sample and the organic phase) is influenced by the segmentation process. The enrichment factor rarely exceeds 20 in commonly used manifolds. The length of the organic (and the aqueous) segments cannot be reduced indefinitely; the lower limit of organic phase segments has experimentally been estimated to be about 1.5 times the internal diameter of the extraction coil tube.[41] The extraction efficiency decreases markedly if the organic phase droplets become too small to form a continuous film on the tubing wall. Also, the segmentation may be unstable due to coalescence of very small segments during transport through the extraction coil.

The degree of the extraction is a function of the residence time in the extraction coil, which can be varied by flow rate or by the extraction coil length. As noted above, the liquid-liquid extraction process requires that the segmented phases remain in contact while the sample analyte approaches a state of thermodynamic equilibrium via its partitioning between the two phases. Extraction is relatively fast in the extraction coil, and generally attains completeness in several seconds due to the high efficiency of the liquid-liquid extraction FIA system.

The influence of the flow rate and manifold parameters on the extraction efficiency naturally depends on the kinetics of the extraction process related to the mass transport process. The extraction rate increases with decreasing segment length and inner diameter of the extraction tube and residence time, so that narrower tubes and short segments provide more effective extraction. The choice of the material of the extraction coil indirectly influences the extraction efficiency since it is higher for the stripping of an analyte from an aqueous phase to an organic one for PTFE, and is very low for glass. On the other hand, the analyte stripping efficiency from an organic phase to an aqueous is better for glass, while for PTFE it begins to drop off at low Q_0/Q_a ratios.

In general, the extraction efficiency is influenced by extraction coil length, inner diameter, coil material, and, principally, the interfacial area between the two phases. Efficiency is increased with increasing interfacial area, decreasing segment length, and decreasing inner diameter of the coil tubing. Very small segments complicate the separation and extraction process. Longer extraction coils and slower flow rates (a longer residence time, t_r) produce a higher extraction efficiency, but in the case of some reactions, a low extraction efficiency is found due to the slowness of the extraction kinetics.[103] It is sometimes better to use the residence time than the extraction coil length L_{EC} to evaluate the influence of liquid-liquid extraction FIA system parameters.

At higher total flow rates, the analyte extraction efficiency decreases due to a decreased contact time between the two phases. The analytical signal increases with decreasing organic phase flow rate Q_0 for surfactants, using a membrane phase separator. The decrease of flow rate of organic phase is limited by the partial solubility of the organic solvents in the aqueous phase[103] and by having to establish a stable film of the organic solvent on the tubing wall.[13,145] The extraction efficiency starts to decrease at some limiting residence time, t_r (around 10 s for surfactants, using a membrane phase separator) in extraction coils for different total flow rates, and the Q_a/Q_0 ratio is limited to 10 to 12. Higher flow rates Q_t decrease the extraction efficiency, whereas very low flow rates of the organic phase result in a higher relative analytical standard deviation s_r; hence, a compromise is usually made.

The greater the difference between the flow rate of the organic phase and the flow rate of the aqueous phase, the poorer the mixing of the two phases (and, hence, the poorer the extraction efficiency). For example, in chloroform-aqueous systems, as the chloroform total flow rate increases, the analyte peak concentration decreases. The limiting flow rate of organic phase (0.6 ml min^{-1}, in this case) gives more reproducible peaks.

Peak height increases with total flow rate at $Q_a/Q_0 = 2$, up to 1 ml min^{-1}, and then remains constant. The peak width and the peak width at half-height $H_{p(1/2)}$ decreases gradually. The peak height and extraction efficiency E(%) gradually increase with the total flow rate Q_t, while the peak height abruptly increases with the Q_a/Q_0 ratio.

Maximum peak height is obtained for a Q_a/Q_0 ratio close to unity. Above this value, the sensitivity drastically drops, probably due to the high total flow through the separator. The residence time in the separator, then, is shorter. The pressure in the system naturally is increased with an increase in the total flow rate; this may induce turbulence in the individual segments, and thus influence extraction efficiency. It was observed that the flow rate must be decreased in tubes having low smaller inner diameters (ID <0.5 mm) since the pressure becomes excessive to the system.

At total flow rates higher than some limiting value (around 1 ml min^{-1}), the baseline is unstable.[101] At very high total flow rates (Q_t >1 ml min^{-1}, $Q_a/Q_0 = 3$), the baseline is noisy and separation of analytes was poor. At very low total flow rates, the time for each sample is very long and sampling frequency is necessarily decreased.

An increase in the recovery of organic phase decreases the analyte peak area. For reaction systems, when Q_a/Q_0 is constant and close to 2, the peak area A_p and the extraction efficiency E(%) increase with a decrease in total flow rate (since the yield of the reaction in slow processes increases with an increase in reaction time). When the total flow rate is constant, the extraction efficiency E(%) also remains constant as the flow rate of aqueous phase or flow rate of organic

phase changes. The analyte peak height H_p increases with a decrease in the Q_0/Q_a ratio, which results in a great improvement in the sensitivity of the determination of the analyte. Peak height increases with sample volume, V_s, while peak width becomes broader and the slope of the calibration graph shows an upward convexity.[101]

In a study of anionic surfactants in wastewater, the analyte peak height increased with increasing extraction coil length (L_{EC} <250 cm), reaching a maximum at some limited length interval (250 to 500 cm), and then decreasing slightly with the length of the extraction coil due to the sample dispersion at L_{EC} >500 cm.[73] At very high extraction coil lengths L_{EC} and longer residence times (t_r >16 s), analyte peak height is not affected by the other parameters. At some limiting extraction coil length (L_{EC} >360 cm), a variation in coil tube inner diameter from 0.35 to 0.70 mm had no effect on analytical signal when an extraction coil volume V_{EC} = 700 µl was held constant.[103]

At different extraction coil lengths, the extraction rate differs for different analytes (e.g., for sodium extraction, the rate is slower than for potassium[101] due to the extraction kinetics of their ion pairs with a crown-ether and an appropriate cationic dye). However, the slow extractability of anionic surfactants is well known and the ion associates extraction in continuous flow analysis requires a longer mixing coil to increase the extractability.[6]

The use of a longer extraction coil tube (several meters, compared to 1 and 2 m) gives the better reproducibility of anionic surfactant peak height and stability of the baseline than obtained using a shorter coil.[104] With increased extraction coil length L_{EC}, peak height increases, but reproducibility of the peak height decreases.[6]

The analytical signal increases with sample volume V_s (up to some limit), and then is constant. Furthermore, peak height increases with sample volume V_s injected into the liquid-liquid extraction FIA system and reaches a constant value (corresponding to a steady state) while the peak width becomes broader. The steady-state method using continuous pumping of the analyte into the system is more convenient and reliable, and it is actually faster than the injection technique (due to the added dispersion in the sample loop with the latter technique).

The peak area did not increase linearly in some cases due to dispersion of the sample during its transport through the system. However, there was good linearity between sample volume and peak area, such that the use of peak area for constructing calibration graphs was usually superior to the use of peak height when large sample volumes were injected.

D. Phase Separators

The phase separation process involves a partitioning of the segmented phases after the extraction has been completed in the extraction coil, in such a manner that the unwanted phase is directed to waste while the other phase(s) is resampled or pumped through the detection system. In most practical separators, the two-phase system cannot be desegmented totally into two pure individual phases. Typically, phase separation efficiency is 80 to 100%.

The phase separator is one of the most important components of a liquid-liquid extraction FIA system. The correct performance of the phase separator is one of the keys to successful development of the separation process, wherein the aqueous and organic phase flow rates must be very precisely controlled. The ideal phase separator should provide complete separation of the phases with very little waste of the resampled solvents.

The phase separator is designed to handle small volume segments of the two immiscible phases, received from the extraction coil in the form of a segmented flow stream. The objectives of the phase separator are to:

1. Continuously separate the phases carrying the extracted analyte and to continuously split them into two or three individual streams in such a manner that one or two of them may be used for determination of the analyte concentration
2. Work properly over a wide range of flow rate ratio Q_a/Q_0 and total flow rate Q_t

3. Recover as much as possible of the desired phase (approaching 100%)
4. Maintain the concentration profile of the analyte and prevent deterioration of the original concentration gradient of the sample
5. Prevent any further sample dispersion and dilution during the separation process (band broadening)
6. Handle the very small segment volumes of both phases (usually 1 to 30 μl)
7. Handle different types of organic solvents

The phase separator must also be made of materials that are chemically inert (glass, stainless steel, fluoroplastics, etc.) to attack by solvents and chemicals. The long-term stability of the phase separator should be good, and it should be easy to operate. Preferably, no adsorption should occur, and the volume of the cavities or grooves in the separator should be kept as small as possible.

It is also necessary to adjust the phase separator so that one (or two) streams contain one phase only, while the waste stream contains the other phase (as well as small amounts of the first phase, including possible emulsions). The phase separator also requires special care to avoid contamination of the detector flow cell with the aqueous phase; however, it frequently suffers from contamination at the beginning of the separation process. Hence, correct operation of the phase separator is critical to good signal baseline stability and S/N ratio in the detector.

Consequently, the phase separator has been an object of considerable study concerning its design, construction,[13,24,32,49,74,91,106] and operational theory.[49,91] Several models have been designed with the aim of improving phase separator characteristics, and currently, almost as many phase separators as applications exist.

The design of phase separators with respect to internal volume is important; a relatively large analyte dispersion can occur when the extracted sample passes through most types of phase separators, influencing the analyte band-broadening process, peak width, time of the analysis, and the sensitivity, selectivity, and precision of the analytical method.

The magnitude of the preconcentration (enrichment) factor in continuous liquid-liquid extraction flow injection analysis is directly proportional to the flow rate ratio Q_a/Q_0, and is limited by the moderate aqueous to organic phase flow ratio that can be handled by the phase separator. Aqueous-to-organic phase flow ratios of not more than 5 to 20 generally must be used to obtain reproducible measurements.

Typical organic solvents (e.g., chloroform, Freon-113, MIBK) separate exceptionally well from water, do not readily produce stable emulsions, and easily pass through the porous Teflon membrane if an overpressure is applied in the waste channel. Therefore, total separation of the organic and aqueous phases should be possible using these solvents over a relatively wide range of the flow rate ratio Q_a/Q_0 and total flow rate Q_t (not true for solvents such as 1-octanol and 1-propanol).

Current phase separators for liquid-liquid extraction flow injection analysis systems can be divided into three groups:

1. Density (chamber) separators, working on the principle of the density difference between the two phases
2. Affinity (heterogeneous material) separators that are constructed from a lipophilic (usually PTFE) and a hydrophilic (e.g., glass, stainless steel, etc.) material, working on the principle of the affinity difference between the phases and the separator materials (as well as the density difference between the two phases)
3. Membrane phase separators that use a lipophilic (and/or a hydrophilic) membrane to exclude one or both phases from the segmented stream

In most cases, the species of interest are extracted from the aqueous segments into the organic segments, and a portion of the organic phase is separated from the segmented two-phase continuous stream. It also is possible to separate the aqueous phase from the segmented stream using a hydrophilic membrane in a multistage or back extraction procedure.

Simultaneous monitoring of both phases has attracted interest recently. This has been used for the simultaneous determination of extractable and nonextractable species using two separators con-

nected in series[31] or a single dual-membrane separator.[51,78] Systems without phase separation using fast reading photometric or fluorimetric detectors also have been studied.[18,102,130,132,133,142–147]

1. Gravity/Density Separators

Density phase separators made of Perspex or PTFE and having an inner volume of the separation chamber of approximately 100 µl utilize the density difference between the solvents to separate the segmented flow stream into two individual streams. Such separators can be used with organic solvents which are heavier or lighter than water by proper choice of an outlet tube (see Figure 19A) to connect the separator to the detector. The geometry of the separator internal separation chamber and capillary system and also the magnitude of the internal volume (tens or hundreds of microliters) of the separator are important to successful separations. The pressure in the detector line should be slightly less than the pressure in the waste line in order to avoid contaminating the flow cell with droplets of the discarded phase.[3]

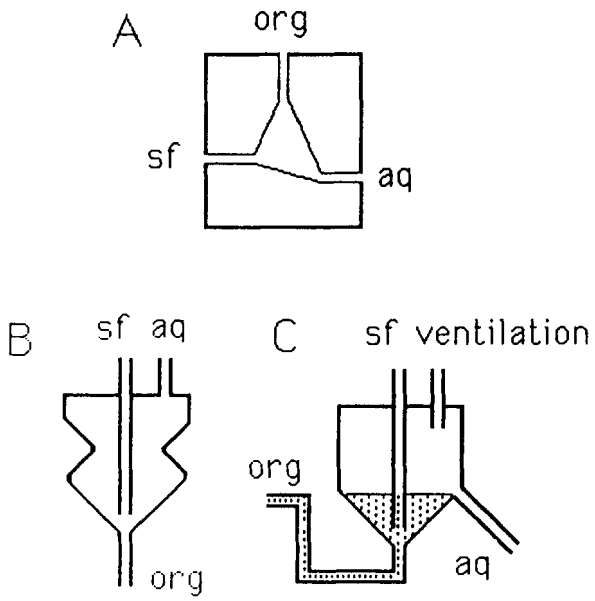

FIGURE 19. Gravity/density phase separators with a conical chamber (A), a separating funnel-type phase separator (B) and an improved phase separator (C).

Improved density phase separators for surfactant analytes were made of glass or fluoroplastics (see Figure 19B and C), from which the heavier organic phase passes to the detector and the aqueous phase (containing traces of organic phase) passes to the waste through two outlet channels (2 mm ID). This type of separator was constructed[32,67] to overcome the changes in permeability of the membrane which occur in the presence of surfactants.[67] The lower portion of the phase separator resembles and functions like a separatory funnel. A difference in the internal volume of the separation chamber ranging from 250 to 350 µl does not influence the phase separation and has little effect on the dilution of the extracted analytes. This improved density phase separator increases the phase separation efficiency and magnifies the problem of the changing permeability of membranes caused by complex matrices containing surface-active substances such as surfactants. It is more efficient than the "tee" segmenter in terms of the quantity of reaction product being separated.[67]

Degassed and chilled solvents are preferably used to prevent vaporization of the organic phase, causing formation of bubbles of organic vapor. Particular care is required to avoid the inflow of aqueous phase into the flow cell, which frequently occurs at the beginning of the operation. Successful separations are also impaired by high contents of methanol or other polar solvents in the extraction system ($\phi > 10\%$).

The utility of a two-phase separator system, consisting of T-piece and membrane separators (used by Kawase et al.[6] for the analysis of anionic surfactants) and having a suppression coil behind the flow cell, prevents evaporation in the flow stream. However, its practical utility for complex samples is questionable since a high content of a polar solvent such as methanol results in baseline drift and noise due to the formation of a cloudy solution in the flow cell.

2. Affinity Phase Separators

This separation device consists of a glass A4 T-connector (Technicon, Tarrytown, NY), with Teflon fibers twisted together into a thread,[1] or the use of Teflon filaments or strips[20] inserted

into the bend from the inlet into the outlet tube intended to transport the organic phase to the detector. This type of lipophilic insert is used to direct the organic segments into the outlet channel where they are rejoined to a continuous stream. A glass-lined stainless steel T-piece[95] and a combined glass/Teflon capillary T-piece[50,66] have also been used (see Figure 20).

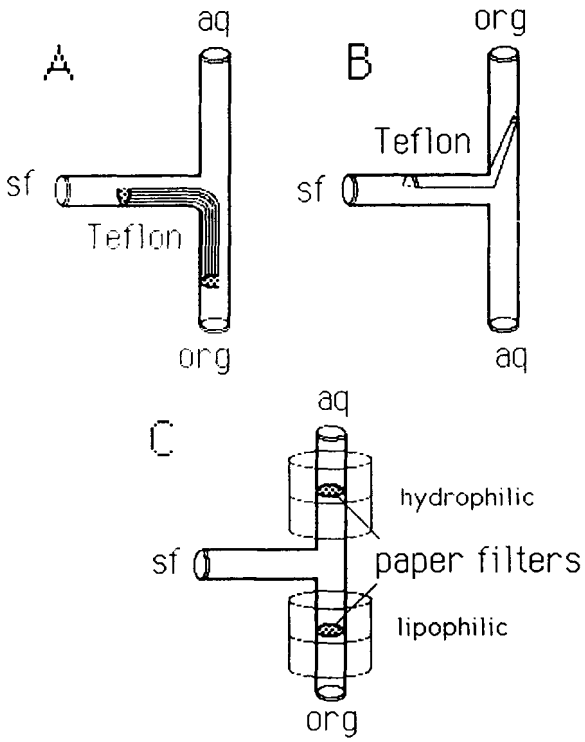

FIGURE 20. Affinity separators with a Teflon fiber bundle (A), Teflon strip (B) and with the hydrophilic and lipophilic paper disks for additional separation of the phases (C).

By the use of differential pumping or by applying a small overpressure produced by a restriction coil or a needle valve, the aqueous phase and excessive organic phase are forced into the second outflow channel of the fitting, and out to waste. In this manner, the aqueous phase is prevented from contaminating the flow cell. Special care must be taken to avoid contamination of the Teflon insert with the aqueous phase, and cleaning by rinsing is a tedious but necessary operation. The Teflon insert should be placed rather close to the wall of the inlet tube and be allowed to bend smoothly and in parallel with the walls of the inlet and outlet channels.

The separation process is facilitated by a significant difference between the densities and interfacial tensions of the two phases. The affinity phase separator works well with organic solvents such as chloroform, MIBK, or Freon-113, but it cannot be used for the separation of organic phases having a low interfacial tension and small density difference with respect to water, such as pentanol and *n*-butanol. High levels of polar solvents such as methanol (ϕ >10%) also impair the separation. It also is very difficult to achieve successful separations using the density or affinity phase separator in complicated extraction systems such as water/methanol/chloroform.

The density difference between the two phases also is used to advantage by letting the heavier and lighter phase out in a downward or upward direction, respectively. Appropriate vertical positioning of the main axis of the phase separator and horizontal positioning of the inlet tube must be arranged for the separation to work properly.

The relatively large volume of the separation chamber causes band broadening. The total flow rate can exceed 5 ml min^{-1}, but the optimum is about 4 ml min^{-1}. Air bubbles do not influence the performance of the T-shaped phase separator.[73] The separation efficiency of the T-shaped phase separator was found to be 85% that of the membrane phase separator.[103]

To overcome the problems caused with the contamination of the flow cell with droplets of water in the organic phase, a T-shape separator with hydrophilic and lipophilic paper disks was constructed.[32] The separator consists of a Daiflon body with three perpendicularly oriented channels, working on the difference in density of the two phases and employing two phase-separating filter papers to complete the separation of the phases (see Figure 20C). The aqueous phase passes to waste through the hydrophilic paper (cellulose) disk pressed between two washers. The organic phase was directed into the flow cell after passing through a lipophilic phase-separating, silicone-treated paper disk, also held between two washers. Both paper disks had a durability of at least 1.5 h, after which they had to be changed. This requirement unfortunately limits the practical applicability of this system.

3. Membrane Phase Separators

Kawase et al.[6,15] were the first to develop a phase separator with a PTFE porous membrane that is permeable to organic solvents, but not to aqueous phase. This is because of its high surface tension to the aqueous phase. The membrane phase separator is (currently) usually preferred to the older separator concepts utilizing differences in affinity or density (which, however, can prove superior with very complicated matrices[67,108] or technological processes). In practice, membrane phase separators are superior to affinity/density phase separators, but they have the disadvantage of requiring frequent membrane replacement.

In order to design an efficient membrane phase separator, the following factors should be considered:

- The membrane material
- The area of the membrane exposed to solvent transfer
- The volume of the cavities or grooves of the phase separator
- The impinging angle of the segmented flow on the membrane
- The long-term stability of the separator
- Ease of operation

The selection of a suitable membrane material for the flow system depends on many factors that may vary during experiments. These include the flow rate, the ratio between the flow rates, the physical and chemical properties of the solvents, chemicals and reagents, the mechanical properties of the material, the number of samples, and the separator design.

Fluoroplastic porous membranes of different pore size (0.5, 0.8, 1, 10 μm [typically, 0.7 to 0.9 μm]) and thickness from different suppliers (e.g., Sumitomo Electric Ind. Co., Japan; Millipore, USA; Norton Chemplast, USA; Enka, Wuppertal, Germany), hydrophobized cellulose acetate or nitrate (Millipore, USA or Whatman, Germany), and hydrophobized paper 1 PS (Whatman) have been used.[32,96,101,103,119,121]

Fluoroplastic porous membranes are more durable, more hydrophobic, and commercially available in a wide choice of pore sizes, thicknesses, total diameters, and backings. Cellulose acetate or nitrate membranes are much less hydrophobic than PTFE membranes, and they also easily adsorb water on their porous surface when dry. Before starting the separation, it is necessary to wet each membrane of the phase separator with appropriate organic solvent to prevent leakage and breakthrough of the solvents through the membrane. This is more important for hydrophobized cellulose membranes than for Teflon membranes. They are also less durable than PTFE membranes.

The silicone-treated, phase-separating filter is less expensive and does not expand or contract with change in pressure. It gives a similar separation efficiency and can be used for separation of the systems with a simple matrix, but its lifetime is significantly shorter than for other types.[32,119,121]

Membrane phase separator designs can be used with a wider variety of water-immiscible solvents, compared to density or affinity phase separators since a density or affinity difference between the two solvents is not strictly required. At low flow rates of both polar and nonpolar solvents, they can be used without any observed contamination (see Table 2). They also can be used when one phase is a mixture of polar solvents (e.g., 50% aqueous methanol).

The membrane phase separator is compatible with high flow rates and flow rate ratios, which can reduce the time of the analysis. As the flow rate ratio Q_o/Q_a is decreased, the "breakthrough" of the aqueous phase occurs at some limiting value[22] at $Q_o = 0.7$ ml min^{-1}, depending on the total flow rate Q_t.

Membrane phase separators can handle only a limited range of flow rate ratios ($Q_a/Q_o < 20$ to 30), partly because small differences in pumping rate can cause the aqueous phase to penetrate the membrane to the organic phase stream. No difference in performance was observed for 0.5-μm pore polypropylene packed Gore-Tex membranes in comparison with 0.2-μm pore Gore-Tex Teflon plumbing tape at slow flow rates. However, unpacked membranes ruptured at a total flow rate of $Q_t > 8$ ml min^{-1}, but polypropylene packed membranes could be used at higher flow rates.[92]

Membranes having pore sizes >1 μm or <0.45 μm were not suitable (see Table 3). The organic phase could easily pass through large

TABLE 2
Some Phase Systems Usable for PTFE Membrane 1 μm (polyethylene backing)[13] in Sandwich Membrane Phase Separator[a]

Phase mixture	ml min^{-1}		
	Q_a	Q_o	Q_s
n-Butanol/15 m*M* nitric acid	0.7	0.6	0.6
n-Pentanol/15 m*M* nitric acid	0.9	1.0	1.0
n-Octanol/15 m*M* nitric acid	1.0	1.0	0.7
Isooctane/water	0.9	1.1	1.0
Chloroform/water + methanol (1 + 1)[a]	0.9	1.1	0.7
Toluene/water[b]	0.9	1.0	0.8
Cyclohexane + *n*-pentanol (8 + 2)/water	1.1	1.1	0.9
Cyclohexane/ethylene glycol	0.9	1.0	0.8

Note: Maximum tolerable flow rates of aqueous Q_a, organic Q_o, and separated Q_s organic phases in ml min^{-1}.

[a] 35 × 2 × 0.3 mm grooves, Perspex bodies.
[b] 35 × 1.5 × 0.5 mm grooves, Teflon bodies.

TABLE 3
Characteristics of Some Membrane Materials[13]

Membrane	Pore size (μm)	Max. tolerable parameters			Membrane	Pore size (μm)	Max. tolerable parameters		
		Q_2	Q_2/Q_0	Q_t			Q_a	Q_2/Q_0	Q_t
PTFE/u	0.02	NB	NB	NB	FP/1	3	1.6	3.2	1.8
FP/u	0.2	4.3	8.6	4.0	Mitex/u	5	0.8	1.7	1.1
FP/l	0.2	3.3	6.6	4.0	Mitex/u	10	0.8	1.7	1.0
FP/l	0.5	2.3	5.2	4.0	Cellulose[a]	1	0.9	1.1	0.8
FP/u	0.5	2.1	4.2	4.0	Cellulose[a]	0.8	0.9	1.1	0.8
FP/u[a]	0.5	0.9	1.1	0.8	Cellulose[a]	5	0.9	1.0	0.6
FP/l	1.0	2.3	4.8	4.0	PTFE tape[a]		0.8	1.0	0.7
FP/l[a]	1.0	0.9	1.1	0.8	FP/u[a]	5	0.4	0.4	0.3

Note: FP: fluoropore; NB: no breakthrough, cellulose acetate and/or nitrate; l: laminated; u: unlaminated.

[a] Maximum tolerable flow rates of aqueous Q_a, organic Q_o, and separated Q_s organic phases in ml min^{-1}; Q_t: total flow rate at $Q_a/Q_o = 1$.

pore size membranes, with a separation efficiency close to 100%; however, after a few hours, the aqueous phase would also pass through the membrane of the smaller pore size.[104] Membranes having pore sizes in excess of 3 μm show instantaneous leakage of the separated solutions.[77]

Small pore size membranes had a lower efficiency (ca. 50% with 0.45 μm). Membranes of intermediate pore size exhibited 100% separation efficiency, with easy adjustment of the backpressure via a needle valve. A constant separation efficiency was found for the 0.2- to 0.5-μm pore size.[103] The influence of the backing of the Fluoropore membranes on the mass transport rate was found to be negligible.[77]

For the membrane phase separator, analyte peak broadening was found to be inversely dependent upon the fraction of the aqueous phase removed from the segmented stream.[1] An almost linear relationship between the peak height and the fraction of the organic phase separated was found. The use of small internal volumes reduced band broadening.

The membrane phase separator causes a lower dispersion than the density or affinity phase separator, especially at higher ratios between the

phase flow rates. A similar dependence of the separation efficiency on flow rate was found for the membrane phase separator and the T-shaped affinity separator. The separation efficiency of the membrane separator was higher — that of the T separator being only 85%[103] or 79%[73] of the corresponding membrane phase separator efficiency. Most parameters were identical for the two types; however, the flow rate range was wider (Q_a of 2.6 to 4.6 ml min^{-1}) for the membrane phase separator (compared to 2.8 to 3.6 ml min^{-1} for A4-T at Q_0 = 0.4 ml min^{-1}).[103]

The analyte peak shape depends on the fraction of organic phase (recovery) that passes through the membrane. The peak height decreases and peak width increases with a decrease in the recovery of the organic phase. When two FALP 04700 membranes were wetted with MIBK prior to use according to the manufacturer's instructions,[73] the analytical signal increased with increasing Q_a/Q_0 (up to 10) at Q_0 constant[7,8] and rapidly decreased at higher values for the membrane and A4-T phase separators. The signal also increased with decreasing organic phase flow rate Q_0 at constant Q_a, with the decrease limited by the dissolution of MIBK in the aqueous phase.

The complex matrix of some samples adversely changes the permeability of the initially hydrophobic membrane (especially samples containing surface-active substances such as anionic surfactants), causing large changes in the separation efficiency and in the sensitivity of the method. It is difficult to achieve successful separations by a membrane phase separator in such complicated systems. Surfactants may be preextracted in the form of ion pairs, surface-active reagents can be introduced as extractants by the merging zone, or intermittent sample pumping techniques can be used to reduce or eliminate this influence. A significant sorption of surfactants on the membrane filter surface can be observed,[121] causing a longer tailing of the peak. This was more evident using silicone-treated phase separating paper filter 1 PS (Whatman).

Wastewaters containing protein substances adversely affect the separation process because the organic phase is unable to pass the membrane of the circular phase segmenter (38/5 μl for a segmented stream and organic phase chambers, GVHP 0.22 μm from PVDF or FALP 1 μm from PTFE membranes, Millipore) when neutral buffered solutions of Methylene Blue are employed. An improved efficiency is obtained at pH 2 (protein-containing substances adsorb on the membrane) since the clogging process is minimized at lower pH by a change in the electrical properties of proteins. The membrane-clogging process decreases the separation efficiency, and additional variations in the back-pressure result in low reproducibility. The membranes are also susceptible to fouling by microparticulate matter present in samples, thus greatly affecting their lifetime and performance.

