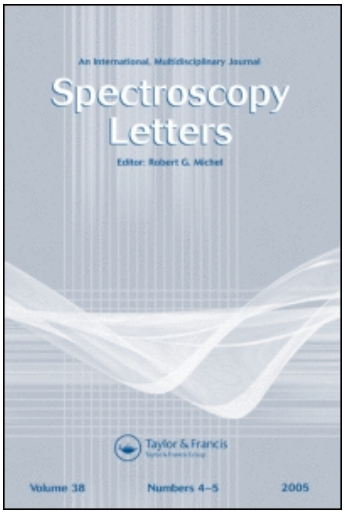

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Turbidimetric and Nephelometric Flow Analysis: Concepts and Applications

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Abstract: A review on flow analysis with turbidimetric and nephelometric detection is presented. A brief discussion of the principles of turbidimetry and nephelometry is given. Particular emphasis is devoted to coupling different flow techniques (flow injection, sequential injection, multicommutation) to these detection techniques. Applications in environmental, pharmaceutical, biological, and food samples are summarized and compared in terms of application range, flow configuration, repeatability, and sampling rate.

Keywords: Flow analysis, nephelometry, turbidimetry

INTRODUCTION

Nephelometry and turbidimetry are closely related analytical techniques based on the scattering of radiation by a solution containing dispersed particulate matter. When a radiation passes through a transparent medium in which solid particles are dispersed, part of the radiation is scattered in all directions, giving a turbid appearance to the mixture. The decrease of the incident radiation, as a result of scattering by particles, is the basis of turbidimetric methods. Nephelometric methods, on the other hand, are based on the

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measurement of the scattered radiation, usually at a right angle to the incident beam. The choice between a nephelometric and a turbidimetric measurement depends upon the fraction of light scattered. When scattering is extensive, owing to the presence of many particles, turbidimetry generally yields more reliable results. Nephelometry is preferred at low concentrations because a small scattered intensity against a black background is easier to measure than a small change in intensity of intense transmitted radiation. It is important to note that scattering associated with both nephelometry and turbidimetry does not involve loss in radiant power; only the direction of propagation is affected.

The intensity of radiation appearing at any angle depends upon the number of particles, their size and shape, as well as the wavelength of the radiation.

Effect of Concentration on Scattering

Turbidimetric analysis consists of the measurement of the decrease in the intensity of the incident radiation that is caused by scattering and is analogous to an absorptive measurement, although the reason for the decrease in intensity is different.

When a beam of radiation of intensity I_0 passes through a nonabsorbing medium that scatters light, the transmitted intensity I is given by the expression:

$$I = I_0 e^{-\tau b}$$

where τ is the turbidity, or the turbidity coefficient, and b the pathlength in the turbid medium. The turbidity τ is often found to be linearly related to the concentration C of the scattering particles. As a consequence, a relationship analogous to Beer's law is applied. That is,

$$S = -\log I/I_0 = kbC$$

where

$$k = 2.303\,\tau/C$$

The equation is employed in turbidimetric analysis in exactly the same way as Beer's law is used in photometric analysis. The relationship between $\log I_0/I$ and C is established with the standard solutions, and the solvent is used as the reference to determine I_0. The resulting calibration curve is then used to determine the concentration of the samples.[1]

Nephelometry is based on the measurement of scattered radiation by sample particles at right angles to the beam. The detector is placed out of the path of the incident radiation from the source. In most cases, the detector is placed at 90 degrees relative to the path of the incident radiation. It measures the intensity of that portion of the scattered radiation that is emitted perpendicularly from the cell in the direction of the detector. For nephelometric measurements, an equation describes the relationship

between the intensity of scattered radiation, the intensity of the incident radiation, and the concentration of the particles that cause the scattering:

$$I = KI_0C$$

The value K is constant only for a particular instrument and when experimental conditions are carefully controlled. The intensity of the scattered radiation is directly proportional to both the intensity of the incident radiation and to the concentration of the analyte. For assays of diluted solutions, it is advantageous to use incident radiation that has a high intensity.[2]

The detected scattered signal may arise from the particles of interest but also from dust, background scatter, or from other molecules (e.g., proteins and lipids) in the sample. Reflection and scatter from optical components of the instrument may also contribute to the background signal. Best performance is obtained in dilute solutions where absorption and reflection are minimal.

Under these conditions, the relationship between concentration of scattering particles and scattered light intensity is almost linear over a very wide range of concentration.

Effect of Particle Size on Scattering

Nephelometric and turbidimetric methods have advantages of being simple, fast, and having high sensitivity. The difficulties arise not from the optical measurement, which is simple, but from the preparation of the suspension. In fact, the fraction of radiation scattered at any angle in colloidal systems depends upon the size and the shape of the particles responsible for the scattering. Because most analytical applications involve the generation of a colloidally dispersed phase in a solution, those variables that influence particle size during precipitation also affect both turbidimetric and nephelometric measurements. Thus, such factors that can affect the results and that must be controlled include the concentrations of the reagents that are used to prepare the suspensions, the rate and order of mixing, and the time after reagents have been mixed and the time before the measurement is made. The pH, the total ionic strength, and the temperature of the solution are other variables that are of critical importance and must be carefully controlled. In order to stabilize the suspensions and prevent the settling of the particles, a protective colloid is usually added. The absence and presence of protective colloids in a suspension also affect the size of the particles. Thus, during calibration and analysis, care must be taken to reproduce all conditions likely to affect particle size.

Effect of Wavelength on Scattering

The wavelength selected for the measurements also has an important effect on scattering. It has been shown, experimentally, that the turbidity coefficient τ

varies with wavelength according to the equation:

$$\tau = s\lambda^{-t}$$

where s is a constant for a given system. The quantity t is dependent on particle size and has a value of 4 when scattering particles are significantly smaller than the wavelength of the radiation; for particles with dimensions similar to the wavelength, t is found to be 2.[1] The latest situation is the usually encountered in turbidimetric analysis.

The wavelength chosen for the turbidimetric or nephelometric assay is also dependent upon the presence of other (interfering) absorbing or fluorescing species in solution. In this case, a wavelength where absorbance or fluorescence by the substances in solution does not occur has to be chosen. If the scattering particles (those that are in the interest of the determination) also absorb radiation, the sensitivity of turbidimetric, but not nephelometric, determinations can be increased by choosing the wavelength at which absorbance occurs. In that case, the instrument measures the sum of absorbance and turbidance, which should also be proportional to concentration.

Equipment

Turbidimetric measurements are usually performed with simple filter photometers, while instruments for nephelometric measurements are similar in design to simple fluorometers. Both instruments comprise a light source that emits in the visible region, a cell compartment, a detector, and a readout device. In the apparatus used for nephelometric determinations the detector is, in most cases, placed at 90 degrees relative to the path of the incident radiation. Detectors that accurately and reliably respond to radiation in the visible region are frequently used. Phototubes are usually used for turbidimetric measurements and phototubes or photomultiplier tubes for nephelometric measurements. A wavelength selector may also be present between the source and the cell compartment, and, for nephelometric measurements, a second wavelength selector can also be placed between the cell compartment and the detector. Laboratories that routinely use this technique for analysis sometimes use instruments that have been specifically designed for turbidimetric or nephelometric measurements, which are usually simpler in design and less expensive than spectrophotometers or fluorometers. Often they use the broad visible continuum emitted from a tungsten filament as the incident radiation, have no monochromator, and apply a phototube or the human eye as the detector. Cells that are used to hold the sample during turbidimetric and nephelometric measurements are identical to the cuvets used for measurements of absorbance and fluorescence. Nevertheless, because scattered radiation from the walls can interfere with the assay, it is sometimes advantageous to coat the exterior of the walls, except those

through which radiation must pass, with a nonreflective black paint. This is particularly important for nephelometric measurements.

