Review

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Chemiluminescence and electrochemiluminescence detection of controlled drugs

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We review the determination of various controlled drugs (opioids, tranquilizers, stimulants, and hallucinogens) using flow-analysis methodologies (flow injection analysis, high performance liquid chromatography, capillary electrophoresis, and microfluidic devices) with chemiluminescence and electrochemiluminescence reagents such as luminol, diaryloxalates, tris(2,2′-bipyridine)ruthenium(II), permanganate, manganese(IV), and sulfite, for industrial, clinical, pharmaceutical, and forensic science applications.

Keywords: chemiluminescence; illicit drugs; controlled substances; narcotics; peroxyoxalate

Introduction

Chemiluminescence is the emission of ultraviolet (UV), visible, or infrared light from an electronically excited intermediate or product of a chemical reaction.[1–9] Once the excited state is reached, the emission process is identical to other modes of luminescence and for practical purposes can be considered instantaneous. The generation of the emitter, however, is dependent on the physical processes of solution mixing and the kinetics and thermodynamics of the chemiluminescent reaction. Flow-based analytical techniques provide an ideal means to reproducibly merge samples (or separated sample components) with chemiluminescence reagents and present the reacting mixture to a photodetector at the time of the greatest emission intensity. The optimal designs of chemiluminescence detectors for flow injection analysis (FIA),[9–11] high performance liquid chromatography (HPLC),[12–15] and capillary electrophoresis (CE)[16–18] have been previously described. A tutorial on the construction of chemiluminescence detectors for FIA and HPLC has also been published in this issue of *Drug Testing and Analysis*.

Electrochemiluminescence (or electrogenerated chemiluminescence) – the emission of light from a chemical reaction that involves at least one species generated at an electrode surface[19–25] – can provide the sensitivity and selectivity of chemiluminescence, with the advantage of precise spatial and temporal control, because the emission of light is often concentrated close to the electrode and may be regulated by varying the applied potential. Furthermore, the active form of some reagents can be regenerated at the electrode, allowing each luminophore to emit many times, which enhances sensitivity. The main disadvantages of electrochemiluminescence are the greater complexity of the detection cell (due to the need to incorporate two or three electrodes) and the possibility of electrode fouling.[9,24,26] However, these issues have been somewhat overcome by the use of inexpensive screen-printed electrodes that have the working, auxiliary, and reference electrodes printed on a disposable substrate.[27]

The instrumentation used to measure the light emitted from chemiluminescent or electrochemiluminescent reactions is relatively simple and inexpensive, but highly sensitive. Limits of detection in the range 10^{-11} M to 10^{-7} M have been reported for a wide variety of important analytes, including many that do not possess a native chromophore or fluorophore. Chemiluminescence is generally more selective than other spectroscopic modes of detection, due to the limited number of compounds that produce a significant emission of light with any particular reagent. Due to these attributes, some of the areas in which chemiluminescence detection has been found to be particularly useful include: simple FIA procedures for process monitoring or rapid preliminary screening; applications requiring high sensitivity at low solution volumes (e.g. CE and microfluidic devices); and in HPLC when the target analytes are present at concentrations below the limits of conventional absorbance or fluorescence detection.

In this review, we discuss the determination of various controlled drugs using flow analysis (FIA, HPLC, CE, and microfluidic devices) with chemiluminescence and electrochemiluminescence detection. It should be noted that this review does not cover chemiluminescence detection for immunoassay and related approaches,[28–34] or gas-phase chemiluminescence detection[35,36] (e.g. chemiluminescence nitrogen detection (CLND)) for supercritical fluid chromatography[37] or HPLC,[38–40] which has been utilized to determine a wide range of street

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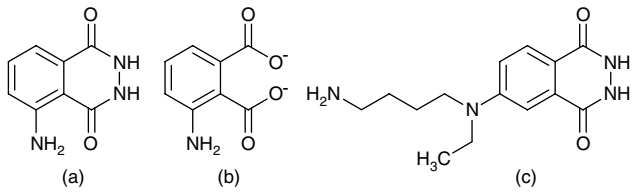

Figure 1. (a) Luminol, (b) 3-aminophthalate dianion, and (c) *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI).

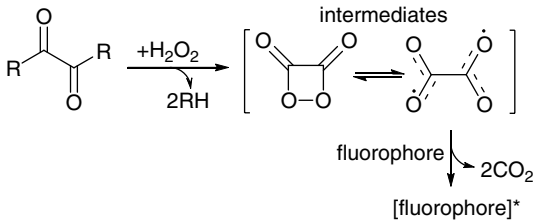

Figure 2. Simplified scheme for peroxyoxalate chemiluminescence.

drugs[39] and pharmaceuticals[40] in complex sample matrices without primary reference standards.

Chemiluminescence and Electrochemiluminescence Reactions

Of the many chemiluminescence reactions that are known, relatively few are commonly utilized for analysis. The following description is limited to reagents that have been used for the detection of controlled drugs using flow-analysis methodology.

Luminol

First reported by Albrecht in 1928,[41] the oxidation of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Figure 1a) in alkaline solution produces blue light (λ_{max} = 425 nm). Under suitable conditions, the reaction is quite long-lived and intense, which is aptly illustrated by its most well-known application: the visualization of latent blood at crime scenes.[42] Like a range of other metal ions and complexes, the haemoglobin in the blood catalyses the oxidation of luminol by hydrogen peroxide.

