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CONTINUOUS SEPARATION TECHNIQUES IN FLOW INJECTION ANALYSIS

A REVIEW

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SUMMARY

After establishing the basic similarities and differences between flow injection analysis (FIA) and high-performance liquid chromatography (HPLC), the objectives pursued with the formation of different types of interfaces in FIA are discussed: pre-concentration, sample clean-up, etc., to improve or facilitate the analytical reaction and/or detection. The types of interfaces most commonly employed in FIA systems are gas–liquid (gas diffusion, distillation, hydride generation), liquid–liquid (extraction, dialysis) and liquid–solid (ion exchange, adsorption, precipitation, dissolution, stripping). The general features of the methods proposed in each of these alternatives are critically discussed, and some representative examples are described. Special emphasis is given to the on-line coupling of HPLC and FIA, showing the potential of this association. Finally, the advantages involved in the joint use of separation units built in unsegmented continuous flow systems and the prospects for this association are discussed.

INTRODUCTION

Nowadays, high-performance liquid chromatography (HPLC), flow injection analysis (FIA) and field flow fractionation (FFF) are commonly used hydrodynamic systems in analytical chemistry. If the instrumental schematic diagrams of HPLC and FIA are compared, it can readily be concluded that there is a remarkable similarity between them: they have important common components such as liquid reservoirs, pump(s), injectors and continuous detection systems. Nevertheless, there are also significant differences, such as working pressure, interface occurrence, versatility, type of analytical problems dealt with, costs, etc. However, the greatest difference is the continuous separation performed in the chromatographic column, which is essential in HPLC and only occasionally needed in FIA (used in about 10% of the papers published so far[1,2].

The formation of interfaces in FIA systems can have two main objectives. On the one hand, it can be used to develop continuous separation processes to improve

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those performed conventionally (liquid–liquid extraction, ion exchange, adsorption, etc.). These separation processes carried out in unsegmented flow configurations are intermediate (both kinetically and thermodynamically) between batch methods in which equilibrium is reached once or several times and chromatographic methods in which equilibrium is reached many times.

On the other hand, the formation of different interfaces in FIA systems can be aimed at non-separative applications, *e.g.*, to improve or facilitate the analytical determination. In this instance, advantage is taken of the chemical reaction between a solid phase and a liquid phase which flows through it. Thus, redox columns have been used to handle reagents sensitive to atmospheric agents[3] or to perform multiple determinations (*e.g.*, NO_2^- and NO_3^-)[4]. The use of immobilized enzymes in packed columns or in wall tubes is a very interesting alternative[5]. Voltammetric and potentiometric stripping techniques performed in a continuous fashion can also be included in this context because they pursue the two above-mentioned objectives in their two basic steps: pre-concentration and determination[6–8].

The different interfaces employed in FIA systems and the corresponding continuous separation techniques used are summarized in Table I. A general and brief description of each is given below. Special importance is given to the coupling of an FIA system with a liquid chromatograph. Finally, the purposes and advantages of the incorporation of non-chromatographic separation techniques into unsegmented flow configurations is critically discussed.

GAS–LIQUID INTERFACES

The gas–liquid separation systems used in FIA can be classified into three groups: (a) *gas diffusion*, in which a gas present in a donor liquid phase or formed by chemical reaction diffuses to the other phase (also a liquid, which acts as an acceptor); (b) *distillation*, in which the gas phase is formed by heating, condensation at a suitable temperature and collection into a second liquid phase; (c) *hydride generation*, in which the gas phase is formed in a chemical reaction and the second one is a gas that transports the sample to the detection system.

TABLE I

CONTINUOUS SEPARATION TECHNIQUES IN FLOW INJECTION CONFIGURATIONS

Interface	*Separation technique*
Gas–liquid	Gas diffusion
	Distillation
	Hydride generation
Gas–solid	
Liquid–liquid	Extraction
	Dialysis
Liquid–solid	Ion exchange
	Adsorption
	Precipitation
	Others

Gas diffusion

The transfer of a gas between two donor streams has scarcely been used in FIA, although it is applicable to a wide variety of analytes, matrices and detection systems (see Table II).

The analyte constituting the gas phase can be present as gas in the donors (O_2, O_3, Cl_2) or can be formed in it by simple chemical reaction induced by an acid (formation of SO_2 or HCN) or a base (formation of NH_3), or may require the aid of relatively high temperatures (formation of acetone from oxidized ketone bodies). Photometry has been the most frequently utilized detection system on account of its suitability for analytes with acid–base properties, which usually diffuse to solutions containing an indicator, whose colour change is a function of the amount of analyte diffused.

Generally, phase separation is isothermal and occurs through a suitable membrane (usually PTFE). Sometimes there is no separation membrane, but this is produced between two parallel rubber sheets supported by Perspex plates. The stream containing the sample spreads throughout the lower sheet, yielding a film that traverses the entire length of the rubber before going to waste. During the transport, the species of interest evaporates and the gas diffuses through the space between the two sheets, being collected in the acceptor stream[9].

The analytical purpose generally in pursued combined FIA–gas diffusion is removal of interferences in complex matrices (biological liquids, foods, vegetable tissues, etc.); nevertheless, enhanced selectivity and increased sensitivity can be achieved by incorporating kinetic discrimination and/or kinetic enhacement into the timing of the system or the reagent concentrations and conditions for a given method, as demonstrated by Pacey *et al.*[10] in the sequential determination of ozone, chlorine dioxide and chlorite and chlorate.

Fig. 1 shows the gas diffusion system used for the determination of ammonia in blood proposed by Svensson and Anfält[11], in which the sample is injected into a distilled water stream which merges with 0.5 *M* sodium hydroxide solution, converting NH_4^+ to NH_3, which diffuses through the PTFE membrane to a stream of phenol red in 10 *M* sodium hydroxide solution, subsequently being monitored at 540 nm (Table II[12–21]).

