

Chapter 1

Molecular Spectroscopy Detection

1. Visible Absorption Spectrophotometry

The absorption of visible and ultraviolet radiation by different chemical compounds results generally from excitation of bonding electrons in the absorbing molecule. In the visible region two main types of electronic transition take place, involving d and f electrons in the case of most transition-metal ions and charge-transfer electrons in the case of complex compounds, where one of the components has electron-donor and the other electron-acceptor properties. The product of radiation excitation, which corresponds to absorption of part of the radiation energy, has a very short life-time and the most common relaxation of the excited particle involves conversion of the excitation energy to heat. The amount of thermal energy produced is usually not detectable. Attenuation of a beam of radiation by an absorbing solution is expressed by transmittance T , which is defined as the ratio of the power of the beam of radiation observed after passing the absorbing solution to the initial power of the beam. The absorbance A of a solution is related to transmittance as follows:

$$A = \log T. \quad (1)$$

A fundamental relationship utilised for analytical purposes in molecular spectroscopy detection is the dependence of absorbance on the cell length b and concentration of an absorbing species c expressed by the Lambert–Beer law:

$$A = abc, \quad (2)$$

where a is a constant called the molecular absorptivity for b expressed in centimetres and c in moles per litre. The large number of known reactions producing species absorbing visible radiation, mainly with the use of organic reagents [1], is a source of very wide application of visible absorption spectroscopy in chemical analysis [2, 3]. It is commonly used in all areas of routine

laboratory chemical analysis, field and clinical tests, in portable instrumentation and in process analysis.

Visible absorption spectrophotometry was already applied in pioneering works on flow injection analysis [4, 5]. Through all the twenty years of development of FIA, spectrophotometry has been and currently is the most common detection used in FIA. Spectrophotometric detectors are principal detectors of each commercial FIA instrumentation.

1.1. Detectors

Each detector used in FIA systems should be designed to monitor as closely as possible the events occurring in the measuring system. In photometric detectors it is facilitated by the smallest possible dead volume and the illuminated volume of the flow cell. A large volume causes poor reproducibility of height and shape of the flow injection peak, whereas a large illuminated volume results in a decrease of detection sensitivity and broadening of peaks [6]. The most often used commercial detectors are flow cuvettes with a geometry that fits conventional spectrophotometers (Fig. 1A), usually with path length 10 mm and a volume of a few to 50–60 μl . In comparative studies it was shown that unfavourable effect of an increase of cuvette volume above 25 μl is especially significant at low flow rates [7]. The optimisation of detector geometry can be made numerically by evaluation of impulse response functions for the FIA system, which show the contribution of the detector to the peak broadening [8]. The application of a capillary flow cell that utilises optical fibres to transmit light with the small illuminated volume ($< 1 \mu\text{l}$) allows one to extend the dynamic range of response and to use it in extraction systems without phase separation [6]. A design of a flow through nanocolorimeter with a cuvette working volume of 115 nl and a light path of 0.5 cm was reported [9]. FIA measurements at path length 0.1 cm were carried out with a crossed-beam thermal lens photometer, which is based on the utilisation of a single laser [10].

Flow-through photometric detectors for FIA can be made using optoelectronic components such as light-emitting diodes (LEDs) as light sources and photodiodes or phototransistors as detectors [11–29]. The commercially available LEDs cover a wide range of wavelengths, from 435 to 1300 nm [12]. The radiation from LEDs has a spectral bandwidth of about 20–70 nm, which is sufficient to substitute for the commonly-used-in-spectrophotometers combination of broadband sources and monochromators. LEDs are stable, inexpensive

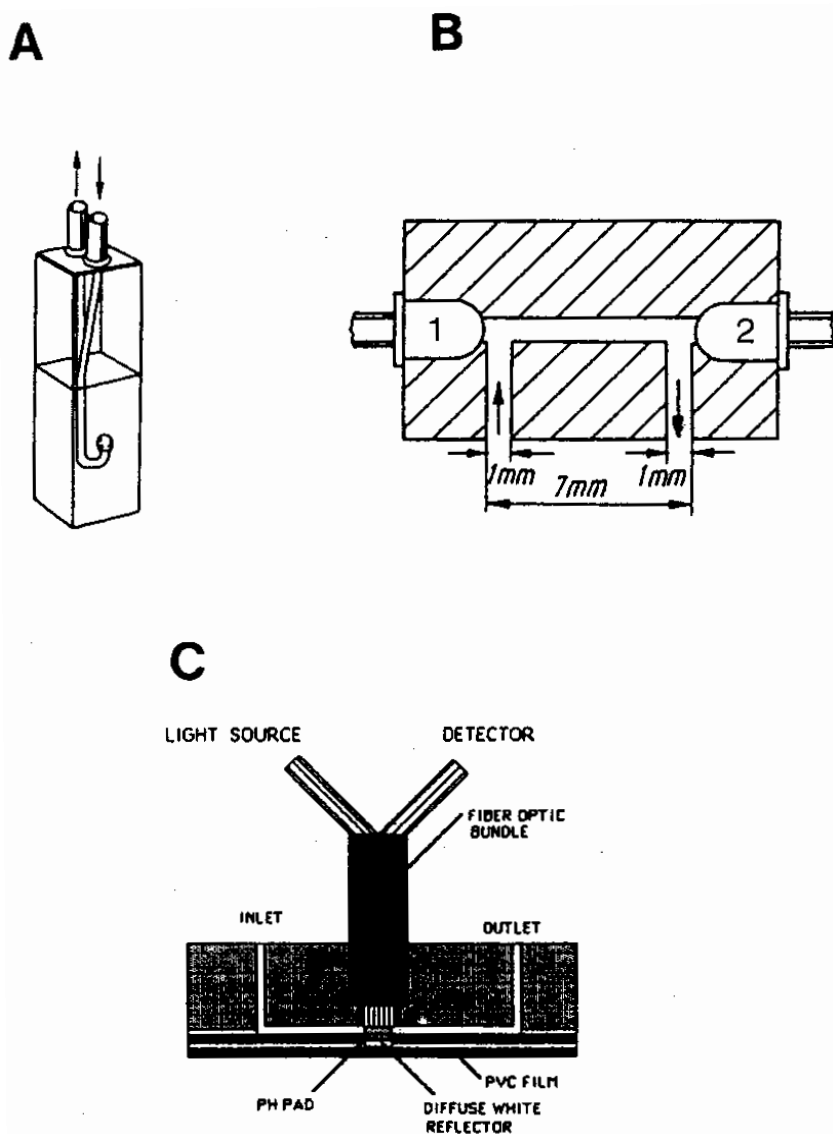


Fig. 1. Flow-through cells used in FIA with spectrophotometric detection: (A) commercial flow-through cuvette for conventional spectrophotometers; (B) flow-through cell with light-emitting diode (1) and phototransistor (2) [13]; (C) flow-through cell with bifurcate optical fibre for the light source and detector and fibrous indicator pad [42]. (Reprinted by permission of copyright owner.)

and easy-to-use light sources and can be easily employed in various designs of flow through detectors. A limited number of available wavelengths when using LEDs means that it rarely fits exactly the maximum of absorption of the measured chromophore. This results in some decrease of the sensitivity of detection. For a 20 nm difference between the maximum of LED emission and the maximum of chromophore absorption a 20% decrease of sensitivity was observed, whereas for a difference of about 50 nm the loss of sensitivity grows up to 40–70% [11]. The application in absorption measurements of laser diodes, which are much more expensive and exhibit the half bandwidth 1–10 nm, does not offer any significant advantage over LEDs [12]. As detectors of transmitted radiation both photodiodes and phototransistors can be used. The latter usually provide 1–2 orders of magnitude greater current output but their response is slower and usually they are more noisy than photodiodes. The simplest construction of an LED-based flow through detector is shown in Fig. 1B. Several other designs, including cells built within the body of an LED [12], were developed. Some applications were also reported for multi-LED cells. The eight LEDs and photodetectors arranged in series allow the observation of the peak formed in the FIA system and analysis of the two-component mixture with different kinetics of colour species formed [17]. An integrated multidiode light source was employed for flow injection spectrophotometry of two- and three-component mixtures [22, 24]. A detector with three LEDs in series was applied in doublet peak measurements in FIA [28]. The compensation of refractive index and turbidity effects were obtained in an LED-based dual-wavelength, double-beam, dual-flow-cell photometric detection system. Detection through the use of fibre optics coupled to the LED photodiode system was employed also in a fabrication of a micro FIA system based on glass substrates by lithographic techniques and etching methodology [29a]. Mobility of reagents and analytes was achieved by exploiting electrokinetic mobility or electro-osmotic flow. The total volume of reactants used was 0.5 μl .