Air bubbles greatly affect the regular performance of the membrane phase separator since air bubbles will pass through the membrane and occupy dead volumes in the joint and recipient portion of the separator (and also in some parts of a detection system), which results in irreproducible measurements. Complete removal of bubbles from the system is a somewhat tedious but necessary operation that results in a decreased sampling rate.[73] Introduction of air bubbles into the extraction system markedly affects the extraction efficiency of the membrane phase separator.[103]

A small overpressure in the waste channel improves the separation by forcing the organic phase to pass through the Teflon membrane (the pressure necessary to force a liquid through a semiporous membrane barrier is directly proportional to the wettability of the membrane material by the passing liquid). This overpressure is usually adjusted by variation of the geometry of the restriction column or coil, or by a needle valve. With sufficient overpressure, the separation becomes complete and no aqueous phase can be found in the organic phase flow. Porous PTFE membranes are preferred to Teflon tape because of their greater mechanical strength and because they require less overpressure. Total separation efficiency (100%) can be achieved by adjusting the overpressure across the membrane by using the needle valve.[104]

Large irregularities in the segmentation pattern may, however, result in occasional organic droplets in the aqueous phase. The separation efficiency is also influenced by the density and the affinity of the organic phase toward the membrane material. In such cases, the spatial orien-

tation (the vertical position[22]) of the membrane phase separator will influence the separation process. Higher differences facilitate the separation process. Interconnection of both flows after separation and detection to the common waste outlet,[65,74] or removing the phases from the separator at a constant flow rate using an additional peristaltic pump, is sometimes necessary to balance the pressure and to diminish the risk of solvent evaporation in the separator. Better reproducibility and lower baseline noise result from this arrangement.

Membranes with backing are more durable than membranes without backing. The total lifetime of the porous Teflon membranes was such that the aging of the material interrupted the continuous liquid-liquid extraction process, and the membrane had to be changed.[73] The lifetime of two 1.0-μm pore size Fluoropore membranes (total lifetime, 2 to 3 h) was greater than that of two 0.5-μm membranes (ca. 1 h) or that of a single membrane of either pore size (1 h and 20 min, respectively). The polyethylene backing membrane provides a working time of several days and can be used at higher total flow rates.[103,107]

Unlaminated membranes performed better than the corresponding laminated membranes of the same pore size. Laminated membranes were also more difficult to seat properly in the phase separator; it was necessary to over-tighten the separator halves to prevent leaking, placing unusual stress on the Kel-F body. The smaller the pore size, the greater the range of useful flow rates. The 0.2-μm unlaminated membrane gave the best results; a flow rate of 0.5 ml min^{-1} of chloroform in combination with an aqueous flow of 4.3 ml min^{-1} of water was possible.[152]

A large membrane area is beneficial for improving the separation efficiency, but sample dispersion usually increases and the lifetime of the membrane decreases with membrane size. The lifetime of circular membranes can be improved by using support screens of different materials (metal, Teflon net, perforated Teflon,[22] borosilicate microfilter glass disks, etc.), or by using polyethylene-backed membranes.

To prevent mechanical destruction of rectangular membranes, different porous support materials are used as a filling material inside the grooves of the sandwich membrane separator (powders or fibers of Teflon, PTFE nets, PTFE or glass supports, or stainless steel nets coated with PTFE). The lifetime is increased several-fold. The area available for mass transport is decreased in both cases.

A phase separator with straight grooves (2 × 40 × 0.8 mm) filled with porous polyethylene material[74] in its lower part (organic phase inlet) and unfilled upper part (2 × 40 × 0.4 mm groove) also prolonged the lifetime of the membranes.

The lifetime of a single membrane is longer at lower Q_t and in the absence of disturbances caused by injection in the segmented flow system.[108] Membranes of GVHP have longer endurance and lifetime (8 h), but problems with the analysis of highly polluted water samples occur; hence, PTFE membranes with a PTFE-coated filter support are preferable in the case of samples with very complicated matrices.

The working principles of the groove cavity type separator and the cylindrical cavity type phase separator differ.[121] In the cylindrical cavity, a thick layer of the organic phase always covers the surface in contrast to the alternating segmented stream flowing along the membrane of the groove type separator, continuously wetted by thin film. The cylindrical type does not work effectively with two-phase systems of low density differences, but works well with systems such as water/chloroform. In the groove type, the density difference does not significantly affect the separation process.

a. Sandwich Phase Separator

The sandwich phase separator consists of two pieces of chemically inert material (e.g., Perspex, Teflon, stainless steel, titanium, etc.), each having a groove facing the membrane (see Figure 21). The two identical grooves, measuring a few centimeters in length, are separated by a strip of Teflon or hydrophobized acetylcellulose membrane which allows the organic phase to pass from one groove to the other. The grooves can have a constant cross-sectional area or (better) a linearly increasing area in the outlet portion and a linearly decreasing area in the segmented por-

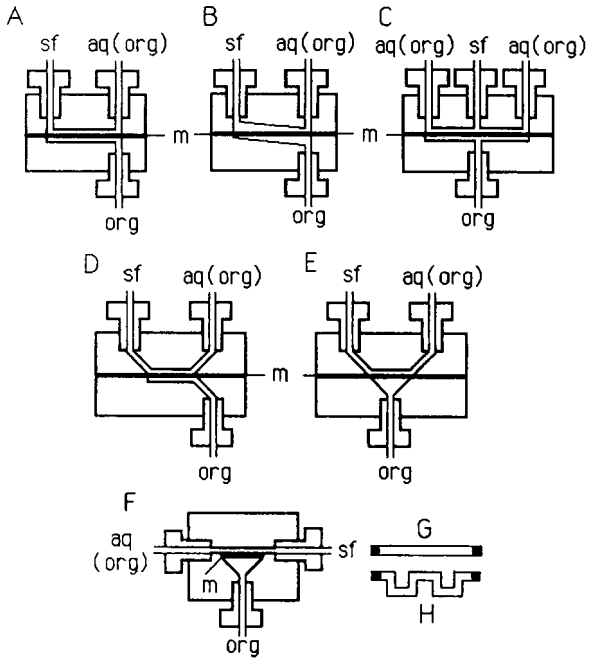

FIGURE 21. Sandwich separators with constant (A, C, D) and linearly increased/decreased (B, E, F) grooves and inlet channel situated at the ends (A, B, D, E, F) or in the center (C) of a donor groove; the separators with the conical acceptor groove and outlet channel situated in the bottom of the groove (E, F); the different possible shapes of the grooves are depicted below (G, H).

tion of the phase separator body, respectively. Sloped grooves (usually 2 mm maximal depth, 2 mm width) provide a better separation efficiency.[100,105,119] Straight, meandering[98] or concentric spiral grooves[74] have also been used to increase the active surface area available for mass transport. The meandering groove provides greater analyte peak heights.[98,106]

The assembly is bolted together using metallic plates and screws. A gasket of suitable material (neoprene,[35] Teflon) or having a mirror-polished surface is sometimes used to prevent leakage of the two phases.

The inlet and outlet tubes are oriented perpendicularly or at some angle to the phase separator main axis. The inlet tube and the outlet to waste are situated on one side, and the outlet taking the appropriate phase to the detector is on the other side of the phase separator body. The tubes are usually located at the ends of the grooves, or the inlet tube can be centrally situated.[35] The separator is positioned to facilitate the separation process based on the density difference between the phases.

The volume and geometry of the phase separator grooves are fundamental parameters for the separation process. The volume should be at least four to five times larger than the volume of an individual segment of the aqueous phase because the groove must not at any time be filled only by the aqueous phase. If this condition is not fulfilled, the aqueous phase will penetrate through the membrane and also damage the membrane. Since a typical segment volume is about 1 to 10 μl, the minimum volume of the inlet groove should be 5 to 50 μl.

The relatively large groove volumes (0.3 ml on each side) of the phase separator and pumping off the organic phase from the separator through the pump provides a high phase separation efficiency and makes it possible to tolerate small irregularities in the phase segmentation. Adding another organic flow into the system compensates for the loss of organic phase resulting from the partial solubility of the organic phase in water and eliminates organic solvent evaporation. This prevents subsequent formation of small bubbles of organic vapors and formation of aqueous droplets after the segment plug, both penetrating through the membrane.[92]

Increasing the membrane area, which can be achieved either by making the grooves shallower and wider, or by making the grooves longer (or both), increases the separation efficiency. The volume of the phase separator must, however, be kept as small as possible since dispersion increases correspondingly.

The extraction rate and the extraction efficiency depend on the groove geometry (length, depth, width, slope), the membrane material, the physical properties of the immiscible liquid phases (their densities, interfacial tension, viscosities, etc.), and on the overpressure across the membrane profile. This type of phase separator (20-μl internal volume) can separate up to 70 or 95% of the organic phase from a segmented flow stream over a wide range of total flow rates (95%[103] and 99% for benzene[110]). The efficiency can be increased by the application of a slight overpressure, but a high overpressure will cause

leakage of the phases or mechanical damage to the membrane.

Several kinds of phase separators employing a porous PTFE membrane (0.8-μm pore size, Sumitomo) were tested by Motomizu and Oshima[93] in order to improve the efficiency and stability of the phase separation. The phase separator bodies were made of poly(chlorotrifluoroethylene) (CTFE) and the membrane chambers inside were made as small as possible. Phase separators having rectangular membranes were found to be more efficient (ca. 100% recovery) than separators having circular membranes (85 or 72%), and the best, smoothest, and most efficient phase separation over a long period of time was obtained with the shaped groove phase separator of type 1A. Segmenters with a cylindrical cavity give a higher sensitivity than that of a groove cavity.

Separators with cylindrical cavities in a "segmented" part and conical cavities in an "unsegmented" part of the main body of a different depth (0.3 to 3.1 mm) and different diameters (0.7 to 5 mm) give an organic phase recovery in excess of 99%, except those with a cylindrical cavity depth under 1 mm, by adjusting the overpressure with a needle valve.[113,121,125] Varying the membrane support ring diameter and the dimensions of the conical cavity, the same recovery is achieved and the sensitivity increases with a diameter increase of up to 4 mm. The organic phase recovery decreases to 40% when the membrane filter comes into contact with the conical cavity walls. The duration of the signal is approximately the same for PTFE membranes of 0.5- and 0.8-μm pore size and for phase-separating paper filter 1 PS (Whatman).

A design using a relatively small membrane area (18 mm^2) without any support screen ensures a high effective area.[100] To ensure a high separation efficiency, the entry to the membrane groove was slanted at an angle, so that the segmented flow was directed toward the membrane surface. A sharp slant with a large angle will improve the impact of the flow on the membrane, but if the groove area is kept constant, the volume will increase accordingly, and this will inevitably lead to increased dispersion. The depth of the 10-mm long groove was made 2 mm at one end and 1 mm at the other, the cavity volume thus being only 27 μl. The resulting slope was sufficient to ensure efficient transfer of the organic phase (efficiency 97%; single layer of membrane lifetime, 20 h; flow rate ratio MIBK/water, 22). A groove inlet configuration seems preferable to the chamber inlet configuration.[74,93]

b. Circular Membrane Phase Separators

In comparison to the rectangular phase separator, a phase separator using a circular membrane has the advantages of commercial membrane availability and ease of manipulation. A typical example is a phase separator having a small circular cavity of 10 mm ID in which the segmented flow is impinging at an angle toward the membrane (see Figure 22). The polyethylene-backed membrane is supported by a Teflon-coated filter screen to improve its durability and long-term stability under the high pressures created by high flow rates. The separation efficiency of one

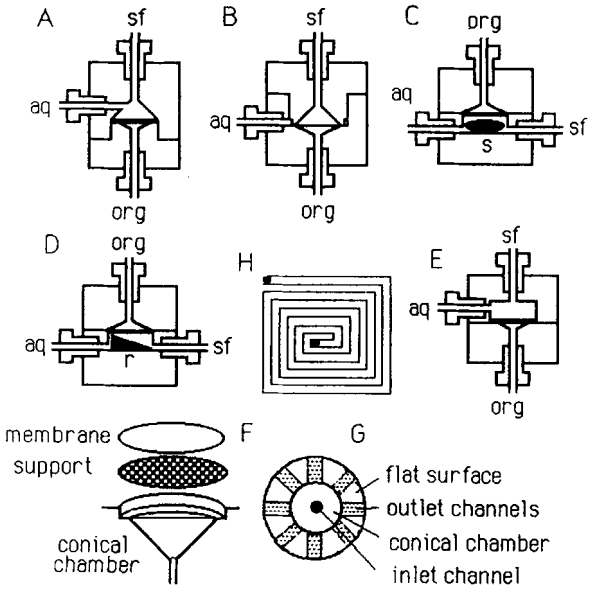

FIGURE 22. Circular membrane separators with conical chambers (A, B) and different orientation of the outflow channel, circular membrane separators with cylindrical donor and conical acceptor chambers (E) with magnetic stirrer (C) or conical Teflon road (D) inside the chamber, eventually, positioning a membrane and a support inside the conical cavity (E), a view of the lower part of the conical chamber (F), and a view of one of several possible forms of an acceptor cavity.

such design[80] was 99%. A membrane phase separator with a rigid support having two cylindrical cavities of a volume of 2.8 μl each was also used.[85]

Supports (perforated Teflon, etc.) are necessary to prevent mechanical damage because the active membrane area is usually larger than in the sandwich phase separator. Positioning the membrane horizontally increases the lifetime of the membrane, as the separation process is forced by gravity.[22] The separation efficiency is usually near 100%.

The total active area of the membrane usually decreases with decreasing total inner volume of the cavities. The remaining area contributes to dead volume, which increases dispersion of the sample. An improved ratio of active/dead area was achieved by using a concentric spiral groove[74] or a special design of the lower portion of the donor chamber (see Figure 22F and G), which has a function similar to that of the multichannel straight groove separator. The segmented flow enters the chamber through a central capillary and passes along the lipophilic membrane to the outflow channels inside the outer part of the chamber. Organic phase penetrates to the acceptor groove, and aqueous phase (with a portion of nonseparated organic phase) is collected inside the "ringlike" orifice and drained off through the outlet channel.

Another phase separator[6] system (Hitachi HPLC gradient mixing chamber, Model 635) consists of two blocks with a gradient mixing chamber (body, joints, ring, and stirring bar are of PTFE), utilizing a porous PTFE membrane which is pressed into the inside flat surfaces of the PTFE ring to make intimate contact with the ring. The stream of segmented phases enters the mixing chamber (9 mm ID, 2 mm depth), and the phases are separated by the PTFE membrane. The stirring bar is rotated during passage through the system (Figure 22C). With an increase in the inner volume of the separation system, sample dispersion increases as the exchange of chloroform on the PTFE membrane surface slows; however, considering the separating capability, the surface area of the membrane should be as large as possible. A stirring bar was introduced into the cavity in order to decrease the inner volume. Improved efficiency and higher and sharper analyte peaks were achieved when the stirring bar was rotated. This was said to be due to the forced exchange of organic phase on the surface of the membrane. It was first used by Kawase et al.[6] in combination with a "tee" separator.

Ogata et al.[24] compared the groove-type phase separator having a more compact design with a circular membrane in which the segmented stream was directed onto the slanted surface at the end of a 3-mm diameter Teflon rod fixed in a circular chamber (Figure 22D). The flow direction was thus toward the membrane where the separation occurred. The CTFE body consisted of an inlet (0.8 mm ID), two outlet channels (0.8 mm for organic phase or 1 mm ID for aqueous phase), and a separating compartment, all made from CTFE. A small piece of cylindrical PTFE rod (r) with the top cut at a 45° angle was fitted to the bottom of the separating compartment, and a polyethylene-backed porous Teflon membrane (FALP Millipore) was sandwiched between the CTFE plug and the orifice. The cut surface was adjusted to point at an angle toward the inlet, allowing the organic phase to be directed to the membrane surface. The organic phase being sucked through the membrane was led to the flow cell through the outlet tube (o) atop the membrane phase separator. The aqueous phase and the excess organic phase are discarded through the outlet after taking a circuitous route around the PTFE rod.

This design causes less dispersion than the classical sandwich membrane phase separator, especially when large volume fractions are passed through the Teflon membrane. A significant decrease of dispersion actually occurs when the volume (0.25 to 0.65 to 0.35 to 1) of the separated fraction increases (35 or 40% peak width of BCG or caffeine). This compares to a decrease of 22 or 20% for the sandwich separator, which is also inferior to the new design with respect to dispersion because of the large surface area and the longer surface of the membrane in the sandwich separator (7 mm^2/70 mm^2 for 2 × 35 mm membrane).

An engraved phase separator having a membrane supported by a Teflon-coated steel grid prevents the membrane from expanding into the acceptor chamber used for the organic phase (Figure 22F to H). The segmented stream enters

the phase separator from below at the center of a circular membrane via a perpendicularly drilled channel, and travels through an engraved coiled channel in contact with the membrane before leaving the membrane area at the periphery. The volume of the receptor chamber for the organic stream is 10 μl.

The coiled groove and straight groove separators had similar properties with respect to sample dispersion (see Table 4). The disadvantage of the straight groove separator is that it is inconvenient to operate. Also, circular membranes are more easily applied than oblong membranes.[74]

A simple phase separator with a cylindrical cavity (4.2 mm diameter, 2.3 mm deep) and conical cavity (5 mm/1.7 mm) can be effectively used for phase separation of solvents having different densities from those of water using fluoroplastic porous membrane filter or less expensive phase separating paper filter, without significant loss of the separation efficiency.[121]

c. Dual Membrane Phase Separators[51,78]

The dual-membrane phase separator consists of two different types of membranes: a hydrophobic membrane of Teflon (10 to 20 μm pore size, Zitex E 249-122, Norton Chemplast), and a hydrophilic membrane consisting of two layers of Whatman No. 5 filter paper. The membranes are placed in two wells on either side of a Kel-F central body piece and are then sandwiched in position by two Kel-F outer body pieces (see Figure 23). The surfaces of the outer pieces lying just behind the membranes are textured in a spoke-like pattern to facilitate uniform flow through the membranes. All three Kel-F bodies are pressed together by stainless steel end plates held with screws.

The organic phase is passed through the Teflon membrane and the aqueous phase simultaneously passes through the paper membrane. The organic and the aqueous phases exiting from the separation chamber (and also the combined aqueous/organic unseparated phase exiting from the top of the phase separator) pass through an adjustable peristaltic pump in order to provide accurate flow control.

When immiscible solvents such as cyclohexane/water are used and when the flow rates are carefully adjusted, breakthrough of the wrong solvent is virtually never encountered, neither with the paper nor with the porous Teflon membranes. It is necessary to wet each membrane with the appropriate solvent before pumping is

TABLE 4
Basic Characteristics of Different Types of Membrane Phase Separators[74,93]

Type	Groove[a]	R (%)	H_p[b] (%)	W_p (%)	Type[c]	A_p[b] (%)	W_p (%)
21B	2/2	100	100	56.7	CG[d]	89	21
21B	1/1	94	100	54.9	CG	98	19
21A	0.5/2	100	100	58.5	GT	30	42
21A	0.5/1	88	96	57.9	SG	100	19
21E	1/1, 0.5/1	100	99	56.7	CP	53	30
22C[e]	1/4.2, 1.6/4.2	100	102	55.8			
21F'	1/4, 2/4	85	94	61.5			
22E	1/3, 2.5/3	72	100	61.5			

[a] Maximum depth/width or diameter of the membrane chamber in millimeters R (%) recovery of the organic phase through a membrane filter.
[b] Peak height or peak area ratio relative to 1A and to SG, respectively; W_p (%) peak width at 1% of the maximum peak height.
[c] Separator (CG: coiled groove, GT: glass T-tube, SG: straight groove, CP: conical/peripheral) used with engraved segmenter.
[d] T-piece segmenter used.
[e] Without a magnetic stirrer, with a conical acceptor chamber.

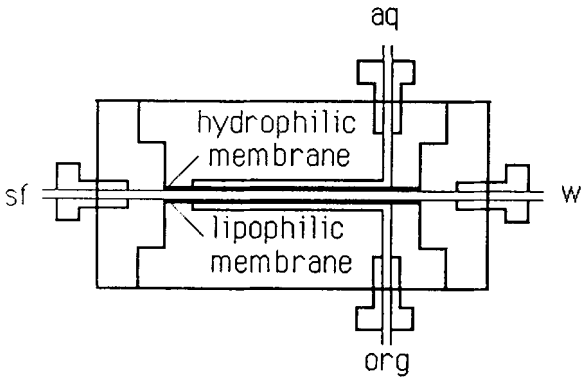

FIGURE 23. A dual membrane phase separator with lipophilic and hydrophilic membranes for simultaneous separation of aqueous and organic phases.

started in order to prevent leakage and breakthrough of the solvents through the membrane. Two single membrane phase separators in series have been shown to work just as well.[31]

d. "Tube-in-Shell" Tubular Membrane Phase Separators

The tubular microporous membrane phase separator, a "tube-in-shell" device (see Figure 24) was made from hydrophobic microporous Teflon tubing (2-μm pore size) with a Teflon tubing inserted at the termini. The segmented stream flows in the central microporous tube rather than through the annular space to prevent potential sample dispersion. The organic phase, wetting the wall of the device, passes through the pores to the annular space. The sample in the aqueous phase passes through the central portion into the detector flow cell. Semipermeable tubing was inserted into a larger diameter Teflon tubing, the active length being several centimeters. The device is suitable for separation of the organic phase from the segmented stream in the second stage of the back extraction procedure, or when the organic phase is used as a carrier only.

A similar design of phase separator was also used for the separation of aqueous phase from segmented flow, the organic phase being monitored, but proper function of the device is questionable. An infrared detector was used for a study of the extraction process of benzyl alcohol from water to carbon tetrachloride.[155]

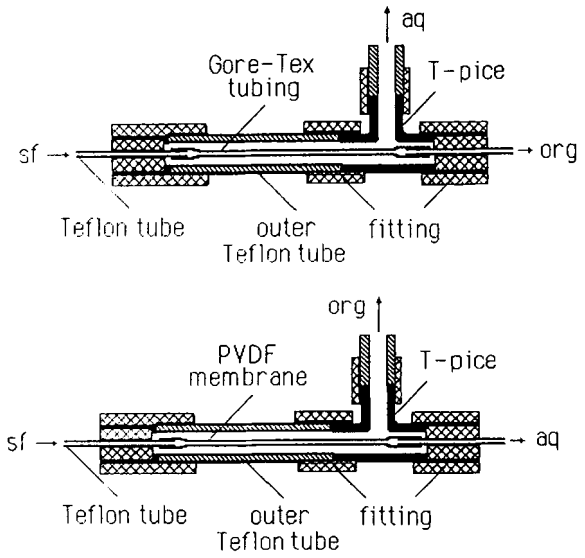

FIGURE 24. A "tube-in-shell" tubular membrane phase separators for separation of the aqueous (A) and organic (B) phases from the segmented flow.

4. Water-Absorbing Column Separators[120]

A liquid-liquid extraction FIA system with a disposable, resin-filled water-absorbing column using, e.g., Sumicagel base gel SP-520 and the inner fibers in a disposable diaper, Sumitimokagaku and "Merries" Kayo, Tokyo) that selectively absorbs the aqueous phase has also been explored. Such absorption materials can be packed (280 or 300 mg) into a glass microcolumn (8 × 10 mm) and conditioned by pumping a 4 + 1 v/v chloroform/methanol mixture for 15 min.

The aqueous phase is injected into a stream of an organic-immiscible solvent, and extraction of the analyte takes place during the transport through the extraction column, followed by the selective absorption of the aqueous phase.[120] Only the organic phase containing the analyte flows into the spectrophotometric detector. The water-absorbing column acts as a phase separator as well as a segmentation remover. The chief advantage of the apparatus (see Figure 25) is its simplicity since no reaction tube or complex phase separator is needed and the extraction efficiency

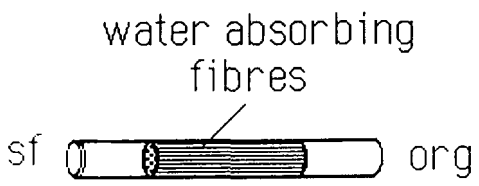

FIGURE 25. A microcolumn phase separator with water absorbing fibers.

is very high. A disadvantage is that a higher column overpressure is sometimes observed and the column may need frequent replacement.

E. Capillary Systems

Reaction and mixing coil geometry is determined by the same rules as in the classical one-phase FIA systems, taking into account minimization of sample dispersion and maximization of the reaction yield.[94,113,125,126] The dispersion process, acting on the sample zone in an aqueous portion of the system, is easily controllable[113] and is not discussed further here.

The dispersion of the sample in the extraction FIA system occurs mainly at the actual injection site and during the movement of the aqueous stream from the point of injection to the phase segmenter. There is also some dispersion in the segmented portion of the extraction FIA system, from separator and segmenter and from connection capillary up to the flow detector. The overall dispersion in two-phase systems must be regarded as an apparent dispersion since the solute concentration in the organic phase also depends on the extraction process in the extraction coil.

The length of the reaction coil L_{RC} is not critical for shorter reaction times.[144] In general, analyte peak height decreases with increasing reaction coil length because of the dispersion of the analyte in the flowing system.[53] A shorter reaction coil is desirable for reducing solute dispersion. Tube length between the mixing point and the segmenter did not affect peak height at L_{RC} <50 cm of 0.5 mm ID,[103] or at 40 cm and 0.35 or 0.7 mm ID,[73] when a fast reaction was used.

A shorter connection tube between the sample injection and mixing point gives better results, and affects less the analyte peak area than the peak height by interfering ions in the liquid-liquid extraction FIA determination of ion associates. The formation of ion associates between voluminous anionic species and metal ions is more significant for poly- than monovalent ions. The higher the charge of a cation and the higher concentration, the more ion associates are formed. The metal ions have to be ejected, which results in the use of longer tubing.[104]

The long reaction time of anionic surfactants with cationic counter ions is well known, however, and efficient extraction of the ion associates in a continuous flow analysis system requires a longer mixing coil to increase the reaction yield.[6] With a higher concentration of foreign cations, peak shape was affected by the length of the connection tube from the reaction coil, so a shorter reaction coil is better due to kinetic factors of side reactions.[104] When a sample is injected into a segmented flow stream, the additional dispersion of the reaction coil tube is eliminated.[106]

A variation in the tube length of the reaction coil from 5 to 40 cm did not affect peak height when a direct determination of metal ions was performed by injecting aqueous sample solutions directly into an organic phase stream acting as a transport medium,[18] or when introducing an aqueous sample and extracting reagent solution directly into an organic extractant stream inside the phase segmenter.[144] The sample was not dispersed and diluted since the film of the organic phase on the walls prevents transfer of the analyte. Peak height in this two-phase system was 1.4 times higher than in a one-phase aqueous/aqueous system. When methanol or water were used as a carrier stream, an extraction coil length over 5 cm changed the signal.[18]

A sample solution in an organic phase injected into a connection tube between an injector and a nebulizer of a flame AAS autonomy system increases the analytical signal with increasing sample volume up to 90 μl, and it is then constant up to 300 μl.[103] The peak area and peak width increase with sample volume V_s up to some limit, and then are constant. No linear relationship existed between the sample volume V_s and the peak height H_p, but there was good linearity between V_s (or number of moles of the analyte injected

into the system) and the peak area (or number of moles extracted into organic phase).

Restriction columns, or restriction coils of different geometry, or needle valves are usually used for regulation of the overpressure across the membrane filter of the phase separator. These also remove the unseparated part of the organic solvent, together with the aqueous phase, into the waste. The length and the inner diameter of the restriction coil effectively regulate the fraction of the organic phase flowing into the flow cell.

The restrictor coil produces a slight back-pressure and, hence, forces the organic phase through the porous membrane. Removing the organic phase flow-through membrane phase separator at a constant flow rate using an additional peristaltic pump or by connecting both flow outlet channels into a single channel[74] improves the reproducibility of the measurements by better than 2%. Membrane clogging and variations in the back-pressure in the restriction coil result in low reproducibility.

F. Detectors and Interfacing

A good flow-through detector should have the following attributes: small volume, low noise level, fast linear response over a wide concentration range, and high sensitivity. Other important features include the detection limit and the detector contribution to the peak width. The selection of suitable detection systems is limited by the presence of trace amounts of one of the immiscible solvents in the other phase since the solubility of solvents is usually very low, but not negligible. Besides the use of classical detection systems which have excellent precision, special types of detection systems were introduced into liquid-liquid extraction FIA to take into account the special tasks of this technique. Optical detector systems are preferred to other types since the influence of the organic phase is less important for such systems. Electrochemical detectors are usually not used, due to the interference of trace concentrations of organic solvents in the aqueous phase.

Since most liquid-liquid extraction procedures (especially those using liquid-liquid extraction of metal chelates with organic analytical reagents) are nonselective, liquid-liquid extraction is often combined with more or less selective detection systems. AAS, atomic emission spectrometry (AES), inductively coupled plasma optical emission spectrometry, fluorimetry, chemiluminescence, and, especially, molecular absorption spectrophotometry are the most popular. These methods can be used to advantage because it is very simple to interconnect the extraction FIA system to a flow-through detector.