In the laboratory, luminol has been used to detect a huge range of organic and inorganic species that act as oxidants, catalysts, enhancers, or inhibitors of the light-producing reaction.[43–46] Alternatively, analytes can be detected after derivatization with luminol analogues, such as *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI; Figure 1c).[47] It is generally accepted that the reaction mechanism involves addition of oxygen (as hydrogen peroxide or superoxide) and expulsion of nitrogen to produce an electronically excited 3-aminophthalate anion (Figure 1b) that emits light to return to its ground electronic state, but the exact pathway is dependent on the reacting species and chemical conditions.[42,47–49]

Peroxyoxalate systems

The light-producing reactions of various oxalates and oxamides with hydrogen peroxide in the presence of fluorescent compounds are known as 'peroxyoxalate' chemiluminescence (Figure 2).[5,15,50] The first example of this class of reactions (involving oxalyl chloride, hydrogen peroxide, and compounds such as 9,10-diphenylanthracene) was reported by Chandross in 1963.[51] Peroxyoxalate reactions are thought to involve electron transfer from the fluorophore to an oxalate biradical intermediate, which subsequently decomposes into CO_2 and $CO_2^{\bullet-}$. The carbon dioxide radical anion can then transfer an electron back to the fluorophore at a higher energy level, leading to the emission of light.[52]

The most effective peroxyoxalate systems therefore combine an oxalate or oxamide that produces relatively high yields of the key intermediate with an efficient fluorophore in organic solvents. Some of the more commonly used reagents are shown in Figure 3. The reaction between oxalates/oxamides and hydrogen peroxide is subject to interrelated general-base and nucleophilic catalysis, for which imidazole is particularly effective.[50]

Peroxyoxalate chemiluminescence has been applied to the detection of hydrogen peroxide and various fluorescent compounds, and extended to other compounds (such as amphetamines[53,54] and tryptamines[55]) using derivatization with efficient fluorophores.[5]

Tris(2,2′-bipyridine)ruthenium(II)

In 1966, Hercules and Lytle reported their observations of orange chemiluminescence from the reduction of tris(2,2′-bipyridine)ruthenium(III) ($Ru(bipy)_3^{3+}$),[56] but the authors themselves credit Jean P. Paris as the first to observe chemiluminescence from a ruthenium chelate in 1962.[56,57] The ruthenium(III) oxidation state of the reagent has limited stability in aqueous solution and therefore is normally prepared from $Ru(bipy)_3^{2+}$ with oxidants such as lead dioxide or cerium(IV) shortly prior to use (Figure 4). Subsequent reaction with a suitable reducing agent produces an emission of light that matches the characteristic photoluminescence of $Ru(bipy)_3^{2+}$.

The single-electron oxidation of $Ru(bipy)_3^{2+}$ can also be performed at an electrode surface either prior to or at the point of detection.[20,22,23,26,57] In the presence of excess 'co-reactant' (such as tripropylamine), the same ruthenium complex can be oxidized and reduced many times and therefore emit many photons of light. This approach, which provides highly sensitive detection of molecules labelled with $Ru(bipy)_3^{2+}$ derivatives, has been successfully employed for a variety of commercial immunodiagnostic applications.[29]

$Ru(bipy)_3^{2+}$ (with chemical or electrochemical oxidation) is a useful reagent for the detection of certain aliphatic amines[6,57] and various other organic[6,57] or inorganic[58] species. In general, the emission intensity from the reaction with amines increases in the order primary < secondary < tertiary, but subtle differences in chemical structure can have a dramatic effect. One class of analyte that has received particular attention with this reagent is the *Papaver somniferum* (opium poppy) alkaloids and their semi-synthetic derivatives.[59] Greenway *et al*. examined the electrochemiluminescence determination of four analytes from this class and found that the limits of detection for the non-phenolic compounds codeine, dextromethorphan and heroin (Figures 5b, 5c, and 5d) were three orders of magnitude superior to that for the phenolic morphine (Figure 5a).[60] This was initially attributed to the coupling reaction of morphine to form pseudomorphine,[60] but later studies suggested other mechanisms for quenching by phenolic compounds.[61,62] Investigations into the relative chemiluminescence response of a range of *P. somniferum* alkaloids with $Ru(bipy)_3^{3+}$ (Table 1) have shown that the