Using a sufficiently strong source of radiation, the absorption measurements can also be carried out in flow cells filled with solid or gel sorbent, which integrates detection and preconcentration steps in FIA [30]. This technique, called *ion-exchange absorptiometry* [31], was successfully employed in FIA [32–37]. Although theoretical considerations indicate most favourable application for this purpose of thin-layer packed cells with a thickness of sorbent up to 0.5 mm [37], a commercially available flow cuvette such as that shown in Fig. 1A was successfully used for such measurements [32–35]. This methodology was employed for determination of various analytes with coloured reaction

products retained on ion-exchangers [32–34], a hydroxypropyl derivative of a Sephadex dextran gel [35] and hydrophobic C18 sorbent [36, 37]. Such determinations were also performed by using a chromogenic ligand immobilised on a cation-exchange resin placed in the flow cell [38]. Transmittance spectrophotometry using reactions taking place at the surface of a filter paper on which a layer of dried reagent mixture had been deposited was also utilised for reactions occurring at a gas-solid interface in the FIA system for determination of bromine and chlorine in the gas phase [39].

In the case of using solid supports in the optical path, mostly for immobilisation of the chromogenic reagent, the reflectance measurement is also employed instead of absorbance measurement. The light is introduced into the flow cell through bifurcated optical fibres. This approach was applied for either the indicator dye reagents immobilised on a cross-linked styrene-divinylbenzene polymer matrix [40], or commercial indicator strips situated in the flow stream at the tip of the optical fibre (Fig. 3C) [41–44]. In reflectance measurements the reflected radiation is a much more complex function of concentration than for Lambert–Beer’s law in absorptive measurements. Only for transport layers on a white opaque background may a reflection vs. concentration dependence resemble a linear relationship, but usually it is affected by radiation scattering, the nature of the reflecting medium, the geometry of illumination and the radiation collection. Such a detection with the immobilised commercial pH indicators was used for sequential determination of both acids and bases [43] and pH of rainwater [44].

Besides various ways of chemistry improvements and optimisation of hydrodynamic parameters of the FIA system, a further improvement in flow injection photometry can be achieved by the use of differential detection with two similar detectors arranged in a series, and separated with a transfer line of suitable length. It was shown that the optimum response is obtained when the dispersed sample volume is approximately equal to the volume of the transfer line between cells [45].

One of the difficulties encountered in some cases in flow injection photometry is interference due to changes of the refractive index of solutions transported through the detector, which causes deformations of the signal, the noisy response resulting in sensitivity and reproducibility deterioration. It is particularly pronounced for a large difference in concentrations between the carrier solution and the sample and in single-line FIA systems with limited dispersion. Instrumentally this effect can be eliminated by carrying measurements at two

wavelengths: one at which the absorbance change is observed due to chemical reaction and another which reflects no influence of colour-forming reaction, but permits observation of the refractive index. As was mentioned above, such measurements can be made with LED detectors [27], with diode-array spectrophotometers [46, 47], or with a dedicated flow cell with optical fibre joints and different filters placed at the end of a multimode coupler [48]. The easiest way to overcome this problem is to use a large volume of the injected sample [49, 50].

1.2. *Measuring Procedures*

The most common configuration of the measuring system in flow injection photometry is the two-line manifold, where the sample is injected into the carrier stream of distilled water, buffer or a chemically inert solution with a similar matrix composition as sample, the reagents then being added by confluence [51]. For a large sample volume such a system is not interfered with by changes of the refractive index differences, but the quality of the pump, the confluence point and the method of downstream mixing are of crucial importance [49]. The insertion into both lines of the air pulse dampers and/or packed bed reactors significantly reduces the amplitude of the baseline noise. In the comparison of different manifolds for the determination of Fe(II) with 1,10-phenanthroline, the double line configuration gave the lowest detection limit [52]. Using the injection valve with the possibility of simultaneous injection of two solutions to different streams, a merging technique of FIA measurement can be employed, which is most often used to reduce the consumption of reagents [53, 54].

Reversed FIA systems with constant aspiration of the sample yield better detectability [52, 55, 56], although there are also opposite observations [57]. Such a procedure is advantageous when the volume of the injected sample is not critical, but rather the consumption of reagents [58, 59]. It can be successfully applied in multicomponent determinations, where to the same sample different reagents can be injected for the determination of different analytes (Fig. 2) [60].

The reagents needed to form a coloured product with analyte, used for sample pretreatment or elimination of interferences, are mostly used in soluble form in the continuously pumped solutions or are injected in reversed FIA systems. Several different ways have also been proposed. The determination of sulphur(IV) based on reaction with formaldehyde and pararosaniline requires one to use a three-line manifold. Utilisation of a passive cation-exchange membrane

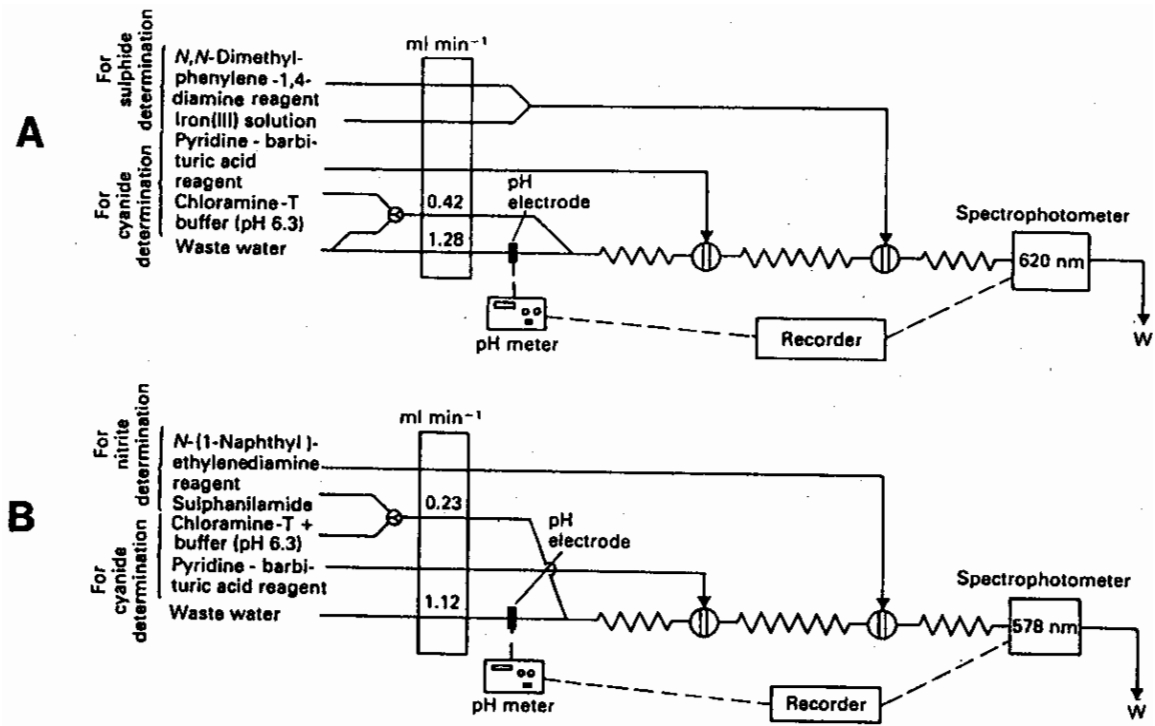


Fig. 2. Reversed flow injection manifolds used for the simultaneous determination of pollutants in waste waters [60]: (A) determination of pH, sulphide and cyanide; (B) determination of pH, nitrite and cyanide. (Reprinted by permission of copyright owner.)

reactor for the introduction of base and a pressurised porous membrane reactor for the introduction of acidic pararosaniline allows one to employ a single flow channel FIA procedure [63]. Similarly, membrane tubings were used for the introduction of acid, molybdate and hydrazine for the determination of phosphate in waters, which allows one to eliminate the need for confluence points in the design of a flow injection manifold [64]. Porous PTFE was used to introduce PAR in the determination of copper [65], whereas a silicon rubber membrane was employed for the introduction of bromine gas in the indirect determination of phenols [66].

Another way of dispersionless introduction of reagents in FIA is application of solid-phase reactors with the use of insoluble or immobilised reagents. Flow injection spectrophotometric procedures were reported for the determination of chloride and bromide using solid mercury(II) thiocyanate and silver thiocyanate minicolumn, respectively [67]. Cerium(IV) arsenate was used as a strongly oxidising solid-phase reactor for the determination of promethazine by monitoring the red product of the oxidised drug [68].

A long list of the developed flow injection spectrophotometric procedures is shown in Table 1. It does not contain catalytic and multicomponent procedures, and also determinations of gaseous analytes, which are discussed below and presented in separate tables.

Table 1. Applications of spectrophotometric detection in the visible region in FIA.