A detection system with two[31,51] independent detectors was used for the simultaneous monitoring of both immiscible phases (see Figure 6B) after their separation using two membrane separators (lipophilic and hydrophilic) connected in series, or with the aid of a dual membrane separator in which the organic phase passed through a Teflon membrane and the aqueous phase passed through a filter paper membrane. Simultaneously, the phases are directed to the detectors, and the analytical signals are fed into digital integrators. Such a system was used for the simultaneous determination of diphenhydramine and 8-chlorotheophilline in pharmaceutical preparations[31] after their separation into cyclohexane and water at pH 10, and for the simultaneous determination of acidity constants.[51]

To decrease the complexity of such systems, both phases were monitored by a single detector (see Figure 26) in which the organic phase flows through the sample flow cell and the aqueous phase stream flows through the reference flow cell.[78] A single photometric double-beam detector was modified to allow electronic switching of the sample/reference designation of both flow

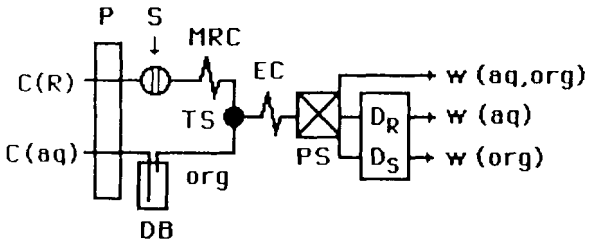

FIGURE 26. A FIE manifold with a dual phase separator and a single detector for simultaneous monitoring of both phases passing sample (D_s) and reference (D_R) flow-cells.

cells by use of a double-pole, double-throw relay. The setting of this relay (either for normal or reversed-flow cell designation) was controlled with an electronic timer after a preselected time delay, to measure both phases as positive signals.

The combination of two spectrophotometric detectors was also used for direct, automated extraction ratio measurements. The organic and aqueous phases were detected by two independent diode-array detectors controlled by computers. The full set of data was treated, and the composition (2:2:1) and a rate constant of the reaction of uranium(VI) with (4-benzoyl-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-thione)-trioctylphosphine were determined.[110]

In-line spectrophotometric detectors situated on a closed-loop system have been used for continuous monitoring of the analytical signal.[92,103] In the first arrangement, the accumulation of the extracted species is monitored directly, and a derivative can be formed inside the loop by addition of a suitable reagent (see Figure 11). The other arrangement involves measurement of the analyte after a preselected delay time, resulting in a transient signal typical of FIA analysis.

A waveguide long capillary cell made of Pyrex glass (1.0 mm ID, 4 mm OD, 1 m long, covered with black polyethylene tube to shield from stray light and to increase the mechanical strength) wrapped with light refractive material and filled with the sample solution[62] was applied as a spectrophotometric detector for the extraction FIA determination of iodide.

An improvement in the system[97] was made when a solvent (such as carbon disulfide) was used which has a refractive index exceeding that of the tube material totally reflects the light inside the tube and propagates it through the capillary with a loss in intensity (apart from absorption by the solution) and applies it to liquid-liquid extraction FIA for extremely sensitive determinations of copper with diethyldithiocarbamate into CS_2. A simple homemade arrangement of a detector based on a tungsten lamp (300 W), an interference filter, and a capillary flow cell (0.53 mm ID, 20, 46, and 200 cm length) connected to the light source, provided a detection system (photocounter and strip chart recorder) and liquid-liquid extraction FIA system, using stainless steel T-joints, which was more useful.

Solid state, flow-through photometric detectors incorporating light-emitting diodes (LEDs) as sources of visible (red, yellow, blue, and green with peak emission wavelengths of 480, 550, 563, 570, 580, 600, 630, 638, 660, 685, and 800 nm are presently available from different suppliers) and UV radiation, and photodiodes or phototransistors as a detector provide a simple, reliable, and low-cost alternative to commercially available spectrophotometers. The LEDs and photodiodes or phototransistors are relatively inexpensive, compact, and commercially available components of "on-tube" detectors, the application of which minimizes sample dispersion within the detection system (Figure 27).

In one of the simplest designs, 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, or both demountable active components are located behind small

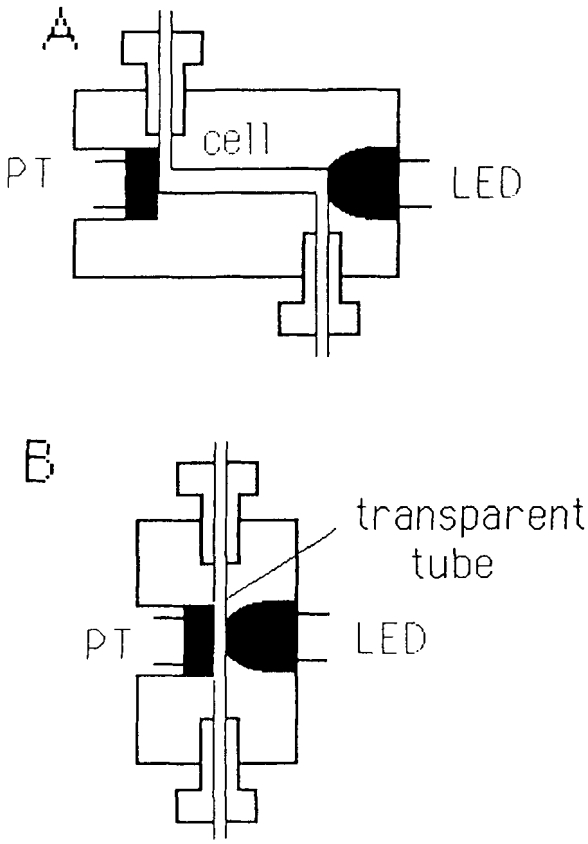

FIGURE 27. Flow through photometric detectors with a light-emitting diode (LED) and phototransistor (PT) in Z- (A) and on-tube (B) configurations.

glass focusing lenses. An alternative "on-tube" design uses nonintrusive solid-state components, and has the advantage of negligible sample dispersion within the flow cell which is realized by use of a perpendicularly oriented (semi)transparent Teflon or quartz tube (Figure 27B). The design can incorporate a beam splitter for compensation of temperature changes and aging of the LED, and therefore reduces long-term drift. A double-beam arrangement uses separate LED-photodiode pairs to monitor the reference and the sample streams.

A fast-reading "on-tube" photometric detection system (approximately 3-ms time resolution) controlled by a computer[132,133,142-147] is very suitable for some applications in separatorless liquid-liquid extraction FIA (Figure 28). The segment length produced by phase segmenters of different geometries, and the behavior of a thin film of an organic solvent formed on the walls of the extraction coil tubing in a continuous liquid-liquid extraction flow system, were studied using this sophisticated system.

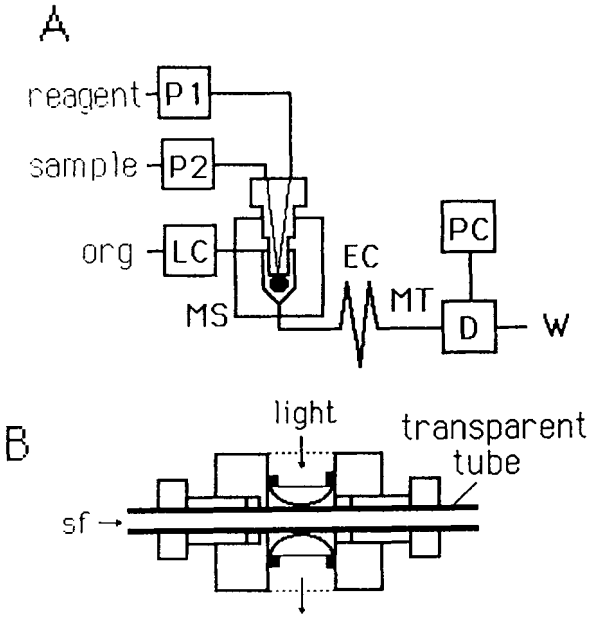

FIGURE 28. A FIE manifold with a dual-channel coaxial segmenter (MS) and an on-tube fast reading photometric detector controlled by a computer (PC) for liquid-liquid extraction without phase separation (A) and a view of an on-tube flow-through cell with transparent fluoropolymer, glass, or quartz capillary. P1, P2, LC: peristaltic and liquid chromatographic pumps, respectively, MT: a measuring transparent FEP tube, sf: segmented flow.

The resulting segmented flow passed through the extraction/measuring tube (Figure 28B). The analytical signal was measured by a detector reading signal values across the transparent FEP capillary tubing. The analog signal was fed into a personal computer via a high-speed data acquisition module using software drivers language support subroutine libraries with the BASIC language interface. Computer programs written in BASIC were used for communication between computer and detector and for data treatment. An integration time of several milliseconds was used to increase the precision of the measurements at the slower reading frequency. All measured values were stored in computer memory, either for post-run mathematical treatment or the data were treated immediately during the experiment. Graphic "on-screen" presentation of the stored data followed, if required.

A similar detection system 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 liquid-liquid extraction FIA and applied for the determination of pesticides in wastewaters. A sophisticated "sorting" computer program allows the separation of analytical signals measured directly on the aqueous and the organic segments, and subsequent determination of extractable species.[130]

A single on-line fluorimetric detector situated on the Teflon tube was used for monitoring fluorescent species in a single-line, liquid-liquid extraction FIA arrangement without any phase separation, when a very small volume of an aqueous sample (10 μl) was injected rapidly in the form of small droplets into the continuously flowing stream of the organic phase.[4]

A laser excitation "on-tube" fluorimetric detector with a time resolution of 2 ms (in which the laser beam of a CW argon ion laser, at 488 nm, was tightly focused to the center of the Teflon tube to eliminate the light scattering from the surface of the tube) was used to study transient phenomena in the flowing system and chelate formation between gallium(III) and lumogallion. Fluorescence was focused by a lens onto

the entrance slit of a double monochromator and detected by a photomultiplier connected to a picoammeter.

Chemiluminescence[53] has an approximately 50-fold higher sensitivity than that of fluorescence, but the precision is lower. The relative chemiluminescence intensities of steroid sulfates depend on their molecular structure (mostly on the presence of hydrophobic functional groups such as oxo and hydroxy groups). It could be used in such cases when no characteristic spectroscopic groups in molecular form are present,[53] using a simple homemade detection system equipped with a 250-μl spiral flow cell in front of a photomultiplier.

Flame AAS detectors are used more frequently for the direct or indirect determination of analytes than graphite-furnace AAS instruments because of their simpler interconnection to the liquid-liquid extraction FIA manifold. Introducing the controlled flow into a flame AAS detection system by peristaltic pumping the appropriate phase stream improves the nebulizer performance, compared with aspiration controlled by the oxidant flow rate in the nebulizer.[16,52,119]

An AAS detector with electrothermal atomization was connected with an extraction FIA apparatus through an electronically controlled system of valves, and used for the determination of heavy metals,[128,129] but the complexity of the system limits its practical use.

IV. SPECIAL TECHNIQUES

A. Unsegmented Systems[65]

An unsegmented system for liquid-liquid extraction in FIA can be used for the separation of the analyte from aqueous sample solutions of the high analyte concentration into the continuously flowing organic phase stream. This system is based on the use of a short connection time of the aqueous and organic phases in a special extraction module with a porous Teflon membrane.

The extraction module is very similar to that used in a dialysis technique (see Figure 3A and B).[94,113] It consists of two halves made from PVDF, each having one inlet and one outlet channel and symmetrically situated grooves of 0.4 and 0.8 mm depth for the organic and aqueous phase streams, respectively. The groove for the recipient stream is filled with support material of a porous polyethylene. A gasket made of Teflon tape with a rectangular cut was adapted to the grooves. The module uses a hydrophobic membrane of PTFE (1 μm porosity) for both immiscible phases, sandwiched between both grooves.

The aqueous sample solution (continuously pumped or injected into the aqueous phase stream at volumes of 40 to 400 μl) is led into an extraction module, together with an organic recipient stream, each to only one side of the membrane. The extracted analyte transfers from the aqueous phase stream across the membrane (which is saturated with the organic solvent) into the organic phase, flowing directly into the detector flow cell. Thus, the segmentation as well as the separation units of the conventional liquid-liquid extraction FIA system are eliminated.

Because of the restricted area and short (but controllable) contact time, only a low extraction efficiency (8 to 18% recovery) is achieved. The extraction efficiency differs for different compounds and is a function of their molecular structure and diffusion coefficients. Extraction efficiency is also influenced by the flow rate ratio of the donor and acceptor streams. Reproducibility and accuracy of the determination decreases with decreasing total flow rate within some limit because small variations in the flow rate produce large variations of the extraction efficiency. The extraction efficiency increases with increasing groove depth by a factor of almost 2 at 0.4 and 0.8 mm. The proposed procedure allows miscible solvent to be used without a significant change in the proportion of the solvents in both phases. Liquid-liquid extraction can be carried out with small sample and organic phase volumes of 40 to 200 μl and 1 ml per sample, respectively.

A microconduit system based on this unsegmented technique[74] has been used since it has a very short startup time (3 to 5 min), typically requires low maintenance, and has reduced reagent, solvent, and sample consumption due to its very small internal volume.

A self-contained module contains engraved integrated conduits for the reaction and mixing

coil, a segmenter, a detachable extraction coil, a membrane phase separator, and a rinsing system for the flow cell. The circular membrane phase separator with a centrally coiled entrance channel and a Teflon-coated stainless steel grid has a 10-μl volume recipient (organic phase) chamber. The separation efficiency is at least 85% for several organic solvents, and drops to ca. 60% in the presence of polar solvents such as methanol.

A similar system with an integral vapor permeation unit of an "tube-in-shell" design was developed to separate volatile organic substances from aqueous samples and preconcentrate them in a stationary (or slowly flowing) stream of organic solvent (methanol, hexane) closed inside a nonporous silicone rubber sample loop. Organic compounds (e.g., alkylated and halogenated aromatic hydrocarbons and phenols) present in the injected sample zone permeate a tubular silicone membrane and are detected by spectrophotometry.[158]

B. Unsegmented System with Liquid Membranes[77]

An unsegmented liquid-liquid extraction FIA system for aqueous/aqueous extraction process with a liquid membrane separation unit (Figure 3A and B) was used for sample cleanup and preconcentration of amines. The separation unit of the liquid-liquid extraction FIA system is similar to that used in the previous unsegmented system and has a function similar to a membrane phase separator. The liquid membrane is sandwiched between two blocks made of Teflon for the acceptor side and made of titan for donor side, in a "dialysis module" with two U-shaped grooves (0.25 mm deep, 1.5 mm wide, and 150 mm long).

An alkaline aqueous sample stream continuously passes a porous hydrophobic Teflon liquid membrane pretreated with an organic solvent (by immersion into it for 15 min). Solvents immobilized in the membrane pores (such as isooctane, *n*-hexadecane, *n*-undecane, and 1-decanol) must be quite insoluble in water since a thin film of a large area comes in contact with large volumes of aqueous solution. The membrane facilitates mass transport between two aqueous solutions (donor and acceptor) by dissolving the extractable analyte in organic solvent immobilized inside the membrane pores.

Porous hydrophobic fluoroplast membranes (Fluoropore FG, FH, FA, FS, Mitex LS and LC, Durapore GV) of different pore sizes (0.2 to 10 μm) were used as solvent supports. The backing of the membranes had negligible influence on the mass transfer rate, but prolonged their lifetime. Membranes with a pore size of over 3 μm showed instantaneous leakage of the two separated aqueous solutions.

The liquid membrane permits analyte transfer from the alkaline donor stream to the aqueous stagnant (or flowing) acidic solution on the acceptor side. The analyte distribution is based on the pH difference, but also on the concentration gradient of an organic analytical reagent. The extractable compounds pass the membrane and a defined fraction is stripped in a small volume of the acceptor groove. The resulting plug of analyte in the aqueous medium is swept from the membrane separator to the detector. Thus, a preconcentration can be achieved if the acceptor stream is closed (arrested) while the sample flow stream is continuously flowing through the donor portion.

The analyte concentration can be determined directly, or after subsequent separation, on an HPLC column. The extraction process is thus simplified since there is neither a segmentation nor a phase separation unit. The mass transport, separation efficiency, enrichment factor, and selectivity of the separation process are influenced by sample volume, support matrix, type of immobilized organic solvent, composition of the donor and acceptor streams, and partition coefficients of analytes between the donor and acceptor stream and the membrane phase. The results obtained agreed with theoretical assumptions.

Solvent extraction was also performed with a hydrophobic microporous membrane wetted by the organic solvent, with the pressure of the aqueous phase maintained at any pressure greater than that of the solvent. Acetic acid was extracted from water into hexane using a Celgard 2400 film. Due to the absence of dispersion and coalescence, this technique produced reasonable extraction rates. When considered in the context of

a hollow microporous hydrophobic fiber extractor, the volumetric extraction rate is likely to be significantly larger than that encountered with conventional extractors.

C. Systems without Phase Separation

A segmented liquid-liquid extraction FIA system was described without phase separation (see Figure 2A) which was based on a rapid injection of microliter volumes (10 μl) of an aqueous sample solution containing an analyte into a continuously flowing stream of an extracting reagent in a suitable organic solvent (dibenzo-18-crown-6 in 1,2-dichloroethane), and applied to the determination of potassium with fluorimetry detection. Although the flow stream was turbid, single, sharp peaks were obtained with good reproducibility.[4]

Systems with a fast-reading "on-tube" photometric detector were used for the determination of pesticides in wastewaters,[130] for the determination of the extractable chelate of Cu(II) with ammonium pyrrolidinedithiocarbamate (APDC),[144] for the determination of nonextractable species,[147] for the simultaneous determination of both extractable and nonextractable species,[146] and for studies of phase segmentation by segmenters of different geometries.[132,133,142-147]

D. Postsegmenter Sample Injection[106]

A relatively large dispersion occurs in all conventional liquid-liquid extraction FIA systems in which samples are introduced into an aqueous phase carrier stream. The dispersion is lower when samples are introduced into the segmented rather than the unsegmented flow stream (see Figure 4A and B) because dispersion of the sample during its transport through the reaction tube and phase segmenter is negligible. The sensitivity, slightly wider range of calibration graph linearity (0 to 10 resp, 0 to 7 μg ml^{-1} of Ni(II) in 20 μl), and sampling frequency (80 U/h) are higher than that obtained by conventional techniques (60 U/h) due to the sharper, higher peaks. The shape of peaks also is more symmetric than that obtained by conventional techniques, ensuring that dispersion both before reaching and within the segmenter is a major contributor to peak broadening.

E. Closed-Loop Systems[92,103]

A novel approach for the on-line preconcentration of analytes from an aqueous solution is by continuous extraction into an organic solvent which is repeatedly circulating in a closed loop. This approach permits the automated preconcentration and purification of analytes. The method is based on continuous circulation of the organic phase in a loop connected with a fresh aqueous stream, which continuously enters the extraction coil via a four-way segmenter and leaves it (to waste) after separation in the membrane phase separator.

The apparatus (see Figure 11) consists of a four-way segmenter, an extraction coil, a membrane phase separator, switching valves, and an on-tube photometric detector. The segmenter merges an aqueous stream (containing sample or blank solutions) and ordinary and secondary "make-up" streams of the organic phase into the single segmented flow stream entering the closed loop system.

This system has a segmented portion (extraction coil), in which alternate segments of the aqueous and organic phases are equilibrated, and an unsegmented portion, in which a membrane phase separator, a detector, and a circulating peristaltic pump are located.

The separated organic phase is repeatedly directed back into the closed loop system or sent to waste by means of the four-way switching valve. This arrangement allows the organic phase to be kept in the loop while the aqueous phase flowing through the extraction coil can be directed to waste. Thus, the analyte is continuously extracted from a large volume of fresh aqueous phase into a relatively small volume of the organic phase circulating in the closed loop system. The analytical signal is continuously monitored using an in-line detector.

The enrichment factor depends on the loaded sample volume (3 to 20 min of loading time), the extraction efficiency, the phase ratio, the loop volume, and the phase separator volume.

Dimensions of the phase separator (0.3 ml on each side), pumping of the organic phase from the separator, and delivery of the secondary low flow rate organic phase stream, increase the reproducibility of the separation process, provide high phase separation efficiency, and allow for handling of any irregularities in the phase segmentation. Secondary "make-up" flow of the organic solvent compensates for the loss of the organic solvent in the system due to the formation of small bubbles of organic vapors, formation of small droplets of aqueous phase, and solubility of the organic solvent in the aqueous phase.

F. Iterative Reversal Systems[111]

A liquid-liquid extraction process without a characteristic separation unit is performed by insertion of a single plug of the organic phase, closed in the loop of the injection valve, into the carrier stream of the aqueous phase containing the analyte. Two liquid-liquid interfaces (menisci) are created and a thin film of the organic phase is formed on the inner wall surface of the injection loop (Figure 29A and B). The aqueous phase stream is subjected to an iterative reversal procedure in the closed loop. The gradual enrichment of the organic phase plug with the extractable analyte (due to mass transfer through the interface boundary) is continuously monitored using an on-loop situated photometric detector.

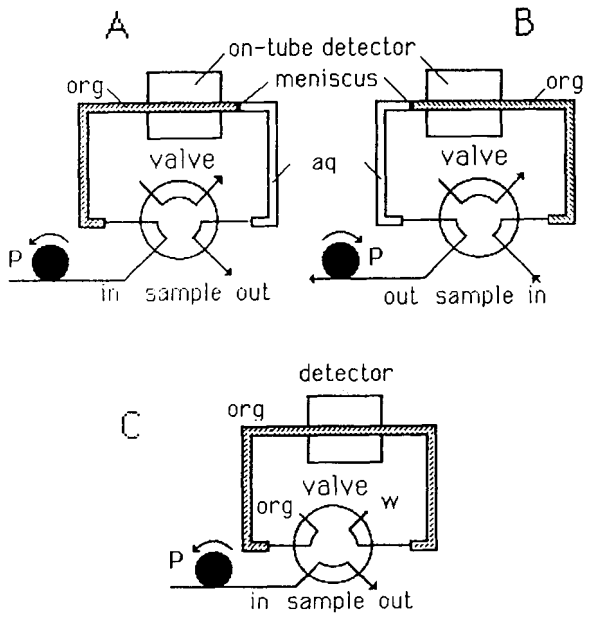

FIGURE 29. An iterative reversal system with the organic phase plug in extremely positions (A, B) inside the sample loop, and in filling position (C); the reversal motion of the peristaltic pump (P) during the measurement cycle is depicted (A, B).

A Gilson Minipuls 2 peristaltic pump controlled by an electronic timer allows the direction and rate of drum rotation, the start of the reversal cycles, the number of cycles, and the cycle duration to be programmed. The aqueous sample merges with the reagent solution to form a reaction mixture in a reaction coil. The closed loop is filled with the organic phase in the filling position. The on-loop detector divides the loop into two uniform subloops (Figure 29C).

The organic phase plug is reverse-transported by the aqueous carrier stream until one of the liquid-liquid interfaces reaches the flow cell. At that moment, the electronic unit reverses the flow direction until the other interface again reaches the flow cell. This reversal process is repeated until a suitable enrichment factor is obtained by mass transport through the interfacial surface. The electronic timer controls the plug movement; thus, at no time do any of the interfaces pass through the flow cell to produce parasitic signals (due to changes in the refractive index or viscosity produced by the two phases). The length of cycle duration also can prevent the aqueous phase from reaching the detector at any moment during the measurements.

Continuous monitoring of the analytical signal allows one to obtain much information from a single measurement, similar to multidetection, allowing a continuous study of the solute transfer between both phases and its dispersion in the organic phase. Continuous monitoring also permits conventional reaction rate measurements for theoretical and practical purposes.

The system is simpler with respect to hydrodynamic and instrumental factors since the segmentation and separation units are eliminated. Preconcentration and dilution of the sample can be used to vary the sensitivity of the determination. The only shortcoming is the use of an electronic component to obtain programmable and reproducible cycles. The proposed method represents an important change in the conventional

approach and in those based on the use of unsegmented systems.[65,77] Its applications are still limited to processes that do not require any chemical reactions after extraction.

G. Zone Sampling Technique[152]

In order to overcome some problems connected with phase separation, the zone sampling technique was used for the isolation of individual segments of organic or aqueous phases (Figure 30). A six-port valve is provided with conductivity probes inserted in the load and waste ports. The extractant segment (aqueous or organic phase) is isolated from the segmented stream by an electronically actuated valve. The valve is switched to the injection position at the moment when the sample loop is completely full of the desired phase. At that time, the conductivity across the probes changes markedly. This change in conductivity is used to actuate the valve to the inject mode and to hold it in that position for a preselected period to complete the injection (Figure 30). The content of the loop is injected into a miscible carrier flowing to the detector. This method provides the advantage of performing further chemistry on the analyte prior to its detection. The system was used for the determination of alkylbenzenesulfonane by ion-pair extraction with the cationic dye, methylene blue, and for the determination of tetrabutylammonium using methylthymol blue.

In some cases, instead of the wetted film bridge phenomenon, adsorption is the primary process in which there is a stationary film along the walls of the extraction coil, or the tubing material itself acts as the adsorption site. In either case, the analyte, as it flows through the tubing, is adsorbed on the walls. Therefore, as the injection time interval increases, more ion-pair molecules accumulate on the material, waiting to be extracted as soon as the incoming organic segment can collect them. Thus, an increase in sensitivity is observed. It has been reported that small radii configurations, such as in a serpentine reactor (effecting sharper turns and flow reversal), have proven to give better mixing. Study has shown the efficiency to be fairly close to the values obtained for coil and straight extraction coils of the same length. In any case, frequent segment breakage in knotted configurations actually make them impractical.

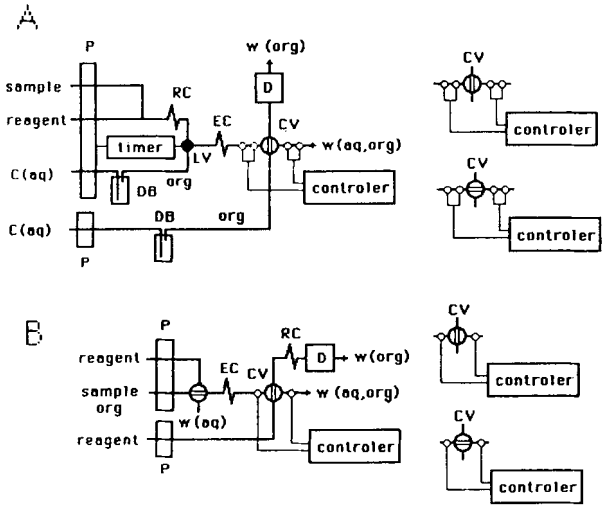

FIGURE 30. FIE manifolds with an intelligent zone sampling for isolation of the organic phase segment (nonconductive) from continuous flow of the aqueous phase (A) and vice versa (B), and isolation and filling positions of the conductivity controlled six-port valve (CV). LV: a timer-operated three-port magnetic Lee valve is used as a segmenter.

V. SOME THEORETICAL ASPECTS OF LIQUID-LIQUID EXTRACTION FIA

A. Segmentation

Segmentation of two immiscible phases is of crucial importance to the results obtained by liquid-liquid extraction FIA. The whole segmentation process can negatively influence sample dispersion, extraction rate, and phase separation.[144] Segmentation may result from two different mechanisms. First, droplets or plugs of one phase may be formed in a continuous flow of the other, which is immiscible with the first. This takes place in a small mixing chamber in the segmenter, at the end of a single inlet tubing capillary, or at the junction of a multichannel inlet capillary system. The mechanism also controls the segmentation in a mixing compartment made of hydrophilic material (a glass T-piece or an A8-T fitting) at moderate flow rates.

The second principle leading to segment formation is the "ripple" process, resulting from the destruction of the thick layer of one of the solvents, which is formed at high pumping rates on the wall of the outlet tubing or on the walls of the mixing chamber in the segmenter. This process dominates at very high flow rates of both phases Q_a and Q_o or at very high flow rate ratios, or when the walls of the mixing compartment are constructed from lipophilic material (fluoropolymer). Also, the walls of a mixing compartment made of hydrophilic material may be covered by a layer of lipophilic impurities, thus changing their hydrophilic character.

Detailed descriptions and studies of the segmentation process and factors controlling segment size and reproducibility of segmentation aimed at developing more efficient phase segmenters and exploring the advantages of improvements in segmentation reproducibility[115,144] were given in previous papers;[13,115,132,144] thus, only basic information is given here.