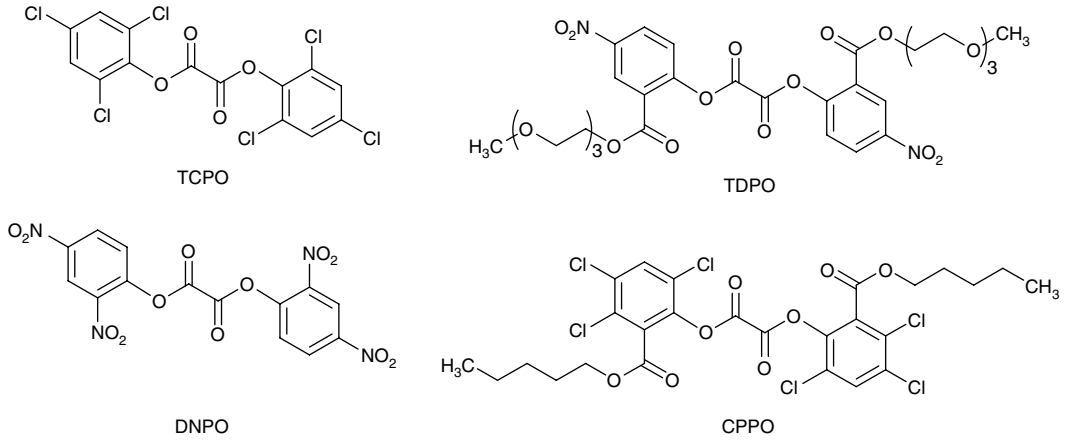

Figure 3. Some peroxyoxalate chemiluminescence reagents used for the detection of controlled drugs: bis(2,4,6-trichlorophenyl)oxalate (TCPO), bis(2,4-dinitrophenyl)oxalate (DNPO), bis[2-(3,6,9-trioxadecyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO), and bis(2,4,5-trichloro-6-carbopentoxyphenyl)oxalate (CPPO).

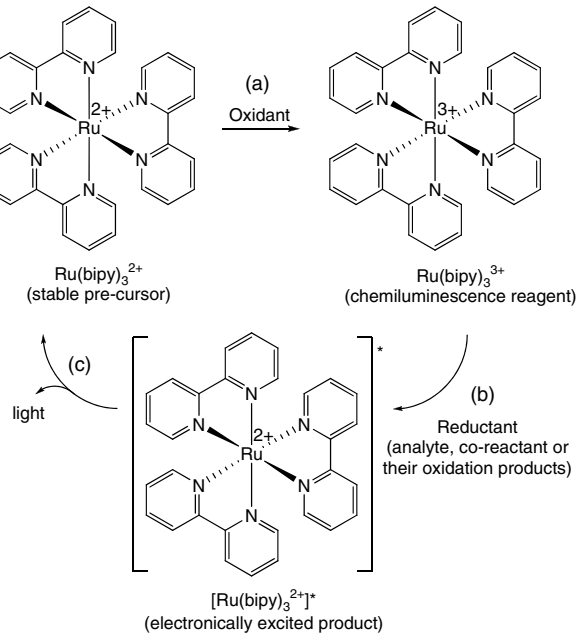

Figure 4. (a) Preparation of tris(2,2′-bipyridine)ruthenium(III), (b) reaction with reducing agent to produce the electronically excited intermediate, and (c) emission of light and re-generation of the starting material.

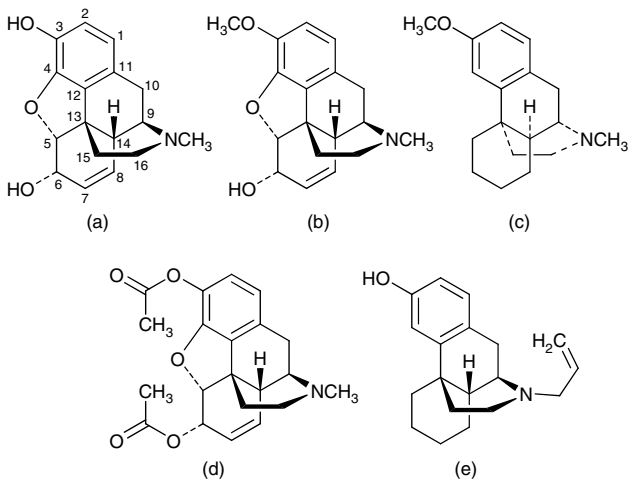

Figure 5. *P. somniferum* alkaloids and related compounds: (a) morphine, (b) codeine, (c) dextromethorphan, (d) heroin, and (e) levallorphan.

greatest intensities were obtained with non-phenolic compounds that possess a morphinan backbone and a tertiary amine.[63,64] Phenolic groups and aromatic or quaternary nitrogens were found to quench the chemiluminescence.[64]

This reagent has been utilized for the chemiluminescence and electrochemiluminescence detection of many other amines, such as opioids not derived from *P. somniferum* (e.g. tramadol, pethidine, methadone, and fentanyl),[65–67] tropane alkaloids (e.g. cocaine, atropine, and scopolamine),[68,69] piperidines and piperazines including phencyclidine (1-(1-phenylcyclohexyl)piperidine; PCP) and ofloxacin,[70,71] and methamphetamine.[72,73]

Acidic potassium permanganate

Reports of chemiluminescence reactions with acidic potassium permanganate can be traced back to the early twentieth century,[74,75] but it was not until the mid-1980s that Townshend *et al*. first utilized this reagent for the chemiluminescence detection of organic compounds.[76–81] In a preliminary screen of various therapeutic and illicit drugs (Table 2) using a batch luminometer, morphine was found to elicit an intense emission.[82] Subsequent

Table 1. Relative chemiluminescence signal for various *P. somniferum* alkaloids with tris(2,2′-bipyridine)ruthenium(III), using FIA methodology. Analyte (1.5 μM, pH 5.8). Reagent: 1 mM $Ru(bipy)_3^{3+}$ in 0.05 M H_2SO_4.[63]