Analyte	Reagent	λ , nm	Concentration range	Reference
Acetoacetate	Hydroxylamine, methyl orange	520	0.1–5 mM	133
Acids, bases	Colorphast pH indicator	640	0.001–1 M	43
Ag(I)	Dithizone	500	5.4–32 ppm	134
Al(III)	Chrome azurol S	546	0.015–0.7 ppm	135
		545	0.06–30 μ M	137
	Chrome azurol S, cetylpyridinium chloride	625	5–400 ppb	136
	Erichrome cyanine	535	1–15 ppm	138
			0.06–0.8 ppm	175
	Pyrocatechol violet	581	3–300 ppb	139
		583	0.045–1.0 ppm	23
	Xylenol orange	506	4.5–7.0 ppm	140

continued

Table 1 (continued)

Analyte	Reagent	λ , nm	Concentration range	Reference
Amines, aromatic	4-N-methylaminophenol, dichromate	530	0.05–20 ppm (as N-NH ₂)	141
Arsenite	Ammonium molybdate, SnCl ₂	690	5–500 ppb	142
B(III)	Azomethine-H	420	0.1–6.0 ppm 1–200 ppb*	89, 143, 250 144
Be(II)	Xylenol orange	493	1.9–4.0 ppm	145
Bi(III)	Pyrocatechol violet	590	30–100 μ M	14
	Lead tetramethyl- enedithiocarbamate	380	0.1–5.0 ppm	146
Bromide	Chloramine T, phenol red	590	0.09–100 ppm	109, 147, 148
	AgSCN, Fe(III)	465	0.01–1.2 mM	67
	KMnO ₄ , N,N-diethyl- <i>p</i> - phenylenediamine	550	0.3–20 μ M	149
Bromine (g)	α -naphthoflavone, As(III)	520	0.5–100 ppm	39
Ca(II)	<i>o</i> -Cresolphthalein	580	5–144 ppm	103, 150
		570	10–250 ppm	154
	Glyoxal- <i>bis</i> (2-hydroxyanil)	520	3–500 ppm	54
	Methylthymol blue	630	40–160 ppm	151
	Zn-EGTA, PAR	505	0.8–7.2 ppm	142
	Dicyclohexano-24-crown-8, propyl orange	420	0.2–100 μ M	153
Cd(II)	Dithizone	520	50–500 ppb	155
	Iodide, malachite green	690	2–200 ppb	156
	1-(2'-pyridylazo)naphthol	560	0.085–2.0 ppm	25
Chemical oxygen demand	Dichromate	445	5–2500 ppm 1.5–100 ppm	78, 79 80
Chlorate	Iodide	370	0.083–0.83	180
Chlorate, chlorite	Iodide	360	0.1–8.3 ppm ClO ₃ ⁻ 0.1–10 ppm ClO ₂ ⁻	166, 167
Chloride	Hg(SCN) ₂ , Fe(III)	480	7–500 ppm 0.2–15 ppm 50–800 ppm	103, 157 158 159
			0.04–1000 ppb	49
	Fe(II), Hg-tripyridyl-s-triazine	600	0.01–10 ppm	160
Chlorine	α -naphthoflavone, As(III)	520	1–100 ppm	39
	Methyl orange	510	1.4–38 ppm	161

continued

Table 1 (*continued*)

Analyte	Reagent	λ , nm	Concentration range	Reference
	<i>o</i> -tolidyne	438	0.08–18 ppm	161
	N,N-diethyl- <i>p</i> -phenylene diamine	515	0.3–14 ppm	162
	4,4'-tetramethyldiamin- othiobenzophenone	640	0.2–1.0 ppm	163
	4-nitrophenylhydrazine, N-(1- naphthyl)ethylene diamine	532	0.03–40 ppm	164
	3,3'-dimethylnaphtidine	535	0.03–1.0 ppm	165
	Iodide, N,N-diethyl- <i>p</i> -phenylene diamine	550	0.5–20 mM	149
Co(II)	4-(2-pyridylazo)resorcinol	565	0.6–500 ppb	13
	Nitroso-R-salt	516	0.5–50 ppm	168
		520	3.5–6000 ppb*	169
CO ₂	Cresol red		440–1760	170
Cr(VI)	1,5-diphenylcarbazine	540	0.1–20 ppm	103, 171
			0.015–4.0 ppm	172
			0.3–2000 ppb	45
			3–700 ppb	75
			10–1000 ppb	73
Creatinine	Picrate	515	3–200 ppm	173
CS ₂ (g)	Diethylamine, Cu(II)	421.5	3–30 ppm	126
Cu(II)	Aquaion	805	0.8–2.4 g/l	174
		810	0.6–30 ppb*	32
	4-(2-pyridylazo)resorcinol	565	3–10 ppb	13
	Bathocuproine	485	1–3700 ppb	45
	Cuprizone	595	0.3–4.0 ppm	175
	CS ₂ , diethanolamine	385	0.1–20 ppm	176
	Lead diethyldithiocarbamate	436	0.04–2.0 ppm	177
	Tetramethylenedithiocarbamate	435	0.12–12 ppm	178
Cyanide	Chloramine T, pyridine,	578	0.3–5.0 ppm	60
	barbituric acid	494	0.02–4.0 ppm	108
	Isonicotinic acid, pyrazolone	548	6–1000 ppb	179
Dissolved	Peroxodisulphate,	552	0.1–2.0 ppm C	86
Organic Carbon	phenolphthalein			
Ethylenedi- amine	Cu(I), pyridine-2-carbaldehyde	475	1.4–84.6 ppm	181

continued

Table 1 (continued)

Analyte	Reagent	λ , nm	Concentration range	Reference	
Fe(II)	1,10-phenanthroline	510	0.1–30 ppm	182	
			0.7–710 ppb	45	
			0.05–2.0 ppm	183	
			0.01–2.0 ppm	52	
	Bathophenanthroline	535	0.1–3.0 ppm	175	
	3-pyridyl-3'-sulphophenylmethanone	580	10–210 ppb	184	
	2-pyrimidylhydrazone				
2-nitroso-5(N-propyl-N-sulphopropylamino)phenol	2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol	753	1–100 ppb	185	
			582	1–100 ppb	50
Fe(III)	Thiocyanate	480	10–400 ppb*	34	
			22–56 ppm	186	
			0.2–10 ppm	187	
Fluoride	La(III), Alizarin complexone	620	0.03–1.2 ppm	188, 189	
			574	0.05–1.2 ppm	59
	Zr, Alizarin red S	520	0.1–10 ppm	83	
Formaldehyde	5,5'-dithiobis(2-nitrobenzoic acid), sulphite	437	10–600 ppm	190	
Gd(III)	1-(2-pyridylazo)-2-naphthol	560	0.9–8.8 ppm	191	
Haloamines	Iodide, starch	590	1–60 ppm	192	
HCl	Bromocresol green	444	0.10–0.16 M	70	
HCN (g)	Chloramine T, isonicotinic acid, 3-methyl-1-phenyl-2-pyrazolin-5-one	630	0.05–65 ppmv	127	
H ₂ O	Karl Fischer reagent	525, 546	0.01–0.2%	193	
H ₂ SO ₄	Bromophenol blue	585	20–900 g/l	106	
Iodide	KMnO ₄ , N,N-diethyl- <i>p</i> -phenylene-diamine	550	0.2–10 μ M	149	
Isoprenaline	Hexacynaoferrate(III)	585	5–50 ppm	94	
Li(I)	14-crown-4-dinitrophenol	420	0.4–2.0 mM	194	
Mg(II)	1-(2-Hydroxy-3-sulpho-5-chloro-1-phenylazo)-2-naphthol-3,6-disulphonic acid	510	0.2–2.4 ppm	195	
			Calmagite	530	24–120 ppm

continued

Table 1 (*continued*)