There are two major variables to examine in connection with the segmentation process: segmentation reproducibility and segment size. While segment size may not affect the extraction efficiency of a fast extraction process or when large sample volumes are introduced into the system, it could theoretically affect the efficiency of slower systems.[13,23] The maximum segment size is determined by the interfacial tension of the organic and aqueous phase, both between each other and between each phase and the tubing material, such that the segment size decreases with decreasing values of $\gamma_{o/a}$.

The segment length in the extraction coil is the principal parameter controlling the size of the contact area between the phases. An irregular segmentation pattern results in losses of the solvent by the wetting process due to a varying film thickness. Serious coalescence of segments of different size is due to differences in linear velocity of the segment having different geometries, due to differences in viscous drag of the wetting phase. This means that the segmentation pattern must be under control and constant during the entire analytical procedure. Thus, during manifold optimization, it is necessary to consider kinetic efficiency, total extraction yield, and peak (sample zone) broadening.

1. Continuous "Droplet-Form" Segmentation

Several assumptions are necessary before a quantitative description of segmentation in a gravity/density segmenter becomes possible:

- Four forces direct droplet formation: gravity, density (uplift), interfacial, and hydrodynamic forces, the orientation of which should determine the value of the resulting force vector and the equivalence of the forces.
- Perpendicular and vertical/horizontal orientations of inlet/outlet flows are assumed for simplicity.
- The droplet volume grows linearly with time and the droplet-forming phase flow rate ($V = Q_{d,t}$).
- The skewing process is fast enough to allow one to neglect mean-time processes.
- No contact exists between the wall surface of the outflow capillary and the confluence chamber wall (actually questionable in the case of a narrow inner diameter chamber made of fluoropolymer).
- The droplet and the profile of the confluence chamber are spherical and circular, respectively.
- No phase losses due to film formation are observed.
- No jet effect occurs at the droplet forming phase flow rate used.

The droplet-forming solvent flows into the stream of the other immiscible phase in the form of droplets or small plugs. Their sides are in contact with the side walls of the inlet tubing at the junction of the mixing chamber of the segmenter (Figure 31). The gravity and density forces are oriented vertically, and the resulting gravity/density force F_{GD} is vertical and parallel to F_G and F_D, with a value equaling

$$F_{GD} = F_G + F_D = V \cdot \Delta\rho \cdot g \qquad (1)$$

where V is the droplet volume, $\Delta\rho$ is the density difference between the two solvents, r_d is the droplet radius, and g is the gravitational constant.

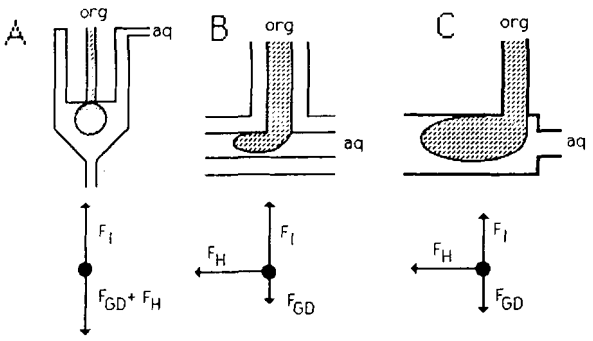

FIGURE 31. Schematic diagram of segmentation in a coaxial (A) and noncoaxial (B, C) segmenter made of glass (B) or fluoropolymer (C) with an enlarged outflow channel; hydrodynamic (F_H), gravity/density (F_{GD}) and interfacial (F_x) force orientations are given below.

The interfacial force F_I can be expressed by the following form of the Tate equation[153]

$$F_I = 2 \cdot \pi \cdot (d_i/2) \cdot \gamma_{0/a} \cdot (1 - \cos \theta) \quad (2)$$

where $\gamma_{0/a}$ is the interfacial tension between the organic and aqueous phases and θ is the solid-liquid-liquid contact angle ($\cos \theta = \Delta\rho \cdot$ h.g. $(d/2)/2 \cdot \gamma_{0/a}$) related to the height of the capillary rise h or to the droplet shape on the surface of the appropriate solid material, and d_i and r_i denote, respectively, the inner diameter and radius of the inlet capillary tubing.

The spherical shape of the droplet is deformed to the resulting distorted spherical or elliptical form by a hydrodynamic force F_H active on the drop area facing the other immiscible phase flow, as a result of the continuous flow Q_n of the phase that does not form droplets (see Figure 31). The hydrodynamic force F_H may be written as $F_H = \Delta P \cdot A_f$, where A_f is the front area of the droplet facing the flow of the other phase and $\Delta P = P_1 - P_2$ is the pressure difference across the profile of the confluence chamber, which is due to the continuous phase flow.[153]

The pressure difference ΔP consists of two terms arising from viscous drag on the flow of the phase that does not form droplets (the Poiseuille term P_P) and changes in the kinetic energy of the same phase as it flows through the narrowing profile of the tubing beneath the drop of the droplet-forming phase (the Bernoulli term P_B). Both terms are linear and quadratic functions of the total flow rate Q_n of the phase not forming droplets. The value of ΔP (assuming a circular chamber cross-section and a spherical droplet profile) is given by the expression

$$F_H = \Delta P \cdot A_f = (P_P + P_B) \cdot A_f$$
$$= k_p \cdot Q_n + k_B \cdot Q_n^2 \quad (3)$$

where k_P and k_B are, respectively, the constant Poiseuille and Bernoulli terms consisting of the viscosity and density of the phase not forming the droplet and the geometry factors of the confluence chamber and droplet.

This force tends to dislodge (skew off) the droplet from the end of the inlet tubing at the moment when the sum (F_R) of the forces pushing it (F_H, F_G, and F_D) is equal to the interfacial force F_I holding the droplet onto the end of the segmenter inlet capillary surface. The resulting force vector must equal zero at the moment when the droplet is cut off. This equilibrium of forces can be expressed by

$$F_G(\alpha_s) + F_D(\alpha_s) + F_I(\alpha_s,\alpha_i) + F_H(\alpha_s,\alpha_i) = 0 \quad (4)$$

where symbols α_s and α_i express the influence of the force orientation in space or among themselves, respectively.

One generalized model for the segmentation process can be used to describe all different kinds of continuous segmenters. The four differently oriented forces (with respect to the vertical axis and to the geometry of the inlet/outlet tubing system, respectively) affect the skewing process. The forces can be oriented axially (coaxial, falling drop, gravity/density segmenters), perpendicularly (T-type segmenters), and/or at different angles among the inlet and outlet tubes (30°, 45°, 60°, 120°, or 150°, etc. for W- or Y-type segmenters). Several extreme cases can be solved where the gravity/density force F_{GD} and hydrodynamic forces F_H are oriented axially or perpendicularly to each other.

a. Coaxial (Dropping) Segmenters

All resulting phase streams are oriented axially among themselves, and the coaxial seg-

menter main axis is vertical (Figures 31A and 32). At low flow rates and low flow rate ratio (Q_a and Q_a/Q_0 or Q_0 and $Q_0/Q_a \rightarrow 0$, respectively), as preferred in this segmenter type, the gravity and density forces predominate in gravity/density coaxial segmenters and the influence of the hydrodynamic force can be neglected. The droplets are spherical. Their size depends primarily on the inner diameter of the inlet capillary d_i, the interfacial tension γ, the density difference between the two liquids $\Delta\rho$, and the value of the gravitational acceleration g. The total droplet volume V grows linearly with the droplet-forming phase flow rate Q_d (organic or aqueous) or the total aqueous phase flow rate $Q_a = \Sigma(Q_a)_i$ and time t, when using the multichannel inlet capillary system, as the droplet is formed. The resulting total droplet volume V can, under ideal conditions (neglecting the influence of hydrodynamic forces and assuming the equivalence of gravity/density and interfacial forces $F_{GD} = F_I$), be expressed by the following equations, valid at the moment when the droplet is cut off, t_{max}:

$$V = Q_d \cdot t_{max} = \sum (Q_2)_i \cdot t_{max}$$
$$= \pi \cdot d_i \cdot \gamma/g \cdot \Delta\rho$$
$$= \pi \cdot d_i \cdot \gamma_{0/\sigma} \cdot (1 - \cos\theta)/g \cdot \Delta\rho \quad (5)$$

The total droplet volume V, the total segment volume V of the reaction mixture in the aqueous medium, and the total segment length L_s (neglecting the film formation process or assuming a stable film thickness), are independent of the flow rate Q_n of the phase that does not form droplets. They are influenced only by the $(1 - \cos\theta)$ term when the values of the other parameters are kept constant.

$$V = V_{max} \cdot (1 - \cos\theta) \quad (6)$$

The maximum droplet volume V_{max} (at equal interfacial and gravity forces) and the corresponding segment length L_{max} are given by the following expressions

$$V_{max} = (4/3) \cdot \pi \cdot r_d^3 = \pi \cdot d_i \cdot \gamma_{0/a}/g \cdot \Delta\rho \quad (7)$$

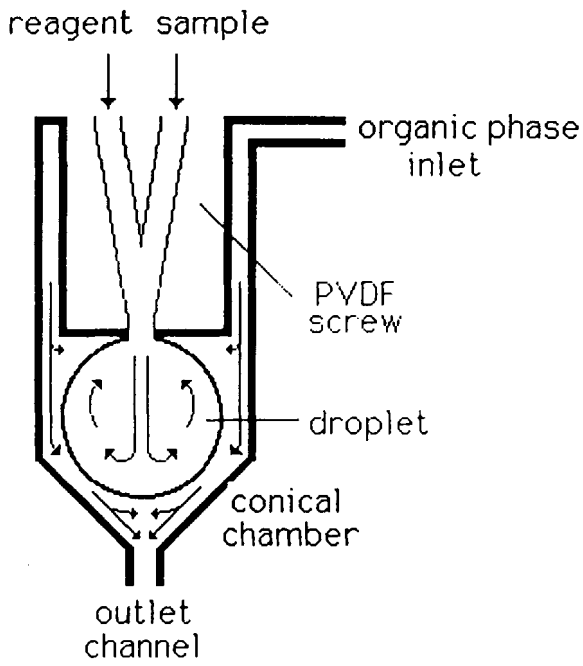

FIGURE 32. Schematic diagram of the segmentation process in a multichannel coaxial segmenter.

$$L_{max} = V_{max}/\pi \cdot (d_e/2)^2 \quad (8)$$

Here, r_d is the droplet radius and d_e is the inner diameter of the outflow measuring or extraction capillary. The actual droplet volume and segment length vary from V_{max} or L_{max} to $V_{min} = L_{min} = 0$ for $\cos\theta = 0$ or 1, respectively.

The normalized droplet volume $V_n = (V_{max} - V)/(V_{max} - V_{min})$ and the normalized segment length $L_n = (L_{max} - L)/(L_{max} - L_{min})$ vary from 0 to 1 independently of the other solvent properties, as can be shown from the dependence of V_n or L_n against the (s) – (1) – (1) interface contact angle θ at the tubing end (see curve 4 in Figure 33 for different θ values). Both values are completely independent of the flow rate Q_n of the phase not forming the droplet, and consequently the dependence of V_n or L_n on Q_2, as an example, is depicted as straight lines (see curve 1 in Figure 33) for $V_n = L_n = 1$.

The actual droplet volume of any defined two-phase liquid-liquid extraction system can thus

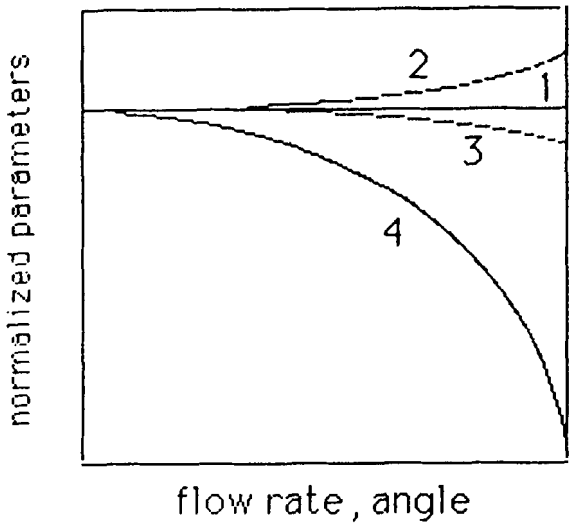

FIGURE 33. Normalized segment volume (V_n) and segment length (L_n) as functions of solid/liquid/liquid contact angle O (curve 4), and of the flow rate for F_H = 0 (curve 1) or assuming negligible influence of the hydrodynamic force in downstream or upstream orientation (curves 3 and 2).

be easily varied by a careful choice of material for the inlet tubing (as it changes the solid-liquid-liquid angle θ_i), by a choice of inner diameter for the inlet tubing, and, to a small extent, by adjusting the flow rates of the two solvents. The droplet volume is also affected by the change in surface tension and by the change in density difference between the two solvents. This can be demonstrated by the changes in segment length resulting from the presence of inorganic salts, surfactants, or methanol. The resultant force vector and the segment volume are affected by the spatial orientation of the end surface of the tubing.[133]

The influence of the hydrodynamic force cannot be neglected at high flow rate of the phase that does not form droplets (Q_a or Q_o) and/or high flow rate ratios (Q_a/Q_o or Q_o/Q_a). The reason for this is due to changes in the confluence chamber cross-section profile at the end of the inner inlet capillary (Figure 31). The Bernoulli term of the continuously flowing phase stream is more important than the Poiseuille term, and the volume of the droplet is influenced mainly by the gravity/density force. To a smaller extent, pressure differences across this profile will also have some influence. The frontal and total area of the droplet facing the flow of the phase not forming droplets is very small, and consequently, the viscosity (Poiseuille) term is less important (or even negligible).

The volume of the resulting droplet is influenced by the flow rate Q_n of the phase that does not form droplets, the flow rate ratio between the phases, and by the construction of the inner capillary system. The force vector F_R is equal to $F_R = F_{GD} \pm F_H$ and is vertically oriented. As a result, a graph depicting the dependence of V_n and L_n on Q_n (see Figure 33, curves 2 and 3) exhibits a small curvature, depending on the orientation of the hydrodynamic force with respect to the axis of the organic phase flow forming droplets (countercurrent, upstream) because of the quadratic term $k_B \cdot Q_n^2$ in Equations 3 and 4. The geometry of the inner capillary system will thus also affect the influence of the flow rate in both cases.

The same conclusions are also valid for single and multichannel segmenters with the flow rates oriented in opposite directions (see Figure 16B and C), with the aqueous phase forming the droplets. The concentration of a particular component in the aqueous reaction mixture, c_i, is governed by the flow rate ratio between the aqueous stream in question and the sum of the flow rates of all streams of aqueous phase, $(Q_a)_i/\Sigma(Q_a)_i$, and the actual concentration of the component in each solution $(c_0)_i$, where

$$c_i = \sum [(c_0)_i \cdot (Q_a)_i] \Big/ \sum (Q_a)_i = \sum [(c_0)_i \cdot V_i]/V \qquad (9)$$

and c_i is independent of the total segment volume V. It can also be varied by changing the dosing time at a constant flow rate ratio of the aqueous phase component streams since the partial volume of each solution in a particular segment, V_i, depends on its flow rate and dosing time ($V_i = t_i \cdot (Q_a)_i$).

The reaction mixture is homogenized during the droplet formation period due to the very intensive mixing pattern produced by the flows of aqueous phase meeting at the end of the inlet capillary system. The homogenization process is forced by the secondary flows occurring due to the viscous drag of the organic phase flow on the

front area of the droplet of aqueous phase. Thus, a practically homogeneous reaction mixture is usually obtained before the droplet is cut off from the capillary system.

The droplets move into the outflow channel after cutting off, together with the continuous flow of the organic phase which is continuously wetting the walls of the mixing compartment and the conical housing. Additional mixing takes place in the conical outflow housing of the multichannel dropping segmenter, while the spherical shape of the droplet is being transformed into a distorted spherical or cylindrical shape inside the reaction coil. A more or less regular segmented flow consisting of independent segments of the two phases is obtained.

The homogeneous reaction mixture is transported through the fluoropolymer capillary system of the FIA analyzer in the form of individual segments of aqueous phase. It is exposed to continuous mixing during passage through the reaction/extraction coil due to an intensive intrasegmental flow, forced by the velocity distribution in a laminar flow profile. Diffusion also aids in distributing the analyte evenly within the segment. The reaction takes place in each segment of aqueous phase, forming a closed reaction system completely isolated from the other aqueous segments by the film of organic phase which prevents analyte carryover. An instantaneous extraction of the solute follows the reaction, increasing the reaction/extraction rate in many cases.

The segment length of the wetting phase not forming droplets and the phase-forming droplets are regulated by their total flow rates and the droplet time t_{max} (V_s) = $Q_n \cdot t_{max}$ or $V_s = Q_d \cdot t_{max}$, respectively) or dropping frequency, $f_d = 1/t_{max} = Q_d/V_s$ or $f_d = 1/t_{max} = Q_d/V_s$, respectively.[143] It grows linearly with the flow rate since the influence of the hydrodynamic forces are trivial. A small curvature of the dependence of $V_s = f(Q_n)$ appears, and a power relationship fits better for higher flow rates and flow rate ratios.

b. Noncoaxial Segmenters

This type of a segmenter consists mostly of a compact segmenter body made of hydrophilic, lipophilic, or combined hydrophilic and lipophilic materials, with an inner triple channel capillary system. The channels can be oriented perpendicularly to each other (T-type segmenters, Figure 31B and C), and/or at different angles among the inlet and outlet tubes (30°, 45°, 60°, 120°, or 150°, etc. for W- or Y-type segmenters), with the immiscible phase flow entering the segmenter horizontally, vertically from the bottom or from the top, and/or at different angles to the main segmenter axis (see Figure 13). Segmentation processes of hydrodynamic and interfacial origin in T-shape segmenters have been described in detail,[46,115] the important role played by a "ripple" segmentation pattern in narrow bore fluoropolymer segmenters was overlooked;[144] thus, only the basic conclusions are given here.

At very low flow rates and low flow rate ratios, the gravity and density forces predominate, and the influence of the hydrodynamic force can be neglected when F_H and $F_G + F_D$ are perpendicular to each other (also at different angles). The resulting total droplet volume, the total segment volume, and the segment length are governed by Equations 5 to 8. They are practically independent of the flow rate Q_n of the phase not forming droplets, and consequently, the dependence of V_n or L_n on Q_2 are depicted as straight lines parallel to the x-axis (similar to curve 1 in Figure 33).

At low flow rates, the gravity force still predominates, but the influence of the hydrodynamic force cannot be neglected. The resulting force vector can be calculated from the condition that the forces acting on the droplet must be equal. The frontal area and the total surface area of the droplet can be expressed from the droplet volume, assuming a spherical droplet shape. The resulting curves are nearly linear and parallel to the X-axis, or, at higher Q_n, slightly deformed toward lower V_n values (similar to curve 2 in Figure 33). This results from the decrease in droplet volume due to the hydrodynamic force acting on the droplet.

At moderate flow rates, the influence of the hydrodynamic force is quite markedly expressed and is comparable with the influence of the gravity and density forces. The resulting curves are strictly nonlinear for different cos θ values, the curvature depending mainly on the F_H/F_{GD} ratio.

At high F_H/F_{GD} ratios, the hydrodynamic force predominates, and the curves are distinctly nonlinear and close to parabolic (Figure 34, curves on right). When F_H/F_{GD} is close to unity, both forces are approximately equal and the curves are slightly concave or convex (but may also be linear).

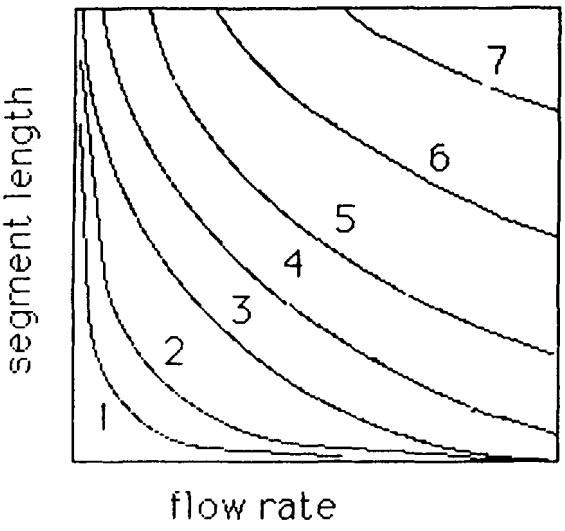

FIGURE 34. The dependence of the segment length on the flow rate of the phase that does not form droplets for different values of F_{GD}/F_H ratio.

The hydrodynamic force predominates at very high flow rates of the phase not forming droplets and at high flow rate ratios. The influence of gravity and density forces is negligible and the volume of the droplet is influenced mainly by the equivalence of hydrodynamic and interfacial forces (Equation 3). The hydrodynamic force F_H grows with increasing total droplet volume as the total droplet area increases and the frontal area of the droplet decreases. Also, the deformation of the spherical shape of the droplet increases under the same conditions, depending on droplet deformability. The frontal area of the droplet, expressed in terms of droplet volume assuming an elliptical shape,[13,46,143] and the Bernoulli term of Equation 3 are influenced both by the total droplet volume and by the flow rate. As the volume increases, the deformation also increases and the frontal area decreases with time. The total surface area increases when the segment shape becomes increasingly ellipsoidal.

The influence of the two terms in Equation 3, combining all the above-mentioned equations, can be expressed as a dependence of the total volume of the droplet V or the total segment length L_s on the flow rate Q_n of the phase not forming droplets. The resulting curves for different values of the constant Bernoulli and/or Poiseuille terms k_B and k_P are clearly nonlinear[46,143] (Figure 34, curves on left).

Quite similar curve shapes can be derived for the Y or W continuous segmenter types since the resulting hydrodynamic force can be expressed in the same way. The resulting force vector predominantly influences the droplet volume or the segment length. However, an exact mathematical treatment of the curves is difficult since the change in the radial velocity profile is complex in nonlinear tubing.

The segment length of the wetting phase (the phase that does not form droplets) is regulated by its total flow rate and droplet time t_{max} ($V_s = Q_n \cdot t_{max}$) or dropping frequency $f_d = 1/t_{max} = Q_d/V_s$. It grows nearly linearly with the flow rate when the influence of the hydrodynamic forces is negligible, and a power relationship is valid for higher flow rates and flow rate ratios.

2. "Ripple-Form" Segmentation Process

The following parameters influence the skewing process (and usually also the segmentation mode):

1. The ability of one of the phases to wet the wall material
2. Possible contamination on the walls of the segmenter chamber
3. The distance between the inlet/outlet tubes (which should approach zero)
4. The flow rate of the phase not forming the droplet and the flow rate ratio (Q_a and Q_a/Q_0 or Q_0 and Q_0/Q_a, respectively), which should both be high
5. The viscosity ratio η_2/η_0 and interfacial tension $\gamma_{0/2}$ of both phases
6. Changes in the geometry of the inner capillary system

Both phases flow continuously into the outlet tube, forming two independent axial laminar flows, one of them having a higher affinity toward the material, thus wetting the walls of the mixing compartment of the segmenter and the outflow tube. The walls are covered by a thin layer of the solvent, forming a more or less stationary film of wetting phase. The driving force for film formation is minimization of the interfacial energy at the solid/liquid interface, which is determined by the relative magnitudes of the surface tension of the inner wall surface of the tubing to the liquids (wetting ability), and the interfacial tension of the liquids. The thickness of the film depends on the flow rate, flow rate ratio, and the alternation frequency and segment length ratio $L_{s(org)}/L_{s(aq)}$. It can, for practical purposes, be expressed by an exponential function in the form[143,153]

$$d_f = k \cdot r_0 \cdot (u \cdot \eta/\gamma_{o/a})^a \qquad (10)$$

where r_0 is the inner diameter of the tubing, u is the linear velocity of the flowing stream (in cm s^{-1}); η and $\gamma_{o/a}$ are viscosity (in poise) and surface tension (in dyn cm^{-1}); and a and k are constant terms which are usually equal to 0.5 or to 0.67.

Once a thick film of wetting phase is formed on the inner wall surface of the two cylindrical parts of the segmenter, it is not stable with time. This is quite independent of the wettability, and any film of a fluid deposited on the inner surface of a narrow cylindrical tube is inherently unstable. It will rearrange so as to decrease the interfacial energy at the liquid/liquid interface (i.e., to reduce the interfacial area), and will eventually produce a ripple, lenses, and finally droplets or plugs of the phases.

The film rearrangement process is forced by many factors. The importance of each of these will depend on the wall material, physical properties of the two solvents, total flow rate, character of the flow, and its velocity distribution. Film rearrangement is also influenced by changes in the flow rate, cross-sectional area, etc. Yet another property affecting film rearrangement is the Rayleigh instability of the film on the inner wall of the tube.[143,153]

A relatively smooth and very thick film of organic phase is formed under the experimental conditions discussed above. The film thickness is relatively uniform at the beginning of the fluoropolymer compartment or the outlet tube. The film will, however, rapidly rearrange (partly inside the mixing chamber) into waves of different amplitudes and will finally break down into droplets or plugs (see Figure 35). The instability with time for the most rapidly growing perturbations of the film thickness can be expressed[153] by

$$\ln[(\delta_f)_t/(\delta_f)_0] = (\gamma_{o/a} \cdot d_f^3 \cdot t)/(12 \cdot \eta \cdot d_e^4) \qquad (11)$$

where $(\delta_f)_t/(\delta_f)_0$ is the growth of the film waves in time t; η is the viscosity of the organic phase; d_e is the inner diameter of the extraction capillary or outflow tube; d_f is the film thickness; and $(\delta_f)_0$ and $(\delta_f)_t$ are the amplitudes of the wave at times zero and t, respectively. According to this theory,[153] when the film is subjected to an infinitesimally small perturbation, a standing wave of length $\lambda = 2 \cdot \pi \cdot d_e \cdot \sqrt{2}$ will start to grow in amplitude until droplets are formed.

Another important aspect of film rearrangement and of the segment formation is its centricity. Under the influence of gravity and density

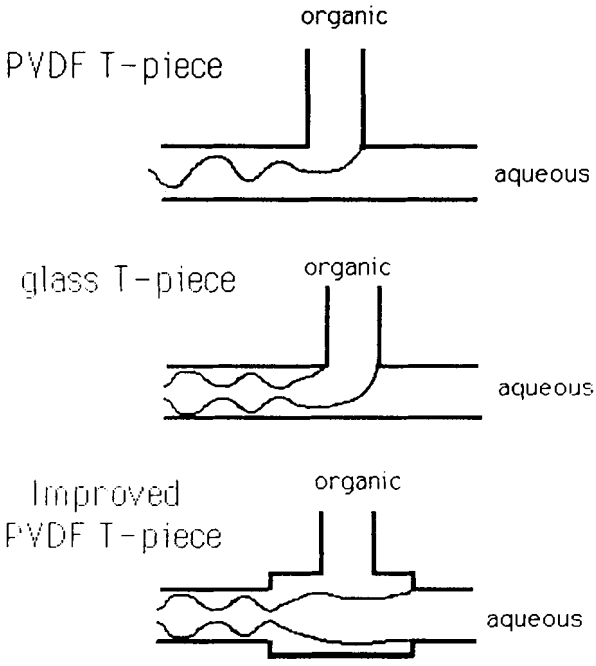

FIGURE 35. The "ripple" segmentation mechanism in a glass (B) and fluoropolymer T-shaped segmenters with a constant (A) and an enlarged (C) outflow channel.

forces, together with the hydrodynamic force, the organic flow tends to wet preferably the bottom or top part of the tube (depending on the direction of the resulting force vector and the spatial orientation of the inlet/outlet tube axis). The asymmetry in centricity forces ripple formation and results in rapid droplet formation, especially in horizontally positioned outlet tubes. Horizontal or vertical orientation of the axis of the outflow channel and/or the axis of the main axial flow influences the resultant force vector, which impedes or forces droplet formation.

The geometrical shapes of the outlet tube and the segmenter compartment strongly influence "ripple" formation during the segmentation process. Coiling the outlet tube will change the parabolic flow velocity distribution across the tubing profile into an asymmetrical velocity distribution, which is deflected toward the outer wall.[113]

Changes in the inner diameter of the outflow branch of the segmenter mixing chamber (together with the short fluoropolymer tubing inserts inside the outflow channel of the segmenter) also change the cross-sectional area of the outflow tube. As a result, the flow of both phases will be speeded up and oriented toward the center of the tube. A secondary flow, perpendicularly oriented to the main axial laminar flow, is thus introduced. The secondary flow produces changes in the flow velocity distribution across the tubing profile, forces the formation of ripple on the film, and increases radial mass transport in the outflow tube. It also increases the influence of the Poiseuille and Bernoulli forces.