Applications of Scattering Methods

Turbidimetric or nephelometric methods are widely used in analysis of water, for the determination of turbidity, and for the control of treatment processes. In addition, the concentration of a variety of ions can be determined using suitable precipitation reagents to form suspensions. Perhaps the best known chemical turbidimetric analysis involves the precipitation of sulfate as barium sulfate under controlled conditions that yield a stable monodisperse suspension. Both techniques can also be used to locate the endpoint of some titrations in which the titrand reacts with the titrant to form a suspension. Generally, the turbidance or the intensity of the scattered radiation increases before the end point and then remains constant. Turbidimetric or nephelometric measurements have been used to locate potential precipitants in commercially prepared soft drinks and alcoholic beverages, to measure potentially equipment-clogging solids suspended in waters that are used in industrial equipment, and as an environmental analytical tool to measure suspended solids in waters.[3,4] Finally, they have also been used to measure suspended particles in gases, like smog and fog.

FLOW ANALYSIS

Flow injection analysis (FIA), introduced in 1975 by Ruzicka and Hansen,[5] is a simple and an alternative method to batch procedures. In a basic FIA manifold, samples are introduced into the system through the injection valve, dispersed in the carrier inside the tubes conduit. Most commonly, the reagent is continuously added through a confluence point located after the injection port and before a coil where reaction takes place. Finally, the reaction product reaches the flow through detector where the detection signal is acquired (Fig. 1A).

In 1990, Ruzicka and Marshall[6] proposed a new flow technique, sequential injection analysis (SIA), based on the same principles of FIA, and conceived as a single pump, a single valve, and a single channel system. The SIA is based on the sequential aspiration of well-defined sample and reagent zones through a selection valve into a holding coil. The flow is then reversed, to propel and mutually disperse these stacked zones through the reaction coil and direct the reaction product to the detector (Fig. 1B). Compared with FIA, these systems allow considerable saving of reagents and a significant decrease on the chemical waste produced, because just the required amounts are aspirated and carrier is not pumped continuously. In addition, different analysis can be performed using the same manifold by

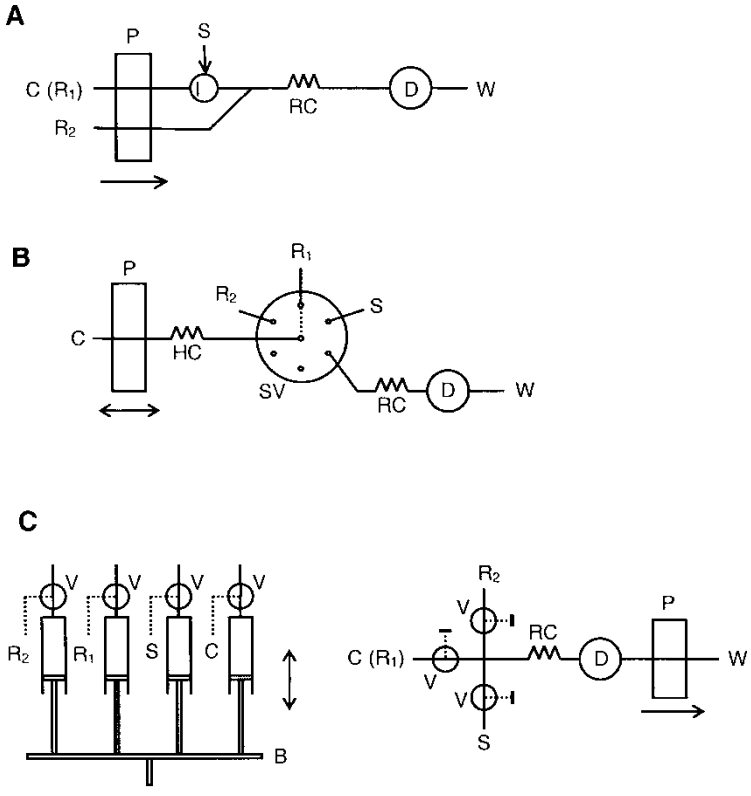

Figure 1. Schematic diagram of flow systems with turbidimetric or nephelometric detection: (A) flow injection analysis, (B) sequential injection analysis, (C) multicommuted flow-injection analysis. I, injection valve; SV, selection valve; V, individual commutation devices (e.g., solenoid valves); S, sample; R_i, reagents; R_1, surfactant, washing solution; R_2, precipitating agent; C, carrier; P, liquid drive; B, pistons bar; D, detector; RC, reaction coil; HC, holding coil; W, waste.

simple reconfiguration of the sequence of events from the computer keyboard. Besides this, the major difference between FIA and SIA methodologies concerns the way that sample and carrier/reagent solutions are mixed inside the tubes. While in FIA the solutions are most commonly mixed in confluence points, giving rise to a concentration gradient of analyte in a constant background of reagent, in SIA efficient mixing is more difficult to achieve due to the absence of confluence points. In fact, in SIA an initial sharp boundary is formed between the adjacent sample/reagent zones stacked in the holding coil. Even after the flow reversal, only a partial overlap of analyte and reagent zones is achieved.[7]

In order to overcome this specific difficulty of SIA, and also to improve the mixing between solutions in flow systems in general, various strategies were

published. In 1985, Pasquini and Oliveira proposed an approach, monosegmented flow (MSFA),[8] in which sample and reagent are introduced between two air bubbles. The bubbles serve to limit the longitudinal sample dispersion and at the same time to enhance the radial mixing. The bubbles are removed before they enter the detection system using a gas-permeable membrane.

Another alternative to overcome mixing difficulties is the multicommuted flow injection analysis (MCFIA), which was first described by Reis et al.[9] associated with the binary sampling approach. This technique is characterized by the use of individual commutation devices (solenoid valves) operating in a simultaneous or a sequential way, where solutions can be accessed randomly. In this approach, small plugs of sample and reagents are inserted in alternative way in the flow system and mutually dispersed while directed to the detector (Fig. 1C). Compared with other flow techniques, the main advantage introduced by the multicommuted approach is versatility based on the use of solenoid valves that can be arranged in multiple configurations. This evidence was pointed out by Zagatto et al.,[10] when it mentioned that multicommutation can unify all concepts already proposed in flow analysis, considering the possibility of accommodating different flow modalities (FIA, SIA) in a system with just solenoid valves.

Turbidimetric and Nephelometric Flow Analysis

Turbidimetry has been widely used as detection method in flow analysis. Besides just automating batch turbidimetric methods, flow techniques such as FIA, SIA, MCFIA, and MSFA, within others, even allowed improvement of the analytical performance of these detectors. Undoubtedly, FIA is the most widely used technique.

The use of a flow system does not affect any of the basic characteristics of batch turbidimetric or nephelometric methods. The equations obtained are still obeyed in the same range and with similar sensitivity.

Although any detector capable of flow-through detection can be interfaced with flow systems, to obtain reproducible signals the detector and the readout device used must have a fast response.

The repeatability of the batch measurements are highly affected by the skillfullness of the operator and, in some cases, by the time at which the detection measurement is made. In fact, the time spent in each measurement can be very high, because at the time of the detection the reaction has to be in steady state. This is particularly observed when the reaction is relatively slow, such as in turbidimetric determinations.

In turbidimetric analysis, the preparation of the standard suspensions is particularly critical, and sample and standard suspensions must be prepared using identical procedures. In fact, as pointed out by Brienza et al.[11] the major problem of turbidimetry is related to processes of solution handling rather than to quality and performance of the measurement instruments.