Analyte	Reagent	λ , nm	Concentration range	Reference
Mn(II)	Formaldehyde	455	0.1–2.0 ppm	197, 198
Mo(VI)	SnCl ₂ , Fe(III), thiocyanate	470	0.05–1.0 ppm 100–600 ppm	199 88
Nd(III)	1-(2-pyridylazo)-2-naphthol	560	0.03–21 ppm	200
NH ₃	Nessler reagent	660	1–200 ppb* 0.2–1.5 ppm	201 183
	Hypochlorite, phenol, nitroprusside	620	0.05–0.5 ppm	202
		695	0.005–1.0 ppm	75
		585	0.1–1.6 ppm	183
		620	0.005–6.0 ppm	96
	Hypochlorite, salicylate, nitroprusside	620	0.03–10 ppm	203
	Phenol red	540	0.085–3.4 ppm	204
	Bromothymol blue	635	0.017–5.0 ppm	21
NH ₃ (g)	Bromothymol blue	520	0.04–1.0 ppm	130
N ₂ H ₄	4-dimethylaminobenzaldehyde	460	0.02–0.3 ppm	183, 205
Ni(II)	Aquoion	410	3.6 g/l	174
	Dimethylglyoxime	445	10–70 ppm*	206
		460	2.5–30 ppm	248
Nitrate	Hydrazine, sulphanilamide, N-(1-naphthyl)ethylene diamine	520	1–10 ppm	207
	Cd, sulphanilamide, N-(1-naphthyl)-ethylene diamine	565	0.1–10 μ M	16
		520	0.4–9 ppm	208
		565	0.1–50 ppm	20
		540	0.7–100 μ M	209
		540, 630	2–440 μ M	48
	UV reduction, sulphanilamide, N-(1-naphthyl)ethylene diamine	540	0.03–100 μ M	210
	Cd, <i>p</i> -aminoacetophenone, <i>m</i> -phenylenediamine	456	0.007–2.9 ppm	211
Nitrite	Sulphanilamide, N-(1-naphthyl)ethylene diamine	565	0.1–10 μ M	16
		535	0.08–0.8 ppm	53
		540	0.1–1.5 ppm	60
	Sulphanilamide, 4-N-methylaminophenol, dichromate	530	3–30 ppm	141
NO ₂ (g)	Sulphanilamide, 4-(1-naphthyl)ethylene diamine	540	50–250 μ g/m ³	131

continued

Table 1 (continued)

Analyte	Reagent	λ , nm	Concentration range	Reference
Ozone	Indigo blue	600	0.03–4.0 ppm	212
	Bis(terpyridine) iron(II)	552	0.06–1.35 ppm	213
Pb(II)	Dithizone	520	0.05–8.5 ppm	214
	Dicyclohexyl-18-crown-6, dithizone 4-(2-pyridylazo) rezorcinol	512	5–200 ppb*	85
Perchlorate	Brilliant green	640	0.036–2.5 ppm	215, 216
Permanganate	Ethylenebis(triphenyl- phosphonium) bromide	545	0.58–25 ppm	215
pH	Merck ColorpHast indicators	580	2.5–7.0 pH	42, 44
	Phenol red	433, 558	8.0–8.2 pH	98
Phenol	4-aminoantipyrine, $[\text{Fe}(\text{CN})_6]^{3-}$	510	0.1–5.0 ppm	217
	4-aminoantipyrine, peroxodisulphate	515	0.005–15 ppm*	218
	3-methyl-2-benzothiazoline hydra- zone, Ce(IV)	470	0.012–15 ppm	219
	<i>p</i> -nitroaniline, nitrite	475	0.03–3.4 ppm	220
Phosphate	Heptamolybdate	450	30–200 ppm	70
	Heptamolybdate, ascorbic acid	660	7.5–75 ppm	4
			0.02–8.0 ppm	19, 75
	Heptamolybdate, Sb(III), ascorbic acid	885	0.05–4.0 μM	55
		660	6–200 ppb*	36
	Heptamolybdate, SnCl_2	670	0.03–2.5 ppm	95
	Heptamolybdate, pyrosulphite	630	95–380 ppb	196
	Heptamolybdate, hydrazine	818	0.012–1.0 ppm	64
Phosphorus	Heptamolybdate, malachite green	627, 750	0.06–30 ppb*	35
	Peroxodisulphate, heptamolybdate, malachite green	650	2–500 ppb	222
Polyphenols	Folin-Ciocalten reagent	750	100–900 ppm	93, 223
Promethazine	Ce(IV)	514	5–400 ppm	68
Propoxur	<i>p</i> -aminophenol, periodate	600	0.12–25 ppm	224
Resorcinol	<i>p</i> -aminophenol, periodate	540	0.016–8 ppm	225
Silicate	Heptamolybdate, oxalic acid, ascorbic acid	886	0.5–100 μM	226
	Heptamolybdate, oxalic acid, SnCl_2	695	10–100 ppb	183

continued

Table 1 (continued)

Analyte	Reagent	λ , nm	Concentration range	Reference	
	Heptamolybdate, oxalic acid, 1-amino-2-naphthol-4-sulphonic acid	820	2–100 ppb	183	
Sn(IV)	Pyrocatechol violet	576	0.3–40 ppb*	227	
SO ₂ (g)	di- μ -hydroxo-bis[bis(1,10-phenanthroline)]	510	0.5–15 ppm (v/v)	230	
	Pararosaniline, formaldehyde	580	25–500 mg/m ³	128	
	Fe(III); 1,10-phenanthroline	508	0.5 μ g/l*	132	
Sulphate	Methylthymol blue, Ba	608	0.1–6.0 ppm	81	
	Dimethylsulphonazo III, Ba	662	1–30 ppm	82	
			0.2–14 ppm	228	
Sulphide	N,N-dimethylaniline, Fe(III)	662	1–45 ppm	229	
	N,N-dimethylphenylene-1,4-diamine, Fe(III)	662	0.5–90 ppm	60	
Sulphite	Pararosaniline, formaldehyde	580	2–200 ppm	232, 233	
			0.00016–3 mM	63	
			568 0.03–70 ppm	234	
		5,5'-dithiobis(2-nitrobenzoic acid)	437	8–300 ppm	190
		Disulphide 5,5'-dithiobis(2-nitrobenzoic acid)	412	0.5–20 ppm	58
	p-aminobenzene, formaldehyde	520	0.2–300 ppm	235	
Surfactants, anionic	Methylene blue	660	4–360 ppm	236	
			0.04–3.5 ppm	237	
			652 0.08–10 ppm	238	
		Ethyl violet	610	0.01–1.0 ppm	239
		Bromocresol purple	588	1–15 μ M	84
		4-(4-dimethylaminophenylazo)-2-methylquinone	560	2–80 μ M	240
		Orange II	490	45–1340 ppm	241
Surfactants, cationic	Bromocresol purple	588	10–50 μ M	84	
Surfactants, nonionic	Tetrabromophenolphthalein ethyl ester potassium salt	609	2–60 ppm	242	
Terbutaline	4-aminoantipyrine, hexacyanoferrate(III)	550	12–150 ppm	243	

continued

Table 1 (continued)

Analyte	Reagent	λ , nm	Concentration range	Reference
Ti(IV)	H ₂ O ₂	410	9–1000 ppm	244
U(VI)	2-(5-bromo-2-pyridylazo)-5-di-ethyl-aminophenol, zephiramine	579	0.1–15 ppm	245
	2-(5-bromo-2-pyridylazo)-5-di-ethyl-aminophenol, fluoride	578	0.5–20 ppm	246
Zn(II)	Xylenol orange	568	26–64 ppm	247
	Zincon	620, 800	0.1–20 ppm	47

*with on-line preconcentration

Among numerous applications of flow injection photometry there are some very original procedures which deserve to be mentioned. Besides widely used reversed FIA, for which was used a mathematical treatment for the development of a conventional standard addition method [69], a sample-to-standard additions method was also reported [70]. The latter eliminates the need to obtain a calibration graph as is commonly practised in conventional FIA determinations. It was also shown that a pH gradient produced in the FIA system when the sample is injected of different pH than carrier solution can be exploited for multicomponent determination, if the colour-forming reagent used forms products with different analytes at different pH values [71].

In the analysis of real samples it is often necessary to adjust the analyte concentration to fall within the linear response of the detector. It can be performed by the use of an appropriate dilution system [72], or by designing a system where the sample is inserted between two reagent segments with different concentrations [73]. In such a system two simultaneous working ranges of concentration are achieved.

An especially large sampling rate can be obtained in the system, where the injected sample is placed between air segments [74]. This was applied in monosegmented continuous flow systems [75, 76]. Air bubbles were removed from the stream prior to the detector in the permeation cell with PTFE membrane [75], or resampling was employed with the removal of a fixed volume from the central part of the monosegmented sample and injection with a second injection valve into the detector line [76]. This approach was successfully

used in the FIA system with solvent extraction without phase separation [25]. A satisfactory limitation of dispersion was also demonstrated in the system where the sample is injected with an air plug positioned at the tailing portion of the sample [250]. The appropriate design of the injection device allows the measurement, where the air phase can be discarded without flowing through the detector, which was described as a *relocating detectors procedure* [251]. Such a concept was illustrated in determinations of aluminium and iron.

Operating with significantly smaller sample volumes, that in conventional FIA systems (100 nl) is possible in the system with electro-osmotic flow and spectrophotometric detection [77]. The advantage of such a system is the separation of the sample matrix, typically uncharged water molecules from charged analyte ions or a charged coloured product.