Distillation

There is only one paper in the FIA literature in which distillation is used with the basic purpose of eliminating the interferents present in a complex matrix, such as waste water for cyanide determination. The method, proposed by Pihlar and Kosta[22], involves the use of a distillation system consisting of a distillation and an absorption unit. The former (borosilicate glass, half-packed with glass helices and wrapped with a heating wire) is entered by the nitrogen stream at the bottom of the distillation column and by a hydrogen cyanide carrier through the condenser into the absorption unit. A 0.1 *M* solution of sodium hydroxide is pumped to the top of the absorption column. A debubbler unit prior to the voltammetric detector removes the gas from the system. Differentiation between total and strongly bound metal cyanide complexes is achieved by UV decomposition of the complexes.

TABLE II

FEATURES OF FIA METHODS INVOLVING GAS–LIQUID INTERFACES

Type	*Analyte*	*Matrix*	*Gas-phase formation*	*Phase separation*	*Analytical objective*	*Detection**	*Special features*	*Ref.*
Gas diffusion	NH_3	Whole blood	pH change	Membrane	Interference removal	P	Use of an indicator	11
	NH_3	Blood	pH change	Membrane	Interference removal	Pot.		12
	NH_3	Plants	pH change	No phase separation	Interference removal	P and Pot.		9
	SO_2	Wine, beer, fruit juices	pH change	Membrane	Interference removal	P		13
	ClO_2			Membrane	Interference removal	P		14
	O_3			Membrane	Interference removal	P		15
	Oxidized ketone bodies	Milk	Heat	Membrane	Interference removal	P		16
	NH_3, urea		Chemical reaction	Membrane	Interference removal	Optosensor	Integrated microconduits	17
	SO_2		Chemical reaction	Membrane	Interference removal	Chem.		18
	O_2, ClO_2			Membrane	Discrimination–separation	Chem.		10

	SO_2	Wine, food		Membrane	Interference removal	Amp.		19
	CN^-	Waste-water	pH change	Membrane	Interference removal	Pot.		20
	ClO_2				Interference removal	Chem.		21
Distillation	CN^-	Industrial water	Heat		Interference removal	Volt.		22
Hydride generation	As^{III}, As^{V}		Chemical reaction		Interference removal	AAS	Second gas-phase speciation	23
	Bi		Chemical reaction		Interference removal	AAS		24
	As, Sb, Bi, Se, Te	Thermal water	Chemical reaction		Interference removal	AAS	Speciation	25
	As	Standard reference	Chemical reaction		Interference removal	AAS		26
	As	NBS orchard leaves	Chemical reaction		Interference removal	MECA		27
	Hg		Chemical reaction		Interference removal	AAS		28

* P = Photometry; Pot. = potentiometry; Chem. = chemiluminescence; Amp. = amperometry; Volt. = voltammetry; AAS = atomic absorption spectrometry; MECA = molecular emission cavity analysis.

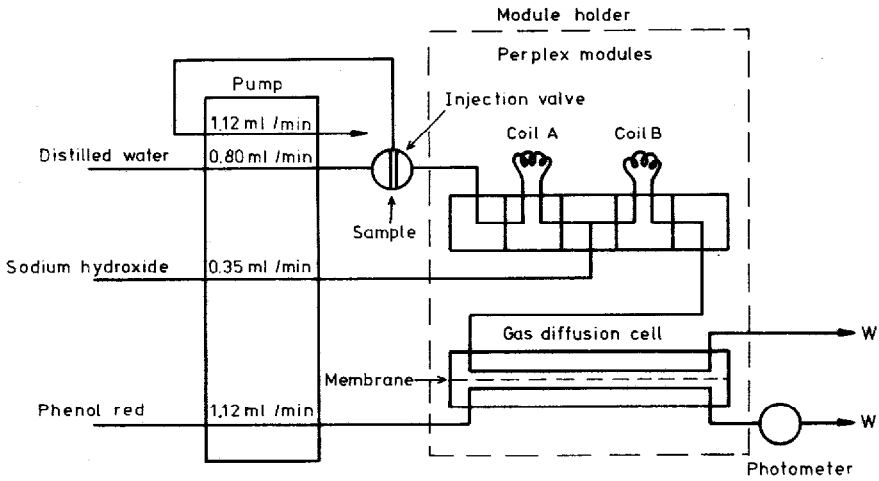

Fig. 1. Schematic diagram of set-up for the determination of ammonia in blood involving a gas-diffusion system (from ref. 11).

Hydride generation

Hydride generation is a *"sui generis"* example of gas–liquid separation systems requiring a chemical agent (usually sodium borohydride) yielding a volatile compound, which is separated from the solution by a gas (second phase) transporting the analyte to an atomic optical detector. The analytical purpose is usually removal of interferences[23–28] and occasionally the performance of speciation studies by exploiting the different rates of formation of hydrides of the different chemical forms in which the analyte occurs[25] (Table II).

GAS–SOLID INTERFACES

There are few methods involving gas–solid interfaces in general and only a direct determinative method for chlorine and bromine in FIA[29], based on the transient signal resulting from two consecutive reactions at a gas–solid interface. The method involves no separation processes.

LIQUID–LIQUID INTERFACES

Separation techniques involving this type of phase have been used to different extents in FIA. Thus, extraction has been utilized relatively frequently[30], whereas dialysis has rarely been adopted. Table III summarizes the most important features of the different FIA methods proposed with both types of separation techniques, which are commented on separately below.