The amount of light scattering in colloidal systems is a sensitive function of the particle size, so any variation in the colloidal solution preparation may result in a lack of particle size uniformity from one determination to the next, altering significantly the turbidimetric or nephelometric measurement. In this context, the flow systems are an attractive tool to improve the reproducibility and precision of turbidimetric determinations. The addition of colloid protectors or surfactants is often required, which, in contrast with batch procedures, is efficiently accomplished in flow-based methodologies. The presence of these agents guarantees the uniform nucleation and prevents the settling of the precipitate, thereby improving the repeatability and reproducibility of the analysis.[11,12] Carryover and memory effects can be lessened in view of better uniformity of the particles, thus reducing washing time and baseline drift. For this task, intermittent addition of a washing solution or a fast washing stream has been exploited.[11]

TURBIDIMETRIC AND NEPHELOMETRIC FLOW ANALYSIS APPLICATIONS

In the following sections, the description of turbidimetric and nephelometric applications using flow methods is given. The applications are organized by the type of analyte: inorganic ions, organic compounds, compounds with immunological importance, and biomass.

Determination of Inorganic Ions

Sulfate

Sulfate is undoubtedly the most popular analyte determined using turbidimetric flow methodologies (Table 1). The method that appears to be almost universal is the barium sulfate turbidimetric procedure (Table 1), being the measurement performed between 410 and 580 nm.[40] Turbidimetric flow procedures with barium chloride, as precipitating agent, have been successfully applied to environmental,[12,13,15–20,22–37,39] clinical,[14,35] and wine[38] samples. Krug et al.[12] were the first authors to adapt the turbidimetric barium sulfate procedure to FIA for the determination of sulfate in natural waters and plant digests, using various types of flow systems with more than one reagent or carrier stream. This was also the first turbidimetric FIA system reported, only a few years after the FIA concept been introduced, which indicates the easy implementation of this reaction to flow systems. Since then, several researchers have developed not only other FIA systems,[13–30,35,36,39] but also SIA,[31,32,34,35,37,38] MCFIA,[33] and MSFA[35] methodologies in order to obtain better precision and sensitivity, shorter analytical cycles, and lower detection limits. Alternatively, a FI

Table 1. Application of turbidimetric and nephelometric flow methods to sulfate determination

Analyte	Flow method	Sample	Reagent	Precipitate	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Sulfate	FIA/tur	Natural waters and plant digests	$BaCl_2$	$BaSO_4$	PVA	10–200 mg L^{-1}	0.85	180	[12]
Sulfate	FIA/tur	Natural waters and predigested plant material	$BaCl_2$	$BaSO_4$	PVA	10–200 mg L^{-1}		250	[13]
Sulfate	FIA/tur	Urine	$BaCl_2$	$BaSO_4$	Gelatin	4–15 mmol L^{-1}	<1.2	120	[14]
Sulfate	FIA/tur	River and sea water	$BaCl_2$	$BaSO_4$	PVA	40–160 mg L^{-1}	1–2		[15]
Sulfate	FIA/tur	Natural waters	$BaCl_2$	$BaSO_4$	Thymol and gelatin	20–500 mg L^{-1}	<2.0	200	[16]
Sulfate	FIA/tur	Surface, ground, and domestic waters	$BaCl_2$	$BaSO_4$	Thymol and gelatin	50–200 mg L^{-1}	<0.95	60	[17]
Sulfate	FIA/tur	Surface, ground, and domestic waters	$BaCl_2$	$BaSO_4$	Thymol and gelatin	Up to 200 mg L^{-1}	<1	60	[18]
Sulfate	FIA/tur	Natural waters and plant digests	$BaCl_2$	$BaSO_4$	PVA	1–30 mg L^{-1} waters 5–200 mg L^{-1} plants	1	120	[19]
Sulfate	Reversed FIA/tur	Effluent water streams	$BaCl_2$	$BaSO_4$	Gelatin	50–200 mg L^{-1}	<2.0	60	[20]

(*continued*)

Table 1. Continued

Analyte	Flow method	Sample	Reagent	Precipitate	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Sulfate	FIA/tur	3% (m/v) cesium iodide solution	$BaCl_2$	$BaSO_4$	PVA	1–100 mg L^{-1}			[21]
Extractable sulfate	FIA/tur	Plant material	$BaCl_2$	$BaSO_4$	Arabic gum	0–35 mg L^{-1}	2	120	[22]
Total sulfur	FIA/tur	Plant material	$BaCl_2$	$BaSO_4$	Arabic gum	0–200 mg L^{-1}	2.1	120	[23]
Sulfate	FIA/tur	Petroleum industry–related waters	$BaCl_2$	$BaSO_4$	—	0–20 mmol L^{-1}		24	[24]
Sulfate-sulfur; sulfur	FIA/tur	Waters and plant materials	$BaCl_2$	$BaSO_4$	Arabic gum	0–300 mg Kg^{-1}	0.01 plant digests 0.3 waters	60	[25]
Sulfate	FIA/tur	Rain waters	$BaCl_2$	$BaSO_4$	PVA	0.50–2.00 mg L^{-1}	2	50	[26]
Sulfate	FIA/tur	Soil	$BaCl_2$	$BaSO_4$	Arabic gum	0–180 mg L^{-1}	0.85	120	[27]
Sulfate	FIA/tur	Fresh and saline waters	$Pb(NO_3)_2$	$PbSO_4$	PVA	2–20 mg L^{-1}	<3	35	[28]
Total sulfur	FIA/tur	Plants	$Pb(NO_3)_2$	$PbSO_4$	—	5.00–25.00 mg S L^{-1}	0.5	400	[29]
Sulfate	FIA/tur, neph	Tap water	$BaCl_2$	$BaSO_4$	—	20–2000 mg L^{-1} tur 20–200 mg L^{-1} neph	4.0		[30]

Sulfate	SIA/tur	Natural waters and industrial effluents	$BaCl_2$	$BaSO_4$	Thymol and gelatin	10–200 mg SO_4^{2-} L^{-1}	<3.9	26	[31]
Sulfate	SIA/tur	Industrial waters	$BaCl_2$	$BaSO_4$	Thymol and gelatin	50–5000 mg SO_4^{2-} L^{-1}	<4.5	20–24	[32]
Sulfate	MCFIA/tur	Plant materials	$BaCl_2$	$BaSO_4$	Tween 80	10–500 mg SO_4^{2-} L^{-1}	2	100	[33]
Sulfate	SIA/tur	Waste waters	$BaCl_2$	$BaSO_4$	Thymol and gelatin	5–200 mg SO_4^{2-} L^{-1}	1.5	12	[34]
Sulfate	SIA, FIA, MCFIA, MSFA/tur	Plant, bovine liver, and blood serum digests	$BaCl_2$	$BaSO_4$	Tween 80	20–200 mg L^{-1}	<3.2	30–40	[35]
Sulfate	FIA/tur	Natural and waste waters	$BaCl_2$	$BaSO_4$	PVA	10–120 mg SO_4^{2-} L^{-1}	<3	40	[36]
Sulfate	SIA/tur	Natural and waste waters	$BaCl_2$	$BaSO_4$	PVA	10–100 mg SO_4^{2-} L^{-1}	<3.3	20–22	[37]
Sulfate	SIA/tur	Wine	$BaCl_2$	$BaSO_4$	PVA	300–1500 mg K_2SO_4 L^{-1}	10	5	[38]
Sulfate	FIA/neph	Unknown waters	$BaCl_2$	$BaSO_4$	PVA	10–80 mg L^{-1}			[39]