Almost all applications of flow injection photometry shown in Table 1 are based on measurement of the increase in absorbance of the developed chromophore, or of the analyte itself. There are, however, also methods based on indirect measurements based on reaction of the analyte with the coloured reagent resulting in a decrease in absorbance, proportional to the analyte concentration. This procedure is employed in the determination of chemical oxygen demand [78–80]. The decoloration of barium complexes with various dyes is used for the determination of sulphate in waters [81,82], the decrease in absorbance of the zirconium/alizarin red S complex is used for fluoride determination [83], and the decrease in absorbance of bromocresol purple is employed for the determination of ionic surfactants [84].

As indicated in Table 1, in numerous methods various ways of on-line sample pretreatment are used in order to improve detectability or selectivity of detection. Some of them are unique and worth being mentioned. The system preconcentration on minicolumn with chelating resin was combined with subsequent selective solvent extraction with the crown ether and following by reaction with dithizone was developed for trace determination of lead [85]. The combination of the on-line UV photo-oxidation with gas diffusion was employed for the determination of dissolved organic carbon [86]. In the FIA system with an electro-osmosis-based fluid propulsion the on-line preconcentration based on the electrostacking effect as used in capillary electrophoresis was reported [87].

Several interesting solutions were developed for adaptation of FIA to the analysis of solid samples. Electrolytic dissolution of steels in a simple electrolytic chamber connected to the FIA system was used for the determination of molybdenum [88]. The determination of total boron in soils was based on

the direct introduction of solid samples into the on-line cell with an ultrasonic probe [89]. The determinations of free sulphur dioxide in wine and dried apple samples can be carried out in the setup, where a gas extraction device to generate and purge gaseous SO₂ was combined with a flow reversal manifold to directly process the gaseous plug generated from the sample [90]. Operations of weighting, dissolution or extraction of the analyte from solid samples or oils were carried out also in robotised FIA systems [91–93].

The flow injection measurement based on transient analytical signal depends on numerous chemical and physical parameters, which should be carefully optimised. Most often it is carried out by single variable optimisation of all essential hydrodynamic and chemical parameters. Among the multivariable methods of optimisation most commonly employed are various versions of the simplex method. The optimisation of the flow injection spectrophotometric method of determination of isoprenaline indicated the advantage of the modified simplex over the univariate procedures in simultaneous optimisation of four variables such as flow rate, tube length, reagent concentration, and pH, or additionally sample size [94]. The univariate method of optimisation involves keeping all but one variable constant and finding the best value of this variable. In the simplex method several variables are changed simultaneously for a search of the most suitable measuring conditions. The multivariable simplex method is especially effective when interactions between the variables can occur. For the optimisation of phosphate determination two procedures of the factorial design and simplex method were compared [95]. In the optimisation of FIA procedures, of great importance is the selection of an appropriate performance criterion. The procedure can be carried out to maximise the peak height or signal-to-noise ratio or the correlation coefficient of the calibration graph. Another criterion can be the peak width. In the optimisation of phosphate determination the response used was peak width/(peak height)² [95]. The optimisation of ammonia determination based on the indophenol blue reaction was carried out using the modified simplex method and the multivariate Powell method with a linear combination of the peak height and residence time as the experimental target function [96]. The Powell algorithm needed fewer evaluations of the objective function than did the modified simplex method, although it does not mean generally that this method of multivariate optimisation is more efficient in absolute terms than the simplex method. A useful way of visualising experimental variables on the performance of an FIA method is to construct a three-dimensional response surface as a plot of instrumental

responses vs. chemical concentrations of flow parameters. They can be obtained in an automated manner on a computer-controlled flow injection methods development system [97].

Measurements of the acidity of solutions are very common in laboratory, environmental and process analysis. With the use of colour indicators either single point pH determination or acid-base titrations can be carried out in FIA systems with spectrophotometric detection. The pH measurements in solutions of low buffering capacity and low ionic strength were realised using the reflectance spectroscopy of an immobilised pH indicator [42, 44]. A sea water pH was measured in the FIA system, where the acid-base adsorption properties of phenyl red injected into a sea water stream were measured [98]. Flow injection determinations of concentrated acids and bases were also done by the use of a buffered carrier stream containing a dissolved acid-base indicator [99, 100]. Within a limited range of linear relationship between peak height and concentration of the analyte was found, and also the simultaneous determination of acids and bases using the same carrier stream was demonstrated. A similar system was developed for a sequential determination of both dilute acids and bases using a single-line manifold with a reflectance cell with immobilised acid-base indicators.

In a single- or double-line manifold, usually with a larger dispersion than in common FIA systems with spectrophotometric detection, determinations corresponding to conventional acid-base titrations can be carried out with colour indicators. These procedures utilise the gradients of analyte concentration formed in the rising and descending parts of the signal of the injected sample and therefore they are called *gradient titrations*. In the single channel version, the titrant containing the indicator is used as the diluent in which the sample is injected. The quantitation is based on relating the time span (i.e. peak width) between the points of the same dispersion on the ascending and descending parts of the peak to the concentration of the analyte [101–103]. The relation between the time span (Δt), and the concentration of the analyte (C_A^0) and the concentration of the titrant carrier solution (C_B) is given by the equation [103]

$$\Delta t = (V_m/Q) \ln 10 \log(C_A^0/C_B) + (V_m/Q) \ln 10 \log(S/V_m n), \quad (3)$$

where S is the injected sample volume, V_m is the volume of the mixing stage (gradient tube or a mixing chamber), Q is the flow rate, and n is the stoichiometric factor between the reacting components. An example of typical signals recorded in flow injection gradient titrations is shown in Fig. 3. The set points

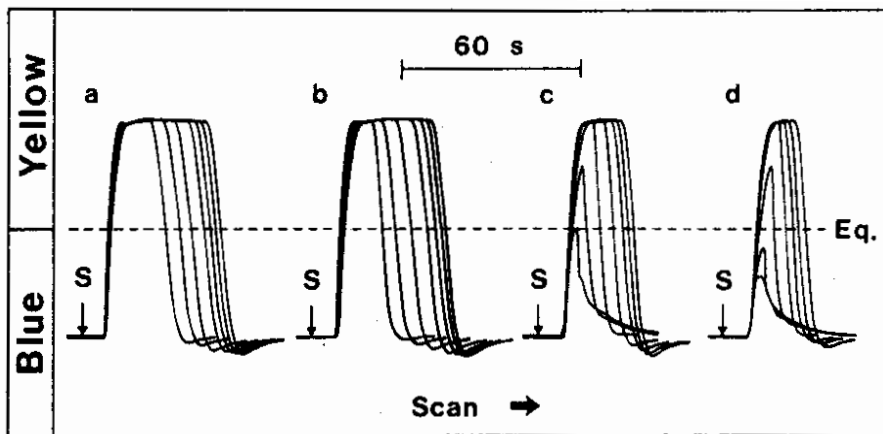


Fig. 3. Gradient titration curves obtained in the flow injection system in the titration of 5, 10, 20, 40, 60, 80 and 100 mM hydrochloric acid with (a) 0.5, (b) 1.0, (c) 5.0, and (d) 10 mM NaOH with spectrophotometric detection using a bromothymol blue indicator in the titrant solution [103]. (Reprinted by permission of copyright owner.)

used for peak width measurement must correspond to the equivalence point in that specific titration system. The width of signal is a logarithmic function of the analyte concentration. The effect of different parameters on the analytical signal in gradient titrations was discussed in detail for acid-base titrations [104]. It was also shown that in the single channel version it is not necessary to include a titrant in such a procedure with a large dispersion system and colour indicator [105]. A largely mechanised instrumental version of this procedure was developed for the determination of sulphuric acid in metallurgical process streams by titration with sodium acetate using a bromocresol blue indicator [106].

1.3. Kinetic Methods of Detection

The flow injection concept of analytical measurement based on recording of transient signal can be especially effectively applied for analytical purposes by the utilisation of kinetic properties on numerous chemical systems. Very early transient redox effect was utilised for FIA by Dutt and Mottola [107]. Into the continuously circulated reagents the analyte was injected, which in a very rapid redox reaction perturbs the concentration of the monitoring species and then

it is followed by subsequent but slower reaction regenerating the monitored species. Such a procedure can be used for determination of chromium(IV) based on oxidation of ferroin with oxalic acid as activator, which can then be indirectly applied in the determination of chemical oxygen demand, and also for determination of V(V), Mn(VII) and Ce(IV) using the diphenylamine sulphonate indicator system.

The formation of the unstable intermediate product in the FIA system can also be exploited for analytical purposes, which was shown for example in the reaction of cyanide with a pyridine-barbituric acid reagent [108]. Such a system requires very detailed hydrodynamic optimisation, and in the mentioned system for cyanide determination a reversed FIA configuration was used.