Extraction

The on-line coupling of a liquid–liquid extraction system to an FIA configuration was simultaneously proposed by Karlberg and Thelander[31] and Bergamin *et al.*[32]. In both instances, the extraction system was located behind the injection valve.

Currently, this separation unit can be placed either its original position or prior to the injection system, in a such manner that the separation process results in a continuous stream of organic phase containing the analyte, which fills the loop of the injection valve[33,34]. As a result of this coupling, configurations of varying complexity according to the particular needs are now available. The following types can be distinguished: (1) *without phase separation*, which is the simplest mode and in which the aqueous sample is injected into a single-channel configuration enclosing the organic stream extractant, which flows through the extraction coil; this is where the formation of an extractable complex between the analyte and the reagent dissolved in the organic phase, which is measured as it passes through the flow cell[35], takes place; (2) *single extraction*, with the separation system located prior to or after the injection unit; (3) *multiple extraction*, in which the separation process is repeated several times by using the same or a different extractant in the successive stages[36,37]; (4) *back-extraction*, which is a multi-stage extraction mode in which the aqueous sample is first extracted into an organic medium and then back-extracted into an aqueous phase, where measurements are performed[38].

The presence of an organic phase within an FIA system requires special precautions because of its dissolution properties. The transport tubing, connectors and extraction system must be made of steel, platinum, glass or PTFE. An organic solvent stream can be created (a) by a peristaltic pump (the PVC flexible tubes commonly used are useless, so that an inert material such as modified PVC, silicone rubber or fluorplast has to be employed), (b) by the displacement technique, which involves pumping an aqueous stream into a closed container (use of a peristaltic pump with ordinary tubing); the container is filled with organic solvent, which is fed at a constant flow-rate towards the FIA system; or (c) by setting a constant pressure with the aid of an inert gas, which forces the organic extract to circulate along the FIA configuration.

Every automatic solvent extraction FIA system has three essential components: (a) a *segmenter*, in which the streams of the two phases involved merge and which is intended to obtain identical alternate segments of both immiscible liquids attaining the extraction coil; (b) an *extraction coil*, in which the transfer of matter between the segments of both phases is carried out, and (c) a *phase separator*, which receives the segmented flow from the coil and continuously splits it into two separate streams in both phases. Of these three elements, the most complex and important is the phase separator, of which several models have been designed with the aim of improving the characteristics of these already available[31,39–45].

The FIA–liquid extraction combination has contributed to the resolution of analytical problems in several areas, especially in environmental, clinical and pharmaceutical chemistry (see Table III), which in general have been devoted to the separation (and sometimes pre-concentration) of the analyte. The applications have been systematized according to the type of analyte to be determined (inorganic or organic) and, within each group, depending on the detection systems utilized.

Fig. 2 shows the two generic types of FIA–liquid extraction arrangements: with the extraction unit located (a) before or (b) after the injection system. Configuration (a) has been used for the speciation of nitrogen as nitrite–nitrate in meats. The determination is sequential and is based on the formation of an ion pair between the copper(I)–neocuproin complex and the nitrate ion. The determination of nitrite

TABLE III

FEATURES OF THE FIA METHODS INVOLVING LIQUID–LIQUID INTERFACES

Type	*Analyte*	*Matrix*	*Features: analyte phase*	*Features: second phase**	*Detection***	*Other features*	*Ref.*
Extraction	Mo	Plants	Aqueous (SCN^-/Fe system)	Isoamyl alcohol	P		32
	Pb, Cd	Aqueous	Aqueous	Chloroform	P	Calculation of extraction constants	58
	Cd	Urine	Aqueous	Chloroform/dithizone	P	New phase separator	39
	PO_4^{3-}	Biological	Aqueous				51
	U	Biological		Tributyl phosphate		Modified T-piece	52
	Zn	Soil	Aqueous	CCl_4/dithizone	P		53
	Ga	Aqueous	Aqueous/lumogallion	Isoamyl alcohol	F	Laser excitation	41
	Zn, Cu, Pb, Ni	Aqueous	Aqueous	MIBK/APDC	AAS		68
	Zn	Biological/ environmental	Aqueous		AAS		55
	Zn	Iron	Aqueous/SCN^-	MIBK	AAS		47
	ClO_4^-	Urine, serum	Aqueous	MIBK	AAS		34
	NO_2^-, NO_3^-	Aqueous	Aqueous	MIBK	AAS		56
	NO_2^-–NO_3^-	Food	Aqueous	MIBK	AAS	Sequential determination	57
	Cu	Aqueous	Aqueous	MIBK/APDC	FAAS		33
	Cd, Cu, Co, Pb, Ni	Diluted samples	Aqueous	Freon 113 (H_2O 3rd phase)		Back-extraction	38
	Amines	Aqueous	Aqueous		P	Calculation of extraction constants	58
	Caffeine	Tablets	Aqueous	Chloroform	P	Calculation of extraction constants	31
	Codeine, acetylsalicyl acid	Tablets	Aqueous	Chloroform	P	Calculation of extraction constants	59