Compound	CL	Compound	CL
Codeine	100	Papaverine	0.5
6-Methoxycodeine	98	Papaveraldine	0.2
2,2′-Biscodeine	23	Cryptopine	0.1
Noscapine	16	Morphine-*N*-oxide	0.05
Thebaine	15	Oripavine	<0.01
Narcotine	11	Laudanidine	<0.01
Narceine	2	Morphine	<0.01
Laudanosine	0.6	Pseudomorphine	<0.01

Table 2. Relative chemiluminescence intensity elicited by a range of drugs with potassium permanganate in acidic solution using a luminometer. Analyte (1 mM, 100 µL) and HCl (1.5 M, 100 µL) solutions were placed into a cuvette, followed by $KMnO_4$ (10 mM, 100 µL) to initiate the reaction.[82]

Compound	CL	Compound	CL
Morphine	100	Streptomycin	0.1
N,*N*-Dimethyltryptamine	40	D-Penicillamine	0.1
Codeine	25	Cannabinol	0
Methacycline	4.5	Diazepam	0
Tetracycline	3.5	Clonazepam	0
Psilocybin	2.7	Lorazepam	0
Oxytetracycline	1.7	Nitrazepam	0
Propranolol	1.7	Flunitrazepam	0
Heroin	0.7	Oxazepam	0
Digoxin	0.6	Chlordiazepoxide	0

Table 3. Relative chemiluminescence intensity for various opioid analgesics with potassium permanganate in acidic solution using FIA methodology. The analytes (0.5 mg/mL, 25 µL) were injected into a carrier stream (tetraphosphoric acid, pH 1.2) that merged with $KMnO_4$ (0.6 mM) immediately prior to entering a glass coil flow cell.[77]

Compound	CL	Compound	CL
Dihydromorphine	100	Norlevorphanol	0.7
Buprenorphine	100	Thebacon	0.7
Normorphine	97	Heroin	0.6
Nalorphine	96	Hydrocodone	0.6
Morphine	96	Thebaine	0.5
Morphine-*N*-oxide	95	Papaverine	0.3
6-Monoacetyl morphine	93	Oxycodone	0.2
Naloxone	57	Phenoperidine	0.1
Benzylmorphine	7.9	Methadone	0.08
Ethylmorphine	3.2	Normethadone	0.08
Norcodeine	2.6	Dextropropoxyphene	0.03
Phenazocine	2.1	Pethidine	0.03
Pentazocine	2.1	Dipipanone	0.02
Codeine	1.9	Norpipanone	0.02
Pholcodine	1.8	Piritramide	0.01
Levallorphan	1.6	Ethoheptazine	0.01
Dihydrocodeine	1.3	Fentanyl	<0.01
Morphine-3-glucuronide	0.8		

examinations by Abbott *et al.*[77] (Table 3) and Barnett *et al.*[83] of a wide range of opioid analgesics using FIA with more optimized chemical conditions, revealed that the prerequisites for an intense chemiluminescence response within this class of compound were a morphinan backbone with a phenolic OH group at carbon-3 and a furan bridge between carbon-4 and carbon-5. This includes compounds such as morphine (Figure 5a), buprenorphine, oripavine, normorphine, naloxone, and 6-monoacetylmorphine, but excludes several close analogues such as codeine and levallorphan (Figures 5b and 5e). Since these preliminary investigations,[76–81] this reagent has been used in the determination of many other compounds (particularly phenols, anilines, indoles, and heterocyclics),[7] but very few can be detected at the exceedingly low concentrations ($\sim 10^{-10}$ M) reported for morphine and related phenolic alkaloids.

Table 4. Relative chemiluminescence intensity for various compounds with soluble manganese(IV) in acidic solution using FIA methodology. Analytes (1 µM) were injected into a carrier stream that merged with 0.5 mM Mn(IV) in 3 M orthophosphoric acid, prior to entering a PTFE coil flow cell. Formaldehyde (0.2 M) was added to the analyte and carrier solutions to enhance the emission intensity.[91]

Compound	CL	Compound	CL
Rhodamine B	100	O^6-Methylcodeine	44
Tryptamine	97	Catechol	38
Serotonin	85	Salbutamol	38
5-Hydroxyindole-3-acetic acid	78	Dopamine	24
5-Hydroxytryptophan	77	Quinol	24
Tryptophan	68	Morphine	23
Fenoterol	61	Hydrazine	21
Pyrogallol	54	Dextramethorphan	6
Pseudomorphine	47	Ascorbic acid	5
Codeine	46	Urea	3

The characteristic red emission of light from reactions with acidic potassium permanganate has been shown to emanate from an electronically excited manganese(II) species.[84,85] The maximum intensity occurs at 734 ± 5 nm (or 689 ± 5 nm if sodium polyphosphate, a commonly used enhancer, is added).[86,87] Chemiluminescence reactions with permanganate are often rapid, reaching maximum intensity within a few seconds,[88] and the rate of slower reactions can be increased by adding manganese(II).[89] The reagent is therefore well suited to HPLC and other flow analysis techniques, but requires detection of the emitted light in close proximity to where the sample and reagent streams are merged.[11,90]

Manganese(IV)