FIA, flow-injection analysis; MSFA, monosegmented flow analysis; SIA, sequential injection analysis; MCFIA, multicommuted flow injection analysis; Tur, turbidimetry; Neph, nephelometry; RSD, relative standard deviation; SR, sampling rate; PVA, poly(vinyl alcohol).

procedure based on $PbSO_4$ colloidal formation in ethanol–water was proposed by Santelli el al. as a turbidimetric method for the determination of sulfate in natural waters.[28] This reaction was also applied by Brienza et al. in the determination of total sulfur in plants using crystal seeding as an alternative approach for improving the rate of crystal growth in turbidimetric flow analysis.[29]

The addition of colloid protectors or surfactants is often required and, in contrast with batch procedures, is efficiently accomplished in flow-based methodologies. These agents can be classified as aqueous solutions of mono- and polyvalent alcohols, such as glycerol, and aqueous solutions of macromolecular material, such as gelatine, various gums, or commercial preparations of surface-active agents.[12] The presence of these agents guarantees the uniform nucleation and prevents the settling of the precipitate, thereby improving the repeatability and reproducibility of the analysis.[11,12,40] For this purpose and for both reactions, different surfactants were used, namely poly(vinyl alcohol) (PVA),[12,13,15,19,21,26,28,36–39] gelatine,[14,16–18,20,31,32,34] thymol,[16–18,31,32,34] arabic gum,[22,23,25,27] and Tween 80.[33,35]

The nucleation of barium sulfate is strongly pH dependent.[11] The pH not only affects the formation or dissolution of the barium sulfate precipitate but also its structure. A precipitate obtained from a solution with a pH in the range 0–1.5 consists of large, well-shaped crystals. At pH 1.5–3, uneven crystals of medium particle size are obtained, whereas at pH 3–7, the precipitate is amorphous.[15] To obtain an acidic medium, hydrochloric acid was frequently applied, and the samples were previously acidified or acidified in the flow systems. Moreover, hydrochloric acid is added to prevent the formation of precipitates of carbonate, chromate, sulfite, phosphate, and oxalate of barium, which may interfere.[40,41]

In turbidimetric flow methodologies, the build-up of precipitate can occasionally occur, which leads to decrease of precision and finally can even block the tubing.[15,41] To overcome this problem, the intermittent addition of an alkaline buffer ethylenediaminetetraacetate (EDTA) washing solution to dissolve the barium sulfate, and, consequently, to reduce the accumulation of the precipitate on the conduit walls and/or on the windows of the flow cell, has been widely exploited.[15,17–23,25,27,31–39]

Although very fast precipitation reactions are concerned, nucleation may be a limiting factor in sample throughput.[11,29] In order to speed up nucleation, improvement of supersaturation conditions involving addition of a nucleant species (often the same as the analyte) has been performed.[29] Addition of sulfate ions into a carrier at a constant concentration or saturation of the streams with barium sulphate result in an extension of the concentration range to lower concentrations, better signal stability, and reduction of the baseline drift.[40] In order to extend the range of the method to low concentrations, several FIA systems with continuous addition of sulfate to the carrier stream[19,22,23,28] or addition of sulfate to the sample before it enters the injection loop[25,26] have been reported. Brienza et al. proposed a

reproducible addition of in-line produced suspensions to improve supersaturation conditions in flow turbidimetry. This crystal seeding leads to a simplification in system design and an improvement in sampling rate and/or sensitivity in procedures usually limited by rate of turbidity formation. The feasibility of the approach was demonstrated in developing a turbidimetric FI procedure for in the determination of total sulfur in plants based on lead sulfate precipitation after adding a confluent stream with lead phosphate nucleant.[29]

Another problem related with the barium sulfate turbidimetric procedure is the possible interference at the wavelength 420 nm caused by the suspended solids, the presence of organic substances, and the intrinsic color of the samples.[18] In order to minimize this difficulty, van Staden[18] proposed a FI procedure with prevalve sample filtration. The interferences are automatically removed by using an active carbon filter located between the sampler and the sampling valve system.

Nephelometric flow injection systems for sulfate determination by precipitation as barium sulfate have also been reported. A liquid-drop windowless optical cell with a reactor without walls for flow injection turbidimetric and nephelometric determination of sulfate has been developed by Liu and Dasgupta.[30] In this approach, problems arising from the deposition of precipitate on flow cell windows were avoided. Gradient dilution techniques were conveniently implemented without precise external timing: with small drops, a single FIA peak is spread over a multitude of drops. In 2003, Jakmunee et al.[39] developed a simple and low-cost flow-through light-scattering detection system for determining the particle mass concentration. The methodology was based on nephelometric detection, using a laser pointer as a light source and a photodiode as a light sensor.

Potassium

Although potassium quantification is generally carried out by flame emission spectrometry, flow turbidimetric determination methodologies using sodium tetraphenylboron (Na-TPB) have also been described (Table 2).

Torres and Tubino[42] proposed a turbidimetric flow injection system for the determination of potassium after precipitation with Na-TPB in alkaline medium. In order to determine low potassium concentrations, an additional potassium solution was continuously added to the carrier. The methodology was applied to the determination of potassium up to 20 mg K L^{-1} in plant leaves, bottle mineral waters, and serum rehydration solution.

A turbidimetric FI system was developed by Lima et al.[43] for the determination of total nitrogen and potassium in vegetable samples using a single spectrophotometer as detector. A solution of Na-TPB prepared in PVA was used as precipitating agent for the determination of potassium. A gas diffusion process was included in the manifold to separate ammonium ions from the rest of the sample and to allow paired analysis. Total potassium

Table 2. Application of turbidimetric flow methods to potassium, nitrogen, phosphate, chloride, and total organic carbon determination

Analyte	Flow method	Sample	Reagent	Precipitate	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Potassium	FIA	Plant leaves, bottled mineral waters, and serum rehydration solutions	Na-TPB	K-TPB	Glycerol	Up to 20 mg K L^{-1}	1	60	[42]
Potassium	FIA	Vegetables	Na-TPB	K-TPB	PVA	78–390 mg K L^{-1}	1.6	70	[43]
Potassium	MCFIA	Fertilizers	Na-TPB	K-TPB	PVA	6.00–60.0 mg K L^{-1}	1–3	240	[44]
Ammonia	FIA	Natural waters and soil extracts	Nessler	$NH_{n-1}Hg_2I_n$	—	0.5–6.0 mg $N\text{-}NH_4^+$ L^{-1}		120	[46]
Total nitrogen	FIA	Plant material	Nessler	$NH_{n-1}Hg_2I_n$	—	0–5% $N\text{-}NH_4^+$ in plant material	<3	100	[47]
Total nitrogen	FIA	Vegetables	Na-TPB	NH_4-TPB	PVA	87–430 mg $N\text{-}NH_4^+$ L^{-1}	<2.1	70	[44]
Phosphate	FIA	Serum samples; organic compounds; plant materials	Molybdate and crystal violet	Blue dye salt	PVA	Up to 1.25 mg PO_4^{3-} L^{-1}	0.56	100	[48]

Phosphate	FIA	Digested plant material	Zinc(II)	$Zn_3(PO_4)_2$	PVA		5–60 mg P L^{-1}	<1.6	180	[49]
Phosphate	SIA	Urine	$CaCl_2$	$Ca_3(PO_4)_2$		—	200–1500 mg L^{-1}	1.1–2.0	15	[50]
	SIA		Ca^{2+} / CO_3^{2-}	$CaCO_3$		—	0.1–0.8 mg L^{-1}	0.97–1.90	12	
Chloride	FIA	River waters	Ag^+	AgCl		—	0–14 mg L^{-1}		15	[51]
Chloride	FIA	Natural waters (river)	Ag^+	AgCl	PVA		Up to 10.00 mg Cl^- L^{-1}		40	[52]
Chloride	SIA	Ground, surface, and waste waters	Ag^+	AgCl	PVA		2–400 mg Cl^- L^{-1}	<3.7	55–57	[53]
Chloride	FIA	Tap, river, deep ocean, and reference waters	Ag^+	AgCl		—	3.0–30 mg Cl^- L^{-1}			[54]
Total organic carbon	FIA	Industrial effluents	$Ba(OH)_2$ solution	$BaCO_3$		—	20–800 mg C L^{-1}		120	[55]

TPB, tetraphenylboron; FIA, flow-injection analysis; MSFA, monosegmented flow analysis; SIA, sequential injection analysis; MCFIA, multicommuted flow injection analysis; Tur, turbidimetry; Neph, nephelometry; RSD, relative standard deviation; SR, sampling rate; PVA, poly(vinyl alcohol).

determination was carried out on the solutions remaining in the donor stream. Analysis can be carried out within concentration range of 78–390 mg K L^{-1}.

