in pH within the sample segment is sufficient for precipitation of $BaSO_4$ at simultaneous continuous rinsing of the flow system with EDTA.

In FIA turbidimetry, satisfactory results have also been obtained for the precipitation of colloidal $PbSO_4$ in the determination of sulphate [340] and $NH_{n-1}Hg_2I_n$ in the determination of ammonia [342].

The addition of an on-line-produced suspension of lead phosphate has been used to improve the rate of crystal growth in a turbidimetric FIA system for the determination of total sulphur by lead sulphate precipitation [341]. This accelerates the turbidimetric process, permits the use of more dilute reagents, and enables more efficient system washing.

In the determination of chloride by precipitation as silver chloride in the system shown in Fig. 7B, light reflectance by the precipitate is used as the method of detection instead of turbidimetry [343]. The flow cell used for this purpose is shown in Fig. 6B. Such an approach generally increases the dynamic range of detection when compared with turbidimetry.

5. Molecular Luminescence Detection Methods

The use of luminescence detection techniques in chemical analysis is based on the formation from the analyte, or with participation of the analyte, of an excited species whose emission spectrum provides analytical information. If the excited state species are formed by the absorption of photons, the subsequent emission of radiation is called *fluorescence*, or more generally *photoluminescence*, for a wide range of electronic transitions. Analytical detection based on fluorescence is called *fluorimetry*. If excited species that emit radiation are formed chemically, the process of emission is described as *chemiluminescence*. In both cases, the emitted radiation allows analytical detection at levels that are one to three orders of magnitude smaller than those usually obtained in molecular absorption spectroscopy. The number of developed conventional methods using fluorescence is much larger than for those using chemiluminescent procedures. Both of these luminescent detection methods usually exhibit a much wider range of response and better selectivity than by absorption detection. Instrumentation for luminescent detection, including flow measurements, is widely available, so these techniques are finding an increasing number of applications in FIA [345].

5.1. *Fluorimetric Detection*

A molecule excited by the absorption of photons can return to its ground state by various deactivation steps, one of which is fluorescence, where the

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emission of radiation takes from 1 ns to 1 μs. Both the structure of the analyte molecule and chemical conditions affect fluorescence. It is most commonly associated with aromatic functional groups with $\pi \rightarrow \pi^*$ transitions, for example, most unsubstituted aromatic hydrocarbons exhibit fluorescence. It is favoured by increasing the rigidity of molecules; so, for example, the immobilisation of fluorescent dyes results in enhanced emission, while the fluorescent intensity of certain chelatin agents increases when they form complexes with metal ions. At sufficiently low concentration of the emitting species, the fluorescent power is a linear function of concentration. Fluorimetric detection is widely applied in the direct determination of various organic compounds. In determinations of inorganic species, the formation of a fluorescent chelate or the diminution of fluorescence resulting from the quenching action of the analyte is utilised.

In FIA fluorimetry, the direct detection of fluorescent analytes is also employed, for example in the determination of four phenothiazine derivatives, where the same excitation wavelength is used but emission is measured at wavelengths characteristic for each analyte [346]. The use of a non-selective reagent for derivatisation, such as *o*-phthaldialdehyde, gives a fluorimetric signal in an FIA system for various amines [347, 348] and amino acids [347, 349]. The use of the same reagent in reaction with sulphite allows the detection of ammonia nitrogen with considerable selectivity over amino acids [350].

In most common procedures, a selective reaction of the analyte that produces a fluorescent product is performed in a simple two- or three-line manifold. Such procedures have been developed, for instance, for the determination of formaldehyde [351], thiamine [352], boron [353], sulphate [354] and thiourea [355]. A list of the numerous other reactions employed is shown in Table 7. In certain cases, for example in the determination of sulphate based on the formation of a ternary complex between sulphate, zirconium and calcein, the method works best after separation of the analyte from its matrix [354]. In the FIA determination of gallium with lumogallion an increase in the fluorescence signal is obtained with the addition of the surfactant polyethyleneglycolmonolauryl ether [356]. Several determinations in simple systems are based on kinetic effects. Trace determination of fluoride is based on its ability to increase the rate of formation of a fluorescent Al(III)-eriochrome red B complex in the presence of hexamethylenetetramine [357]. Catalytic-fluorimetric procedures have been developed for the determination of copper(II) [358], iodide [359] and vanadium (V) [360].