All these factors introduce secondary forces which speed up the ripple formation process. When the segmenter is in a horizontal position, segments are formed in the segmenter mixing chamber or immediately after reaching the beginning of the fluoropolymer outflow tube, whereas in a vertical position with the outflow axis oriented from the top to the bottom of the chamber, segments are formed approximately 1 to 20 cm from the segmenter. Thus, the ripple segmentation mode is faster and less reproducible with a horizontal outflow axis than with a vertical outflow from top to bottom. Also, coiling the outflow tube, especially near the segmenter outflow channel, has a positive effect on segment formation and its reproducibility.

B. Film Formation

The high extraction efficiency in the extraction coil is explained by the mechanism of liquid-liquid extraction FIA in narrow capillary tubes, which is mainly due to the formation of a very thin film of one solvent on the inner wall of the capillary system[41,115,144,145] surrounding segments of the other phase. This can be directly observed for glass and thin wall fluoropolymer tubings, and indirectly for steel.

If one of the phases in a liquid-liquid segmented system forms a film on the tubing wall, surrounding the other segments, the film-forming phase will consist of a continuum throughout the extraction coil (see Figure 36C and F). All segments of the film-forming phase are in the contact with each other since the film more or less completely covers the walls of the extraction coil. The existence of a bridging film between adjacent

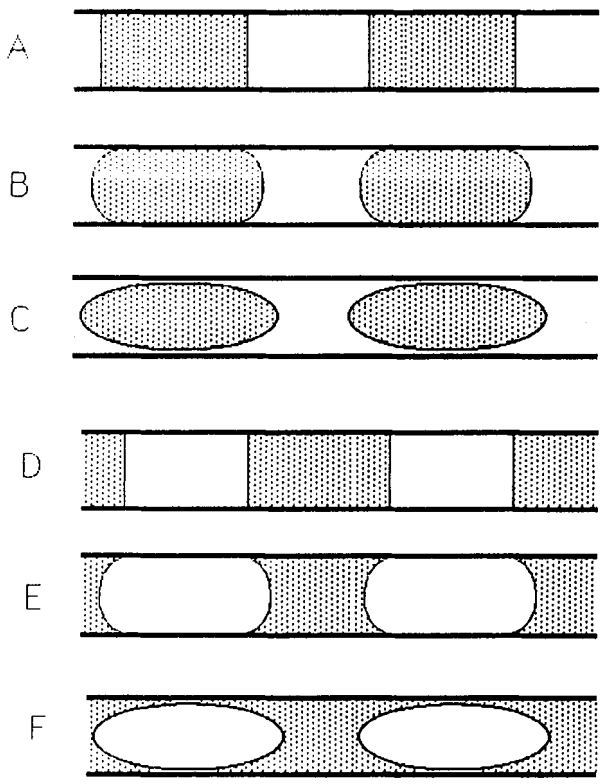

FIGURE 36. The shape of the segments of aqueous (empty) and organic (filled) phases in a glass (A to C) or fluoropolymer (D to F) tubing inside a sample loop of the loop injector (A, D) and at zero (B, E) and very high (C, F) total flow rates Q_t.

segments of one of the phases actually stabilizes the entire segmented stream.[41,121,145] On the other hand, transfer of solute from one segment to the adjacent segment of the same phase may also be observed in the extraction coil. Consequently, during passage through the extraction coil, the sample in the film-forming phase is spread to more segments than into which it was originally introduced and dispersion occurs. This is due to mass transfer "backwards" from one organic segment into subsequent organic segments via the wetting film. The film is thus important to the peak broadening process (i.e., for the dispersion and dilution of the sample). The thicker the film, the greater the peak broadening.

The investigation of segmented flow in thin-walled Teflon tubes through a microscope with 10× magnification (using an attached SLR camera and a flashlight producing short duration flashes) shows[41] that there is no film when the segment train is still. The organic phase does not wet the tubing wall and the contact angle is greater for a chloroform/water system. When a flow velocity is applied, the contact angle approaches zero and a thin film appears. On increasing the flow rate, the film gradually becomes thicker. Hence, film thickness is a function of the flow velocity. When the flow is stopped again, the film disappears and breaks up into small lenses (or for the case of very thin films and short segments, it is just taken up by the main segments).

The behavior of a thin film of an organic solvent formed on the walls of the extraction coil tubing in a continuous liquid-liquid extraction flow system was also studied using a fast reading "on-tube" photometric detection system (approximately 3-ms time resolution), controlled by a computer.[145]

A marked increase in segment length of the aqueous phase with increasing total flow rate was found during studies of the segmentation process in liquid-liquid extraction FIA.[133] A loop injector was used for introducing distinct, reproducible volumes of one phase into the continuous flow of the other one as a segment volume standard. The segments of very precisely defined volumes of one immiscible phase in a continuously flowing stream of the other phase were used for determining the wetting film thickness, by measuring the lengthening of the aqueous segments in a continuous flow of an organic solvent.[145] The film thickness d_f was calculated from the segment lengthening at different chloroform flow rates, and was found to depend polynomially on the linear flow velocity $d_f = (u^a)$.[145]

A plot of the analytical signal against time measured for the segments of the organic phase in the continuous flow stream of the aqueous phase (or for the aqueous segments in the continuous flow of the organic phase at very low flow rates and low flow rate ratios) shows a characteristic, nearly rectangular shape, which is deformed at the higher flow rates (see Figure 37).

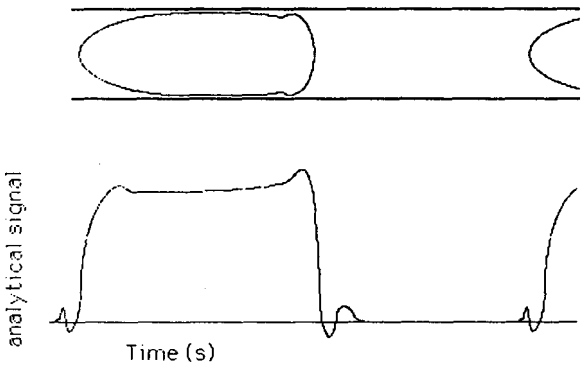

FIGURE 37. A segment shape of the aqueous phase in fluoropolymer tubing at moderate flow rate Q_t and appropriate signal shape with the typical spikes and deeps at the ends of the "trapezoidal" form (organic phase used for baseline establishment).

The slopes of the nearly horizontal parts of the curves are close to zero for very long segments of either phase or for very low flow rates. They are mainly positive for shorter organic phase segments or higher flow rates, which also tend to move the position of the plateau toward slightly higher values (especially at very high flow rates and high frequencies of segment alternation). The film formation process results in an increase in both baseline noise and baseline signal. A more or less clear deformation of the horizontal parts of the curves was evident, and may be attributed to film formation or film destruction, as well as to a fluctuation of the film thickness with time.

At high flow rate of the organic phase Q_0, the aqueous segments have a nearly rectangular wave shape, with spikes of varying

amplitude at the ends of the segment plateau[41,102,115,130,133,144–147] as a result of the sharper concave ends of the segments. The abrupt changes in the optical parameters on surfaces of the menisci result, in some cases, in the formation of negative "deeps" on the baseline at the beginnings or at the ends of the aqueous segments (Figure 37).

The typical shapes of the organic segments in the aqueous phase flow stream (or of the aqueous segments in the organic phase stream), seen at different flow rates, express the lengthening of ascending and descending parts of the curves for both segments. More convex or concave forms of the ascending and descending parts of the curves at the higher flow rates are due to deformation of the strictly cylindrical shape that the segments have in the injection loop, to their distorted cylindrical or semi-ellipsoidal shape inside the extraction coil. The characteristic paraboloid form of the leading and trailing ends of the segments is brought about by film formation at the ends of the segments and by deformation of the segments due to the action of hydrodynamic forces.

The length of the aqueous segments is markedly increased with increasing flow rate of the organic phase stream. This influence is stronger for the shorter segments than for longer ones over the entire range of the flow rates of the organic phase Q_0. The relative changes in segment length depend polynomially on the flow rate, with some evidence of a plateau at higher flow rates of the two solvents. The ratio between analytical signal area and signal duration increases for the aqueous segments with the flow rate Q_0, and then remains practically constant at higher flow rates.

Segmentation reproducibility is impaired by increasing the length L_{EC} of the extraction capillary and the total flow rate Q_t, with a minimum occurring at moderate flow rates. The segmentation pattern is very regular over a very wide range of Q_a, Q_0, and L_{EC}.

Better segmentation reproducibility is achieved by injecting the aqueous phase into the flowing organic phase than by injecting the organic phase into the aqueous phase stream since in the former case a stable film of organic phase already exists on the tubing walls prior to injection. The lower wetting ability of water on the Teflon tubing material of the loop capillary is also important, especially for the rinsing process. The segmentation reproducibility is better for longer than for shorter segment lengths, but it is satisfactory in most cases.

Small droplets of the organic or aqueous phase are formed at the leading or trailing ends of the segments as a result of incomplete rinsing of the sample loop at the low flow rates. The same phenomenon has also been observed at very high flow rates ($Q_a > 8$ ml min^{-1}), probably as a result of destruction of the very narrow cylindrical segments, or destruction of the very thick film of organic solvent formed on the Teflon tubing walls at high flow rates when the interfacial forces are lower than the Poiseuille forces.[143] This results in a less reproducible segmentation pattern.

These processes were observed during the earlier research studies and have been the subject of very detailed investigation, including peak height and peak area depression,[41,115] sample dispersion and peak broadening,[6,41,54,115] and kinetics of the extraction process.[85,114,121] Considering the extent of very good work on the various parametric effects, there is an increasing need for a detailed fundamental description of the wetting film behavior.

1. Film Formation Theory

When a plug of a very precisely defined volume of one immiscible solvent is introduced into a continuously flowing stream of the other solvent, the original cylindrical shape of the solvent plug inside the loop injector changes to a spherical, an ellipsoidal, or a deformed tubular segment shape inside the reaction/extraction tubing (see Figure 36). The resulting segment shape depends primarily on the ratio between the segment volume and the inner radius of the tube, the total flow rate, the interfacial tension of the solvents, the surface tension of the solvents, the tubing material, and other factors.

Depending on the material of the extraction capillary coil, the solvent displaying greater affinity to the tubing material will cover its inner walls with a very thin film of relatively stationary nature. The film of organic solvent will surround the deformed spherical, ellipsoidal, or tubular

aqueous segments in fluoropolymer tubing, whereas the segments of organic phase will be surrounded by an aqueous film in glass or metal tubing.

As cited earlier, the driving force for the film formation is the minimization of the interfacial energy at the solid/liquid interface, which is determined by the relative magnitudes of the surface tension of the inner wall surface of the tubing to the liquids (wetting ability) and the interfacial tension of the liquids. The film thickness is related to the nature of the film-forming phase in such a way that higher viscosity and/or lower interfacial tension result in a thicker film. The influence of the properties of the film-forming phase depends on the $(\eta/\gamma_{0/a})^a$ factor. For a flow velocity $u = 11$ cm s^{-1}, a pentanol film is about eight times thicker than a chloroform film.[41] This difference offers an explanation for the great difference in dispersion between FIA systems that are similar except with respect to the type of organic solvent.

In this case, the velocity, surface tension, and viscosity are important parameters. The quotient $(\eta/\gamma_{0/a})^a$ for $a = 1$ is 58, for $a = {}^2/_3$ is 15, and for $a = {}^1/_2$ is 8 times greater for pentanol than for chloroform. Using the d_f relationship with $k = a = {}^2/_3$, the calculated values of d_f for pentanol and chloroform are between 3 and 6 times higher than that obtained experimentally, but the quotient seems to be useful for predicting the relative influence of the phase forming a film.[44]

Another factor influencing the formation of the wetting film are the hydrodynamic forces connected with the mass transport due to the velocity distribution of the laminar flow across the tube profile, as the segment of nonwetting solvent forms a compressible bolus flowing through the stream of the other solvent. Besides other factors influencing the segment shape, the mean value of the film thickness (or the geometrical parameters of the "ringlike" orifice between the segment and the tubing walls) of one liquid behind a single plug of another (immiscible) liquid moving through a capillary (when one phase preferentially wets the capillary surface), evidently depends on the linear velocity u of flowing stream (or its total flow rate Q_t).

The linear velocities in FIA are generally low, and the prevailing flow pattern is laminar. Consequently, the flow velocity near the tubing wall is zero, while in the center of the tube it is twice the mean value taken along a radius. The film forms part of a relatively stationary phase along the wall, forcing a secondary internal flow to circulate within each segment.

There is no exact theory for film formation in liquid-liquid extraction FIA systems. However, several equations describing the film formation in gas/liquid systems[148,149] have been derived and also applied to liquid/liquid system.[7,41,143] These equations have the form

$$d_f = k \cdot r_0 \cdot (u \cdot \eta/\gamma_{0/a})^a \qquad (12)$$

where r_0 is the inner radius of the tubing, u is the linear velocity of the flowing stream (in cm s^{-1}), η and $\gamma_{0/a}$ denote viscosity (in poise) and surface tension (in dyn cm^{-1}), and a and k are constants which are usually equal[154,155] to ${}^1/_2$ or to ${}^2/_3$.

The film formed by one phase on the tubing walls as a result of a linear velocity distribution across the tubing diameter or due to the wetting properties of the solvent, causes changes in the segment geometry.[41,115,145] Assuming a constant volume V_s of the segments of one immiscible phase (in this case aqueous) in the continuous flow stream of the other phase, we can predict the lengthening of the aqueous phase segment caused by the wall film (or by changes of the nearly cylindrical shape of the segment at zero flow rate to some ellipsoidal or deformed cylindrical shape; see Figure 36), the ends of the segments displaying a more or less characteristic convex (concave) shape at higher flow rates.[41,115,145]

The segment length L_s can be expressed as a function of the segment volume V_s and the outer radius of the cylindrical segment r_s, which depends on the inner radius of the tubing r_0 and the mean value of the film thickness d_f ($r_s = r_0 - d_f$), in the form:

$$L_s = V_s/\pi \cdot r_s^2 = V_s/\pi \cdot (r_0 - d_f)^2 \qquad (13)$$

Here, the segment length L_s is inversely proportional to the second power of the outer radius of the segment r_s. With increasing film thickness d_f, L_s will increase as a result of the decreasing

outer radius of the segment at higher flow rate or linear velocity. The relative segment lengthening decreases with increasing tube inner radius.

Assuming that the $(\eta/\gamma_{0/a})^a$ term is constant during the experiments (and by inserting the film thickness value d_f from Equation 12 into Equation 13, the segment length can be expressed as a function of the linear velocity u as

$$L_s = V_s/\pi \cdot (r_0 - k_u \cdot r_0 \cdot u^a)^2 = L_0/(1 - k_u' \cdot u^a)^2 \quad (14)$$

or as a function of the total flow rate Q_t (Q_a in our case) in the form

$$L_s = V_s/\pi \cdot (r_0 - k_q \cdot r_0 \cdot Q_t^a)^2 = L_0/(1 - k_q' \cdot Q_t^a)^2 \quad (15)$$

Using different values for a and the k_u, k_u', k_q, or k_q' constants (a = 0.1 to 2, k = k' = 0.1 to 1), we can calculate the theoretical influence of the linear velocity u (or the total flow rate Q_t) on the segment length L_s (or on its relative lengthening $(L_s - L_0)/L_0$). The resulting curves are strictly nonlinear, their shape depending on both constants. The intercept with the L_s or $(L_s - L_0)/L_0$ axis corresponds to the original segment length L_0 at zero linear velocity [or total flow rate ($u \rightarrow 0$, $Q_t \rightarrow 0$)], or it is equal to zero, respectively. The segment length L_0 can also be calculated from the originally injected volume V_s of the appropriate immiscible solvent as $L_0 = V_s/\pi \cdot r_0^2$.

The length of the aqueous segments is increased in all cases as a result of the formation of a regular film of the organic phase on tubing walls made of lipophilic material, and of the convex form of both ends of the segments. Shorter segments are more strongly affected by film formation, and the film is completely destroyed when very long aqueous segments alternate with relatively very short organic segments. The analytical signal measured on the aqueous segments decreases with decreasing alternation frequency of the aqueous segments, or when a single segment of organic phase is introduced into a continuous stream of water. This is in agreement with visual observations[41,115,145] and with some experimental results.[152]

As noted, the thickness of the film depends on the flow rate and flow rate ratio, as well as on the alternation frequency and segment length ratio $L_{s(org)}/L_{s(aq)}$. All experimental evidence points to power relationships with a <1; the L_s values reaching a more or less evident plateau at higher flow rates Q_t or linear velocities u as a result of a polynomial dependence of the film thickness d_f on the two parameters.[145] A study of this equation implies that the quotient η/γ could be useful in estimating and comparing film-forming properties for different solvents. The coil material also influences film formation and its parameters. Tubing materials such as PE, PTFE, etc. allow the organic phase to form a film that improves the conditions for efficient extraction.[68]

Using η = 0.0058 P and $\zeta_{0/a}$ = 32.8 dyn cm^{-1} for chloroform, the values of the constant a can be calculated, ranging from 0.4 to 0.7, depending on the segment length[145] (values ranging from 0.34 to 0.5 have been found for shorter or longer water segments in an air-segmented continuous flow analysis SCFA; see References 154 and 155). This value is probably inversely proportional to the segment length, as the Poiseuille force increases with the length of tube distortion.

The thickness of the film is generally expected to be linearly dependent on flow rate or the linear velocity, with the value of the constant a equal to 1. These conclusions are due to an erroneous interpretation of the experimentally found dependence of the film thickness (in microns) as a function of the linear velocity u (in centimeters per second) for pentanol/water or chloroform/water systems, assuming a linear dependence of the film thickness (a = 1) on the linear velocity u or total flow rate[41] Q_t.

The experimentally found film thickness values (d_f = 7 and 14 μm for u = 11 and 23 cm min^{-1} and d_f = 14.6 μm for u = 3.5 cm min^{-1} are given in References 41 and 115, respectively with d_f and the u. d_f c. · 30 μm estimated for pentanol in a pentanol/aqueous system for u = 5 cm s^{-1} in 0.7 mm PTFE tube[41]) are usually lower than those theoretically calculated. This may be due to partial destruction of the film by altering segments of the other immiscible solvent, and overlooking the influence of the leading and trailing end shapes on the mean value of the film thickness.

Film thickness was also calculated using Bretherton's equation[156] for 0.0058 P, 32.8 dyn cm^{-1}, u = 3.5 cm s^{-1} as 14.8 μm.[115] The shape of the segment ends was also measured directly from photographs of the segments flowing through the extraction coil at 3.5 cm s^{-1}. The ends were semi-ellipsoids with a major axis of 1.5 mm (radius of the tube) and a minor axis of 0.97 ± 0.06 mm (0.15/0.09), independent of the segment length.

Film formation also has an influence on the baseline value, as was observed for segment shapes at different flow rates of the aqueous phase at constant organic phase flow rate. The length of the aqueous segments increases with increasing total flow rate Q_t, and the film is destroyed at a certain defined value of the ratio of the lengths of the aqueous and organic segments. Above that value, the baseline is identical to that of water flow. At very high flow rates, and when the aqueous and organic segments alternate with a very high frequency, the role of the film formation is very important. The film thickness becomes high and the baseline increases rapidly with increasing flow rate. At a very high organic flow rate with short water segments, the baseline signal cannot be compensated, and segment length measurement is not possible.[133,143-147]

At a very high total flow rate Q_t, it is impossible to visually observe deviations in the flow pattern in the extraction coil, but there is a definite risk that the small organic phase segments are disrupted so that an irregular segmentation pattern results. This might lead to an inferior contact between the two phases and a decrease in the extraction efficiency. The typical physical disruption of organic phase segments was not assured experimentally; nevertheless, it might be the cause of the observed deviations.

The ability of a segment to resist disruption should depend on its segment length. The force that tends to disrupt the segment is proportional to the linear flow velocity u. Both the segment length and u are inversely proportional to the cross-sectional area of the tube. This means that the conditions for segment disruption will depend principally on the total flow rate and the flow rate ratio, and not on the tube diameter. This might explain simultaneously appearing maximum values for different coil ID values; thus, the concentration ratio cannot be increased indefinitely just by increasing sample flow rate and flow rate ratio, Q_a/Q_0 (furthermore, at a very high total flow rate Q_t, the efficiency begins to decrease[17]). Reproducibility decreases at Q_0 <0.3 ml/min^{-1}, Q_a ca. 4 ml/min^{-1} (10 to 2% at 0.15 with respect to 0.30). The baseline was found to be unstable and noisy for benzene/chlorobenzene[104] and benzene/dichlorobenzene[101] mixtures (1 + 3 and 1 + 1 v/v, respectively) at Q_0 and Q_2 >0.9 ml min^{-1}; thus, 0.8 ml min^{-1} was used for the reagent, the carrier, and the organic phase flow rates.[101]

C. Extraction

Liquid-liquid extraction in FIA is efficiently performed, despite having relatively large organic (and especially aqueous) phase segments. This can be attributed to the formation of a very thin, relatively stationary film of one solvent on the inner wall of the capillary tube system, produced by the solvent having the greater affinity for the capillary coil material.

The extractable analytes are transferred from the relatively homogeneous solution of the higher analyte concentration in one immiscible solvent into segments of the other phase via the segment interface. The analyte is transported to the segment interface by diffusion and by the internal flow within the segment. The interfacial area available for the extraction consists of menisci between the segments and the film of organic phase surrounding the aqueous phase segments in Teflon tubing (or the film of the aqueous phase surrounding the organic phase segments in the glass or metallic tubing). Since the contact area becomes very large relative to the segment volumes, the solute transfer is very efficient. The extraction equilibrium is reached after some necessary time delay, which depends on the extraction rate, extraction efficiency, and other factors.

Two basic mass transport mechanisms must be distinguished — transport through the vertical interfaces (menisci) between the two phases (axial extraction), and transport through the horizontal interfaces (the wetting film) on the tubing wall (radial extraction). Which of these mechanisms will predominate is determined by the ratio

between the interfacial area and volume, as well as by other factors.[41,104,105,133,143] The size of the interfacial contact area is influenced mainly by the segment length in the extraction coil, requiring that the segmentation pattern is controllable and constant during the entire analytical procedure.

The first of these two mechanisms plays an important role in ordinary liquid-liquid extraction FIA using relatively short segments of both phases. When efficient segmentation is not possible or when long segments of one immiscible phase are introduced into the continuous flow of the other phase, the second (film) mechanism plays an important role in effecting the extraction. However, because the extraction process was found to be relatively poor using a long aqueous sample zone and no regular segmentation, the first mechanism would play the more important role in liquid-liquid extraction FIA.[106] Most probably, an integrated model of the liquid-liquid extraction will overcome the existing problems connected with these two extreme situations.

The model of liquid-liquid extraction that must be considered involves the aqueous segment running through a sheath of organic solvent on the inner surface of PTFE tube. Extraction takes place instantly and the analyte concentration changes gradually due to dispersion.[115] It is assumed that, in a straight tube solvent extraction setup, laminar flow occurs. The cross-sectional profile is parabolic, with the linear velocity being zero at the walls, while the flow in the center of the tubing is twice the mean value of the linear velocity. The difference between the local flow velocities drives the liquid in the segments into a circulating toroidal flow. Small eddies are also formed within the segments, improving the mixing. The model[85] involves a species X being extracted from an aqueous segment to the organic phase, with the organic phase forming the film on the walls (Figure 38). It is assumed that there is an even distribution of the analyte within the aqueous segment at the start of the extraction process.[85]

Eventually, diffusion zones (defined to be the regions in both aqueous and organic segments adjacent to the liquid-liquid junction where the transport of species X takes place) are formed. Their thickness is affected by the amount of convective mixing in the segments. In the aqueous segment, species X is more concentrated in the bulk region (Figure 38, marked off by broken lines) than in the diffusion zone. The opposite holds true for the organic segments, where the concentration of X is greater in the diffusion zone than in the bulk region. In order to describe the actual extraction process, one should take into consideration the mass transport of species X from the bulk to the interface in the aqueous segment and its transport from the interface to the bulk of the organic segment.

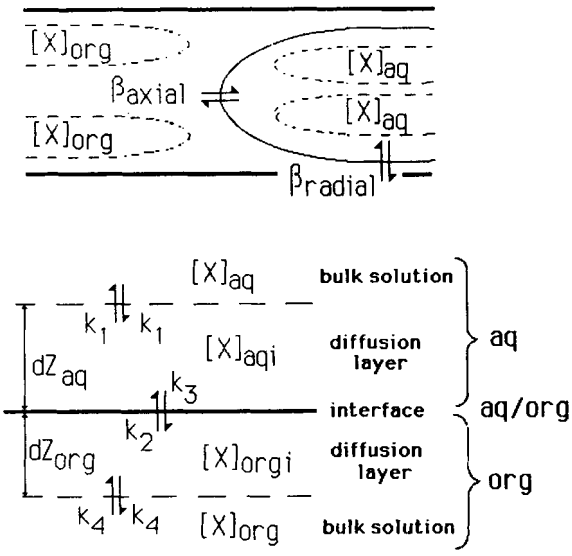

FIGURE 38. Schematic diagram of the transport phenomena in liquid-liquid extraction FIA systems, k_1 to k_4 are diffusive mass transfer constants, β are mass transfer coefficients (see text for more details).

From a kinetic standpoint, the extraction process is assumed to be first order. The diffuse mass transfer constants of X to and from the diffusion zones for the aqueous and organic phases (see Figure 38) are represented by k_1 and k_4, respectively. Both are defined as D/dZ, where D is the relevant diffusion coefficient and dZ is the thickness of the diffusion layer. Consequently, the greater the convection, the smaller the dZ value and the greater the k_1 and k_4 values. The rate constant for the transfer of X from the aqueous diffusion zone across the liquid junction to the organic diffusion zone is k_2. The rate constant for backward transfer is k_3.

The concentration of the analyte in the bulk regions of the aqueous and the organic phase are

represented by $[X]_{aq}$ and $[X]_{org}$, respectively, while the analyte concentration in the interface is symbolized by $[X]_{aqi}$ and $[X]_{orgi}$. The interface area/volume ratio is S/V. The flux across the individual interfaces can be described by $Q_1 = k_1([X]_{aq} - [X]_{aqi})$, $Q_2 = k_2[X]_{aqi} - k_3[X]_{orgi}$, and $Q_3 = k_4([X]_{orgi} - [X]_{org})$.

At steady-state conditions, $Q_3 = Q_2 = Q_1 = (V/S)\, d[X]_{org}/dt$. Working through the equations will eliminate $[X]_{aq}$, $[X]_{aqi}$, and $[X]_{orgi}$. For the case in which $V_{aq} = V_{org}$ (where the volume of both phases is equal), this gives

$$1 - (1 + 1/K_D)[X]_{org}/C = \exp\{-t_c(S/V)k_1k_4(k_2 + k_3)/(k_1k_3 + k_1k_4 + k_2k_4)\} \quad (16)$$

The distribution constant K_D is equal to k_2/k_3. Residence time in the extraction conduit from phase segmentation to phase separation is represented by t_c, while C is the initial concentration in the aqueous phase. When mass transport to the interface (k_1 and k_4) is fast enough, compared to the transport across the interface, several terms can be eliminated, and

$$1 - (1 + 1/K_D)[X]_{org}/C = \exp\{-t_c(S/V)(k_2 + k_3)\} \quad (17)$$

Although this simplified model does not take into account all possible kinetic aspects of an extraction, it can be concluded that the ratio between the interface area and the phase volume (S/V) is very important.

When applied to an experimental flow system, the following significant points are made:

- The extraction rate is greatly improved by increasing the (S/V) ratio; by decreasing the inner tubing diameter, a maximum surface contact between phases can be achieved, thereby increasing the probability of the analyte to cross.
- Mass transfer to and from the interface increases with flow rate (directly related to flow velocity), likely due to an increase in the convective constituent of intrasegmental mixing.
- The extraction rate is also increased by decreasing the segment length, not so much because of an increase in the boundary areas, but rather largely attributed to eddies formed at points on the tubing wall; the number of eddy points increases with the number of segments per unit extraction conduit length.

In coiled tubes, however, the role of a secondary flow is taken into consideration. Centrifugal force is developed as a result of the helical geometry of a coil. It acts most strongly on the fastest moving regions of the flow at the tube center. This portion of the fluid is transported radially outward and is replaced by fluid flowing tangentially along the wall. This secondary flow makes intrasegmental mixing in coiled tubes more efficient, such that mass transfer is increased. This process can be correlated with the dimensionless velocity parameter De^2Sc, where $De^2Sc = [(d_t^3u^2l)/(\eta \cdot D)](1/d_c)$, in which De and Sc are the dimensionless Dean and Schmidt numbers, d_t is the inner diameter of the tubing, u is the average linear velocity of the fluid, η is the kinematic viscosity, D is the diffusion coefficient of the solute, and d_c is the coil diameter measured at the tube axis.