	Anionic surfactants	Industrial water	Aqueous	Chloroform	P		43
	Anionic surfactants	Sewage water	Aqueous	Toluene	P		60
	Cationic surfactants	Aqueous	Aqueous	Chloroform	P	Different segmentors	40
	Non-ionic surfactants	Aqueous	Aqueous	1,2-Dichloroethane	P		61
	Caffeine, surfactants	Aqueous	Aqueous	Chloroform	P		62
	8-Dichlorotheophilline, diphenyldramamine	Tablets	Aqueous	Cyclohexane	P	Measurements in both phases	42
	Procyclidine	Tablets	Aqueous	Chloroform	P	Theoretical studies of quantitativity	63
	Drugs	Biological	Aqueous			Adsorption problems	64
	Vitamin B_1	Tablets	Aqueous	Chloroform	F		65
	Steroids	Aqueous	Aqueous/lucigenin	1,2-Dichloroethane	Chem.		66
	Anionic surfactants	Waste-water	Aqueous	MIBK	AAS	Increase of sensitivity	67
Dialysis	Zn	Aqueous			Volt.	Theoretical	71
	Ca	Milk	Aqueous		P		72
	SO_4^{2-}	Urine	Aqueous	Reagent solution	P		73
	Cl^-, PO_4^{3-}	Serum	Acidic solution		P		69
	Glucose	Milk, waste-water, fermentation broth			P	New dialysis probe	74
	Glucose, urea	Urine, serum	Buffer	Basic medium	P, Pot	Enzyme reactor	70
	Galactose	Serum	Aqueous		P	Enzyme reactor	75
	Glucose	Serum	Sample	Sample	Chem.		76
	Glucose	Plasma			Chem.		77
	Galactose, lactose, dihydroxylactose	Urine, milk	Aqueous	Aqueous	Amp.	Enzyme reactor	81
	Glucose	Plasma	Phosphate buffer	Phosphate buffer	Volt.		78
	CO_2	Plasma	Aqueous acid	Base indicator	P		79
	Li	Serum	Aqueous	Borax solution	P	Ion-selective electrode	80
	Metal ions	Aqueous	Ligand solutions	Ligand solutions		Theoretical	82

* APDC = 1-pyrrolidinecarbodithioic acid; MIBK = methyl isobutyl ketone.
** For abbreviations, see Table II.

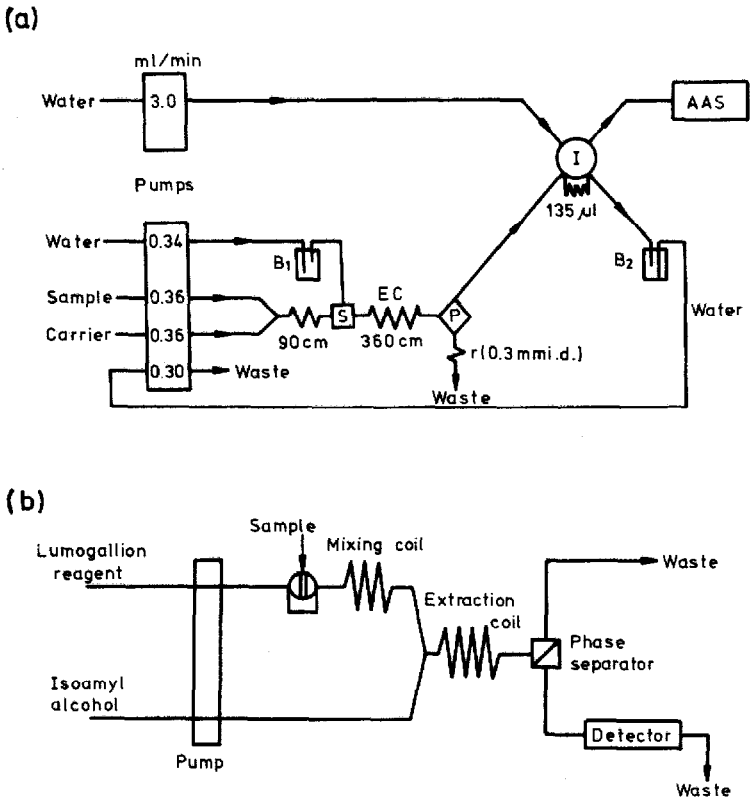

Fig. 2. General types of FIA–liquid–liquid extraction arrangements: (a) before the injection system (with displacement bottle); (b) after the injection system.

requires its prior oxidation to nitrate with Ce^{IV}[46]. A manifold such as that in Fig. 2b has been designed for Imasaka *et al.*[41] for the determination of galliun based on the formation of a fluorescent complex with lumogallion, this complex being extracted with isoamyl alcohol.

Combined extraction–FIA has also been applied to non-analytical aspects, such as the calculation of the extracted analyte fraction[47], the peak area[48] and height[47] as a function of the flow-rate and other parameters characteristic of the chemical system such as acidity constants based on the use of a dual-membrane phase separator[49]. The contributions in this area are still not very numerous, although a greater development of these aspects aimed at the study of reaction mechanisms and kinetics with the aid of laser-induced excitation[41] or multi-extraction systems[36,37] and fast scan detectors[36] can be predicted (Table III)[50–68].

Dialysis

FIA–liquid–liquid separation by use of a suitable membrane has been almost exclusively applied in the area of clinical analysis. The purpose of this separation technique has usually been removal of interferences (only once has it been used as a dilution method[69]). Table III lists the main contributions of combined dialysis–

FIA, among which the paper by Gorton and Ogren[70] is a representative example. They determined glucose and urea in serum using the corresponding immobilized enzymes. A schematic diagram of the configuration used for the determination of urea is shown in Fig. 3. The sample is injected into a donor buffer, which is driven towards waste once the analyte has passed through the membrane, from which it is led by the acceptor (phosphate buffer, pH 6) to the tubing zone containing the reactor packed with urease immobilized on controlled-pore glass. The detection system (ammonia-selective electrode) requires the use of a basic stream merging with the main channel after the enzymatic reactor to make the pH of sample plug adequate for the release of the monitored product. The theoretical aspects of these separation techniques in its association with unsegmented flow systems have been dealt with by Bernhardsson *et al.*[71] (Table III[72,82]).

SOLID–LIQUID INTERFACES

Separation techniques involving the presence of liquid and solid phases have been used in conjunction with FIA almost from the beginning of this technique (nearly exclusively ion exchange until very recently). More recent is the use of these phases involving adsorptive processes, precipitation as a separation and pre-concentration technique being the latest innovation in this area.