The turbidimetric determination of potassium in fertilizers using Na-TPB in PVA was elected by Vicente et al.[44] to demonstrate the feasibility of exploiting a tandem stream with large initial slugs in a MCFIA system. Comparing with the other reported flow systems, sampling rate undergoes a remarkable increase because three samples are simultaneously processed inside the analytical path. Analysis can be carried out at a rate of 240 samples per hour between 6.0 and 60.0 mg K L^{-1}.

Nitrogen

In 1856, Nessler[45] introduced a reagent consisting of mercury (II) iodide and potassium iodide in alkaline solution for the qualitative and quantitative determination of ammonia. Since then, Nessler's reagent has been extensively referred as the most sensitive test for ammonia; however, it is only accurate if a number of conditions are carefully controlled. The turbidimetric FIA systems applied to nitrogen determination are summarized in Table 2.

A turbidimetric FIA system for the determination of ammonia in low concentrations using Nessler's reagent was first developed by Krug et al.[46] The method was based on the reaction between ammonia and Nessler's reagent with the formation of a brown precipitate measured at 410 nm. The effects of reagent composition, flow rate, temperature, and protective colloids in the FI system are discussed in detail. Both natural waters and soil extracts can be analyzed in the range 0.5–6.0 mg N-NH_4^+ L^{-1}.

In order to investigate the feasibility of isothermal distillation in flow injection analysis, Zagatto et al.[47] proposed a turbidimetric FI system with the Nessler reagent for the determination of total nitrogen in plant material. The merging zones approach was employed to add Nessler's reagent in a discrete way so as to avoid baseline drift, which happens when this reagent is added continuously,[48] and to diminish reagent consumption. The influence of surfactant, flow rates, alkalinity, ionic strength, collector stream pH, reagent concentration, and sample volume in ammonia distillation are discussed.

In 1997, Lima et al.[43] developed a turbidimetric FI system for the determination of total nitrogen and potassium in vegetable samples using a single spectrophotometer as detector. Sodium tetraphenylboron (Na-TPB) was used as precipitating agent and poly(vinyl alcohol) (PVA) as surfactant. Ammonium ions were withdrawn from the sample by diffusion of volatile ammonia from the donor to the acceptor. Total nitrogen determination was carried out on the solution in the acceptor stream after its injection into the turbidimetric flow path where the ammonium tetraphenylboron precipitation occurred. Analysis can be carried out within concentration range 87–430 mg N-NH_4^+ L^{-1}.

Phosphate

The majority of manual and automated methods for orthophosphate determination, in a great variety of samples, are based on the spectrophotometric determination of phosphorus as phosphomolybdenum blue.[49] Nevertheless, as an alternative to colorimetric procedures, different turbidimetric methodologies have been proposed (Table 2).

Burns et al.[48] developed a FI manifold with a mixing chamber for the determination of phosphate with molybdate and crystal violet. The insoluble blue dye salt is kept in colloidal solution with PVA and measured at 560 nm. The system was applied to the determination of phosphate in serum samples and after appropriate mineralization to organic compounds and to plant materials.

A simple, fast, and low-cost FIA system was proposed by Diniz et al.[49] for the turbidimetric determination of orthophosphate in digested plant material. The determination was based on the precipitation of orthophosphate with zinc in buffer medium (pH 6.0). PVA was added in all solutions as a colloidal protector in order to increase both sensitivity and reproducibility and consequently to reduce the washing time. Orthophosphate was determined in the concentration range from 5 to 60 mg P L^{-1} with an analytical frequency of 180 h^{-1}.

In 2001, Simonet et al. proposed two SIA systems for the turbidimetric determination of phosphate in urine samples.[50] One method was based on the calcium phosphate crystallization, and the other on the inhibitory action of phosphate on the calcium carbonate crystallization. As urine samples with high calcium content ($\geq$400 mg L^{-1}) can interfere in the method based on the calcium phosphate crystallization, a cation exchange resin was incorporated in the manifold. Phosphate could be determined within the range of 0.2–1.5 g L^{-1} and 0.1–1.8 mg L^{-1} for calcium phosphate and for the inhibitory method, respectively.

Chloride

The spectrophotometric mercury thiocyanate/iron (III) method has been largely used for chloride determination.[52] However, because this methodology requires the use of a highly toxic reagent, an effort to replace it has been recommended. As there are few other spectrophotometric methods for chloride determination, the turbidimetric procedure involving silver nitrate with the formation of silver chloride becomes attractive as it is environmentally less harmful and it is easily implemented in flow analysis, requiring similar instrumentation (Table 2).

Zaitsu et al.[51] were the first to propose a turbidimetric FI procedure for the determination of chloride in river water. The method was based on the turbidimetric measurement at 440 nm of a silver chloride suspension in nitric acid medium. A prior separation step involving ion-exchange was required. The method was applicable for chloride concentrations up to 14 mg L^{-1}.

In 1997, Sartini et al.[52] also presented a FI procedure involving the silver chloride precipitation for the automated turbidimetric determination of chloride in river waters up to 10 mg L^{-1}. For accuracy improvement, in-line cation exchange was accomplished by means of a resin minicolumn. Studies aiming at the inclusion of the approaches of crystal seeding and the addition of surfactants were also carried out.

Mesquita et al.[53] developed a SIA system using the silver chloride reaction for the turbidimetric determination of chloride in different types of water, where chloride concentration differs significantly. It was possible to determine chloride between 2 and 400 mg L^{-1} by simply changing the sample aspiration time. The novelty of this work when comparing with the previous FI applications is the possibility of the determination of chloride over a wide range of concentration, with a single system. In addition, a considerable saving of reagents is achieved due to noncontinuous consumption.

Zenki et al.[54] proposed a closed-loop FI system with turbidimetric detection for a repetitive determination of chloride. The system of recycling consists of a single manifold and is superior because of its simplicity, which is an advisable feature for routine purposes. The method was applied to the determination of chloride in tap, natural, and reference waters between 3.0 and 30 mg L^{-1}.

Total Carbon

The total organic carbon (TOC) is one of the most important parameters for acquiring knowledge about water and waste water quality because it concerns theoretically all organic compounds.[55] However, the determination procedure is complex and time-consuming. In order to develop a simple, robust methodology with higher analytical frequency, Paniz et al.[55] proposed a FI turbidimetric system with a gas–liquid transfer microreactor for the determination of TOC and its fractions in industrial effluent samples. Samples were decomposed into glass vials in a microwave oven, and a fraction of CO_2 was injected into a carrier gas and pumped to a glass microreactor. This device was specially developed to ensure a quantitative reaction with a barium hydroxide solution. The resulting suspension was removed from the microreactor, pumped to the flow cell, and the transient signal was recorded. With minor modifications, the system allows the determination of different carbon fractions. The dynamic range was 20–800 mg C L^{-1} and the maximum analytical frequency was 120 determinations per hour (Table 2).