Like the previous model for straight tubes, extraction in coiled tubes is treated as a first-order process and is assumed to be governed by mass transfer to and from the aqueous/organic interface.[115] The integrated form of the extraction rate expression is $\ln[A_{eq,0}/A_{eq,0} - A_{t,0})] = K_{obs}.t$, where $A_{eq,0}$ and $A_{t,0}$ are either the steady-state analyte absorbances or peak areas, due to the analyte in the organic phase at equilibrium and at time t. The observed extraction rate constant K_{obs} is a function of both the mass transfer within the individual segments and the interface area (S). It is related to S/V (where V represents the volume of the segment) and its ratio as $K_{obs} = (S/V) \cdot \beta$ (where β is the overall log mean mass transfer coefficient and has the unit of velocity), and its reciprocal is often viewed as the resistance to mass transfer.

This coefficient is related to individual mass transfer coefficients by the expression $\beta = \{(1/\beta_{aq}) + (1/\beta_{org}K_D) + (1/\beta_i)\}^{-1}$, where β_{aq} and β_{org} refer to mass transfer coefficients to the interface through the aqueous phase, and away from the interface through the organic phase, respectively. K_D is the distribution coefficient of the

solute, and β_i refers to the transfer coefficient across the interface itself (see Figure 38). In the absence of surface-active solutes, the $1/\beta_i$ term can be neglected. In liquid-liquid extraction FIA systems, K_D is usually large, such that $\beta_{org}K_D$ is much larger than β_{aq} and the organic phase acts like a "sink" for the solute. Thus, the extraction rate is governed by mass transfer within the aqueous phase only where $\beta = \beta_{aq}$.

Since the solute leaves the aqueous phase through both ends and sides of the aqueous segments, β_{aq} can be treated as the sum of the products of aqueous phase mass transfer coefficients, one for axial mass transfer to the ends, one for radial mass transfer to the sides of the aqueous segment, as

$$\beta_{aq} = \beta_{aq,\ axial}(S_{ends}/S_{seg}) + \beta_{aq,\ radial}(S_{side}/S_{seg}) \quad (18)$$

From experimental data, some general trends are observed:

1. The extraction rate is enhanced by a high S/V ratio, best achieved with a small tubing diameter.
2. The extraction rate increases with decreasing segment length. Similar to the earlier model, the increase in S accompanying a decrease in segment length accounts for only a small fraction of the increase in K_{obs}. This phenomenon can be explained in terms of β_{aq}. When the segment is sufficiently reduced that the ends of the segment are on the order of a tube diameter apart, they interact hydrodynamically to increase the radial velocity component of the circulation within the segment. In effect, β_{aq} increases as convective intrasegmental mixing increases.
3. Increasing the flow rate increases the extraction rate with respect to time, brought about by the increase in intrasegmental mixing. Although a corresponding increase in the thickness of the stationary film is also expected, the mass transfer rate is not affected since the rate-determining mass transfer process occurs in the nonfilm-forming aqueous phase.
4. Based on Equation 18, a tighter coil (smaller d_c) increases the secondary flow and eventually increases mass transfer. This effect is more pronounced for long segments than for short segments due to the smaller velocity differential of short segments in a coiled tube. Unlike long segments, the axial flow profile in short segments is less than parabolic; hence, the centrifugal forces are more uniformly distributed across the tube.

As can be seen from the above models, they have a number of common aspects. They cover two extreme situations existing in liquid-liquid extraction FIA systems, associated with the use of tubes of different inner diameters and segments of different geometry. It is clear that there is an increasing need to formulate more general models which also cover intermediate situations of FIA. Such models will probably describe all of the possible segment geometries, fast to slow chemical reactions that occur, and differences in the extraction rates. It should be noted that the main part of the extraction process occurs in the extraction coil. However, some portion of the extraction occurs in the segmenter and phase separator, where relatively intense mixing of both phases occurs due to various factors (differences in manifold geometry, flow rates, changes in character of the flow through the phase separator and segmenter, and changes in the space orientation of the flows).

The enrichment factor (the ratio between the flow rates of aqueous phase containing the sample and the organic phase) is influenced by the segmentation process. The enrichment factor rarely exceeds 20 in commonly used manifolds. The length of the organic (and aqueous) segments cannot be reduced indefinitely. The lower limit of organic phase segment length has been experimentally estimated to be 1.5 times the internal diameter of the extraction coil tube.[41] The extraction efficiency decreases drastically if the droplets of organic phase become too small to form and maintain a continuous film on the tubing wall. Segmentation may be unstable due to coalescence of very small segments during their transport through the extraction coil.

The efficiency of the reaction or extraction can be expressed in the following form:[93]

$$m = Q_0/1000 \int_{t_1}^{t_2} C_0 * dt$$

$$= Q_0 \cdot \text{const.} \int_{t_1}^{t_2} A * dt \qquad (19)$$

where m is the number of moles extracted, C_0 is the molar concentration of the sample component extracted into the organic phase; Q_0 is the flow rate of the organic phase passing through the membrane (milliliters per minute) and flow cell; t_1 and t_2 are the times when a peak is initiated and finished, respectively; and A is the absorbance (when C_0 is calculated from the measured absorbance A, path length ℓ, and molar absorptivity ϵ, where $C_0 = A/\ell \cdot \epsilon$). The peak area is defined as

$$A_p = A_1 * H_p * W_p \qquad (20)$$

where A_1 is the absorbance corresponding to 1 cm of the peak height H_p, and W_p is the peak width. The apparent extraction efficiency E(%) can be expressed as $E(\%) = 100\ m/C_0 * V_s$, where E(%) is the percent of analyte extraction, C_0 is the concentration of the analyte in the sample solution (moles per liter), and V_s is the volume of sample injected. The extraction efficiency and peak height obtained using narrow tubing were better than those obtained with larger bore tubing.[93]

In some cases, the segmentation can break down at higher flow rates and flow ratios (e.g., pentanol at 23 cm s^{-1}; *n*-butanol 5 cm s^{-1}; MIBK at 7 ml min^{-1}). The liquid-liquid extraction efficiency decreases dramatically in the MIBK/aqueous system if the aqueous flow rate is above 7 ml min^{-1} and the phase volume ratio is simultaneously increased above 15. For pentanol/water in PTFE tubing, partial breakdown of the segmentation has been observed at a flow velocity of 23 cm s^{-1} and a phase ratio of unity. In a butanol/aqueous system[44,65] segmentation has been observed to breakdown at a flow velocity as low as 5 cm s^{-1} (i.e., 0.7 ml min^{-1}). This is also true for chloroform/aqueous methanol (1 + 1) breakdown.[44] Segmentation breakdown is more likely for phases apt to form thick films than for those forming thin films. The thicker film depletes the segments of organic phase, making them shorter and more likely to be disrupted.

The detector signal increases up to Q_0 = 6.5 ml min^{-1} and Q_a/Q_0 15 at tube ID values of 0.7, 1.0, and 2 mm; thus, total Q_t seems to be the cause of the decline in extraction efficiency rather than the coil volume, inner diameter, and coil length. The signal is greatest for a flow rate ratio of 5:2 (MIBK), which corresponds to a Q_a of 2.2 ml min^{-1} at Q_0 = 0.42 ml min^{-1}. The peak height increases as the Q_a/Q_0 increases, up to 5.2, and rapidly decreases above this value. This subsequent decrease can be attributed to the observable irregular segmentation between the two phases in the extraction coil. At $Q_a > 2.2$ ml min^{-1}, the peak height of the organic phase has a maximum at 0.4 to 0.73 ml min^{-1} for Q_0; at $Q_0 >$ or $Q_a <<$, the liquid-liquid extraction efficiency decreases since segmentation and separation of the phases are incomplete.[49]

D. Dispersion

An injected aqueous analyte is not only present in originally injected segment and in an adjacent segment of organic phase, but, to some extent, also downstream (and partly upstream) from the injected aqueous segment. Hence, liquid-liquid extraction occurs other than only at the boundary between the organic/aqueous phases containing the sample. The segments of an immiscible fluid and of an aqueous sample injected into a segmented flow do not remove the dispersion since intersegmental transfer and mixing occurs.

It is well established that for FIA liquid-liquid extraction systems, when one uses different organic phases and tubing materials the dispersion can vary greatly. In some cases (for example, chloroform and water in Teflon tubing), using low to medium flow rates, no dispersion of the sample can be detected, although long extraction coils have been tested; however, for pentanol and water in the same tubing, a large dispersion occurs.[41] One should also be aware of other causes for analyte dispersion, such as adsorption on the tubing walls.[152]

Each part of the liquid-liquid extraction system contributes to the total dispersion. The concept of dispersion has to be applied to all components and operations which cause changes in the concentration profile of the sample. The total dispersion consists of a combination of partial terms connected with the components, which ultimately is multiplicative.

When, however, the analyte appears only in the organic phase, and the objective is to transport it over some distance with minimum dispersion, segmentation of the organic phase with water is recommended when using glass or metal tubes. The aqueous phase will then form a film, thereby encapsulating the organic phase segments so that no intersegment mass transfer occurs.[68,143,147] In liquid-liquid extraction FIA, it was found that the movement through the extraction coil of a solute dissolved in the film-forming phase is retarted by this film so that some solute is transferred to subsequent segments, resulting in a band broadening of the peak.[41] However, if the solute is in the nonfilm-forming liquid, minimal peak dispersion is observed.

Tubing made of PTFE is preferentially wetted by organic phase, making this the film-forming phase. As can be seen from several studies, the drug pheniramine, which spends most of its time in the organic phase, undergoes significant broadening in the extraction coil; however, phenylephirine, which remains in the aqueous phase, undergoes no measurable broadening. These considerations suggest placing the organic phase separator between the extraction coil and a delay coil, and placing an aqueous phase separator after the delay coil to reduce the dispersion, rather than vice versa.[78,80]

The dispersion process is less significant for thinner films, thus the dispersion decreases with decreasing flow rate[41,68,85] as the peak width is decreased. The coil material also influences the dispersion process since PTFE or PE tubing allows the organic phase to form a thick film which improves the extraction efficiency.

It also is important to increase the exchange between the stationary layer and the bulk of the segments since this will reduce the dispersion. To promote this, the tubing diameter of the extraction coil should be small, thus giving a short diffusion distance between the stationary layer and the bulk of segment.

Therefore, physical evidence of the existence of a film, studies of the conditions under which it occurs, and useful approaches for measuring the film thickness are of crucial importance to furthering the understanding of the dispersion phenomena in segmented liquid-liquid extraction systems.

Within the extraction coil, two different modes of mass transfer from one segment to the adjacent segment of the same phase may be distinguished. A solute in the phase forming a thin film on the tube walls can thus be dispersed during its transport through the extraction coil by intersegmental mixing. The amount of solute transported by the film determines the magnitude of dispersion, and consequently, the film thickness is a major factor.

The extraction process can be very rapid so that the species originally present in a segment at a high concentration moves across the interposed segment of the other phase into the next segment of the same phase,[4] resulting in band broadening. The film plays an important role in the analyte peak-broadening process, i.e., in the dispersion and dilution process of the sample. The thicker the film, the greater the peak broadening.

When an extraction is performed in a FIA system so designed that the extraction coil is just long enough to achieve equilibrium, the extractant will spend roughly half of its time in both phases. This means that for a fixed organic phase the tubing material should be chosen in such a way that the phase forming the thinnest film "wets" the tubing surface. For example, the comparison between pentanol and chloroform shows the influence of viscosity and surface tension.[41] When the organic phase is fixed, the surface tension also is fixed, suggesting that the tubing material should be chosen so that the phase with the lowest viscosity wets the tubing walls.[41]

The most critical component with respect to overall dispersion is the phase separator. An almost linear relationship between analyte peak height and fraction of organic phase transported

through the flow-through detector was found.[80] This observation emphasizes the importance of an efficient phase separator.

Hence, when the fraction of the organic phase flowing into the flow cell is decreased, the analyte peak height will also decrease. Simultaneously, the fraction of the sample reaching the detector cell is decreased (similar to one-phase FIA). If analyte concentration is being measured, a decrease in the fraction of organic phase does not necessarily lead to a decrease in sensitivity if the dimensions and dispersion of the detection system is decreased accordingly.

The volume of the separator probably cannot be made smaller than twice that of the sample volume V_s (5 μl gives L_s = 8 mm in 0.7-mm ID tube at 30 μl s^{-1}). In a manifold when liquid-liquid extraction is allowed to reach equilibrium, fluctuations in L_s are unlikely to change the analytical precision. Conversely, in a manifold where the extraction coil is short so that extraction is incomplete, the precision of the method can be influenced[85] by fluctuations in L_s.

For shorter tubes, the use of 0.5-mm tubing produces a stronger signal than obtained with tubing of 0.8 mm. This can be explained by the greater efficiency of the liquid-liquid extraction process with lower ID tubing, which is due to both convective flow patterns and the organic phase film on the walls of the tubing.[20,41,85,106]

A segmented liquid-liquid extraction FIA system without phase separation based on rapid injection of microliter volumes (ca. 10 μl) of an aqueous sample solution containing potassium ions into a continuously flowing stream of an extracting agent in a suitable organic solvent (dibenzo-18-crown-6 in 1,2-dichloroethane) was used for the fluorimetric determination of potassium. Although the flow stream was turbid, single, sharp peaks were obtained with good reproducibility. Applications of FIA without phase separation also were found to be very useful.

VI. APPLICATIONS OF LIQUID-LIQUID EXTRACTION FIA

It might be assumed that the development of a new liquid-liquid extraction FIA method for the determination of an analyte would be a straightforward adaptation of an existing manual procedure to the liquid-liquid extraction FIA system. However, most all the literature represent equilibrium data obtained under equilibrium conditions. Also, whenever masking of interferents is necessary, manual procedures add masking reagents to the sample solution well in advance of analyte extraction, allowing complexation sufficient time to occur. In flow systems, all reactions take place nearly simultaneously, in less than tens of seconds.

Hence, liquid-liquid extraction in continuous flow analysis differs from that in manual batch analysis as equilibrium is seldom achieved due to the limited sample residence time, the interfacial area between both phases, and the kinetics of the liquid-liquid extraction process. Thus, kinetic factors play a very important role.

However, available equilibrium data can serve as a good basis for the selection of the FIA experimental conditions, with the final choice made from experiments performed under exactly the same dynamic conditions as those used for the analytical procedure.

A. Inorganic Analytes

1. Metals

Alkali metals (Li, Na, K) in different types of water samples are determined by spectrophotometric or fluorimetric methods (see Table 5) based on the liquid-liquid extraction of an ion associate since no suitable chelating reagent exists. Colored electroneutral ion associates formed between the metal ion, crown-ether derivatives, and anionic chromophoric species (mostly azo- or triphenylmethane dye) are usually extracted from alkaline solution of pH around 10 into a benzene/chlorobenzene (mono- or dichlorobenzene) mixture.

Picrate anion, polynactin, anionic aminonaphthalene sulfonate (magnesium salt), Tropeolin 00, tetrabromophenolphthalein ethyl ester anion ($TBPE^- H^+$), and four analogous 4-(4-dialkylaminophenyl)azo-2,5-dichlorobenzenesulfonate azo dyes (Ethyl Orange derivatives) as counter anions and three analogs of 18-crown-6 ethers were examined as suitable agents for the determination of sodium and/or potassium.[4,101,105] Methods with $TBPE^- H^+$ and mono- or dibenzo-

TABLE 5
FIE Determination of Metal Ions

Ion	Matrix	Conditions	Method	Ref.
Li	Water	Dichloromethane	MAS	45
	Standard	Chloroform/crown ethers	MAS, ISE	112
K		1,2-Dichloroethane	FL	4
	River water		MAS	75
	River water	b/Clb, A	MAS	105
Na, K	River, tap water	b/Clb, B, Si-column	MAS	101
Ca	River water	b/Clb, A	MAS	135
Cu		MIBK, APDC	FAAS	17
		Dioxotetramine	ICP	90
	Water	Chloroform, DDC-Zn	FAAS	92
	Standard, water	CS_2, DDC, of	MAS	97
	Water NPS	MIBK, APDC	FAAS	119
	Standard	Chloroform, APDC	MAS	136
	Standard	Chloroform, APDC	MAS	144
Zn	Biological, environment		FAAS	30
	Iron	MIBK, SCN	MAS	47
	Water	MIBK, SCN	FAAS	52
	Soil	Dithizone	MAS	55
Cd, Pb	Water	CCl_4, dithizone, ec	MAS	12
Cd	Urine	Chloroform, dithizone, PS	MAS	32
Pb	Water, plant		MAS	27
	Urine	MIBK, APDC	FAAS	79
	Soil	MIBK, I^-	MAS	100
Fe, Cd, Ag			AAS	33
Co		$(phen)_4P^+$	MAS	122
Ni	Water	Chloroform, FDD	MAS	106
U	Ore leachates	Heptane, TBP, BrPADAP	MAS	35
	Nuclear waste	MIBK, BrPADAP	MAS	103
	Water	Basic study	MAS	110
Ga	Water	Isoamylalcohol, Lumogallion	FL	18
Mo	Plant	Isoamylalcohol, CNS + Fe	MAS	3
Tl, Au		Rhodamine G	MAS	137
Rh		Crownethers, Brilliant Green	MAS	127
HM		MIBK, *n*-butylacetate	FAAS	16
	Water	MIBK, APDC	FAAS	28
	Blood serum		ICP	37
	Water	Freon 113	GAAS	36
	Water	Freon 113, APDC, DDDC, be	AAS	40
	Water	CCl_4, dithizone		
	Soil		FAAS	69
	Polluted soil		ICP	86

TABLE 5 (continued)
FIE Determination of Metal Ions

Ion	Matrix	Conditions	Method	Ref.
	Water	Freon 113, APDC	GAAS	109
	Seawater	Freon 113, APDC	GAAS	128
	Water	Freon 113, APDC	GAAS	129

Note: HM: heavy metals, DDC: diethyldithiocarbamate, B: benzo-18-crown-6 + tetrabromophanolphthelein ethyl ether (105), A: benzo-18-crown-6 + 4-(4-dialkylaminophanylazo-2,5-dichlorobenzenesulfonate (101), DDDC: diethylammonium-*N,N*-dithiocarbamate, APDC: ammoniumpyrrolidindithiocarbamate, FDD: furan-2,5-dione-dioxime, NAS: spectrophotometry, FAAS: flame atomic absorption spectrometry, GAAS: AAS with an electrothermal atomization, ISE: potentiometry with ion-selective electrodes, ICP: inductively coupled plasma atomic emission spectrometry, FL: fluorometry, ec: extraction constants, be: back extraction, b/Clb: benzene/chlorobenzene (1 + 1 v/v), of: optical fiber flow cell, PS: introduction of a new phase separator.

18-crown-6 ether derivatives are more selective and sensitive, but inferior to that obtained with Tropeoline 00 or Ethyl Orange dichloro derivatives. Sodium and potassium ions were determined spectrophotometrically as the ion associate after separation on an "on-line" column packed with silica gel. A segmented liquid-liquid extraction FIA system without a phase separator was used for fluorescence measurement of potassium with dibenzo-18-crown-6 ether and aminonaphthalene sulfonate after rapid injection of aqueous sample solutions into the continuous stream of the solution of the reagents in the organic phase.[4]

Tetrabromophenolphthalein ethyl ester anion ($TBPE^- H^+$) and six analogous 4-(4-dialkylaminophenyl)azo-2,5-dichlorobenzenesulfonate azo dyes (Tropeolin 00, Methyl, Propyl and Ethyl Orange and its halo-derivatives) as counter anions and dicyclohexano-24-crown-8 and 18-crown-6 ether and its mono- and dibenzo-analogs were also examined as a suitable agents for the determination of calcium. Propyl Orange and 24-crown-ether were used for the spectrophotometric determination of calcium in river water.[135]

Heavy metal ions are usually separated and preconcentrated in the form of suitable electroneutral metal chelates formed with organic or inorganic analytical reagents (e.g., carbamates, dithizonates). They can also be extracted in the form of ion associates formed between the metal ion, an organic analytical reagent (such as 18-crown-6 ether derivatives), and a coloring reagent, or between a charged metal chelate and a voluminous counterion (e.g., surfactants, organic dyes, etc.), as nonspecific reagents.[10,127] Metal ions are determined using AAS and emission spectrometric or less selective spectrophotometric methods after more or less selective liquid-liquid extraction (see Table 5). Fluorimetric and other optical methods play a less important role.

Lead and cadmium in the form of dithizonates were extracted from citrate (pH 8) and phosphate (pH 9) buffer solutions into carbon tetrachloride, and determined using a spectrophotometric procedure.[12] A method of determination of the extraction constant was also developed, with continuous monitoring of chelate concentration by injecting small volumes of the aqueous phase into the organic phase stream. Lead was determined in glaze and petrol samples in the concentration range of 0.2 to 1 mg l^{-1} after the elimination of zinc interference with ferrocyanate.

Lead and copper can be determined with dithizone after liquid-liquid extraction into carbon tetrachloride or MIBK, respectively, and injection of a sample in the organic phase, into the aqueous carrier stream. The improvements in the detection limit and overall sensitivity (enrichment factor, ca. 1.5) are mainly due to the favorable extraction volume ratio (50:1) that can be achieved with the single line arrangement.[52] Similar results were obtained for the preconcentration of copper(II) ions from ammonium citrate buffer of pH 4 into chloroform. The method is based on the replacement of Zn(II) ions by copper(II) to form more stable metal chelate, $Cu(DDC)_2$. Copper in the form of carbamates can

be determined via either a molecular spectrometric[136] or AAS[119] method.

Lead was determined[100] in soil extracts after "on-line" extraction with iodide into MIBK. A 60-fold enhancement in sensitivity compared to direct aspiration of the aqueous samples was achieved. The lead content in urine of exposed and unexposed adults was determined by flame AAS in an acetylene-air flame after "off-line" liquid-liquid extraction as a lead chelate with APDC from a slightly acidic solution, into MIBK.[79]

Synthetic samples containing 2% Fe and various concentrations of Zn were analyzed by liquid-liquid extraction of Zn in the form of $Zn(SCN)_2$ into MIBK, after reduction of Fe(III) to Fe(II) with ascorbic acid. In this way, Fe(II) remains unextracted and the results obtained for Zn are essentially free of iron spectral interferences.[55]

A waveguide capillary cell[62,97] as a spectrophotometric detector allows the determination of copper in water samples after liquid-liquid extraction of its metal chelate with DDDC into carbon disulfide[97] from citrate buffer of pH 9. Sample zones were sandwiched between zones of the color-developing agent in an aqueous solution, and segmented by CS_2.

Several metal ions (Cu, Ni, Pb, and Cd) were determined in aqueous samples[28] as metal chelates with APDC in MIBK using an "in-line" automated system with flame AAS.[17] The organic extract was led into the loop of an injector and directed into the AAS nebulizer by an aqueous carrier stream. An increase in sensitivity of 15 to 20 was achieved, in comparison with the direct aspiration of the aqueous samples. Neither back extraction nor dispersion of the organic plug occurred.

Various trace elements (Zn, Cd, Co, Ni, Mn, Pb, Fe, Cu, Cr, Na, and K) were determined via simple injection of the aqueous sample solution into a continuous stream of MIBK or *n*-butylacetate passing to an air-acetylene flame for AAS. Sensitivity enhancement factors of 1 to 4.8 (1.4, typically) relative to values obtained in the aqueous stream were obtained for all metals, except for chromium(III) and chromium(VI) when using MIBK and *n*-butylacetate (but not for methanol, probably because of excessive dilution effects).[37]

Trace amounts of metal ions (Cd, Cu, Pb, Co, and Ni or Cd, Cu, Fe, Pb, Ni, and Zn, respectively) in distilled, saline, lake, and seawater samples were extracted[36,40,128,129] from aqueous solution (acetate buffer of pH 6 or 5.5) as their carbamate chelates (APDC and DDDC) into Freon-113, and back-extracted into an acidic aqueous solution containing mercury(II) in 0.5 *M* nitric acid as a stripping reagent. The aqueous solution was collected in plastic cups after separation of the Freon-113 and analyzed by "off-line" graphite-furnace AAS. An enrichment factor for the extraction/stripping method on the order of 15 to 30 was achieved, and recoveries were 90 to 100%, except for copper (80 to 95%).

Uranium(VI) was determined spectrophotometrically using an ethanolic solution of 2-(5-bromo)-2-(pyridylazo)-5-diethylaminophenol (BrPADAP) in the presence of benzyldimethyltetradecylammonium chloride (Zephiramine), or with the same reagent in methanol and pyridine (10% v/v) in the form of the metal chelate. Uranium was extracted with a solution of tributylphosphate in heptane and aluminum nitrate in ammoniacal buffer as a salting-out solution,[35] or from slightly acidic nitrate medium as a solvated molecular species of the composition $UO_2(NO_3)_2 \cdot n$ MIBK $\cdot m$ H_2O using MIBK in the presence of ammonium nitrate and large amounts of ammonia as a salting-out solution.[105] The selectivity was improved by the complexing solution. The distribution ratio D of uranium(VI) with TOPO (trioctylphosphine oxide) and HBMPPT (4-benzoyl-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-thione) and the stoichiometry of the chelate $UO_2(BMPPT)_2(TOPO)$ were determined in 0.01 *M* $LiClO_4$ media by simultaneous detection of the separated phases using diode array detection.[110]

Gallium was determined with lumogallion (4-chloro-6-(2,4-dihydroxyphenylazo)1-hydroxybenzene-2-sulfonic acid) after extraction into isoamylalcohol from acetate buffer medium of pH 3.3, using an "on tube" laser-excited fluorimetric measurement. Mg(II), Co(II), Zn(II), Ni(II), and Fe(II) scarcely affect the determination. Negative errors caused by Al(III), Fe(III), Cu(II), and Cr(VI) are totally reduced by the liquid-liquid extraction FIA procedure and by masking Fe(III) and Cu(II) ions with ascorbic

acid and thiourea, respectively, up to a tenfold molar excess of these ions.[18]

Molybdenum in plant material was determined spectrophotometrically using the thiocyanate method after extraction from 3 *M* HCl into isoamylalcohol.[3] Interference by Fe(III) ions was eliminated by Sn(II) reduction in acid solution (3 *M* HCl).

Cobalt(II) ion was determined spectrophotometrically as an ion associate of tetrathiocyanatocobaltate with ethylene-bis(triphenylphosphonium) bromide from a neutral medium of pH 6 (citrate, dihydrogenphosphate, borate, and diethylbarbiturate buffer solution) into chloroform.[122]

2. Inorganic Anions

Voluminous inorganic anions, such as perchlorate, permanganate, nitrate, etc., can be determined by direct spectrophotometric or indirect AAS method after their liquid-liquid extraction into organic solvents in the form of ion associates with cationic organic dyes of the triphenylmethane group, or with metal chelates of organic analytical reagents (see Table 6).

Direct determination of phosphorus in water as orthophosphate is based on the continuous liquid-liquid extraction of the ion associate formed between molybdophosphate and Malachite Green in a strongly acidic medium of 0.8 *M* sulfuric acid, into benzene-MIBK mixture (1 + 2 v/v). The use of a relatively high sulfuric acid concentration prevents silicate interference.[25,93]

Phosphorus in the form of 12-molybdophosphate was determined after on-line liquid-liquid extraction into *n*-butylacetate.[25] The resulting organic phase was injected into an aqueous carrier stream in a single-line FIA system, with detection by flame AAS. The observed baseline noise arises from small droplets of the aqueous phase containing an excess of molybdate reagent suspended in the organic phase, rather than from co-extracted isopolymolybdate.

Perchlorate was determined spectrophotometrically after extraction of its ion associate with Brilliant Green from a neutral medium of pH 6 into benzene.[116] The determination was carried out in chlorate samples after selective destruction and removal of bromides, iodides, nitrates, and thiocyanates.

An indirect AAS method[50] for perchlorate determination in biological fluids is based on the continuous extraction of a copper(II)/6-methylpicolinealdehyde azide ion associate with perchlorate ion into MIBK. The method is selective, as iodide, bromide, nitrate, and tungstate can be tolerated at weight ratios of 10, 1000, 200, and 10, respectively. The lesser interference from hypochloride, chlorate, and chloride permits the determination of perchlorate in several mixtures of chloro anions. Only EDTA and thiocyanate interfere seriously at ratios of more than 2 or 1, respectively.

Permanganate and dichromate ions were determined spectrophotometrically[117,123] as ion associates with ethylene-bis(triphenylphosphonium) bromide by extraction from a neutral medium of pH 6 (citrate, dihydrogenphosphate, borate, and diethylbarbiturate buffer solution) and 10% NH_4F (w/v) solution, into chloroform.