Differences in the rate of reaction between various analytes and the same reagent can be utilised for designing multicomponent measuring schemes, which is discussed below. Such effects can be advantageous for the improvement of the selectivity of FIA determination in comparison to conventional steady-state measurements. The determination of bromide based on oxidation with chloramine-T to bromine, followed by reaction with phenol red, is possible under FIA conditions in the presence of a large excess of chloride, because of the much slower formation of chlorinated products [109].

The use of conventional kinetic methods of determination (initial rate, fixed and variable time) is possible in the FIA system of such a design, where the entrapment of the sample plug into a closed loop allows repetitive passage through a single detector until the sample is completely dispersed into the carrier [249]. Such a setup enables the calculating kinetic parameters and also the measurement of concentrated samples without a prior dilution.

Numerous sensitive spectrophotometric FIA methods were developed with the use of catalytic effects, mainly for the determination of trace amounts of transient metals (Table 2). The determination of Cu(II) based on catalysing the Fe(III) reaction with thiosulphate in the presence of thiocyanate can be carried out in the closed-loop system with electrochemical removal of the catalyst by reducing to Cu(0) and simultaneous regeneration of the monitored species by anodic oxidation of Fe(II) [110]. In most developed methods, however, a simple FIA measurement with sample injection is used. In the determination of selenium, which catalyses the oxidation of phenylhydrazine by potassium chlorate, the direct FIA system was compared with reversed and stopped flow configurations [111]. The best detection limit was found for the reversed system, and the widest linear range of responses for direct FIA measurement.

Table 2. Catalytic methods in FIA with spectrophotometric detection in the visible region.

Analyte	Catalysed reaction	λ , nm	Concentration range	Reference
Ag(I)+Hg(II)	$[\text{Fe}(\text{CN})_6]^{4-} + \alpha, \alpha'$ -bipyridyl	536	1–64 ppb Ag 0.5–75 ppb Hg	112
Cu(II)	Fe(III)+thiosulphate with SCN-	480	5–250 ppm	110
	Fe(III)+thiosulphate	525	0.1–1.8 ppm	116
Fe(II)+Fe(III)	Leucomalachite green + $\text{S}_2\text{O}_8^{2-}$ + 1,10-phenanthroline	618	0.3–15 ppm Fe(II) 0.6–15 ppm Fe(III)	114
Fe(III)	<i>p</i> -phenetidine + periodate	540	0.7–500 ppb	117
	N,N'-dimethyl- <i>p</i> - phenylenediamine + H_2O_2	554	0.08–100 ppb	117
Formaldehyde	Brilliant green + sulphite (inhibition)	615	0.02–3.0 ppm	115
Mn(II)	N,N-diethylaniline + periodate	475	0.02–1.0 ppb	118
	Malachite green + periodate	615	10–70 ppb	119
	N,N-diethylaniline + periodate	615	0.002–15 ppb*	120
	Leucomalachite green + periodate	620	0.04–12 nM*	121
Mo(VI)	Iodide + H_2O_2	350	0.7–1000 ppb	122
			1.0–40 ppb	123
Mo(VI)	Iodide + H_2O_2 with starch	580	0.04–3.2 μM Mo 0.06–3.2 μM W	113
Se(IV)	Phenylhydrazine + chlorate + chromotropic acid	360	0.15–50 ppm	111
Sulphate	ZrOCl ₂ + methylthymol blue	586	50–500 ppm	124
V(V)	4-aminoantypyrine + N,N-dimethyl- aniline + bromate + tiron	555	0.05–2.0 ppb	125

*with on-line preconcentration

Differences in catalytic effects have found several applications in FIA systems for multicomponent determinations. Hg(II) and Ag(I) exhibit different catalytic effects on the ligand substitution reaction between hexacyanoferrate(II) and α, α' -bipyridyl with thiourea as an activator, which was utilised for simultaneous determination in the system, where flow was stopped and absorbances were determined at 100 and 200 s after sample injection [112]. Simultaneous differential rate determination of Fe(II) and Fe(III) was based on their different behaviour in the redox reaction between leucomalachite green and peroxodisulphate with and without the presence of the activator

1,10-phenanthroline [114]. For this purpose the original procedure exploiting the formation of a double peak as a result of injecting a large sample zone, sandwiched between reagent zones of appropriate composition, was developed.

As the example of application of inhibiting properties in flow injection photometric systems the determination of formaldehyde based on its inhibition of the brilliant green-sulphite reaction [115].

1.4. *Determination of Gaseous Analytes*

In this area of application of flow injection photometry, especially interesting systems from the point of view of practical applications are closed-loop FIA systems. Repetitive processing of air samples for sulphur dioxide determination was based on the reaction of SO₂ at a gas-liquid interface with dinuclear complex of iron(III) with 1,10-phenanthroline [230]. The method does not require a pretrapping of SO₂ and the main reagent was electrochemically regenerated by controlled potential electrolysis. Most often, however, determinations of gaseous components of air or process gases are carried out in the FIA systems with a cell absorbing the gaseous analyte. Such cells are usually equipped with a planar or tubular membrane of various porosity and permeability, through which the analyte diffuses into the receiver stream. The analysed gas is pumped by a fixed period of time, after which the receiver solution containing diffused gas is injected into the FIA system with an appropriate colour-forming reagent. Most favourable is to incorporate the absorbing membrane cell, called also a permeation denuder, into the sample loop of the injection valve. Two possible configurations of such a system are shown in Fig. 4. In one of them the receiver solution is also used as a carrier solution in FIA measurements, whereas in the second one the absorbing solution is injected into another carrier or reagent solution.

Hollow-membrane fibre gaseous collectors of analyte were used in determination of carbon disulphide in air [126] and trace HCN in process gas streams. CS₂ is absorbed in the ethanol stream, which then merges with reagents, forming coloured chelate of Cu(II) with dithiocarbamate. HCN is collected with a silicone rubber tube in a caustic solution, which then merges with a stream of chloramine-T and a mixture of isonicotinic acid and 3-methyl-1-phenyl-5-pyrazolin-5-one. The system with planar permeation denuder and polypropylene membrane was utilised for determination of SO₂ in air with analyte preconcentration in the recipient channel [128]. In a dual-line manifold EDTA solution was used as the absorber stream, which was merged with the

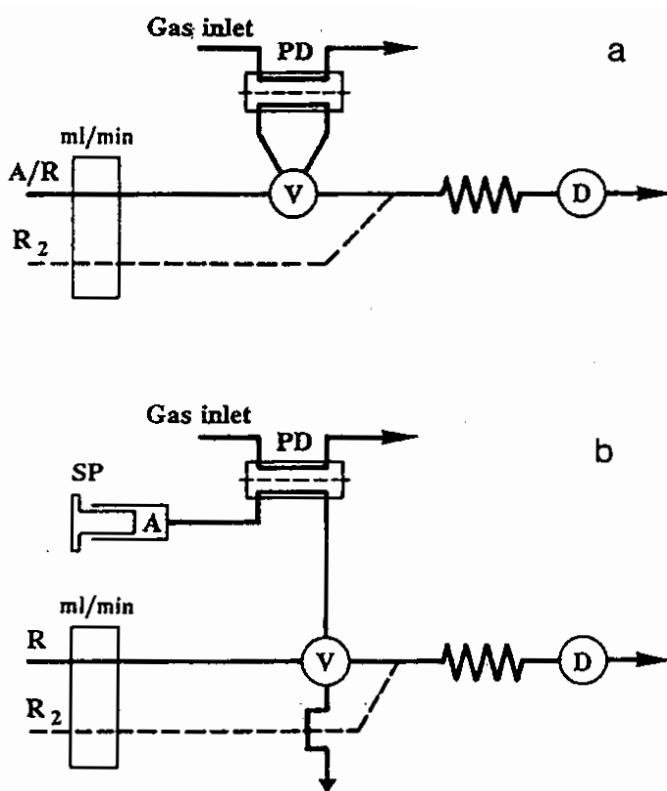


Fig. 4. Arrangements of the sampling systems for the determination of gaseous analytes in FIA with a permeation module used (a) instead of the sample loop in the injection valve, and (b) located in the loading channel of the injection valve [128]. V — valve; D — detector; A — absorber stream; R — reagent stream; SP — syringe pump; R — optional additional reagent channel. (Reprinted by permission of copyright owner.)

premixed pararosaniline-formaldehyde reagent stream. A planar membrane for absorbing gases was used also in flow through modules functioning simultaneously as gas absorber and spectrophotometric detector with optic fibre light transmission. The detector with reflectance measurements of the light used earlier for enzymatic determination of creatinine [129], was employed also for the determination of ammonia in air in a stopped-flow system [130]. Such a detection/reaction cell is equipped with a hydrophobic membrane, which separates acceptor and donor sides. It serves also as a reflector and light is delivered

to the cell and the signal returned to the detector by a bifurcated optical fibre placed perpendicularly to the membrane. The reflected light was measured as absorbance and the collected signal was passed through a filter before reaching the detector. Ammonia was detected by monitoring the colour change of an acid-base indicator. In another design of the gas diffusion/detector module used for the determination of atmospheric nitrogen dioxide, a light beam was introduced via an optical fibre mounted parallel to the membrane surface, which allows absorptive spectrophotometry detection [131]. Nitrogen oxide was absorbed through a microporous polypropylene membrane in solution containing N-(1-naphthyl)-ethylenediamine in HCl.