Reactions with other manganese oxidants can also produce the same characteristic red luminescence from manganese(II).[86] A soluble manganese(IV) reagent for chemiluminescence detection has been prepared by reducing potassium permanganate with sodium formate and dissolving the solid manganese dioxide in 3 M orthophosphoric acid.[8,91,92] A considerable enhancement in emission intensity is obtained by adding formaldehyde to the reaction mixture.[92] Although the light-producing reaction pathways for this reagent are related to that of permanganate, the selectivity of the two reagents is quite different (compare Tables 3 and 4).[7,8] One notable example is the limits of detection for opium alkaloids morphine and codeine, which were 5×10^{-8} M and 1×10^{-8} M using manganese(IV),[92] but 1×10^{-10} M and 3×10^{-7} M using permanganate.[77,93]

Other oxidants

There are many reports of chemiluminescence from the reaction of various organic compounds with oxidants such as cerium(IV),[94–96] hexacyanoferrate(III),[97,98] and hypohalites.[3,99] Unlike permanganate and manganese(IV) in acidic solution, the emitting species in these reactions often appears to be derived from the organic analyte, or generated by energy transfer from a reaction intermediate to a more efficient luminophore (e.g. rhodamine B) that has been added to enhance emission intensity.

Oxidation of sulfite

Under suitable conditions, the oxidation of sulfite is accompanied by a weak emission of light,[100] which is often ascribed to the formation of electronically excited sulfur dioxide (first postulated over three decades ago by Stauff and Jaeschke[101]). The emission intensity is significantly enhanced by a variety of compounds (such as riboflavin,[102] quinine,[103] noscapine,[104] papaverine,[105] atropine,[106] carbofuran[107] and 3-cyclohexylaminopropane sulfonic acid[102]), which can be exploited for quantitative detection. In cases in which the compounds or their oxidation products are fluorescent, the mechanism of enhancement generally involves energy transfer to the more efficient fluorophore, but the light-producing pathways for other enhancers are yet to be fully elucidated.[108,109]

Detection of Controlled Substances

Opioids

The chemiluminescence detection of opioids (particularly opium poppy alkaloids and their semi-synthetic derivatives) has been dominated by two reagents: acidic potassium permanganate and $Ru(bipy)_3^{2+}$.[59] As described in the preceding section, these reagents offer somewhat complementary selectivity for this class of analyte. Some of the best detection limits using permanganate have been obtained with phenolic morphinan compounds that contain a furan bridge (e.g. morphine, oripavine), whereas $Ru(bipy)_3^{2+}$ is much more effective for non-phenolic morphinan compounds with tertiary amine functionality (e.g. codeine, heroin). Using various flow analysis methodologies, limits of detection as low as 5×10^{-11} M for morphine with permanganate,[89,110] and 5×10^{-10} M for codeine with $Ru(bipy)_3^{2+}$[111] have been reported. Moreover, these two reactions have been utilized as standard, rapid chemiluminescence systems to evaluate microfabricated devices[112–115] and other analytical instrumentation;[11,88,90,110] examine reagent stability,[89,116] structure[113,117–119] and immobilization;[113,114,120–122] and explore new chemiluminescence-based analytical approaches.[123–125]

These reagents have also been used to determine morphine, codeine and other opioids in applications including: (1) monitoring the industrial-scale extraction of alkaloids from opium poppies;[63,83,111,126–130] (2) analysis of pharmaceutical preparations and seized illicit drugs;[60,78,122,124,131,132] and (3) the determination of these drugs and their metabolites in biological fluids[65,79,133–137] and in the larvae of insects commonly found on the bodies of drug overdose victims.[138,139]

(1) Analysis of opium poppies and process liquors

Several commercially important pharmaceutical opiates (morphine, codeine, oripavine, and thebaine) are obtained from opium poppies through a proprietary series of aqueous and non-aqueous extractions. To maximize efficiency, the concentration of the alkaloids must be monitored throughout this process. HPLC with UV absorbance detection is the conventional workhorse approach for these measurements, but the complexity of the plant-derived sample matrix limits the chromatographic resolution of the target analytes even after 20–30 min separation. Simple FIA and sequential-injection analysis (SIA) procedures with acidic potassium permanganate chemiluminescence have been applied to rapid monitoring of morphine, but this approach is restricted to morphine-rich process streams in which the concentrations of other phenolic morphinan alkaloids are not significant.[83,126,127] Codeine can be monitored using FIA with $Ru(bipy)_3^{3+}$ in the presence of relatively high levels of morphine (Table 1), but not in samples containing significant levels of thebaine.[63]

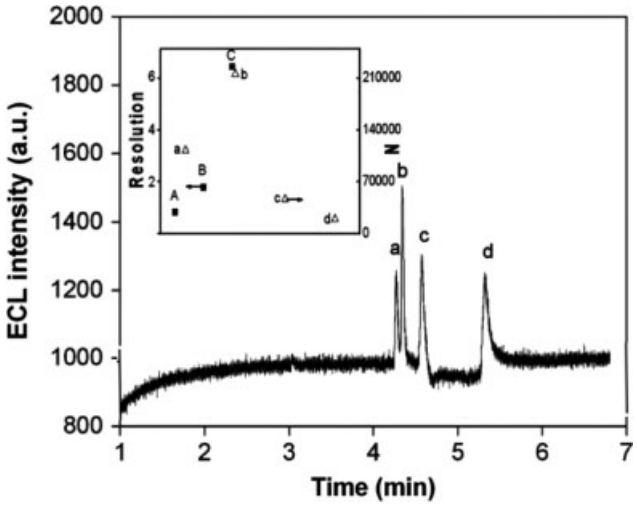

Figure 6. Electropherogram of standard solution of (a) 2×10^{-5} M thebaine, (b) 2×10^{-5} M codeine, (c) 1×10^{-4} M narcotine and (d) 4×10^{-5} M morphine, using end-column $Ru(bipy)_3^{2+}$ electrochemiluminescence detection. Reprinted from Gao *et al.*[129] (© Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