Determination of Organic Substances

Organic substances (Table 3) can be determined turbidimetrically either as ion associates with voluminous organic dyes or metal chelates or as their chelates

Table 3. Application of turbidimetric and nephelometric flow methods to the determination of organic compounds

Analyte	Flow method	Sample	Reagent	Precipitate	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Levamisole hydrochloride	FIA	Pharmaceutical samples	HgI_4^{2-}	Ion-association complex	—	7–32 mg L^{-1}	0.9	80	[56]
Chlorhexidine	FIA	Pharmaceutical formulations	Thymol blue	Ion-association complex	—	10.5–63.0 mg L^{-1}	1.5	53	[57]
Diphenhydramine hydrochloride	FIA	Pharmaceutical preparations	Bromophenol blue	Ion-association complex	—	50–230 mg L^{-1}	0.3	51	[58]
Amitriptyline	FIA	Pharmaceutical formulations	Bromocresol purple	Ion-association complex	—	30–200 mg L^{-1}	1.4	39	[59]
Phenformin	FIA	Pharmaceutical preparations	Tungstate	Tungstate poly-anion	—	120–122 mg L^{-1}	0.8	67	[60]
Thiamine	FIA	Pharmaceutical formulations	Silicotungstic acid	$[Thi]_2[Si(W_3O_{10})]_4$	PEG	5.0×10^{-5} to 3.0×10^{-4} mol L^{-1}	<1	90	[61]
Homatropine methylbromide	FIA	Pharmaceutical preparations	Silicotungstic acid	$[Hom]_4[Si(W_3O_{10})]_4$	—	8.1×10^{-5} to 2.2×10^{-4} mol L^{-1}	<1.5	70	[62]
Cyclamate	FIA	Low-calorie soft drinks and artificial sweeteners	$BaCl_2$	$BaSO_4$	PVA	0.015–0.120% (w/v)	5.9	45	[63]
Dipyrone	FIA	Pharmaceutical formulations	Ag^+	Ag^0 colloidal suspension	—	5.0×10^{-4} to 2.5×10^{-3} mol L^{-1}	1.8	45	[64]
Dodecylbenzene sulfonic acid	FIA / SIA / neph	Commercial sample detergents	*o*-tolidine	Ion-association complex	—	1.6–300 mg L^{-1}	1.2–2.6	68/20	[65]

(*continued*)

Table 3. Continued

Analyte	Flow method	Sample	Reagent	Precipitate	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
L-lysine	FIA	Pharmaceutical preparations	L-glutamic acid	L-glutamic acid (inhibition assay)	—	0.5–20 mg L-lys L^{-1}	2.5		[66]
L-arginine and L-ornithine	FIA	Pharmaceutical preparations	L-histidine	L-histidine (inhibition assay)	—	0.2–12 mg L-arg L^{-1} 0.5–20 mg L-orn L^{-1}	2.3 L-arg 2.6 L-orn	7	[67]
L and D-aspartic acid	FIA	Pharmaceutical preparations; racemic sample of L and D-aspartic acid	L and D-histidine	L and D-histidine (inhibition assay)	—	3–40 mg L-asp L^{-1} 4–40 mg D-asp L^{-1}	2.1 L-asp 2.5 D-asp		[68]
D and L-glutamic acid	FIA	Pharmaceutical preparations; racemic sample of L and D-glutamic acid	L and D-histidine	L and D-histidine (inhibition assay)	—	Up to 40 mg L^{-1}	2.6–2.9		[69]
L and D-histidine	FIA	Synthetic samples	L and D-glutamic acid	L and D-histidine (inhibition assay)	—	5–100 mg L-his L^{-1} 8–100 mg D-his L^{-1}	3		[70]
Phytic acid	SIA	Food samples	Calcium oxalate	Calcium oxalate (inhibition assay)	—	0.05–0.6 mg L^{-1}	2.0	20	[71]

PEG, poly(ethyleneglycol); Thi, thiamine; Hom, homatropine; FIA, flow-injection analysis; MSFA, monosegmented flow analysis; SIA, sequential injection analysis; MCFIA, multicommuted flow injection analysis; Tur, turbidimetry; Neph, nephelometry; RSD, relative standard deviation; SR, sampling rate; PVA, poly(vinyl alcohol).

with metal ions. Turbidimetry, in most cases, avoids liquid/liquid extraction procedures and application of organic solvents. The methods are faster and simpler than the conventional methodologies.[40]

Nonkinetic Methods

Calatayud and Falco[56] developed a turbidimetric FI system for the determination of levamisole hydrochloride, a levo-isomer of tetramisole hydrochloride of the anthelmintic drug family, in pharmaceutical samples. The method is based on ion-association compounds and deals with quantification of levamisole with tetraiodomercurate (II) as precipitating agent. The usual extraction into an organic phase is avoided.

Chlorhexidine, a bactericidal drug, is a member of the biguanide family, several members of which are found in pharmaceutical formulations. Calatayud et al.[57] proposed a FI methodology with turbidimetric detection based on the formation of an ion pair between chlorhexidine and thymol blue that avoided the extraction step. Studies of chlorhexidine–dye and chlorhexidine–Cu(II) were carried out to determine the best precipitate for this determination.

Diphenylhydramine hydrochloride, usually found in many pharmaceutical preparations, is a conventional antihistaminic of the H_1 type (receptor antagonists) with pronounced sedative properties. It also has antiemetic, anticholinergetic, and local anesthetic properties. An ion associate of diphenylhydramine hydrochloride with bromophenol blue has been employed for the FI turbidimetric determination of diphenylhydramine in pharmaceutical preparations (tablets).[58] A single-channel manifold in which the sample solution was injected into the carrier–reagent stream was used, with a monitoring wavelength of 650 nm. In order to establish the most suitable precipitate for this determination, several diphenylhydramine–dye systems were evaluated. A number of interfering substances were also studied.

Amitriptyline is an odorless white powder with a bitter and burning taste. In 1990, Calatayud and Pastor proposed a FIA procedure with turbidimetric detection for the determination of amitriptyline hydrochloride in pharmaceutical preparations.[59] The method was based on the formation of an ion-association compound with Bromocresol purple, and liquid–liquid extraction was required.

Phenformin is a hypoglycemic drug used in the treatment of diabetes mellitus. Calatayud and Sampedro[60] developed a turbidimetric FI system for the determination of phenformin in pharmaceutical preparations. After studying some phenformin–counteranion compounds in order to determine the suitable precipitate, tungstate was selected as reagent. The method is based on the direct injection of the sample into a tungstate reagent stream and the subsequent detection of the formed white precipitate at 700 nm.

Thiamine (vitamin B_1) is a white crystalline powder, hygroscopic, and with a nutlike taste used clinically in the treatment or prevention of

beriberi. Costa-Neto et al.[61] developed a FI merging zones system for the turbidimetric determination of thiamine in pharmaceutical preparations. The proposed method was based on the precipitation of thiamine with silicotungstic acid in acid medium to form a precipitate in suspension (thiamine silicotungstate) that is determined turbidimetrically at 420 nm. An improvement of sensitivity, repeatability, and baseline stability of the FIA system was obtained by adding poly(ethylene glycol) as colloidal protector.