Nitrates or nitrites (0.13 to 2.2 mg l^{-1}) in water samples were determined in the form of their ion associates with neocuproine (bis-(2,9-dimethyl-1,10-phenanthroline) copper(I) chelate, after extraction into MIBK.[64] Using the same extraction principle, the sequential determination of both anions in meat samples is possible.[66] Total nitrate was determined after oxidizing nitrite to nitrate by cerium(IV) sulfate, with an apparent increase in sensitivity (since the sensitivity of the nitrite method is 10 times lower), and/or the reduction of nitrite to nitrogen by sulfamic acid. The nitrite was determined by the difference. Very high concentration of anions can be tolerated.[66] All interferents increase the signal because of additive interference, except for oxalate, which causes a decrease of the peak height. The most serious interference from chloride can be controlled by precipitation with silver sulfate or with an ion-exchange resin in the Ag form. Chloride ion, in addition, was determined with a detection limit of 4.6 mg l^{-1} by using chromotropic acid.

Iodide[62] can be detected, based on iodine self-absorption at 540 nm. Iodide ions were oxidized to iodine under acidic condition using sodium nitrate (2% m/v) in 1% acetic acid, and the solution was injected into a carbon disulfide flow stream and detected using an automated spectro-

TABLE 6
FIE Determination of Voluminous Inorganic Anions

Anion	Matrix	Conditions	Method	Ref.
Perchlorate	Urine, serum	MIBK, A	AAS, i	50
Perchlorate	Std.	Benzene, D	MAS	116
Nitrate/nitrite	Water	MIBK, B	AAS, i	64
Nitrate/nitrite	Meat	MIBK, B	AAS, i	66
Orthophosphate	Biological	F	MAS	25
Phosphate	Water	G, off D	FAAS	51
Orthophosphate	Water	Benzene/MIBK, C	MAS	93
Permanganate	Std.	Chloroform, E	MAS	117
Iodide	Water	CS_2, nitrate		62
Anions				34
Silicate				42
Dichromate	Std.	Chloroform, H		123
Fluoride	Water		ICP	139

Note: A: [(6-methylpicolinealdehyde azine)-copper(I)$_2$], $(ClO_4)_2$; B: bis-[2,9-dimethyl-1,10-phenanthrolinato)copper(I)], neocuproine; C: molybdophosphate + Malachite Green; D: Brilliant Green; E: ethylene-bis-(triphenylphosphonium) bromide; F: ?; G: *n*-butylacetate/molybdate; H: tetramethylene-bis-(triphenylphosphonium) bromide, i: indirect AAS determination; off D: off-line detection; std.: standard solution analysis.

photometric detection system with a 5-m hollow fiberoptical cell.[62,97]

B. Organic Analytes

1. Surfactants

Direct spectrophotometric methods for the determination of ionic surfactants are based on the formation of the stoichiometric, electroneutral ion associate of the composition $(T^- \cdot R^+)$, $(T^+ \cdot R^-)$, etc., between a charged surfactant anion T^- or cation T^+ and a cationic or anionic organic analytical reagent R^+ or R^-, respectively, or with a suitable cationic metal chelate. The ion associates are sparingly soluble in water but easily extractable into nonpolar organic solvents such as chloroform, benzene and its chloro- or alkyl-derivatives, or MIBK (see Table 7).

The sensitivity of the determination is governed by the molar absorptivity of the chromophore (usually organic dye or metal chelate). Triphenylmethane, thiazine, and azo dyes are the most frequently used cationic dyes. The extractability of the ion associate of the cationic dyes decreases in the order: Ethyl Violet, Brilliant Green, Hoffmann's Violet, Crystal Violet, Malachite Green, New Fuchsin, Methylene Blue, and Pararosaniline, and the extraction efficiency of the solvents decreases in the order: MIBK, 1,2-dichloroethane, chloroform, dichlorobenzene, monochlorobenzene, benzene, and toluene.[6,15,29,48,74,83,104,108,138]

The most suitable (and also most frequently used) are pairs of Crystal Violet with a chloroform and toluene mixture (1 + 1), Azure B with chloroform, and mainly Methylene Blue with chloroform or dichlorobenzene, because of their high molar absorptivity and less interference from diverse compounds. Only voluminous anions such as perchlorate, nitrate and thiocyanate cause greater interferences. Inhibition of the liquid-liquid extraction of the anionic surfactant in the presence of a nonionic surfactant is mostly due to kinetic factors[108] or to the formation of a stable ion associate between the ionic surfactants.

The blue ion associates of the Methylene Blue with anionic surfactants are extracted from acidic or neutral media into chloroform[6,65,74,108] or dichlorobenzene.[104] The presence of salting-out substances such as sodium[104] or potassium sulfate[108]

TABLE 7
FIE Determination of Surfactants

Type	Matrix	Conditions	Method	Ref.
Anionic	Water	Chloroform, MB	MAS	6
	River water	Dichlorobenzene, MB	MAS	8
	River water	Dichlorobenzene, MB	MAS	29
	Sewage water	Toluene, EV	MAS, ns	48
	Water	Chloroform, MB	MAS, ns	65
	Wastewaters	MIBK, A	AAS, i	73
	Water	Chloroform, MB	MAS, mc	74
	River water	Chloroform, B	MAS	83
	River water	Chloroform, MB	MAS	104
	River water	Dichlorobenzene, MB	MAS	104
	Water	Chloroform, MB	MAS	108
	Water	Chloroform, MB	MAS, ip	111
	Water	Chloroform, Q	MAS	124
Cationic	Water, std.	Chloroform, OII	MAS	15
	Water	MIBK, C	AAS, i	107
Nonionic	Water, std.	ClEt, TBPEK	MAS	67

Note: MB: Methylene Blue; EV: Ethyl Violet; OII: Orange II; A: Cu(I)-phenanthroline; B: 1-alkyl-4-(4-diethylaminophenylazo)pyridinium chloride (6×10^4 l mol^{-1} cm^{-1}); C: tetrathiocyanatocobaltate(II); TBPEK: tetrabromophenolphthalein ethyl ester, potassium salt, liquid-liquid extraction as $[T^{\circ} \cdot K^{+}]^{+} \cdot TBPE^{-}$; Q: 4-(4-*N,N*-dimethylaminophenylazo)-2-methylquinoline; i: indirect determination; ClEt: 1,2-dichloroethane; ns: nonsegmented, mc: microconduit; ip: iterative procedure.

favors the extraction process and minimizes baseline noise and analytical signal of the blank solution. Pre-extraction of red or reddish-blue degradation products of the Methylene Blue from alkaline solution (pH >8) has a similar effect.

The relative molar extractability are partially influenced by the extraction rate, which depends on the surfactant structure and its alkyl chain length and molecular mass. Alcohols such as methanol, ethanol, or *n*-propanol are often used to reduce this influence, and to enhance the extraction process, as they act both as ion-pack solvating and dissolving agents.

The methods are very sensitive and selective since the effect of most of the interferents is depressed in strongly acidic media (pH 2), in contrast to neutral media. Applying an amplification method[111] to the analysis of trace concentrations of anionic surfactants, the sensitivity can be varied from 0.01 to 1.16 μg ml^{-1}. The determination of anionic surfactants in the concentration range of c(T) = 0.04 − 4 μg ml^{-1} with an s_r <3%, is possible.

Indirect methods of anionic surfactant determination[73] are based on the ion-associated liquid-liquid extraction of anionic surfactants with cationic metal chelates of the organic analytical reagents [such as ethylenediamine-copper(II), 1,10-phenanthroline-copper(II), and thiourea-copper(II)] into a suitable organic solvent (MIBK, Freon, etc.). The overall concentration of anionic surfactant is determined by indirectly measuring the metal content present in the organic phase by flame AAS. Indirect methods are usually more sensitive than spectrophotometric procedures and are free from interferences of both ionic and nonionic surfactants and other potential interferents.

Ionizable organic compounds (glutamic acid, succinic acid, phenol, etc.) forming ion associates with 1,10-phananthrolino-copper(II) chelate, inorganic cations [such as Co(II), Ni(II), Zn(II), and Fe(III)] which can replace copper(II) in the chelate, voluminous inorganic anions (perchlorate, iodide, nitrate, etc.) that can produce a positive interference by forming an ion associate with the chelate, and certain anions (such as oxalate, thiosulfate, etc.) which can destroy the chelate by forming soluble copper(II) complexes, can be tolerated at higher concentration (1 mg ml^{-1}). The exceptions are Zn(II), Fe(III),

iodide, and perchlorate, which cause serious interferences (tolerance ratio to anionic surfactant is 25). Chloride ion does not affect the liquid-liquid extraction efficiency.

A direct spectrophotometric method for cationic surfactant determination[8,16] is based on the liquid-liquid extraction of ion associates (formed between cationic surfactants such as tetraalkylammonium or alkylpyridinium cations and anionic voluminous Orange II dye)[16] into chloroform. An indirect AAS method may be used, based on measurement of the cobalt concentration in the organic phase (MIBK), present in the form of tetrathiocyanatocobaltate(II)/cationic surfactant ion associate, after its liquid-liquid extraction from acetate buffer media of pH 4.8. The method is very sensitive, precise, and mainly very selective and free of the interferences from nonionic and anionic surfactants, inorganic anions (carbonates, sulfides, cyanides, etc.) forming precipitates with cobalt ions, inorganic cations displacing Co(II) from the chelate, ionizable organic compounds (glycol, urea, etc.) forming ion associates with chelates, or cationic surfactants and ions commonly accompanying cationic surfactants in water samples (chloride, magnesium, calcium) since they can be tolerated at relatively high concentrations. Only copper(II) is an exception, with a maximum tolerable ratio of about 4.

The determination of nonionic surfactant[67] of the general type $RO(CH_2CH_2O)_nH$ (where R is an alkylphenyl group and n is the number of moles of oxyethylene group) is based on extraction of the colored ion associates (formed between the cationic coordination product of the alkylphenyl chain with potassium ion and an anionic chromophore, such as the potassium salt of tetrabromophenolphthalein ethyl ester) from borate buffer of pH 9.3 into 1,2-dichloroethane. Each nonionic surfactant varies in its response to the reagent since the reaction is predominantly dependent on the number of oxyethylene groups present in the molecule, the molecular mass, and the structure of the side chains of the nonionic surfactant. Triton® X-100 (n = 10) gave the greatest response, compared to Antarox CO-430 n (n = 4), which gave the least.

2. Drugs

Spectrophotometric methods for the determination of drugs in pharmaceutical preparations, tablets, sprays, etc. (see Table 8) are based on the principle of direct extraction of self-absorbing nonionic compounds. Drugs can also be determined after liquid-liquid extraction of a colored ion-associates formed between ionic drug species and suitable ionic coloring reagent (such as picrate and organic dyes containing a triphenylmethane group). Chloroform is usually used as an extracting agent, but 1,2-dichloroethane, cyclohexane, and isooctanol also play an important role. Fluorimetry and chemiluminescence are useful in the liquid-liquid extraction FIA determination of drugs.

Spectrophotometric determinations of caffeine[1] in coffee, tea, and Coca Cola and of acetylsalicylic acid preparations containing sodium dodecyl sulfate (SDS), potato starch, calcium carbonate, and citric acid were performed using a single-line extraction FIA manifold after selective extraction into chloroform from 0.16 *M* sodium hydroxide. The acetylsalicylic acid remains in the aqueous phase because of its ionization, while caffeine is readily extracted under these experimental conditions. SDS interference was eliminated by addition of tetrapropylammonium bromide to the organic phase to form easily extractable ion associates.

Codeine[5] in the same matrix was extracted as its yellow-orange picrate ion associate into chloroform from an aqueous carrier stream buffered at pH 6.5 (phosphate buffer). The determination of codeine in the presence of acetylsalicylic acid HA (which is also distributed into chloroform from acidic solutions) is based on a suitable pH choice of 6.5, at which the amounts of both picric acid and salicylic acid extracted into chloroform are low enough, while the percentage of the codeine present as the ion associate with picrate anion is sufficiently high.

Enalapril (1.5 to 60 $\mu g\ ml^{-1}$), an important secondary amine (1-(*N*-(*S*)-1-carboxy-3-phenylpropyl-L-proline-1 ethyl ester maleate) used to control hypertension based on its inhibition of angiotensin-converting enzyme, is extracted into

TABLE 8
FIE Determination of Drugs and Other Types of Organic Substances

Compound(s)	Matrix	Conditions	Method	Ref.
Caffeine	Pharm. tabl.	Chloroform	MAS, ec	1
	Coffee, tea, Coca Cola	Chloroform	MAS	
	Aq	Chloroform	MAS	65
				75
Codeine	Pharm., tabl.	Chloroform	MAS	5
Thiamin (B_{12})	Pharm.	Chloroform	MAS, FL	10
				11
Procyclidine HCl	Pharm., tabl.	Chloroform	MAS, ec	22
Diphenyldramine + 8-chlorotheophyline	Dramamine tabl.	Cyclohexane	MAS, bp	31
3,5-Dimethylphenol + *p*-toluidinium ion	pK_{ai} detn.		MAS	51
Steroid sulfate + bile acids	Biol. mat.	ClEt, BTB	FL, CL	53
Terodiline	Blood serum	*n*-Heptane		57
Enalapril	Pharm.	ClEt, LG	MAS	58
Phenylephirine HCl + phenyramine	Nasal spray	Chloroform	MAS	78
Drugs	Biol.	?	MAS	64
Berberine + benzethonium	Drugs	TBPEK	MAS	140
Quaternary ammonium	Std.	Chloroform	MAS	152
PAH	Shale oil	DMSO/pent.	FL/HPLC	20
		DMSO/aq	FL/HPLC	23
		DMSO/aq	FL/HPLC	39
Free fatty acids	Vegetable oils	Toluene		19
				88
Halocarbons	Seawater		GC/MAS	72
Amines		Aq/aq	MAS/HPLC	77
Bittering comp.	Beer	Isooctane	MAS	81
Pesticides		*n*-Heptane	MAS-UV/HPLC	96
	Std.	Chloroform	MAS	130
Partition coeff.	Std.	1-Octanol	MAS	102
	Std.	1-Octanol	MAS	146

Note: PAH: polycyclic aromatic compounds; DMSO: dimethylsulfoxide, liquid membranes; ClEt: 1,2-dichloroethane; DMSO/aq: dimethylsulfoxide with water; DMSO/cyclohexane: water in DMSO/cyclohexane; DMSO/pent.: DMSO pentane; BTB: Bromothymol Blue; LG: lucigenin (*N,N'*-dimethyl-9,9'-biacridinium dinitrate) and H_2O_2; TBPEK: tetrabromophenolphthalein ethyl ester, potassium salt; ec: determination of extraction constants; CL: chemiluminescence; bp: simultaneous monitoring of both phases.

dichloromethane and chloroform from acetate buffer solution of pH 3 to 4 (pH 3.2 was used) as its yellow ion associated with Bromothymol Blue, without interferences of any degradation products of Enalapril (diacid and diketopiperazine derivatives are major products) or excipients (sugars, cellulose, and magnesium stearate) in the pharmaceutical dosage form.[58] Procyclidine hydrochloride, a synthetic antispasmodic drug used in the treatment of Parkinsonism, was determined[22] in tablets as an ion associated with picrate by extraction into chloroform from aqueous samples at pH 2.

Diphenhydramine and 8-chlorotheophylline

were determined in Dramamine tablets by simultaneous monitoring of both phases after their separation by a dual membrane separator[31] of Teflon and paper membranes, using two detection systems. At pH 10 (ammonia buffer), the former is quantitatively extracted into cyclohexane and the latter remains in the aqueous phase. Solvent pH was chosen to be above the acid/base dissociation constant pK_a of 8-chlorotheophylline, and below the pK_a of the diphenhydraminium ion; thus, both components can be ionized and determined.

Simultaneous determination of phenylephrine hydrochloride and pheniramine maleate in nasal spray[78] after quantitative extraction of the former into chloroform at pH 13 in the form of the neutral free base (while the latter remains in the aqueous phase as an anionic phenolate) was performed using two porous membrane phase-separators and a single photometric detector. Interfering species such as thimerosal, maleate, and benzalkonium, present in the spray, were removed from the injected aqueous sample solution using miniature on-line ion exchange microcolumns packed with Amberlyst A 26 and Amberlyst 15 macroporous anion and cation exchange resin, respectively.

Terodiline (*N-tert*-butyl-1-methyl-3,3-diphenylpropylamine), a drug with anticholinergic and calcium antagonistic properties used in the treatment of urinary incontinence, was determined in human serum using capillary gas chromatography in an "off-line" configuration. The samples were injected into an organic stream, alkalized, segmented with organic phase, and extracted into heptane.

Chemiluminescence and fluorescence detectors were used[53] for the determination of steroid sulfates, steroid glucuronides, bile acid 3-*O*-sulfates, and free glucine- and taurine-conjugated bile acids, using lucigenin (*N,N'*-dimethyl-9,9'-biacridinium dinitrate) as an extracting and chemiluminescent reagent. The extracted ion associate of steroid sulfate-lucigenin (whose chemiluminescence properties are produced by the addition of either hydrogen peroxide or an organic reducing compound) was mixed with alkaline hydrogen peroxide solution, and the resulting analytical signal was monitored. The polarity of the solvents affects both the efficiency of the extraction and the fluorescence intensity. Nonpolar solvents drastically decrease the fluorescence intensity to zero (e.g., hexane, carbon tetrachloride, or 10% 1,2-dichloroethane).

3. Other Organic Substances

Nonionic species can be separated and preconcentrated by direct extraction into nonpolar solvents using segmented or unsegmented liquid-liquid extraction FIA systems. Such substances are determined as a sum of the extractable compound; preconcentrated and partly separated by a multiple liquid-liquid extraction method including more than one extraction step realized by one or several organic solvents); or are selectively determined by successive liquid chromatographic separation after a nonselective preconcentration and separation step. Voluminous organic ions are determined after extraction of the ion associate formed between the ionic analyte species and a suitable organic or inorganic coloring reagent (with organic dyes belonging to the most useful species). Spectrophotometric or fluorimetric detection is generally preferred (see Table 8).

Organophosphorus pesticides, fenthion and azinphos methyl, were determined using a continuous liquid-liquid extraction FIA system coupled on-line using liquid chromatography with UV detection after their extraction from aqueous samples with *n*-heptane. Diazinon was not extracted significantly. Other pesticides and related compounds did not interfere.[98] A fast reading "on-tube" spectrophotometric detector operated by a computer was also used for pesticide determinations, with a sophisticated computer "phase separation".[130]

Bitterning compounds were extracted from degassed beer (after acidification with 0.1 *M* HCl) into isooctane (1 + 2 v/v), and determined by measuring the absorbance of the separated organic phase at 275 nm against pure isooctane.[81] Amines were separated from complex matrices of the alkaline aqueous sample solutions into an aqueous recipient stream using a liquid membrane phase separator and spectrophotometric or chromatographic quantitation.[77]

Three single step liquid-liquid extraction pro-

cedures were connected together by multichannel pumping and resampling for the isolation and speciation of polycyclic aromatic hydrocarbon (PAH) compounds from complicated sample matrices. The individual PAHs were determined in shale oil utilizing video fluorimetry, combined with liquid chromatographic separation after a liquid-liquid extraction FIA preconcentration step.

The separation and preconcentration of halocarbons such as chloroform, bromodichloromethane, dibromochloromethane, bromoform, trichloroethylene, tetrachloroethylene, carbon tetrachloride, and 1,1,1-trichloroethane were performed in a liquid-liquid extraction FIA system in a coated glass coil, internally into pentane, and the phases were separated with the aid of a membrane phase separator. The organic phase was collected in the sample loop of the injector valve of a gas chromatograph. The individual halocarbons were then separated and quantified[72] by capillary gas chromatography equipped with a ^{63}Ni electron capture detector kept at 275°C.

VII. CONCLUSIONS

Liquid-liquid extraction flow injection analysis represents one of the most progressive techniques of analytical flow systems since it is very applicable to important problems related to selectivity and sensitivity improvement in many branches of analytical chemistry. Since Karlberg's and Bergamin's pioneering works a substantial number of papers have been published, the rate of which continues to be high (see Figure 39).

Liquid-liquid extraction FIA has contributed to the solution of analytical problems in a great variety of areas, especially in clinical biochemistry, pharmaceutical chemistry, and environmental chemistry, which require the separation and preconcentration of analytes from very complex matrices. Despite the use of toxic organic solvents and recent developments in other separation and preconcentration techniques such as membrane or microcolumn techniques, the role of liquid-liquid extraction FIA in some areas remains indisputable.

The method is also suitable to resolving nonanalytical problems connected with the calcula-

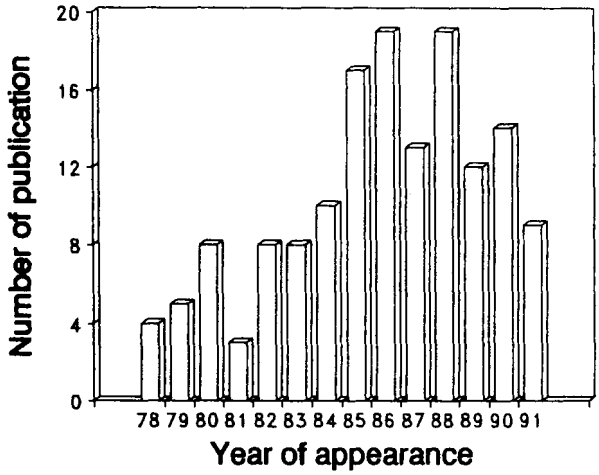

FIGURE 39. Number of publications and the years in which they were published.

tion of the extracted solute fraction,[49,110] peak area,[54] peak height[49] as a function of the total flow rate Q_t, and other parameters. Broad examples include calculation of the acidity and extraction constants based on the use of a single or dual membrane phase separator,[51] the determination of 1-octanol/water partition coefficients,[102,146] the evaluation of reaction mechanisms and kinetics,[85,114] and studies of various aspects of the extraction process[85] with the aid of laser-excited fluorescence,[18] fast scanning detectors,[20] or multiextraction systems.[20,23,39]

The possibility of performing extractions on very small volumes of samples (only tens of microliters are needed) is extremely valuable in many cases (e.g., in the field of clinical chemistry, where this parameter is often the limitation in the application of many analytical techniques). Overall volumes involved in liquid-liquid extraction FIA may be reduced further to a few microliters since organic solvents, reagents, and samples are expensive. Disposal problems and exposure risks for the technicians are also limited because of the consumption of much smaller amounts of reagents and solvents than required by manual methods.

Requirements on the safety of the working area due to contamination are less using the totally closed FIA system, and the combination of high sampling rate, short starting-up time, low maintenance level, reproducible behavior over several days, and on inherently small sample di-

lution and good reproducibility are the real features of this methodology.

The liquid-liquid extraction FIA method is usually simpler, easier to handle, and more rapid and accurate than batch procedures. Nevertheless, the sensitivity of the method is generally lower than that of batch analysis, unless it is employed as a preconcentration technique. Kinetics plays the decisive role in the continual separation process, as the equilibrium is exceptionally related to residence time. Sample dispersion and a low flow rate ratio acceptable to most components of the liquid-liquid extraction system are other important factors. Kinetic aspects, on the other hand, can improve selectivity by considering the kinetic discrimination of some chemical reactions.

The time required for sample preparation, calibration, and measurement is short, and the sampling frequency is sufficiently high (30 to 60 samples per hour, typically) for most practical applications. The method can be easily adapted to a wide range of concentrations by simply modifying the detection system, the sample volume, or the flow rate ratio of the sample to the organic phase stream. The method can also be fully automated.

Recent improvements in phase segmenters and the development of the systems without phase separation via mathematical treatments of data measured on both phases opens up a whole new area of possibility where studies of liquid-liquid extraction can be performed directly on each individual segment of the organic and aqueous phase, thereby avoiding problems connected with phase separation, sorption of extractable species on tubing walls, etc. Finally, it may even be possible to eliminate the phase segmentation and/or phase separation processes altogether if an appropriate unsegmented system is used, or the reproducibility of segmentation is sufficient to allow precise timing of the measurement periods.

These sophisticated systems can also be very useful in segmented postcolumn derivatization in liquid and ion chromatography, especially when using the multichannel coaxial segmenter for direct introduction of an effluent and a derivatizating reagent directly into the organic phase stream. The use of fast-reading "on-tube" detectors simplify postdetector derivatization requirements.

The merits of these combined instruments can be expressed in terms of their increase in sampling frequency, their decrease in sample size, their reduced solvent and chemical consumption, the possibility of measuring samples containing high salt concentrations, the decrease in interference effects from the matrix, the use of new calibration techniques, the reduction of hazards associated with the use of inflammable solvents, the reduction of solvent evaporation into the laboratory atmosphere using a closed system environmental, and mainly, the indisputable selectivity and sensitivity increase due to the preconcentration and separation process of this methodology.

ACKNOWLEDGMENTS

My thanks to Prof. Folke Ingman of the Department of Analytical Chemistry, The Royal Institute of Technology, Stockholm, for his fruitful cooperation and valuable discussion during the early stage of the work, and especially for his collaboration on the text of Sections III.B and V.A. My thanks also to Prof. Purnendu K. Dasgupta of the Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, for his interest during the finalization of the manuscript and my gratitude to the authors and publishers of papers that have been cited, for their work. Finally, my thanks to my wife, Zdenka, and my sons, Petr, Pavel, and Zdeněk, for their patience and encouragement during the preparation of this review.

REFERENCES

1. **Karlberg, B. and Thelander, S.,** Extraction based on the flow injection principle. I. Description of the extraction system, *Anal. Chim. Acta*, 98, 1, 1978.
2. **Růžička, J. and Hansen, E. H.,** Flow injection analysis. X. Theory, techniques and trends, *Anal. Chim. Acta*, 99, 37, 1978.
3. **Bergamin, F. H., Madeiros, J. X., Reis, B. F., and Zagatto, E. A. G.,** Solvent extraction in continuous flow injection analysis. Determination of molybdenum in plant material, *Anal. Chim. Acta*, 101, 9, 1978.
4. **Kina, K., Shiraishi, K., and Ishibashi, N.,** Ultramicro solvent extraction and fluorimetry based on the flow injection method, *Talanta*, 25, 295, 1978.