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Table 7. Applications of molecular fluorescence detection in FIA.

Analyte	Reagent used or catalysed reaction	λ, nm (excitation, emission)	Concentration range	Reference
Adrenaline	Iodine	350, 510	1–25 ppm	373
Al(III)	Acid alizarine garnet R	366, 460	0.013–7 ppm	362
	Lumogallion, Brij-35	484, 552	0.15–15 nM	364
	Salicylaldehyde picolinoylhydrazone	383, 468	1–90 ppb	380
Albumin	Sodium 8-aniline-1-naphthalene sulphonate	370, 470	349	
Amino acids	*o*-phthaldialdehyde	360, 550	0.01–10μM	347
Amines	*o*-phthaldialdehyde,2-mercaptoethanol	340, 432	10–50 μM	348
Ammonia	*o*-phthaldialdehyde, sulphite	360, 420	0.02–20 μM	350
B	Chromotropic acid	313, 350	0.005–100 μM	359
Be(II)	3-hydroxy-2-naphthoic acid	355, 455	0.08–0.78 ppb	363
Berberine	—	355, 517	0.004–1 μM	371
Ca, Mg	Calcein	300, 515	1–50 μM	374
Ce(III), Ce(IV)	—	260, 350	30–500 ppb	379
Coumarins	—	350, 418	1–23 μM	375
Cu(II)	2,2′-dipyridyl ketone hydrazone, O_2	350, 429	8–300 ppb	358
	2,2′-dipyridyl ketone hydrazone, H_2O_2	350, 427	0.2–300 ppb	369
C(II), Hg(II)	dipyridyl ketone phenylhydrazone, H_2O_2	350, 430	1–6 ppm	377
Cyanide	Pyridoxal	355, 435	0.1–20 ppm	368
Eu(III)	Thenoylfluoroacetone, trioctylphosphine oxide	352, 613	1.5–150 ppb	381
Fe(III), Mn(II)	Salicylaldehyde thiosemicarbazone, H_2O_2	357, 437	40–600 ppb	378
Fluoride	Zr(IV), calcein blue	323, 438	0.2–20 ppb	367
	Al(III), eriochrome red B	470, 595	0.01–3.8 ppm	357
Formaldehyde	2,4-pentanedione, ammonium acetate	410, 480	0.1–330 μM	351
Ga(III)	Lumogallion	490, 520	22–108 mM	356
			4–87 μM	370

continued

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Table 7 (*continued*)

Analyte	Reagent used or catalysed reaction	λ, nm (excitation, emission)	Concentration range	Reference
Glycine	*o*-phthaldialdehyde	337, 455	0.002–8000 ppb	349
Iodide	As(III), Ce(IV)	254, 350	3–400 nM	359
Kynurenic acid	—	370, 465	0.01–6 μM	361
Metal ions	8-quinolinol-5-sulphonic acid	369, 520	0.1–10 μM	376
Nitrate	Copperized Cd, 3-amino-1,5-naphthalenedisulphonic acid	365, 470	0.01–200 μM	366
Oxalate	Zr(IV), flavonol	350, 480	10–360 μM	382
Paracetamol	$Fe(CN)_6^{3-}$, N, N'-dimethylformamide	331, 427	0.04–17.6 ppm	372
Phenothiazine derivatives	—	310–360, 382–523	0.01–4 ppm	346
Sulphate	Zr(IV), calcein	410, 505	0.8–25 ppm	354
Thiamine	Hg(II)	370, 465	0.2–7 μM	352
Thiourea	Ta(III)	227, 419	0.5–10 μM	355
V(V)	Sodium 4,8-diamino-1,5-dihydroxyanthraquinone-2,6-disulphonate	524, 582	0.2–2 ppm	360
Zn	*p*-tosyl-8-aminoquinoline	377, 495	0.01–500 nM	365

Background fluorescence in the analysis of natural samples can be eliminated by the separation of the analyte from the matrix by solid-phase extraction on an appropriate sorbent. This procedure has been employed, for example, in the determination of urinary kynurenic acid [361]. Discrimination against riboflavin in the fluorimetric kinetic determination of Al(III) has been obtained in a microcomputer-controlled FIA system, where individual peak profiles are corrected for dispersion by comparing signals in a non-reacting reference solution and a reacting sample solution [362].