Both CE and HPLC with chemiluminescence detection have been employed for the selective detection of multiple opiate alkaloids.[92,111,128–130,140] Morphine, oripavine, and pseudomorphine were determined in process samples using CE with acidic potassium permanganate chemiluminescence.[130] The determination of codeine, 6-methoxycodeine and thebaine using CE with $Ru(bipy)_3^{2+}$ chemiluminescence has also been demonstrated.[128] CE with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection has been applied to the determination of codeine, thebaine, morphine and narcotine in opium poppy traditional medicine.[129] In contrast to the selectivity described in other chemiluminescence and electrochemiluminescence studies with $Ru(bipy)_3^{2+}$,[60,63] the reported limit of detection for morphine was over two orders of magnitude superior to that for the non-phenolic alkaloids.[129] Whilst this discrepancy was not necessarily supported by the respective peaks in the electropherograms in that paper (e.g. Figure 6), it should be noted that the optimum pH and most appropriate buffer for the greatest chemiluminescence and electrochemiluminescence emission with this reagent are analyte dependent.[60,64,117] The detection reservoir of that study was buffered at pH 9.18,[129] which is much closer to the optimum for morphine (pH 9.0) than codeine (pH 4.0).[60]

Separation times for these CE procedures were between 5 and 14 min and the detection limits ranged from 1×10^{-9} M to 1×10^{-6} M.[128–130] However, a faster and more sensitive HPLC approach has been developed, in which process samples were separated on a commercially available monolithic column.[111] Codeine and thebaine were determined within 2 min using a flow rate of 3 mL min^{-1}, solvent gradient (acetonitrile in an aqueous solution of trifluoroacetic acid), and $Ru(bipy)_3^{2+}$ chemiluminescence detection (Figure 7a). Morphine and oripavine were determined within the same timeframe using methanol as the organic modifier and permanganate chemiluminescence

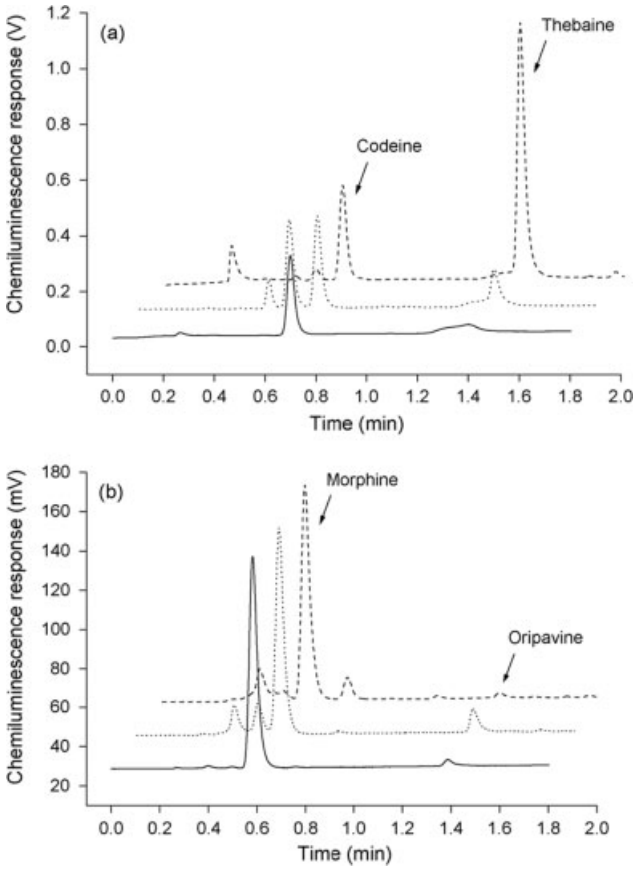

Figure 7. Typical chromatograms for industrial process samples using monolithic column HPLC with (a) $Ru(bipy)_3^{2+}$ and (b) permanganate chemiluminescence detection. Reprinted from Costin *et al.*[111] (© Elsevier 2007. Reproduced with permission).