5. **Karlberg, B., Johansson, P. A., and Thelander, S.,** Extraction based on the flow injection principle. II. Determination of codeine as the picrate ion-pair in acetylsalicylic acid tablets, *Anal. Chim. Acta,* 104, 21, 1979.
6. **Kawase, J., Nakae, A., and Yamanaka, M.,** Determination of anionic surfactants by flow injection analysis based on ion-pair extraction, *Anal. Chem.,* 51, 1640, 1979.
7. **Růžička, J. and Hansen, E. H.,** Flow injection analysis, *Chem. Tech.,* 9, 756, 1979.
8. **Kina, K.,** Determination of anionic surfactants by flow injection analysis based on ion-pair extraction, *Dojin,* 14, 9, 1979 (in Japanese).
9. **Růžička, J. and Hansen, E. H.,** Flow injection analysis principles, applications and trends, *Anal. Chim. Acta,* 114, 19, 1980.
10. **Karlberg, B. and Thelander, S.,** Extraction based on the flow injection principle. III. Fluorimetric determination of vitamin B1 (thiamine) by the thiochrome method, *Anal. Chim. Acta,* 114, 129, 1980.
11. **Johansson, P. A., Karlberg, B., and Thelander, S.,** Extraction based on the flow injection principle. IV. Determination of extraction constants, *Anal. Chim. Acta,* 114, 215, 1980.
12. **Klinghoffer, O., Růžička, J., and Hansen, E. H.,** Flow injection analysis of traces of lead and cadmium by solvent extraction with dithizone, *Talanta,* 27, 169, 1980.
13. **Nord, L. and Karlberg, B.,** Extraction based on the flow injection principle. V. Assessment with a membrane phase separator for different organic solvents, *Anal. Chim. Acta,* 118, 285, 1980.
14. **Kina, K. and Ueno, K.,** Flow injection analysis opens new aspects in analytical chemistry, *Kagaku, Kyoto,* 36, 662, 1980 (in Japanese).
15. **Kawase, J.,** Automated determination of cationic surfactants by flow injection analysis based on ion-pair extraction, *Anal. Chem.,* 52, 2124, 1980.
16. **Fukamachi, K. and Ishibashi, N.,** Flow injection-atomic absorption spectrometry with organic solvents, *Anal. Chim. Acta,* 119, 383, 1980.
17. **Nord, L. and Karlberg, B.,** An automated extraction system for flame atomic absorption spectrometry, *Anal. Chim. Acta,* 125, 199, 1981.
18. **Imasaka, T., Harada, T., and Ishibashi, N.,** Fluorimeric determination of gallium with lumogallion by flow injection analysis based on solvent extraction, *Anal. Chim. Acta,* 129, 196, 1981.
19. **Ekström, J. G.,** An automated method for determination of free fatty acids, *J. Assoc. Off. Anal. Chem.,* 58, 935, 1981.
20. **Shelly, D. C., Rossi, T. M., and Warner, M. I.,** Multiple solvent extraction system with flow injection technology, *Anal. Chem.,* 54, 87, 1982.
21. **Fang, Z.,** Development of flow injection analysis, *Turangzue Jinzhan,* 10, 48, 1982 (in Chinese).
22. **Fossey, L. and Cantwell, F. F.,** Characterization of solvent extraction/flow injection analysis with constant pressure pumping and determination of procyclidine hydrochloride in tablets, *Anal. Chem.,* 54, 1693, 1982.
23. **Rossi, T. M., Shelly, D. C., and Warner, I. M.,** Optimization of a flow injection analysis system for multiple solvent extraction, *Anal. Chem.,* 54, 2056, 1982.
24. **Ogata, K., Taguchi, K., and Imanari, T.,** Phase separator for flow injection analysis, *Anal. Chem.,* 54, 2127, 1982.
25. **Ogata, K., Taguchi, K., and Imanari, T.,** Determination of orthophosphate by flow injection analysis based on solvent extraction, *Bunseki Kagaku,* 31, 641, 1982 (in Japanese).
26. **Ranger, C. B.,** Flow injection analysis. A new approach to near-real-time process monitoring, *Autom. Stream Anal. Process Control,* 1, 39, 1982.
27. **Mortatti, J., Krug, J. F., and Bergamin, F. H.,** Spectrophotometric determination of lead in natural waters and plant material by flow injection analysis, *Energ. Nucl. Agric., Piracicaba,* 4, 82, 1982 (in Portugese).
28. **Nord, L. and Karlberg, B.,** Sample preconcentration by continuous flow extraction with a flow injection atomic absorption detection system, *Anal. Chim. Acta,* 145, 151, 1983.
29. **Mise, T.,** Determination of anionic surfactants by flow injection analysis based on solvent extraction, *Miyakojo Kogyo Koto Semmon Gekko Kenkyo Hokoku,* 17, 7, 1983 (in Japanese).
30. **Ogata, K., Taguchi, K., and Imanari, T.,** Flame atomic absorption spectrometry coupled with solvent extraction/flow injection analysis, *Chem. Pharm. Bull.,* 31, 1419, 1983.
31. **Fossey, L. and Cantwell, F. F.,** Simultaneous monitoring of both phases in the solvent extraction/flow injection analysis of Dramamine tablets, *Anal. Chem.,* 55, 1882, 1983.
32. **Burguera, J. L. and Burguera, M.,** Determination of cadmium in human urine by extraction with dithizone in a flow injection system, *Anal. Chim. Acta,* 153, 207, 1983.
33. **Jones, E. A.,** The determination by flow injection analysis of iron, sulfate, silver and cadmium, *Techn. Rep. Mintek,* M111, 32, 1983.
34. **Hemmings, P.,** Automated Flow Injection Analysis for Anions in Waters, Ph.D. thesis, University of Birmingham, Birmingham, U.K., 1983.
35. **Lynch, T. P., Taylor, A. F., and Wilson, J. N.,** Fully automatic flow injection system for the determination of uranium at trace levels in ore leachates, *Analyst (London),* 108, 470, 1983.
36. **Bäckström, K., Danielsson, L.-G., and Nord, L.,** Sample workup for graphite furnace atomic absorption spectrometry using continuous flow extraction, *Analyst (London),* 109, 323, 1984.
37. **McLeod, C. W., Worsfold, P. J., and Cox, G. A.,** Simultaneous multi-element analysis of blood serum by flow injection/inductively coupled plasma atomic emission spectrometry, *Analyst (London),* 109, 327, 1984.

38. **Müller, H. and Müller, V.,** Principles and applications of flow injection analysis, *Z. Chem.*, 24, 81, 1984 (in German).
39. **Rossi, T. M., Shelly, D. C., and Warner, I. M.,** Optimization of a flow injection analysis system for multiple solvent extraction, *Energy Res. Abstr.*, 9(No. 8101), 1984.
40. **Bengtsson, M. and Johansson, G.,** Preconcentration and matrix isolation of heavy metals through a two-stage solvent extraction in a flow system, *Anal. Chim. Acta*, 158, 147, 1984.
41. **Nord, L. and Karlberg, B.,** Extraction based on the flow injection principle. VI. Film formation and dispersion in liquid-liquid segmented flow extraction systems, *Anal. Chim. Acta*, 164, 233, 1984.
42. **Ogata, K., Soma, S., Koshiishi, I., Tanabe, S., and Imanari, T.,** Determination of silicate by flow injection analysis coupled with supression column and solvent extraction system, *Bunseki Kagaku*, 33, 535, 1984.
43. **Imato, T. and Ishibashi, N.,** Experimental studies of dispersion in FIA-solvent extraction process, *J. Flow Injection Anal.*, 1, 23, 1984 (in Japanese).
44. **Nord, L.,** Extraction in Liquid-Liquid Segmented Flow Systems Applied to the Mechanization of Sample Pretreatment, Ph.D. thesis, Royal Institute of Technology, Stockholm, 1984.
45. **Wu, Y. P. and Pacey, G. E.,** Spectrophotometric determination of lithium ion with the chromogenic crown ether 2″,4″-dinitro-6″-trifluoro-methylphenyl-4′-aminobenzo-14-crown-4, *Anal. Chim. Acta*, 162, 285, 1984.
46. **Cantwell, F. F. and Sweileh, J. A.,** Hydrodynamic and interfacial origin of phase segmentation in solvent extraction/flow injection analysis, *Anal. Chem.*, 57, 329, 1985.
47. **Sweileh, J. A. and Cantwell, F. F.,** Sample introduction by solvent extraction/flow injection to eliminate interferences in atomic absorption spectroscopy, *Anal. Chem.*, 57, 420, 1985.
48. **Hirai, Y. and Tomokumi, K.,** Extraction-spectrophotometric determination of anionic surfactants with a flow injection analysis, *Anal. Chim. Acta*, 167, 409, 1985.
49. **Bäckström, K., Danielsson, L.-G., and Nord, L.,** Design and evaluation of a new phase separator for liquid-liquid extraction in flow systems, *Anal. Chim. Acta*, 169, 43, 1985.
50. **Gallego, M. and Valcárcel, M.,** Indirect atomic absorption spectrometric determination of perchlorate by liquid-liquid extraction in a flow injection system, *Anal. Chim. Acta*, 169, 161, 1985.
51. **Fossey, L. and Cantwell, F. F.,** Determination of acidity constants by solvent extraction/flow injection analysis using a dual-membrane phase separator, *Anal. Chem.*, 57, 922, 1985.
52. **Tyson, J. F., Adeeyinwo, C. E., Appleton, J. M. H., Bysouth, S. R., Idris, A. B., and Sarkissian, L. L.,** Flow injection techniques of method development for flame atomic absorption spectrometry, *Analyst (London)*, 110, 487, 1985.
53. **Maeda, M. and Tsuji, A.,** Fluorescence and chemiluminescence determination of steroid and bili acid sulfates with lucigenin by flow injection analysis based on ion-pair solvent extraction, *Analyst (London)*, 110, 665, 1985.
54. **Sweileh, J. A. and Cantwell, F. F.,** Use of peak height for quantification in solvent extraction/flow injection analysis, *Can. J. Chem.*, 63, 2559, 1985.
55. **Sun, L., Li, L., and Fang, Z.,** Flow injection analysis of soil available zinc by solvent extraction with dithizone, *Fenxi Huaxue*, 13, 447, 1985 (in Chinese).
56. **Oguma, K. and Kuroda, R.,** Simultaneous determination by flow injection analysis, *J. Flow Inject. Anal.*, 2, 98, 1985 (in Japanese).
57. **Nord, L., Johansson, P. A., and Brotell, H.,** Flow injection extraction and gas chromatographic determination of terodiline in blood serum, *Anal. Chim. Acta*, 175, 281, 1985.
58. **Kato, T.,** Flow injection spectrophotometric determination of enalapril in pharmaceuticals with bromothymol blue, *Anal. Chim. Acta*, 175, 339, 1985.
59. **Karlberg, B.,** Liquid-liquid extraction in FIA, *FIAstar Newslett.*, 2, 1, 1985.
60. **Betteridge, D., Wade, A. P., and Howard, A. G.,** Reflections on the modified simplex-I, *Talanta*, 32, 709, 1985.
61. **Matschiner, H. and Sivers, P.,** Flow injection analysis special solutions for general problems, *Wiss. Fortschr.*, 35, 142, 1985 (in German).
62. **Fujiwara, K. and Fuwa, K.,** Liquid core optical fiber total reflection cell as a colorimetric detector for flow injection analysis, *Anal. Chem.*, 57, 1012, 1985.
63. **Hansen, E. H.,** Flow Injection Analysis, D.Sc. dissertation, Technical University of Denmark, Copenhagen, 1986.
64. **Gallego, M., Silva, M., and Valcárcel, M.,** Determination of nitrate and nitrite by continuous liquid-liquid extraction with a flow-injection atomic-absorption detection system, *Fresenius Z. Anal. Chem.*, 323, 50, 1986.
65. **Sahleström, Y. and Karlberg, B.,** An unsegmented extraction system for flow injection analysis, *Anal. Chim. Acta*, 179, 315, 1986.
66. **Silva, M., Gallego, M., and Valcárcel, M.,** Sequential atomic absorption spectrometric determination of nitrate and nitrite in meats by liquid-liquid extraction in a flow injection system, *Anal. Chim. Acta*, 179, 341, 1986.
67. **Whitaker, M. J.,** Spectrophotometric determination of nonionic surfactants by flow injection analysis utilizing ion-pair extraction and an improved phase separator, *Anal. Chim. Acta*, 179, 459, 1986.
68. **Karlberg, B.,** Flow injection analysis or the art of controlling sample dispersion in a narrow tube, *Anal. Chim. Acta*, 180, 16, 1986.
69. **Fang, Z.,** The automation of soil chemical analysis

by flow injection analysis, *Proc. Multinatl. Instrum. Conf., Beijing, China*, p. 800, 1986.
70. **Luque de Castro, M. D.,** Flow injection analysis. A new tool to automate extraction processes, *J. Autom. Chem.*, 8, 56, 1986.
71. **Fang, Z.,** Flow injection techniques in atomic spectroscopy, *Fenxi Huaxue*, 14, 549, 1986 (in Chinese).
72. **Fogelqvist, E., Krysell, M., and Danielsson, L.-G.,** On-line liquid-liquid extraction in a segmented flow directly coupled to on-column injection into a gas chromatograph, *Anal. Chem.*, 58, 1516, 1986.
73. **Gallego, M., Silva, M., and Valcárcel, M.,** Indirect atomic absorption determination of anionic surfactants in wastewater by flow injection continuous liquid-liquid extraction, *Anal. Chem.*, 58, 2265, 1986.
74. **Sahlestrom, Y. and Karlberg, B.,** Flow injection extraction with a microvolume module based on integrated conduits, *Anal. Chim. Acta*, 185, 259, 1986.
75. **Iwachido, T., Onoda, M., and Motomizu, S.,** Determination of potassium in river water by solvent extraction flow injection analysis, *Anal. Sci.*, 2, 493, 1986.
76. **Feješ, J. and Melnik, S.,** Optical methods of detection in flow injection analysis, *Chem. Listy*, 80, 1009, 1986 (in Czech).
77. **Audunsson, G.,** Aqueous/aqueous extraction by means of a liquid membrane for sample cleanup and preconcentration of amines in a flow system, *Anal. Chem.*, 58, 2714, 1986.
78. **Lucy, C. A. and Cantwell, F. F.,** Simultaneous determination of phenylephrine hydrochloride and pheniramine maleate in nasal spray by solvent extraction flow injection analysis using two porous membrane phase separators and one photometric detector, *Anal. Chem.*, 58, 2727, 1986.
79. **Burguera, J. L., Burguera, M., La Cruz, D. L., and Naranjo, O. R.,** Determination of lead in the urine of exposed and unexposed adults by extraction and flow injection/atomic absorption spectrometry, *Anal. Chim. Acta*, 186, 273, 1986.
80. **Bäckström, K., Danielsson, L.-G., and Nord, L.,** Dispersion in phase separators for flow injection extraction systems, *Anal. Chim. Acta*, 187, 255, 1986.
81. **Sahlestrom, Y., Twengstrom, S., and Karlberg, B.,** An automated flow injection extraction method for determination of bittering compounds in beer, *Anal. Chim. Acta*, 187, 339, 1986.
82. **Valcárcel, M. and Luque de Castro, M. D.,** Continuous separation techniques in flow injection analysis, *J. Chromatogr.*, 393, 3, 1987.
83. **Motomizu, S., Hazaki, Y., Oshima, M., and Toei, K.,** Spectrophotometric determination of anionic surfactants in river water with cationic azo dye by solvent extraction flow injection analysis, *Anal. Sci.*, 3, 265, 1987.
84. **Attiyat, A. S.,** Study of sample solvent/carrier combination for flow injection analysis atomic absorption spectrometry, *J. Flow Inject. Anal.*, 4, 26, 1987.
85. **Nord, L., Bäckström, K., Danielsson, L.-G., Ingman, F., and Karlberg, B.,** Extraction rate in liquid-liquid segmented flow injection analysis, *Anal. Chim. Acta*, 194, 221, 1987.
86. **Scokart, P. O., Meeus-Verdinne, K., and De Borger, R.,** Speciation of heavy metals in polluted soil by sequential extraction and ICP spectrometry, *Int. J. Environ. Anal. Chem.*, 29, 305, 1987.
87. **Sakai, T.,** Flow injection analysis coupled with solvent extraction, *Bunseki Kagaku*, 34, 260, 1987 (in Japanese).
88. **Canham, J. S. and Pacey, G. E.,** Automated free fatty acid determination using flow injection analysis solvent extraction, *J. Am. Oil Chem. Soc.*, 64, 1004, 1987.
89. **Bäckström, K., Danielsson, L.-G., Ingman, F., and Huazhang, Z.,** Studies of two-phase equilibria by liquid-liquid segmented flow extraction of dithiocarbamic acid into various solvents, *Talanta*, 34, 783, 1987.
90. **Kumamaru, T., Nitta, Y., Matsuo, H., and Kimura, E.,** Inductively coupled plasma atomic emission spectrometric determination of copper by suction-flow on-line liquid-liquid extraction of its macrocyclic dioxotetramine chelate, *Bull. Chem. Soc. Jpn.*, 60, 1930, 1987.
91. **Persaud, G., Xiu-min, T., and Cantwell, F. F.,** Behavior of solute adsorbed at the liquid-liquid interface during solvent extraction with porous-membrane phase separators, *Anal. Chem.*, 59, 2, 1987.
92. **Atallah, R. H., Růžička, J., and Christian, G. D.,** Continuous solvent extraction in a closed-loop system, *Anal. Chem.*, 59, 2909, 1987.
93. **Motomizu, S. and Oshima, M.,** Spectrophotometric determination of phosphorus as orthophosphate based on solvent extraction of the ion associate of molybdophosphate with malachite green using flow injection, *Analyst (London)*, 112, 295, 1987.
94. **Valcárcel, M. and Luque de Castro, M. D.,** *Flow Injection Analysis. Principles and Applications*, John Wiley & Sons, New York, 1987.
95. **Karlberg, B.,** Flow injection extraction in theory and practice, *Fresenius Z. Anal. Chem.*, 329, 660, 1988.
96. **Farran, A. and De Pablo, J.,** Continuous-flow extraction of organophosphorus pesticides coupled on-line with high-performance liquid chromatography, *Anal. Chim. Acta*, 212, 123, 1988.
97. **Fujiwara, K.,** Application of a wave-guide capillary cell in the determination of copper by flow injection analysis, *Anal. Chim. Acta*, 212, 245, 1988.
98. **Růžička, J. and Hansen, E. H.,** Homogeneous and heterogeneous systems. Flow injection analysis today and tomorrow, *Anal. Chim. Acta*, 214, 3, 1988.
99. **Karlberg, B.,** The theoretical and practical development of flow injection extraction, *Anal. Chim. Acta*, 214, 29, 1988.
100. **Fang, Z., Zhu, Z., Zhang, S., Xu, S., Guo, L., and Sun, L.,** On-line separation and preconcentration in flow injection analysis, *Anal. Chim. Acta*, 214, 41, 1988.
101. **Motomizu, S. and Onoda, M.,** Flow-injection de-

termination of sodium and potassium by separation on a silica column and extraction spectrophotometry with benzo-18-crown-6 and tetrabromophenolphthalein ethyl ester, *Anal. Chim. Acta*, 214, 289, 1988.

102. **Gluck, S. J.,** Determination of octanol/water partition coefficients by flow injection analysis, *Anal. Chim. Acta*, 214, 315, 1988.
103. **Atallah, R. H., Christian, G. D., and Hartenstein, S. D.,** Continuous flow solvent extraction system for the determination of trace amounts of uranium in nuclear waste reprocessing solutions, *Analyst (London)*, 113, 463, 1988.
104. **Motomizu, S., Oshima, M., and Kuroda, T.,** Spectrophotometric determination of anionic surfactants in water after solvent extraction coupled with flow injection, *Analyst (London)*, 113, 747, 1988.
105. **Motomizu, S., Onoda, M., and Oshima, M.,** Spectrophotometric determination of potassium in river water based on solvent extraction of the complex formed with a crown ether and an anionic azo dye using flow injection, *Analyst (London)*, 113, 743, 1988.
106. **Toei, J.,** Segmented flow injection solvent extraction analysis, *Analyst (London)*, 113, 1861, 1988.
107. **Martinez-Jimenez, P., Gallego, M., and Valcárcel, M.,** Indirect atomic absorption spectrometric determination of some cationic surfactants by continuous liquid-liquid extraction with tetrathiocyanatocobaltate, *Anal. Chim. Acta*, 215, 233, 1988.
108. **Del Valle, M., Alonso, J., Bartoli, J., and Marti, I.,** Spectrophotometric determination of low levels of anionic surfactants in water by solvent extraction in a flow injection system, *Analyst (London)*, 113, 1677, 1988.
109. **Bäckström, K. and Danielsson, L.-G.,** Design and evaluation of an interface between a continuous flow system and a graphite furnace atomic absorption spectrometer, *Anal. Chem.*, 60, 1354, 1988.
110. **Nekimken, H. L., Smith, B. F., Jarvinen, G. D., Peterson, E. J., and Jones, M. M.,** Computer controlled flow injection analysis system for on-line determination of distribution ratios, *Anal. Chim.*, 60, 1390, 1988.
111. **Canete, F., Rios, A., Luque de Castro, M. D., and Valcárcel, M.,** Liquid-liquid extraction in continuous flow systems without phase separation, *Anal. Chem.*, 60, 2354, 1988.
112. **Attiyat, A. S., Christian, G. D., Xie, R. Y., Wen, X., and Bartsch, R. A.,** Comparative evaluation of neutral and proton ionizable crown ether compounds as lithium ionophores in ion-selective electrodes and in solvent extraction, *Anal. Chem.*, 60, 2561, 1988.
113. **Růžička, J. and Hansen, E. H.,** *Flow Injection Analysis*, John Wiley & Sons, New York, 1988, 186.
114. **Lucy, C. A. and Cantwell, F. F.,** Kinetics of solvent extraction-flow injection analysis, *Anal. Chem.*, 61, 101, 1989.
115. **Lucy, C. A. and Cantwell, F. F.,** Mechanism of extraction and band broadening in solvent extraction-flow injection analysis, *Anal. Chem.*, 61, 107, 1989.
116. **Burns, D. T., Chimpalee, N., and Harriott, M.,** Flow injection extraction-spectrophotometric determination of perchlorates with brilliant green, *Anal. Chim. Acta*, 217, 177, 1989.
117. **Burns, D. T., Chimpalee, N., Harriott, M., and McKillen, G. M.,** Flow injection extraction spectrophotometric determination of permanganate with the ethylene-bis(triphenylphosphine) cation, *Anal. Chim. Acta*, 217, 183, 1989.
118. **Kubáň, V., Komárek, J., and Čajková, D.,** Use of liquid-liquid extraction in flow injection analysis with detection using atomic absorption spectrometry, *Chem. Listy*, 84, 376, 1989 (in Czech).
119. **Kubáň, V., Komárek, J., and Čajková, D.,** Determination of copper by flow injection analysis with flame atomic absorption detection after liquid-liquid extraction preconcentration, *Collect. Czech. Chem. Commun.*, 54, 2683, 1989.
120. **Toei, J.,** Potential of a modified solvent extraction flow injection analysis, *Talanta*, 36, 691, 1989.
121. **Canete, F., Rios, A., Luque de Castro, M. D., and Valcárcel, M.,** Kinetics of ion-pair extraction in continuous flow systems, *Anal. Chim. Acta*, 224, 169, 1989.
122. **Burns, D. T., Chimpalee, N., and Harriott, M.,** Flow injection spectrophotometric determination of cobalt by extraction as tetrathiocyanatocobaltate(II) with the ethylenebis(triphenylphosphonium) cation, *Anal. Chim. Acta*, 225, 123, 1989.
123. **Burns, D. T., Chimpalee, N., and Harriott, M.,** Flow injection extraction spectrophotometric determination of dichromate with the tetramethylenebis(triphenylphosphonium) cation, *Anal. Chim. Acta*, 225, 241, 1989.
124. **Dasgupta, P. K. and Lei, W.,** Solvent extraction in continuous flow systems with intelligent zone sampling, *Anal. Chim. Acta*, 226, 255, 1989.
125. **Karlberg, B. and Pacey, G. E.,** *Flow Injection Analysis, A Practical Guide*, Elsevier, Amsterdam, 1989.
126. **Burguera, J. L. and Burguera, M.,** *Flow Injection Analysis in Atomic Absorption Spectrometry*, Marcel Dekker, New York, 1989.
127. **Koshima, H. and Onishi, H.,** Separation of rhenium by extraction with crown ethers and flow injection extraction spectrophotometric determination with brilliant green, *Anal. Chim. Acta*, 232, 287, 1990.
128. **Bäckström, K. and Danielsson, L.-G.,** A mechanized continuous system for the concentration and determination of Co, Cu, Ni, Pb, Cd and Fe in seawater using graphite furnace atomic absorption, *Mar. Chem.*, 28, 33, 1990.
129. **Bäckström, K. and Danielsson, L.-G.,** On the design of a continuous flow two step extraction sample workup system for graphite furnace atomic absorption spectrometry, *Anal. Chim. Acta*, 232, 301, 1990.
130. **Thommen, C., Fromageat, A., Obergfell, P., and Widmer, H. M.,** Application of a capillary flow cell

to sophisticated flow injection analysis, *Anal. Chim. Acta*, 234, 141, 1990.
131. **Ballesteros, E., Gallego, M., and Valcárcel, M.,** On-line coupling of a gas chromatograph to a continuous liquid-liquid extractor, *Anal. Chem.*, 62, 1587, 1990.
132. **Kubáň, V., Danielsson, L.-G., and Ingman, F.,** Comparison of segmenters for liquid-liquid extraction flow injection analysis, *Talanta*, 37, 1169, 1990.
133. **Kubáň, V., Danielsson, L.-G., and Ingman, F.,** The design of coaxial segmenters for liquid-liquid extraction flow injection analysis, *Anal. Chem.*, 62, 2026, 1990.
134. **Haloax, J. J., Wiese, G., and Bennekom, W. P.,** Selective and sensitive on-line flow extraction, preconcentration and normal phase liquid chromatography, *Anal. Chim. Acta*, 239, 171, 1990.
135. **Motomizu, S., Oshima, M., Yaneda, N., and Iwachido, T.,** Spectrophotometric determination of calcium with dicyclohexano-24-crown-8 and propyl orange by solvent extraction flow injection method, *Anal. Sci.*, 6, 215, 1990.
136. **Szpunar-Lobinska, J. and Trojanowicz, M.,** Flow injection extraction spectrophotometric determination of copper with dithiocarbamates, *Anal. Sci.*, 6, 415, 1990.
137. **Koshima, H. and Onishi, H.,** Flow injection method for the separation of thallium and gold on a Sephadex column and their extraction photometric determination with rhodamine B, *Anal. Sci.*, 6, 421, 1990.
138. **Kubota, H., Katsuki, M., and Motomizu, S.,** Batchwise and flow injection methods for spectrophotometric determination of anionic surfactants with 4-(-4-N,N-dimethylaminophenylazo)-2-methylquinoline, *Anal. Sci.*, 6, 705, 1990.
139. **Mauroori, J. L. and Miyazaki, A.,** Indirect inductively coupled plasma atomic emission determination of fluoride in water samples by flow injection solvent extraction, *Anal. Chem.*, 62, 2457, 1990.
140. **Sakai, T.,** Solvent extraction spectrophotometric determination of berberine and benzethoinium in drugs with tetrabromophenolphthalein ethyl ester by batchwise and flow injection methods, *Analyst (London)*, 116, 187, 1990.
141. **Clark, G. D., Whitman, D. A., Christian, G. D., and Růžička, J.,** Sample handling and preconcentration in flow injection analysis, *Crit. Rev. Anal. Chem.*, 21, 357, 1990.
142. **Kubáň, V.,** Liquid-liquid extraction in flow injection analysis, *Radioakt. Ziv. Prostr.*, 9, 13, 1990 (in Czech).
143. **Kubáň, V. and Ingman, F.,** Phase segmentation in liquid-liquid extraction continuous flow analysis, *Crit. Rev. Anal. Chem.*, 22, 37, 1991.
144. **Kubáň, V. and Ingman, F.,** Design of a multichannel dropping segmenter for liquid-liquid extraction continuous flow injection analysis, *Anal. Chim. Acta*, 245, 251, 1991.
145. **Kubáň, V.,** Study of the film formation process in liquid-liquid extraction flow injection analysis, *Collect. Czech. Chem. Commun.*, 55, 1228, 1991.
146. **Kubáň, V.,** Determination of 1-octanol/water partition coefficients by flow injection extraction without phase separation, *Anal. Chim. Acta*, 248, 493, 1991.
147. **Kubáň, V., Danielsson, L.-G., and Ingman, F.,** Application of the multichannel coaxial dispenser in segmented continuous flow analysis (SCFA), *Talanta*, in print.
148. **Forman, W. B.,** *Continuous Flow Analysis. Theory and Practice*, Marcel Dekker, New York, 1976.
149. **Foreman, J. K. and Stockwell, P. B.,** *Automatic Chemical Analysis*, Horwood, Chichester, 1975.
150. **Valcárcel, M. and Luque de Castro, M. D.,** *Automatic Methods of Analysis. Techniques and Instrumentation in Analytical Chemistry*, Vol. 9, Elsevier, Amsterdam, 1988.
151. **Apffel, J. A., Brinkman, U. A. Th., and Frei, R. W.,** Design and application of a post-column extraction system compatible with miniaturized liquid chromatography, *Chromatographia*, 18, 5, 1984.
152. **Lindgren, C. C. and Dasgupta, P. K.,** Flow injection and solvent extraction with intelligent segment separation. Determination of quaternary ammonium ions by ion-pairing, *Talanta*, in press.
153. **Coulson, J. M. and Richardson, J. F.,** *Chemical Engineering*, 3rd ed., Vol. 1, Pergamon Press, Oxford, 1976.
154. **Bartle, K. D., Wooley, C. L., Markides, K. E., Lee, M. L., and Hansen, R. S.,** *JHRaCC*, 10, 128, 1987.
155. **Snyder, L. R. and Adler, H.,** Dispersion in segmented flow through glass tubing in continuous flow analysis; the ideal and nonideal model, *Anal. Chem.*, 48, 1017 and 1022, 1976.
156. **Chen, Jing-Den,** Measuring the film thickness surrounding a bubble inside a capillary, *J. Colloid. Interface Sci.*, 109, 341, 1986; **Bretherton, F. P.,** The motion of long bubbles in tubes, *J. Fluid Mech.*, 10, 166, 1961.
157. **Curran, D. J. and Marden, S. K.,** Coaxial phase separator for on-line extraction in flow-injection systems, *Anal. Chim. Acta*, 247, 67, 1991.
158. **Melcher, R. G.,** Flow-injection determination of membrane-selected organic compounds, *Anal. Chim. Acta*, 214, 239, 1988.
159. **Kubáň, V.,** Application of flow injection extraction in chemical analysis, *Talanta*, submitted.
160. **Motomizu, S. and Korechika, K.,** Flow injection determination of copper based on the solvent extraction of the ion associate of dibromocuprate(I) with cationic dyes, *Nippon Kagaku Kaishi*, 795, 1991.