On-line columns have been used in FIA fluorimetry for the preconcentration of trace amounts of Be(II) [363] and Al(III) [364], and also to separate zinc from interfering alkali and alkaline earth metal ions and to concentrate zinc from sea water prior to the reaction with the organic indicator ligand *p*-tosyl-8-

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aminoquinoline [365]. A copperised cadmium column has been used to reduce nitrate on-line prior to its reaction with naphthalenedisulfonic acid to give a fluorescent product [366].

Several different manifolds, including a reversed FIA system, have been compared for the trace determination of fluoride based on the formation of a fluorescent ternary complex with Zr(IV) and calcein blue [367]. For process monitoring, a system with continuous sample aspiration was found to be most suitable. An increase in the sensitivity of FIA measurements can be obtained using a stopped-flow procedure. This has been employed in the determination of cyanide based on its reaction with pyridoxal and pyridoxal-5-phosphate [368], and in a Cu(II) determination based on its catalytic effect on the 2,2′-dipyridylketone hydrazone/hydrogen peroxide reaction [369]. This method is not free of interferences, but the use of the stopped-flow technique essentially lowers their effects. An increase in sensitivity and selectivity can also be obtained through the on-line use of solvent extraction. This has been utilised in the determination of gallium with lumogallion [370], and for determining the herbal medicine berberine, which yields a much stronger fluorescence intensity in organic solvents than in water [371].

Fluorimetric FIA determinations of pharmaceutical analytes have been satisfactorily carried out in the systems with on-line reactors packed with various reactive solid phases. Hexacyanoferrate(III) retained on an anion-exchange resin is used for the oxidation of paracetamol, whose oxidation product is reacted with N,N′-dimethylformamide in order to enhance the fluorescence [372]. In a similar manner an open-tubular PVC reactor with iodine impregnated on the inner wall is employed in the determination of adrenaline [373].

Various strategies are applied in multicomponent determinations with fluorimetric detection. The above-mentioned determinations of four phenothiazine derivatives were based on the measurement of emission at different wavelengths [346]. The determination of traces of calcium and magnesium can be based on the fluorescence of the calcein complexes after the separation of analytes from their rare earth ion matrix on an on-line ion-exchange column [374]. To discriminate between calcium and magnesium, 8-quinolinol is used as a masking agent for magnesium and EGTA for calcium. The effects of pH have been utilised in the determination of mixtures of coumarins [375] and metal ion mixtures [376]. In the former system, scopoletine and umbelliferone are determined in an FIA system at two different pH values with a double-injection valve. The analytes exhibit pH-dependent excitation spectra, so they can be

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determined individually at the same wavelength using simultaneous injections of analyte mixture and different buffers. Ternary and quaternary mixtures of zinc, cadmium, lead, magnesium and aluminium can be analysed through the formation of fluorescent 8-quinolinol-5-sulphonic acid complexes at different pH. A pH gradient created in an FIA system has been used for this purpose, with the fluorescence-time scans produced being processed using a partial least squares algorithm [376].

Two-component determinations of metal ion mixtures have been developed in FIA fluorimetric systems using catalytic effects. Determinations of Cu(II) and Hg(II) have been based on their catalytic effects on the oxidation of various hydrazones. The system is used in stopped-flow mode, with two sample segments being merged before the detector after undergoing different chemical reactions (Fig. 8) [377]. Sequential and differential catalytic-fluorimetric methods have also been developed for the simultaneous determination of Mn(II) and Fe(III) based on their catalytic action on the oxidation of salicylaldehyde thiosemicarbazone by hydrogen peroxide [378].

Native cerium(III) fluorescence has been exploited for the simultaneous determination of Ce(III) and Ce(IV) in an FIA system with a zinc reductor minicolumn and sample splitting between two branches of the manifold [379].