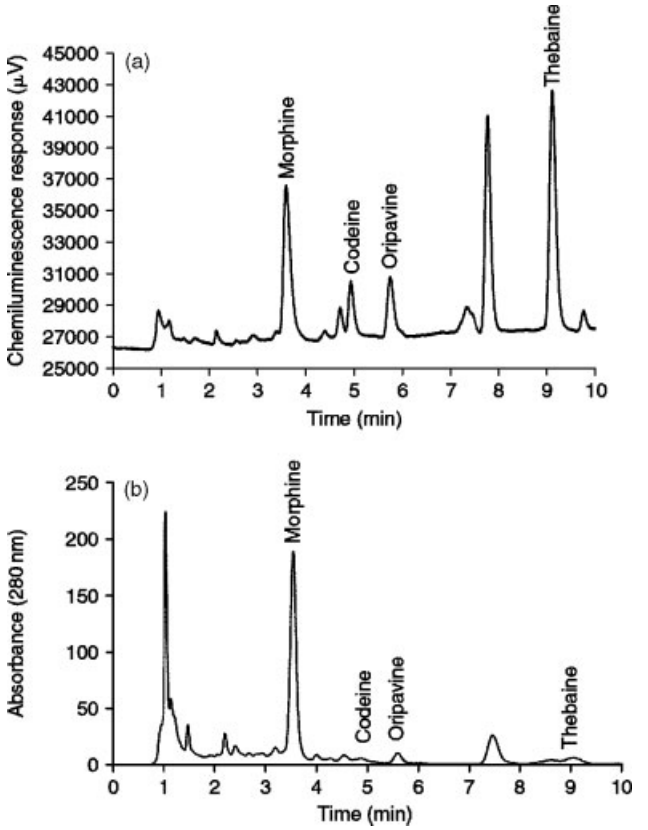

Figure 8. Chromatograms for industrial process samples using HPLC with (a) dual reagent chemiluminescence detection and (b) UV-absorbance detection. Reprinted from Lenehan *et al.*[140] (© CSIRO, 2004).

detection (Figure 7b). Limits of detection for the four analytes were between 1×10^{-10} M to 1×10^{-9} M. Samples were taken from different points in the process line and diluted between 250- and 125000-fold so that the target analytes were within the linear calibration range. The results were in good agreement with the standard procedure involving ion-pairing HPLC with UV-absorbance detection.[111]

Two chemiluminescence approaches to determine the four analytes of interest within a single chromatographic run have been reported.[92,140] The first was based on a dual-reagent system containing both permanganate and $Ru(bipy)_3^{2+}$.[140] In this approach the permanganate served to detect morphine and oripavine as well as oxidize the other reagent to its reactive ruthenium(III) state for the detection of codeine and thebaine. Limits of detection for all four alkaloids were within one order of magnitude (between 5×10^{-7} M and 3×10^{-6} M),[140] but much poorer than those achieved with the separate reagents.[111] Nevertheless, the dual chemiluminescence reagent provided better sensitivity than UV absorbance for the detection of these analytes in process samples (Figure 8).

The second approach involved chemiluminescence detection with manganese(IV) (enhanced by 1 M formaldehyde), for which a similar response is obtained from each of the target analytes.[92] Limits of detection between 5×10^{-9} M and 5×10^{-8} M were established using FIA. However, this reagent has not yet been applied to process samples and due to its broader selectivity may be subject to greater interference from concomitant species.

(2) Analysis of pharmaceuticals and illicit drugs

Several FIA procedures for the determination of opioids and related compounds in pharmaceutical formulations and other simple matrices have been reported: buprenorphine with permanganate;[78] noscapine and papaverine based on their enhancing effect on the chemiluminescence oxidation of sulfite;[104,105] codeine with $Ru(bipy)_3^{2+}$ (electrochemiluminescence);[60] and codeine and noscapine with tris(1,10-phenanthroline)ruthenium(II) (and cerium(IV)).[141] Limits of detection for these procedures were generally around 10^{-8} M. Miniaturized integrated devices for electrochemiluminescence detection with immobilized $Ru(bipy)_3^{2+}$ (or related complexes) have also been applied to the determination of codeine in pharmaceutical preparations.[113,114] The limits of detection in these systems were orders of magnitude poorer than the previous FIA investigation,[60] but still more than sufficient for this application.

A rapid method for the detection of heroin based on FIA with a tris(2,2′-bipyridine)ruthenium(II) electrochemiluminescence sensor has been proposed.[142] The immobilized reagent exhibited good stability and the limit of detection was 1×10^{-6} M. A selectivity study showed that various biological molecules did not interfere, but the only tertiary amines investigated were codeine, morphine, and papaverine (of which codeine strongly interfered). Considering the range of tertiary amines and other compounds detected with this reagent,[6,57] there is significant potential for

interference from compounds unrelated to heroin. However, as suggested by the authors, this sensor could be combined with chromatographic or electrophoretic separation. A related FIA-electrochemiluminescence approach for the determination of codeine and morphine in samples from illegal suppliers has been reported; the limits of detection were 5×10^{-9} M and 3×10^{-8} M, respectively.[122]

We have found that codeine, strychnine, and chloroquine strongly interfere with an FIA procedure to rapidly screen seized drug samples for the presence of heroin based on the chemiluminescence reaction with $Ru(bipy)_3^{3+}$.[131,143] However, the target analyte can be clearly distinguished from these interferents by a quick, base-catalysed hydrolysis to morphine and subsequent chemiluminescence reaction with permanganate, providing an unambiguous two-step FIA test for heroin.[131,143] This concept was subsequently adopted in an SIA procedure, in which the sample was sandwiched between the two reagents.[124]

A CE procedure with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection for the determination of heroin and cocaine on banknotes[132] is discussed in the *Stimulants* section with other approaches to detect cocaine. The use of $Ru(bipy)_3^{3+}$ as a spray reagent for the detection of heroin on surfaces (such as polymer bank notes) has been proposed.[144] A few other opiate alkaloids were found to elicit an intense response, but heroin could be distinguished from the tested compounds (except 3-monoacetylmorphine, a by-product of heroin synthesis) based on the time of emission.[144] Like the FIA-electrochemiluminescence procedures previously described,[122,142] there is significant potential for interference, but the effect on this presumptive chemical test will be dependent on the frequency and quantity that the interferences are encountered on the surfaces under investigation.