The absorption of the gas analyte can be also carried out in a layer of foamed hydrophobic material containing both micropores and macropores. Biporous PTFE absorbers were applied in FIA determination of SO₂ in air [132]. An aqueous solution of Fe(III) and 1,10-phenanthroline was used as a reagent, in which Fe(III) was reduced by SO₂ to form coloured chelate.

1.5. *Multicomponent Determinations*

A very favourable feature of the analytical method is the possibility of multicomponent determination. In this respect chromatographic methods and some spectroscopic methods have a predominant role in modern chemical analysis. There are also numerous developments in FIA to adapt this methodology for multicomponent analysis [252]. In flow injection visible spectrophotometry such determinations can be carried out in manifolds with one or several detectors, or with detectors enabling simultaneous detection at several wavelengths. Basic configurations of manifolds used to achieve this are shown in Fig. 5.

In the simplest configuration the sample injected with one injection valve is split into separate branches of the manifold with different sample pretreatment and separate detectors, such as it was used for simultaneous determination of nitrate and nitrite [253]. The splitting of the sample segment can also be realised in combination with dialysis of the sample with a double-line dialyser, which was reported for simultaneous determination of chloride and calcium [254]. Multicomponent determinations can also be carried out in the system of several parallel manifolds with separate detectors and simultaneous, independent injection of the sample solution into each carrier stream by coupled injection valves [158, 255, 256]. An exceptionally complex system was developed for simultaneous determination of inorganic sulphur species in aqueous samples of importance to the petroleum industry using absorptive and

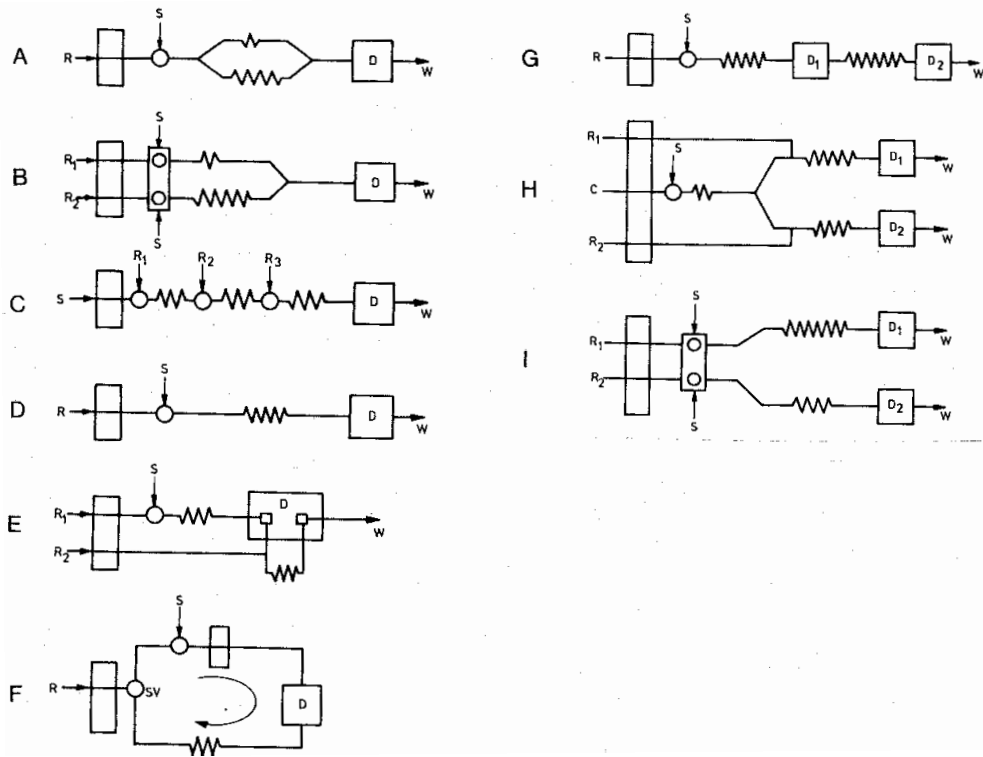


Fig. 5. Typical manifolds for multidetection flow injection systems with a single (A–F) and two (G–I) detectors [252]. (A) with splitting and confluence points; (B) with dual injection and a confluence point; (C) reversed FIA system with sequential injection of the reagents; (D) zone penetration system; (E) with two flow cells connected in series in a double-beam photometer; (F) closed-open system; (G) in-series detectors; (H,I) parallel detectors. R — reagents; S — sample injection point; D — detector; C — carrier; SV — selecting valve; W — waste. (Reprinted by permission of copyright owner.)

turbidimetric detectors [256]. Systems more difficult to realise due to the necessity of careful balance of back-pressure are FIA setups with sample splitting followed by confluence of the streams and a single detector. Such a system was developed for simultaneous determination of chloride and nitrate [257].

A difference in the kinetics of reaction of various species with the reagent has also been utilised for this purpose. Several systems were reported for the determination of two-component mixtures of alkaline earth metals based on the difference between the dissociation rates of the cryptand (2.2.2) complexes with phthalein complexone as the chromogenic reagent [258–260]. Hg(II) and Zn(II) were determined simultaneously utilising more rapid reaction of the Hg-Zincon complex with DCTA complexone than the Zn-incon complex [17]. The different rates of the dissociation of the citrate complexes of Co(II) and Ni(II) followed by measuring the absorbance of PAR complexes were used as a basis for the kinetic simultaneous determination of these analytes [261]. Kinetic determinations of these metals in three different configurations of the FIA system were based also on the different formation rates of their complexes with 2-hydroxybenzaldehyde thiosemicarbazide [262]. The best results were obtained in the setup with splitting up of the sample segment, then the confluence point and a single detector. A kinetic procedure for sequential determination of silicate and phosphate was based on the differences in the rates of formation of heteropolyacids and molybdenum blue [263].

For the simultaneous determination of two components doublet peaks can be utilised, which are formed when the centre of a large volume of the sample zone remains unmixed. Ni(II) in the presence of Fe(II) was determined by direct spectrophotometry of aquoion at the centre of the sample zone, whereas Fe(II) was first oxidised on-line to Fe(III) and then complexed by thiocyanate to form a red complex [264]. The same determination with time-based selectivity was performed with simultaneous injection of sample and reagent, called the *zone penetration* system [265]. To the same kind of measuring systems belongs the system where the sample containing Fe(II) and Fe(III) is inserted between zones of water and ascorbic acid, with subsequent addition of 1,10-phenanthroline [266]. The signal provides a plateau region corresponding to Fe(II) followed by a peak of total iron.

Simultaneous sample injection into different points of a non-branched manifold allows one to obtain different pretreatment of analytes in the injected sample zones. This was reported for the determination of Fe(III) and Ti(IV) with tiron [267]. Simultaneous determination of Cu(II) and Zn(II) was

developed in the FIA system, where two sample plugs were injected into the same carrier [268]. One of them was for zinc determination and merged with a plug of a masking agent and another was used for the sum of copper and zinc. A similar function is obtained in the system with multicommutation, where in a single-line manifold by using several injection valves a certain sequention of sample and reagent zones is introduced. This was demonstrated for determination of iron and chromium using salicylic acid and diphenylcarbazide as chromogenic reagents [248].

There are also examples of determination of two components by successive injection of the sample solution into FIA systems of changeable configurations after each injection, or at least with a change of reagent for each injection. In such a case none of the manifolds shown in Fig. 5 was used. Cr(III) and Cr(VI) were determined with or without on-line oxidation of Cr(III) [269], Pb(II) and Bi(III) were determined with arsenazo III after pH changes of the carrier solution [270], whereas Ca and Mg were determined in the system with a buffer containing or not containing the masking agent for Ca [271].

The different stability of complexes of various metals with the same ligand at different pH can be utilised in FIA systems, where on the interface between sample zone and carrier a pH gradient is formed. This was employed for determinations of Pb(II) and V(V) with PAR [71, 272] and Cu(II) and Zn(II) with zincon [273].