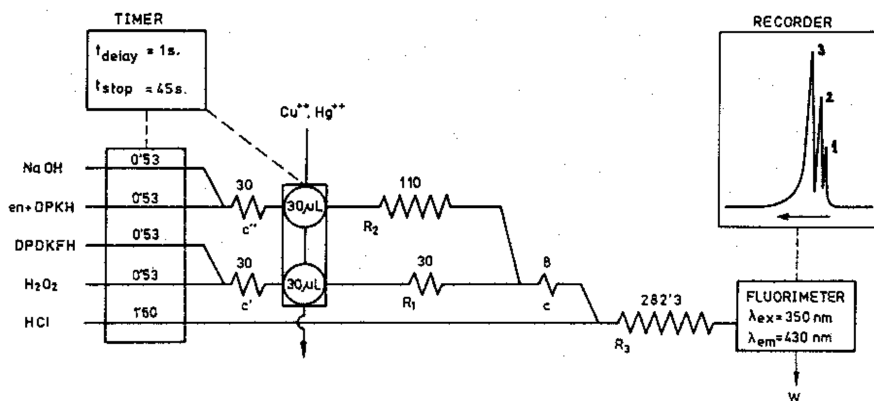

Fig. 8. Flow injection manifold used for the simultaneous catalytic-fluorimetric determination of copper and mercury [377]. The flow rates are given in ml.min^{-1}, reactor lengths in cm. DPKH — 2,2′-dipyridyl ketone hydrazone; DPDKFH — dipyridylketone phenylhydrazone; 1 — baseline; 2 — Cu(II); 3 — Hg(II). (*Reprinted by permission of copyright owner.*)

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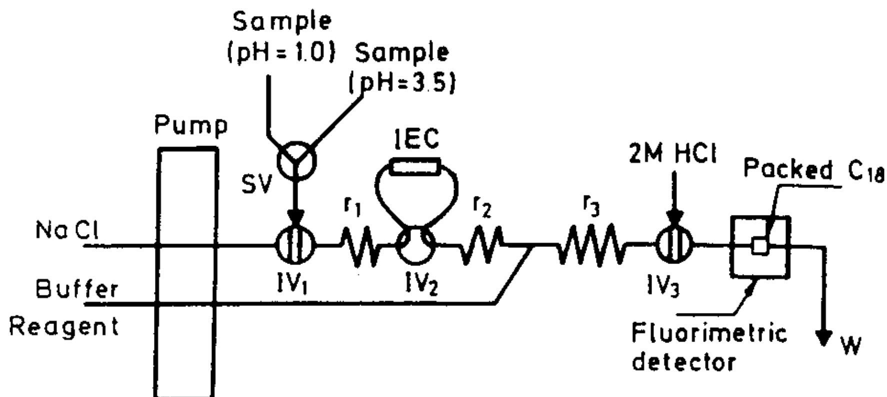

Fig. 9. Manifold for the fluorimetric speciation of aluminium based on the formation of the fluorescent Al-salicylaldehyde picolinohydrazone complex [380]. SV — switching valve; IV — injection valve; r — reactors; IEC — ion-exchange column; W — waste. (*Reprinted by permission of copyright owner.*)

A fluorimetric system with a C18 sorbent packed in a flow cell has been developed for the speciation of five different forms of aluminium [380] (Fig. 9). By adjusting the injected sample to different pH values and using an anion-exchange column, three forms of aluminium (i.e. acid reactive aluminium, total monomeric aluminium and non-labile monomeric aluminium) are determined and two other forms can be calculated. Table 7 lists developed applications of FIA fluorimetry.

5.2. *Chemiluminescence Detection*

Instrumentally, chemiluminescence measurements are simpler than fluorimetric ones, as the only sources of light are species excited by suitable chemical reactions. No wavelength-restricting devices are necessary, and the detector consists of a suitable flow cell and a photomultiplier tube. The simplicity of instrumentation, the good sensitivity of detection and the large number of known chemiluminescent systems have led to many applications of this type of detection being developed for FIA systems. Added to these advantages is the ability to design detectors and systems for solution manipulation which are specifically for chemiluminescent detection.