Later on, the same research group proposed another system for the determination of homatropine.[62] Antimuscarinic compounds are drugs that play an important role in the central nervous system. The most widely used are areatropine, scopolamine, homatropine, and homatropine methylbromide (HMB). A FI turbidimetric procedure exploiting merging zones for determining HMB in pharmaceutical preparations was proposed. The determination was based on the precipitation reaction of HMB with silicotungstic acid in acidic medium and the precipitate was measured at 410 nm.

Sodium and calcium cyclamates are additives widely used as non-nutritive sweetener in many diet and medicinal products. They are no longer permitted as a food additive in many countries including Canada, the United States, and in European countries due to their conversion to cyclohexylamine, which is a strong carcinogen. However, they are available in other countries as a sweetener. In 2005, Llamas et al.[63] proposed a FI turbidimetric in-direct method for determination of cyclamate in low-calorie soft drinks and artificial sweeteners without pretreatment. It was based on the oxidation of the sulfamic group, which is present in cyclamates, to sulfate by addition of nitrite. Then, a precipitate of barium sulfate was obtained by reaction with barium chloride, in presence of PVA in perchloric acid solution, at 30°C. The analytical signal was measured at 420 nm.

Dipyrone is a white crystalline powder, soluble in water and ethanol, which presents anesthetic and antipyretic properties. A FI procedure using a solid phase reactor with AgCl immobilized in a polyester resin was developed by Marcolino-Jr et al.[64] in 2005 for determining dipyrone in pharmaceutical formulations. The determination is based on the reduction of Ag^{+} ions of the solid phase reactor to Ag^{0} by dipyrone. A colloidal suspension of Ag^{0} produced is transported by carrier solution (0.01 mol L^{-1} NaOH) and turbidimetrically detected at 425 nm. The concentration of dipyrone injected is proportional to the quantity of Ag^{0} produced.

Simple light scattering methods (batch, FI, SI) for the determination of anionic active matter in detergents based on a novel reaction were reported by March et al.[65] in 2005. The methods were based on formation of a solid phase by association of anionic surfactants and protonated *o*-tolidine. Measurements were carried out with a conventional spectrofluorimeter at 400 nm, and dodecylbenzene sulfonic acid (DBS) was selected as the reference anionic surfactant. Influence of the main parameters affecting the characteristics of the methods was studied by the univariate method. The methods were applied to commercial samples and results successfully compared with a volumetric recommended method.

Kinetic Methods

Some organic substances act as crystallization inhibitors for organic molecules with similar chemical structures (or a slightly different bulk component of molecular crystal). The inhibitory effect can be assigned to selective interactions with the foreign molecule at specific points in the crystallizing substances that induce marked changes in the crystallization rate at very low inhibitor concentrations. These processes have found application in analytical chemistry, mostly in the determination of amino acids.[66]

Several studies concerning the determination of different amino acids using turbidimetric flow analysis methodology have been reported,[66–70] as alternatives to spectrophotometric, liquid chromatographic, and chemiluminometric or electrochemical detection.[66] The high selectivity and sensitivity of crystal growth inhibitory processes make these systems potentially useful for the enantiomeric resolution of inhibitory substances.[70]

Ballesteros et al.[66] developed a FI turbidimetric method for the discrimination of L- and D-lysine enantiomers by the inhibitory action of L-lysine on the crystallization of L-glutamic acid. A multidetection flow system including an open-closed loop and a single detector permits the determination of kinetic parameters for the crystallization of L-glutamic acid in the presence of 2-propanol. L-lysine can thus be determined in the presence of D-lysine concentration or other amino acids with no need for a prior separation. The proposed method was applied to the determination of L-lysine in pharmaceutical preparations.

A FI method for the determination of L-arginine and L-ornithine based on the inhibition of L-histidine crystallization was also presented by Ballesteros et al.[67] The open-closed system permits turbidimetric multidetection of the signal in the crystallization of L-histidine in the presence of an organic solvent (2-propanol). The proposed method permits the selective determination of L-arginine and L-ornithine in pharmaceutical preparations in the presence of their D-enantiomers and other L-amino acids without the need for a prior separation.

Hosse et al.[68] proposed a FI system for the enantiomeric discrimination of L- and D-aspartic acid that enables the turbidimetric multidetection of the signal produced in the crystallization of histidine from a supersaturated solution. The presence of L- and D-aspartic acid delays the growth of L- and D-histidine crystals, respectively, the delay being proportional to the concentration of aspartic acid. The method was applied to the determination of L-aspartic acid in pharmaceutical preparations and the resolution of a racemic sample of L,D-aspartic acid.

A FI turbidimetric method for the indirect determination of D- and L-glutamic acid by the inhibitory effect of these substances on the crystal growth of D- and L-histidine, respectively, in the presence of an organic solvent is proposed by Ballesteros et al.[69] This continuous method allowed the sequential determination of D- and L-glutamic acid in a multidetection flow system, including an open-closed loop and a single spectrophotometer.

The methodology was applied to the determination of L-glutamic acid in pharmaceutical preparations and the determination of D- and L-glutamic acid in a racemate of DL-glutamic acid.

In 1998, Rodríguez et al.[70] developed a FI turbidimetric method for the sequential determination of L- and D-histidine in synthetic samples, containing both enantiomers in variable concentration ratios. The method was based in the rate of crystal growth of L- and D-glutamic acid caused by the adsorption of foreign species (of L- and D-histidine, respectively) at a specific point of the crystal surface.

This kinetic-turbidimetric detection approach was also applied to the determination of acid phytic in food samples using a SI system.[71] The method was based on the diminution of the calcium oxalate crystallization reaction rate in the presence of phytic acid. Such a crystallization rate has been evaluated from the increase of turbidity with time.

Immunologic Reactions

The antigen–antibody interaction is a bimolecular association similar to an enzyme–substrate interaction, with an important difference: it does not lead to an irreversible chemical modification in either the antibody or in the antigen. The association between both involves various nonconvalent interactions. Antibody (precipitins) and the soluble antigen interacting in aqueous solution form a lattice that eventually develops into a visible precipitate.[72]

The first quantitative determination of proteins based on an immuniprecipitin reaction was reported by Heidelberger and Kendall in 1935. The current importance of the immunoprecipitin technique for the analysis of proteins has been emphasized by the development of an automated immunoprecipitin analyzer and the subsequent use of laser nephelometry to increase the sensitivity of the method. FIA provides an attractive high-speed, low-cost alternative to the existing instrumentation for the study of immunoprecipitin reactions[72] (Table 4).

Immunoprecipitation reactions using FIA with merging zones was applied to the determination of human serum immunoglobulin G (IgG) in serum samples and human IgG antiserum.[73–78]

A stop-flow merging zones FI system for monitoring the precipitin interaction between yeast mannan (the model antigen) and concanavalin A (the model antibody) was first developed by Worsfold[73] in 1983. In this paper, the suitability of the FIA for the study of biochemically specific interactions is also discussed.

In 1984, a study of a model immunoprecipitin reaction between concanavalin A and yeast mannan using a microcomputer-controlled stop-flow merging zones FIA manifold with turbidimetric detection was reported by Worsfold and Hughes.[74] The system described could be used routinely for immunoprecipitin analysis in clinical laboratories, IgG in human serum, and also to study kinetic aspects of such reactions.