Ion exchange

Combined FIA–ion exchange has been preferentially devoted to the pre-concentration of minor species in complex samples (industrial, rain and sea waters, soldering smokes, biological fluids, etc.), although it has also been utilized as a separation technique and to facilitate the determination of different analytes in the same sample by then sequential elution after keeping them on a suitable active agent. Taking into account that the analytes most frequently determined are metal cations, it is obvious that the commonest active agents used are chelating resins of different types. Table IV shows the main species determined by methods involving the use of FIA–ion exchange, classified according to the type of analyte: cationic species (individual and mixtures), anionic species and conjugate acid–base forms. A representative example of the versatility of combined FIA–ion exchange and its easy adaptation to the resolution of various problems is the paper by Olsen *et al.*[83] on the determination of heavy metals in sea water by atomic-absorption spectrometry with their prior separation and concentration in a micro-column of chelating resin incorporated into three different FIA configurations of increasing complexity to overcome

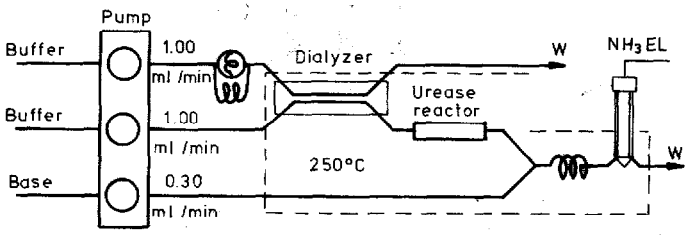

Fig. 3. Configuration for determination of glucose in serum with sample dialysis (from ref. 70).

TABLE IV

FEATURES OF THE FIA METHODS INVOLVING LIQUID–SOLID INTERFACES

Type	*Analyte*	*Matrix*	*Analytical purpose*	*Active agent*	*Detection**	*Special features*	*Ref.*
Ion exchange	NH_3	Rain water	Separation	Resin (Amberlite R-120)	P	Column in sample loop, alternating flow	85
	NH_3	River water	Separation	Amberlite IRA-400	F		86
	Ni		Pre-concentration	Chelating resin	AAS	Multi-functional valve	87
	Ca		Separation	Chelating resin	AAS		88
	Cu		Pre-concentration, separation	8-Quinolinol	Pot.		89
	Mn, Pb, Cu	Soldering smokes	Pre-concentration	Chelating resin (Dowex A-1)	AAS	Series of injection valves (sample–eluent)	90
	Zn, Cd		Pre-concentration	Chelating resin	P	Study of resin selectivity	91
	Zn, Cd		Simultaneous determination	Amberlite IRA-400	Chem.	Sequential elution	92
	Pb, Cd, Cu		Pre-concentration	Chelex-100	AAS	Integrated microconduits	84
	Ba, Be, Cd, Co, Cu, Mn, Ni, Pb		Pre-concentration	Chelex 100	ICP-AAS	Simultaneous determination	93
	Ni, Cu, Pb, Cd	Tap, sea, polluted water	Pre-concentration	Chelating resin	AAS	Two alternating columns	94
	Cd, Pb, Cu, Zn	Sea water	Pre-concentration	Chelex-100	AAS	Series injection and selecting valves	83
	Cd, Pb, Cu, Zn	Sea water	Pre-concentration	Chelex 100 and 8-quinolinol	AAS	Series injection and selecting valves	95

	Pyrophosphate, orthophosphate		Simultaneous determination	TSK-gel SAX	P		96
	Anions		Simultaneous determination	Basic resin	P		97
	Br^-	Sea water, serum, chloride reagents	Separation	Amberlite XAD-2	P		98
	Acid–base forms		Salt interference substraction	Resin			99
	Acid–base forms		Determination		Cond.		100
	Polyphosphates		Separation	TXK-gel SAX	P	Hydrolytic catalysis	101
	Peroxides	Introduction of NH_3	Introduction of NH_3	Cation-exchange membrane	F	Enzyme membrane	102
Adsorptive pre-concentration	Cr^{III}	Urine	Separation	Activated alumina	ICP-AES		103
	Oxyanions		Pre-concentration separation	Activated alumina	ICP-AES		104
	Chlorpromazine	Urine	Pre-concentration	Carbon paste electrode	Volt.		105
	Doxorubicin		Pre-concentration	Carbon paste electrode	Volt.		106
	CN^-, Cl^-		Determination	Pt electrode	Amp.		107
Precipitation	NH_3, Cl^-, $C_2O_4^{2-}$		Theoretical aspects	Fe^{3+}, Ag^+, Ca^{2+}	AAS	With and without continuous dissolution of precipitate	108
	Cl^-	Waters	Determination	Ag^+	AAS	Normal and reversed FIA modes	109
	Cl^-, I^-	Foodstuffs	Simultaneous determination	Ag^+	AAS	Mixture resolution	110

* F = Fluorimetry; Cond. = conductimetry; ICP-AES = Inductively coupled plasma atomic emission spectrometry; others as in Table II.

the shortcomings successively encountered. The single-channel manifold used, featuring two series of injection valves located prior to the column (Fig. 4a), is the simplest alternative to implementing the pre-concentration step. The propelling system involves gas pressure. The carrier, ammonium acetate drives the sample injected by valve I_1 to the micro-column through a coil and the by-pass of valve I_2, where the analytes are retained. The second step is the injection of the eluent through valve I_2. The shortcomings of this configuration are the appearance of a pre-peak due to the sample matrix, disturbances arising from changes in the compactness of the resin in changing from NH_4^+ to H^+ and lack of homogenization between the sample and