The most frequently used reagent for chemiluminescence detection in FIA is luminol (5-amino-2,3-dihydro-1,4-phthalazine dione), which, when oxidised

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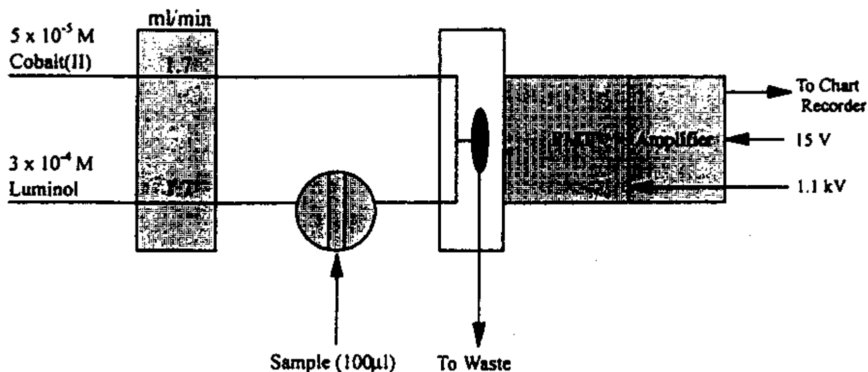

Fig. 10. Flow injection manifold for the chemiluminescence-based determination of hydrogen peroxide [383]. The distance from the injection valve to the zone merging point is 20 cm and the distance from the point of merging to the flow cell is 2.0 cm. (*Reprinted by permission of copyright owner.*)

to 3-aminophthalate, produces blue chemiluminescence. Some transition metal ions, and also a number of organic species, have a catalytic or an inhibiting effect on the luminol reaction with hydrogen peroxide or oxygen, and this is employed for analytical determination. Luminol, in the presence of Cu(II) [382] or Co(II) [383], can be used for the very sensitive detection of hydrogen peroxide. The simplest system used for the determination of H_2O_2 in sea water is shown in Fig. 10. Several procedures have been developed for the determination of transition metal ions. Determinations of Co(II) [383] and Fe(II) [384] are based on the catalytic effect of these ions on luminol oxidation by hydrogen peroxide. In the latter case, additional on-line preconcentration on 8-quinolinol column is employed. The same effect has been utilised in the determination of gold as tetrachloroaurate(III) in a reversed micellar system [385]. Determinations of Zn and Cd have been based on their inhibition of the cobalt-catalysed generation of chemiluminescence from luminol [387]. In determinations of inorganic anions, luminol has been used in procedures reported for bromide [388], nitrate [389] and nitrite [390]. The determination of bromide is based on the homogeneous catalysis of the bromine generated from the bromate-bromide-acid decomposition of hydrogen peroxide, which is necessary for luminol chemiluminescence. The nitrate determination is carried out in the system with on-line photoactivation to give peroxonitrite, which oxidises luminol. In all procedures for the determination of anions, cation-exchange columns are used for the removal of traces of transition metal ions.

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The selective determination of chlorine dioxide in an FIA system with a gas-diffusion module has been based on the oxidation of luminol with hydrogen peroxide as a catalyst [212, 391]. A determination of protein is based on the measurement of the decreasing catalytic activity of Cu(II) in the reaction between luminol and hydrogen peroxide [392]. FIA systems with a gas-diffusion step and chemiluminescence detection have been developed also for determinations with reagents other than luminol. The simultaneous determinations of nitrate and nitrite are carried out in a setup where nitrogen oxide formed by the reduction of analytes produces chemiluminescence by reacting with ozone [393]. Selectivity of detection is obtained by selecting appropriate reducing agents. The selective determination of chlorine in a system with a gas-diffusion unit has also been reported, and is based on the reaction of hypochlorite ion with 2,4,5-triphenylimidazole [394].

A simpler design for chemiluminescence FIA systems can be achieved by using immobilised reagents. Two such systems have been developed for the determination of hydrogen peroxide, using the immobilised fluorophore 3-aminofluoranthene [395], or bis(2,4,6-tri-chlorophenyl)oxalate packed into a bed reactor [396]. In the first case, the detection is based on the chemiluminescent compound 1,1′-oxalyldiimidazole, and the immobilised compound serves as an active intermediate, which is excited to the first singlet state. In the second case, the immobilised peroxyoxalate is a chemiluminescent reagent, and detection is carried out in the presence of perylene as sensitiser. A lower limit of detection (6 nM) is obtained for the second system.

Apart from the above-mentioned determination of nitrate and nitrite, the speciation determination with chemiluminescence detection has also been developed for iron [397]. This detection method is based on the reaction of brilliant sulphoflavin with hydrogen peroxide and Fe(II). In the FIA system, iron is preconcentrated on a cation-exchange column, and the determination of total iron is carried out after the reduction of Fe(III) with ascorbic acid.