Reversed FIA systems of various designs were successfully applied in simultaneous determinations of Cr(III) and Cr(VI) [61, 62, 274]. Systems with successive injection of specific reagents were developed for determinations in the same aspirated sample pH, sulphide and cyanide or pH, nitrite and cyanide (Fig. 2) [60]. Then the use of two selection valves providing easy change of buffer and chromogenic in the measuring system allows the determination of Cu(II), total iron and Al(III) in the same sample at the same wavelength using cuprizone, bathophenanthroline and eriochrome cyanine R, respectively [175]. Yet another application of the reversed FIA system was developed for determination of creatine and creatinine [278]. The sample was merged with a picrate stream. A continuous signal was proportional to the creatinine concentration, whereas peaks obtained after injection of 1-naphthol and biacetyl were proportional to the creatine concentration.

An entirely different concept of multicomponent determinations in FIA systems with spectrophotometric detection is based on simultaneous measurements at different wavelengths and appropriate processing of experimental

data. It is advantageous with regard to the possible simplicity of the FIA manifold, but requires a more sophisticated detector and a larger number of calibrating solutions. The number of wavelengths used should be at least the same as the number of determined species. With the use of a flow-through detector with an integrated multidiode light source with LEDs of three wavelengths, mixtures of Al(III) and Zn(II) [22], and also Al(III), Fe(III) and Zn(II) [24] using xylenol orange were analysed. For this purpose, however, the most suitable detector is a diode array mainly used in HPLC [275]. Multiwavelength detection can be used to enhance the selectivity, as was demonstrated for the determination of the anti-cancer agent teniposide using 50 wavelengths [276]. In determinations of a single analyte the diode array detector can be employed to increase the concentration range of available responses by performing measurements at two different wavelengths corresponding to the maximum of absorption and far from it. This was applied for determination of nitrite [277]. Multicomponent applications of diode array detectors were reported for two-component mixtures of metal ions reacting with one [277–280] or a mixture of chromogenic reagents [281–284], for three-component mixtures of cations [284], and organic compounds [285, 286]. In most cases a limited number of wavelengths were used corresponding to the number of analytes, although measurements in a very large spectrum and their interpretation were reported [279, 281]. It was also shown that by using second-derivative spectra a better resolution can be obtained than by direct absorption measurements [279]. Applications of flow injection photometry to multicomponent analysis are presented in Table 3. Some other examples are discussed in Chapter 7.

Table 3. Multicomponent determinations in FIA systems with spectrophotometric detection.

Principle of differentiation of analytes	Analytes	Manifold type*	Concentration range	Reference
Reaction kinetics	Ag(I), Hg(II)	D	1.0–64 ppb Ag 0.5–75 ppb Hg	112
	Ca, Sr	B, G	0.2–2.0 mM	259
	Ca, Mg	D	0.04–0.16 mM	260
	Co(II), Ni(II)	H	2–8 ppm	261
	Fe(II), Fe(III)	B	1–10 ppm	114
		A, G, H	0.1–1.0 mM	262
	Mg, Sr	B, G	0.2–2.0 mM	259

continued

Table 3 (continued)

Principle of differentiation of analytes	Analytes	Manifold type*	Concentration range	Reference
Zone penetration	Phosphate, silicate	C	0.25–1.5 ppm P 2.5–15 ppm Si	263
	Zn(II), Hg(II)	D	15–50 μ M	17
	Fe(II), Ni(II)	D	2.7–5.4 mM Fe 0.17–0.24 mM Ni	264
		C	1.0–6.0 mM Fe 0.05–0.35 mM Ni	265
pH gradient	Fe(II), Fe _{total}	D	0.1–12 ppm	266
	Cu(II), Zn(II)	D	0.1–3.0 ppm Cu 0.4–12 ppm Zn	273
		D	0.03–0.15 mM Pb 0.025–0.11 mM V	71
			D	6.0–10 ppm Pb 0.5–3.0 ppm V
Different chemistry in branched manifold			Ca, chloride	H
	Chloride, NH ₃	I	0.2–20 ppm	158
	Chloride, nitrate	A	10–100 μ M	257
	Fe(II), Fe(III)	I	0.5–120 ppm	255
	Nitrate, nitrite	H	0.1–20 mM nitrate 0.05–2 mM nitrite	253
			Sulphide, sulphite, thiosulphate	I
Different chemistry in single-line manifold	Cr(III), Fe(III)	D	20–60 ppm Cr 25–200 ppm Fe	248
	Fe(III), Ti(IV)	C	11–105 ppm Fe 1.2–12.4 ppm Ti	267
			Bi(III), Pb(II)	
Sample injections to altered manifolds	Ca, Mg		8–120 ppb Ca 1–30 ppb Mg	271
	Cr(III), Cr(VI)		0.055–4 ppb Cr(III) 0.018–2 ppb Cr(VI)	269
			Cu(II), Zn(II)	

continued

Table 3 (*continued*)

Principle of differentiation of analytes	Analytes	Manifold type*	Concentration range	Reference	
Injection of reagents	Al(III), Cu(II), Fe(III)	C	0.06–0.8 ppm Al	175	
			0.3–4.0 ppm Cu		
			0.1–3.0 ppm Fe		
	Cyanide, sulphide, nitrite	C	0.3–5.0 ppm cyanide	60	
			0.5–5.0 ppm sulphide		
			0.1–1.5 ppm nitrite		
	Cr(III), Cr(VI)	F	1.0–3.0 ppm Cr(III)	274	
			0.6–1.2 ppm Cr(VI)		
			B	0.5–3.0 ppm Cr(III)	62
				0.2–1.2 ppm Cr(VI)	
	Creatine, creatinine	C	2–30 ppm	297	
Difference in absorption maxima in the same chemical conditions	Al(III), Zn(II)		0.2–25 ppm	22	
			Al(III), Fe(III), Zn(II)	1.0–10.0 ppm	24
	Co(II), Cu(II), Ni(II)		5–50 μ M	284	
			<i>o</i> -, <i>m</i> -, <i>p</i> -cresols	0.1–100 ppm	285
	Cu(II), Fe(III)		16–25 ppm Cu	277	
			1–7 ppm Fe		
			2–8 μ M	278	
			1–42 μ M	282	
			0.4–35 μ M	283	
	Fe(II), Fe(III)		0.2–15.5 ppm Fe(II)	281	
			3–20 ppm Fe(III)		
	Fe(III), free acid		0.1–1.6 M Fe	280	
			0.5–6 M acid		
1-, 2-naphthols			1–50 ppm	285	
Ni(II), Zn(II)			1.0–7.0 ppm	279	
Nitrophenylhydrazines			0.5–30 μ M	286	

*according to Fig. 5

1.6. *FIA or Liquid Chromatography?*

The testing of detection conditions in FIA systems is a very often used method for the optimisation of working conditions for the chromatographic determinations [e.g. 287, 288]. The FIA system combined with a chromatographic setup can be used for effective analyte preconcentration in HPLC [289] or GC [290]. In the first of these applications transition metal ions were

preconcentrated on microcolumn with Chelex-100, whereas in the second one preconcentration and ethylation of organotin species was carried out on a microcolumn with C18 sorbent.

Incorporation of a separation column in the flow system between the injection valve and the detector, and then acquisition of signals corresponding to several analytes, is a typical arrangement of the column chromatographic setup. Regardless of the size of a column, used pressure or flow rate, such a system should not be considered as a flow injection system, which sometimes can be found in the literature. Such systems are low pressure liquid chromatography systems with post-column reaction detection. They were developed, for instance, for determination of inorganic polyphosphates [291], for speciation of dissolved organic and inorganic phosphorus in environmental samples [292], for simultaneous determination of silicon and phosphorus in biological standards [293], and for determination of silicate, phosphate and arsenate [294]. Sodium and potassium were separated on a column packed with silica gel as complexes with crown ether [295]. Cobalt(II) preconcentrated on a chelating column is then separated from the excess of Mn(II) and Fe(III) on a strongly acidification resin, but chromatographic signals for both Co(II) and Mn(II) are recorded [296].

2. Detection in the Ultraviolet Region

Absorption in the UV region (185–400 nm) generally results from the excitation of π , α and non-bonding electrons to higher energy levels. Absorbing compounds containing these electrons mostly include organic molecules, but also some inorganic ions, e.g. nitrite, nitrate, azide or carbonate. Spectrophotometric measurements in the so-called vacuum ultraviolet ($\lambda < 185$ nm) are much more difficult, because components of the atmosphere absorb strongly in this region, and so it is not used for analytical purposes. The instrumentation for measurements in the visible and UV ranges differs in the sources of radiation used and materials for cuvettes. Deuterium or hydrogen lamps are the most common UV radiation sources, while the cuvettes used are made of quartz, fused silica or certain polymeric materials that pass UV radiation.

The similarity between the detection processes in the visible and UV regions means that their applications in FIA involve the same instrumental designs, measuring procedures and interpretation of experimental data. The majority of the applications reported involve procedures which use conventional UV/VIS