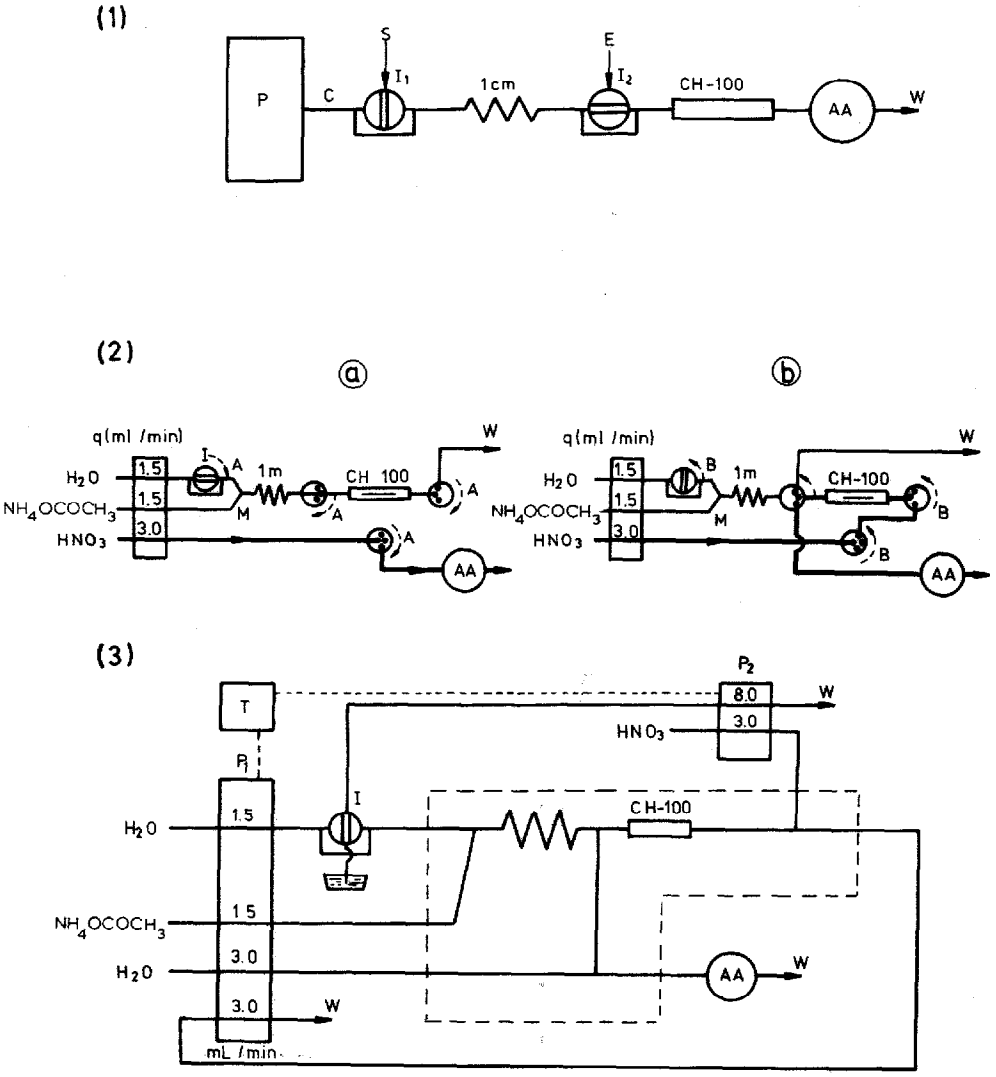

Fig. 4. FIA–ion exchange configuration of increasing complexity: (1) single-channel manifold; (2) with elution; (b) in the reverse direction of the retention (a); (3) automated manifold.

carrier in the central zone of the sample bolus, which is very acidic and hinders the retention step. These shortcomings are overcome by configuration (b), which uses a merging point for the ammonium acetate stream and elutes the analytes in the reverse direction of the retention by selecting valves whose purpose is illustrated in the figure. The automation of the method is carried out by a manifold with a single injection valve and a system with two pumps and a timer synchronizing the stop and start of the pumps (start of pump 1 during the pre-concentration process and that of pump 2 during the elution). The sample matrix does not reach the detector in either instance and the micro-column is regenerated in the elution step.

A new and spectacular contribution in this area is the combination of FIA and ion exchange by the use of integrated microconduits[84] (Table IV[85–102]).

Adsorptive pre-concentration

Two active agents have been used for the adsorptive pre-concentration of an analyte in FIA: activated alumina and electrode surfaces (carbon paste or platinum). Adsorption on alumina has been used for the pre-concentration of the Cr^{III} ion in biological samples (urine) in its determination by inductively coupled plasma–atomic emission spectroscopy[103], and for the pre-concentration of oxy anions (arsenate, borate, chromate, molybdate, phosphate, selenate, vanadate) with the same detection system[104]. Pre-concentration on a carbon paste electrode prior to the voltammetric determination of the analyte has been used for the analysis of drugs such as chlorpromazine[105] and doxorubicin[106] in urine samples. Finally, the pulsed amperometric determination of electro inactive adsorbates, such as chloride and cyanide at platinum electrodes[107], shows the potential association of FIA with this separation and pre-concentration technique.

Precipitation

This technique, widely used in classical analytical chemistry, has scarcely been automated owing to the intrinsic difficulties involved. Recently, our research team has established automatic precipitation methods in unsegmented continuous configurations incorporating a filter to develop indirect atomic absorption determinations. A schematic diagram of the two basic systems employed is shown in Fig. 5. The simplest alternative (Fig. 5A) is the injection of an anion (analyte) into a carrier of a precipitating cation (reagent). The precipitate formed is retained in the filter and a negative FIA peak is obtained. Another configuration involving the washing and dissolution of the precipitate formed has been employed to improve the analytical possibilities (Fig. 5B); a positive FIA peak is obtained in this instance. The performance of this continuous precipitation system has been tested with three types of precipitates: gelatinous [iron(III) hydroxide], curdy (silver chloride) and crystalline (calcium oxalate)[108]. The determination of chloride in different types of waters[109] and chloride–iodide mixtures in foodstuffs[110] has been satisfactorily carried out by this novel methodology.