In addition to the use of luminol and peroxyoxalate, several applications in FIA chemiluminescence have been reported for lucigenin. Luminescence is produced by the addition of either hydrogen peroxide or an organic reducing agent to an alkaline solution of lucigenin. FIA systems have been reported for the determination of reducing sugars [398] and ascorbic acid [399]. In the determination of sugars, the chemiluminescence is significantly enhanced when the analyte is incubated with sodium periodate prior to the reaction with lucigenin. The determination of ascorbic acid is carried out in a merging

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zones configuration with a cation-exchange column for the removal of transition metal ions and a photoreactor. Detection is based on the chemiluminescent reaction of lucigenin with the products from the photooxidation of the analyte, sensitised by toluidine blue.

Significant changes in chemiluminescent reactions can be caused by the use of ordered surfactant assemblies such as micelles, reversed micelles or bilayer aggregates. This has been employed in the determination of total chromium using flavin mononucleotide chemiluminescence [400]. After oxidation by hydrogen peroxide, this system exhibits chemiluminescence in the presence of Cu(II) and Cr(VI). The use of cationic surfactant micelles decreases the signal for Cu(II), but not for Cr(VI), and this is exploited in the determination of chromium.

Several other examples of FIA chemiluminescence systems are included in Table 8.

Table 8. Applications of chemiluminescence detection in FIA.

Analyte	Reagents	λ, nm	Detection limit	Reference
Ascorbic acid	Lucigenin, toluidine blue	440	0.2 nM	399
Au(III)	Luminol, hexadecyltrimethylammonium chloride		0.01 ppb	386
Bromide	Luminol, bromate	417	0.0625 ppb	388
Cd, Zn	Luminol, Co(II)	425	3 (Cd); 5 (Zn) ppb	387
Chlorine	Rhodamine GG		0.1 μM	401
	2,4,5-triphenylimidazole		75 ppb	394
Chlorine dioxide	Luminol, H_2O_2		5 ppb	391
Co(II)	Gallic acid, H_2O_2	643	0.04 ppb	402
		643	8 pM	403
	Luminol, H_2O_2	425	0.01 ppb	384
Cr(III), Cr(VI)	Flavin mononucleotide, H_2O_2, tetradecyldimethylbenzylammonium chloride		50 nM	400
Cyanide	Uranine, didodecyldimethylammonium bromide		1.0 ppb	404

continued

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Table 8 (*continued*)

Analyte	Reagents	λ, nm	Detection limit	Reference
Dansylalanine	Bis(2,4-dinitrophenyl)-oxalate, H_2O_2		0.5 nM	405
Fe(II)	Luminol, H_2O_2		0.1 pM	389
Fe(II), total Fe	Brillant sulphoflavin, H_2O_2		0.45 nM	397
H_2O_2	Luminol, Co(II)		10 nM	382
	Luminol, Co(II)	440	5 nM	383
	1,1′-oxalyldiimidazole, 3-aminofluoranthene		10 nM	395
	Bis(2,4,6-trichlorophenyl) oxalate, perylene		6 nM	396
Hydrazine	Hypochlorite, Ni(II)		0.5 nM	406
Morphine	Permanganate, tetraphosphate	430	0.1 nM	407
Nitrate	Luminol		70 nM	389
Nitrate, nitrite	TI(III), iodide, ozone		0.7 ppb (NO_3^-) 0.35 ppb (NO_2^-)	393
Nitrite	Luminol, H_2O_2	454	1 nM	390
Proteins	Luminol, Cu(II), H_2O_2		0.1 ppm	392
Pyrogallol	Periodate, hydroxylamine	455	0.1 μM	408
Steroids	Lucigenin			398
	Ce(IV), sulphite		13–19 ppm	409
Sugars	Lucigenin, periodate		1 μM (glucose)	398
Sulphide	Fluoresceine, hypochlorite	520	40 ppb	384
Sulphite	Permanganate, riboflavin phosphate	510	90 ppb	410
Surfactants, non-ionic	Hypochlorite, rhodamine B		3 ppm	411

6. Other Molecular Spectroscopic Detection Methods

The advantages of flow injection methodology has led to its widespread application in analytical procedures involving less common spectroscopic

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