Table 4. Application of turbidimetric and light-scattering flow methods to immunological methods and to the determination of biomass

Analyte	Flow method	Sample	Reagent	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Concanavalin A	FIA/tur		Yeast mannan	—	Up to 10.0 mg mL^{-1}	<5.3		[73]
Concanavalin A	FIA/tur		Yeast mannan	—	0.1–20.0 mg mL^{-1}		50	[74]
Antibody Ig G	FIA/tur	Human serum	Goat anti-human Ig G antiserum	—	0–3556 mg Ig G dL^{-1}	2.0–6.8	40	[75]
Antibody IgG	FIA/tur	Human serum	Goat anti-human Ig G antiserum	PEG	Up to 2844 mg dL^{-1}	<6	40	[76]
Monoclonal antibodies (mab)	FIA/tur	Fermentation of mouse–mouse hybridoma cells	Anti-mouse IgG	—	1–1000 mg L^{-1}	2		[77]
IgA	FIA/tur	Human serum	Sheep anti-human IgA	—	0.09–0.36 g L^{-1}		40	[78]
Pullulanase isoenzyme	FIA/tur	Fermentation of *Clostridium thermosulfurogenes*		—	10–1000 U L^{-1}	1.5		[79]
Antigen anti-A Mab, a monoclonal antibody of the IgG type	FIA/tur	Mammalian cell cultivation processes	Solution of the antibodies (anti-mouse IgG)	—				[80]

(*continued*)

Table 4. Continued

Analyte	Flow method	Sample	Reagent	Surfactant	Working range	RSD (%)	SR (h^{-1})	Ref.
Total prothrombinase complex (prothrombin, factor V, factor X_a, Ca^{2+}, phospholipids)	FIA/tur	Human plasma (venous blood)	Calcium thromboplastine	—	10–100% of total clotting activity	<2.8	50	[81]
Fibrinogen	FIA/LS	Human plasma	Ammonium sulfate and guanidine hydrochloride	—	1–20 mg L^{-1}	<1.33	80	[82]
Biomass	FIA/tur	Bacterial and yeast fermentation broth		—	15–4000 mg L^{-1}	0.95	90	[83]
Total biomass	SIA/tur	Unfiltered yeast fermentation broth		—	0.2–80 g L^{-1}	3		[85]
Biomass	FIA/tur	Microalga bioreactor		—		5.9		[86]

PEG, poly(ethyleneglycol); LS, light scattering; FIA, flow-injection analysis; MSFA, monosegmented flow analysis; SIA, sequential injection analysis; MCFIA, multicommuted flow injection analysis; Tur, turbidimetry; Neph, nephelometry; RSD, relative standard deviation; SR, sampling rate; PVA, poly(vinyl alcohol).

An immunological reaction between human serum immunoglobulin G (IgG) and goat anti-human IgG was developed using automated stop-flow merging zones FIA manifolds by Worsfold et al. Turbidimetric detection was used to monitor the rate of reaction.[74,75] Serum samples and human reference serum were analyzed and their IgG concentrations interpolated from a second-order fit[75] or from the linear[76] calibration data. In order to enhance the formation of large molecular aggregates and to increase the sensitivity, polyethylene glycol was introduced to the carrier stream.[76]

Freitag et al.[77] proposed a stop-flow merging zones FI system for real-time monitoring of specific proteins in fermentation processes. The method is based on the formation of aggregates between the proteins to be determined and their antibodies, with the subsequent turbidimetric determination. The analyzer was used to measure monoclonal antibodies produced in fermentations of mouse–mouse hybridoma cells and to quantify pullulanase isoenzymes produced in a fermentation of *Clostridium thermosulfurogenes*.

An automated merging zones FIA procedure for the determination of IgA in human serum via its interaction with sheep anti-human IgA was developed by Wang et al.[78] The FIA coupled with turbidimetric detection provided a precise, rapid, and simple system for the study of immunoprecipitin interaction.

An online assay for a thermostable pullulanase and antithrombin III is described by Freitag et al.[79] The assay is based on the formation of aggregates between the protein to be measured and the antibodies raised against the protein. A stop-flow merging zones FIA manifold was used to monitor pollulanase activity of *Clostridium thermosulfurogenes* cultures.

Hitzman et al.[80] used an assay with turbidimetric detection for the online or offline monitoring of mammalian cell cultivation. A FI system with merging zones and stop-flow approach was applied. Reference channel was also incorporated where no immunoreactant was supplied so that medium blank absorption could be assessed. The difference of peak high within the two channels was used to establish linear regression model and to calculate the sample concentrations.

Romero et al.[81] developed an automatic FI method for the evaluation of the hemostasy process based on the estimation of the extrinsic coagulation pathway (prothrombin, factor V, factor X_a, Ca^{2+}, phospholipids). A stop-flow merging zones manifold was proposed, and the clotting reaction rate was monitored at 340 nm.

A light-scattering method for the determination of fibrinogen in human plasma is presented by Silva et al.[82] The method is based on the analyte precipitation in the presence of ammonium sulfate in glycine hydrochloride buffer. The approach was developed by using a flow-injection manifold where the light scattered by the solid suspension formed was monitored in spectrofluorimeter with an incident wavelength of 340 nm.

Determination of Biomass

In order to make microbial processes most efficient, several parameters that give information about physical and chemical environment, as well as about growth and production, have to be determined continuously.[83,84] FIA is a very promising method for online process control, due to its versatility, the simplicity of experimental setup, low cost, and good reproducibility. The combination of suitable sampling devices with FIA systems is a prerequisite toward online control of bioreactor processes. It includes problem-orientated pretreatments of the sample and allows the application of FIA to the control of almost all kinds of bioreactors.[84] Biomass is a basic parameter in bioreactor operation that is often used as an indirect measure of product formation, subtract consumption, and process disturbances.[85,86] Traditional direct determinations by counting the cell number under the microscope or determining cell dry weight are both tedious and time-consuming and are not suitable for online bioprocess control.[84,85] The use of turbidity of the fermentation broth as analytical signal for bacterial and yeast fermentations biomass measurement is the usual method of noninvasive biomass estimation. The turbidimetric FI methods applied to biomass determination are summarized in Table 4.

An automated FI analyzer for measuring the concentration of biomass, glucose, and lactate during lactic acid fermentations was described by Benthin et al.[83] Biomass concentrations were determined by absorbance (turbidity) measurements. Traditionally, the absorbance of the broth is measured by continuously diluting the broth to the range of linear response. Despite automatic washing procedures, these analyzers are more or less liable to clogging and forming deposits on the optical surfaces. Applying the FI principles, these problems can be minimized. The sample was injected into a small stirred mixing chamber (MC) with subsequent detection at 565 nm. In the MC, rapid and reproducible dilution of the sample occurs, and consequently potential matrix effects from the viscosity of the fermentation broth are reduced. The analyzer is calibrated by injection of potassium permanganate standard solution and the absorbance values converted to biomass concentration (g cell dry mass L^{-1}) by a linear relationship between the measured absorbance and measured biomass concentration during batch fermentation.

In 1994, Baxter et al.[85] developed a SI system for the determination of total biomass from yeast (*Saccharomyces cerevisiae*) fermentation. The assay uses both turbidimetric (absorbance) and nephelometric measurements at a wavelength that is not absorbed by the liquid medium. In contrast with the FI system previously described, the biomass is determined without pretreatment or dilution of the original sample. The assay uses a SIA system to sample a precise volume of biomass obtained from the bioreactor and to deliver it to a flow cell where it is quickly mixed and the analytical signal detected.

A FI system for the online determination of biomass in a microalga (*Pavlova lutheri*) bioreactor was developed by Meireles et al.[86] The device

was fully computerized and was based on diluting small aliquots of the culture followed by measuring optical density (turbidity); this figure was then accurately correlated with biomass, in terms of both cell number and ash-free dry weight, during the entire culture time. The growth rate and biomass productivity of *P. lutheri*, cultivated under batch and semicontinuous modes, were monitored as experimental testing model.

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