HPLC–FIA COUPLING

The state of the art of on-line post-column reaction detectors in HPLC has recently been reviewed by Frei *et al.*[111]. One of the shortcomings of this configuration indicated in this interesting paper is the need for the post-column addition of re-

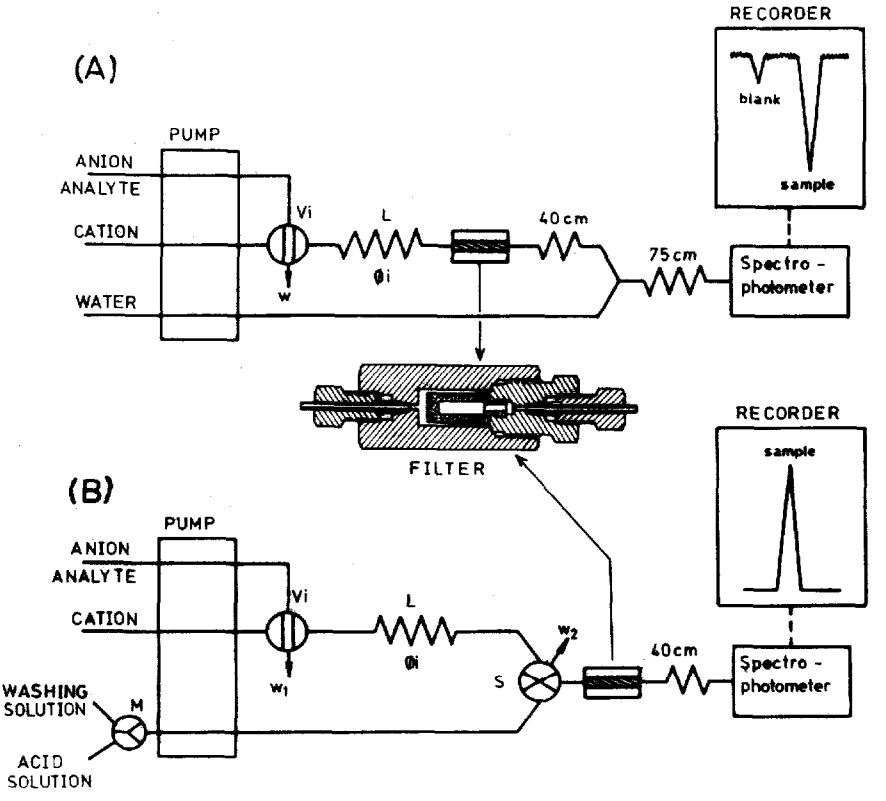

Fig. 5. (A) Precipitation system and (B) precipitation–redissolution in a non-segmented flow configuration.

agents. The incorporation of the reagent can be performed in three different manners: (a) through a constant stream of it, which is mixed with the effluent from the chromatographic column; (b) through its injection into a carrier mixing with the effluent; and (c) by means of solid-phase reactors into which the reagent, generally a catalyst, is immobilized. An additional pump is needed in the first two alternatives to establish the reagent or carrier flow. Strictly, only when there is a complementary post-column injection can an FIA system coupled to a liquid chromatograph be considered. There are other possibilities of post-column pumpless reaction units (electrochemical, photochemical, thermal reactors, etc.) in addition to solid-phase reactors.

The basic components of an HPLC–FIA coupled system are two injection valves, two pumps, a chromatographic column, a reactor, and a continuous detector, in addition to the reservoirs of the eluent(s), carrier(s) and reagent(s). There are two manners of implementing this association depending on the situation of the injection valve of the FIA subsystem (see Fig. 6): (A) when it is located before the confluence of the carrier with the chromatographic effluent and (B) when the valve is at the confluence point itself. Restrictor coils are frequently employed to prevent the formation of air bubbles. There are few analytical methodologies based on these two modes.

Several FIA systems in which the injection can be alternatively substituted by

a confluence with the chromatographic effluent have been described. The total concentration of analytes is determined by means of the FIA system injecting the sample through the FIA valve, whereas the discrimination between analytes (multiple determination) can be performed by incorporating the effluent in the post-column system which operates without an injection valve and thus acts as an open-tubular reaction detector. Inorganic polyphosphates[112], polyphosphoric acids in phosphorus smokes[113], phosphate and phosphonate (using two photometric detectors arranged in parallel[114] or in series[115] and the complexing abilities of ligands for metal ions[116,117] have been determined with this dual configuration. A real on-line HPLC–FIA coupling with two injection valves is only justified when there are specific problems involved. Such is the case with the determination of phosphinate, phosphonate and phosphate[118], in which a previous reagent ($NaHSO_3$) is needed to oxidize P^{I} and P^{III} to P^{V}, which yields the analytical reaction with the chromogenic re-

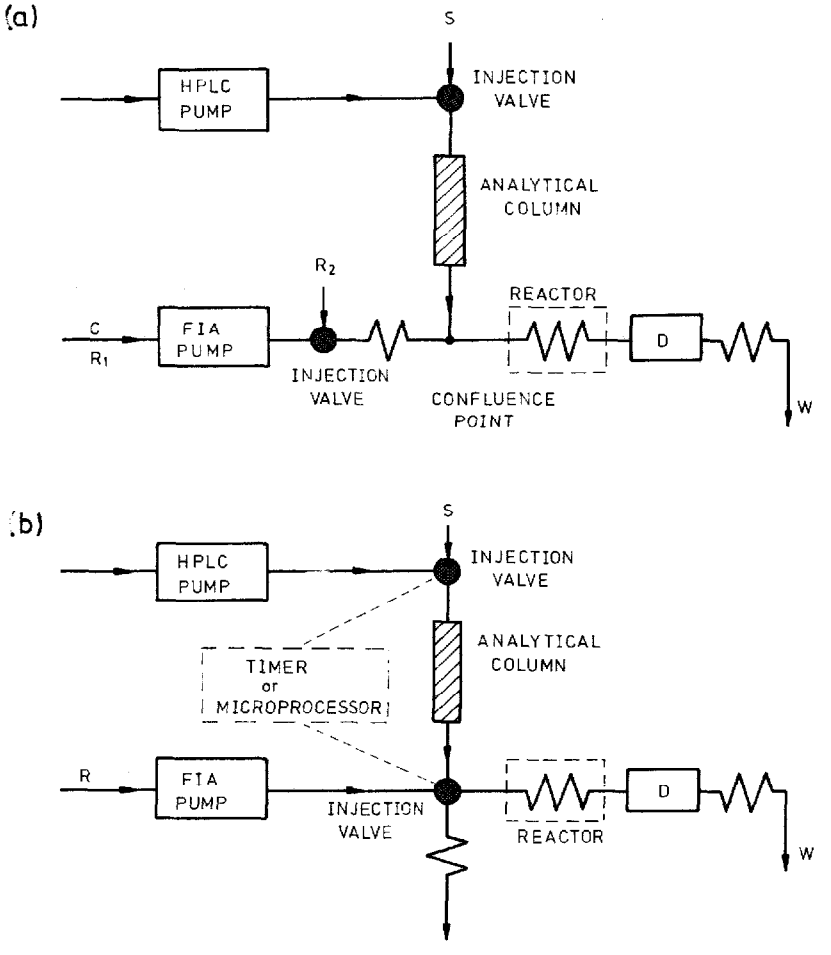

Fig. 6. General arrangement of HPLC–FIA combination: (A) with FIA injection prior to the confluence with the chromatographic eluate; (B) with injection of the chromatographic eluate. C = carrier; R = reagent; S = sample; D = detector; W = waste.

agent (Mo^{VI}–Mo^{V}). As the sulphite solution tends to corrode stainless steel and to disturb the flow-rate of the reciprocating pump, it is introduced with a loop-valve injector to avoid contact of this reagent with the pump.

The use of two valves in a on-line HPLC–FIA coupled system has other alternatives, as stated before. The chromatographic effluent fills the loop of a sampling valve, which injects samples of it into the reagent stream (Fig. 6B) at regular intervals. The automatic synchronous operation of the two valves is mandatory in this instance. Mixtures of reducing sugars with photometric detection[119] and of amino acids with amperometric detection[120] have been resolved by means of this configuration.

CONCLUSION

Liquid–liquid extraction is by far the most frequently employed separation technique in FIA systems, as can be seen in Fig. 7. About 20% of the papers published in this context deal with the use of ion-exchange mini-columns. The importances of gas diffusion and dialysis are similar to each other but less than that of the previous technique. Separation through membranes of molecules (dialysis), gases (gass diffusion) and immiscible liquids (extraction) is the foundation of over 60% of the continuous separation processes developed in FIA configurations.

In addition to the use of solid–solid reactive interfaces to develop certain analytical methodologies, as stated in the Introduction, the main purpose of the incorporation of separation techniques in unsegmented flow systems is related to the improvement of sensitivity (pre-concentration), selectivity (sample clean-up, multiple determinations) and, in some instances, to facilitate the analytical reaction and/or detection, which would be impractical without a prior separation technique. Another advantage over conventional batch non-chromatographic separation techniques is the higher sampling rate achieved, which is of great relevance in routine determinations.

It is interesting to note the decisive role played by kinetics in these continuous separation processes. In general, physico-chemical equilibrium has not been attained by the time the detection of the sample zone takes place, in contrast to the corresponding batch and air-segmented continuous flow methods. On the one hand, one

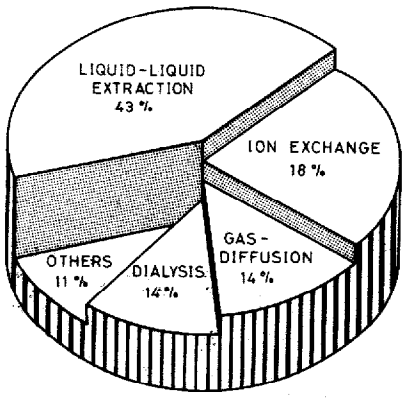

Fig. 7. Percentage distribution of the continuous separation techniques associated with FIA systems.

can assume that this should result in a decreased precision, but, in fact, the relative standard deviations of both alternatives (non-kinetic–batch and air-segmented and kinetic–FIA) are virtually identical. On the other hand, a less evident but most favourable phenomenon occurs: kinetic discrimination, which increases the selectivity level when the process is performed in a continuous fashion[121]. Nevertheless, the sensitivity is generally lower, unless it is designed as a pre-concentration procedure.

Despite the advantages, comparatively few FIA systems with continuous separation have been used so far, which can be attributed to the occurrence of a large number of experimental factors that influence these dynamic systems, which is an initial "barrier" to their development. Nevertheless, it suffices to test any of the above-described configurations to become immediately aware of the few technical and instrumental difficulties involved. It is logical to predict an increase in the number of papers and applications in this context in the next few years, particularly in the field of atomic spectroscopic techniques[122].

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