(3) Analysis of biological fluids and insects

The first analytical application of permanganate chemiluminescence detection for organic analytes was an HPLC method to determine morphine (a major metabolite of heroin) in blood and urine, which involved solid phase extraction and separation on a styrene-divinylbenzene column (10 min), with *N*-ethylnormorphine as an internal standard.[79,145] Limits of detection, however, were considerably poorer than those previously reported using FIA methodology. The method was later improved using an ODS column and separation conditions that were more compatible with permanganate chemiluminescence, to provide limits of detection of 4×10^{-9} M (20 pg on-column) for morphine and 5×10^{-8} M for 6-monoacetylmorphine.[133] Nalorphine, a commercially available morphine derivative, was used as the internal standard. Further improvements could perhaps be derived from advances in separation and detection described in the more recent HPLC-chemiluminescence procedure for the analysis of process samples.[111] Similar monolithic column HPLC methodology for the determination of morphine with permanganate chemiluminescence detection has been utilized for entomotoxicology – the study of bioaccumulated drugs or toxins in flesh feeding arthropods (mainly flies and beetles) that remain when soft-tissue toxicological samples are no longer available from a decomposing body.[138,139]

A flow analysis system with a molecular imprinted polymer column and permanganate chemiluminescence detection has been applied to the rapid screening for morphine in the urine of heroin users, with a limit of detection of 7×10^{-9} M.[135] The use of the molecular imprinted polymer increased the tolerance to potential interferents (such as epinephrine, ascorbic

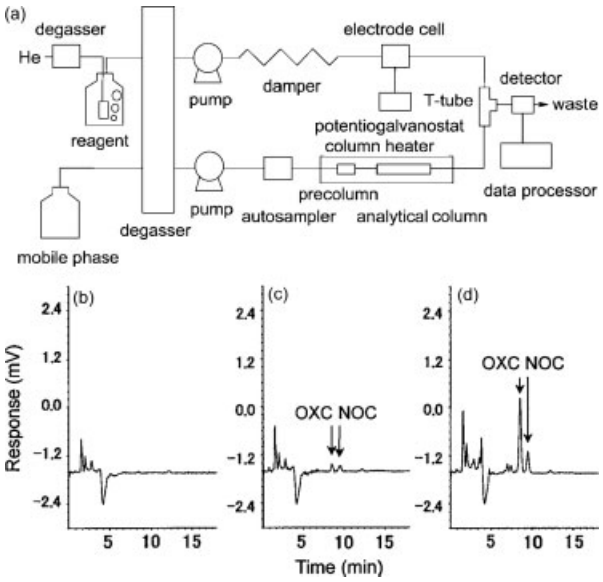

Figure 9. (a) Flow manifold for the determination of oxycodone and noroxycodone using HPLC-chemiluminescence, and chromatograms for (b) blank plasma, (c) plasma spiked with 0.514 ng/mL oxycodone and 1.04 ng/mL noroxycodone, and (d) plasma obtained from a patient 5 h after consuming a single 5 mg oxycodone tablet. (Reprinted from Gemba *et al.*[147] with permission of the publisher (Taylor & Francis Group, http://www.informaworld.com).

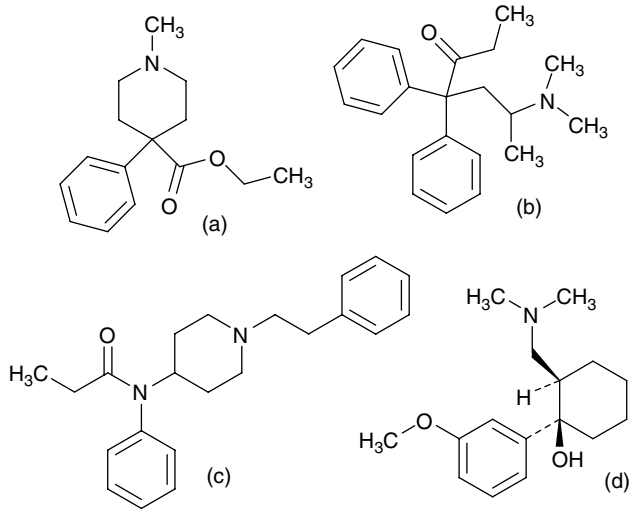

Figure 10. Synthetic opioids (not derived from *P. somniferum*): (a) pethidine, (b) methadone, (c) fentanyl, and (d) tramadol.

acid and codeine) by approximately two-orders of magnitude. Permanganate chemiluminescence has also been used to detect heroin, 6-monoacetylmorphine and morphine after electrophoretic separation.[146] A two- to five-fold improvement in sensitivity over UV absorbance detection was noted, but the procedure was not applied to real samples.

An HPLC procedure involving solid-phase extraction, reverse-phase separation and $Ru(bipy)_3^{2+}$ chemiluminescence detection has been developed for the determination of oxycodone (an analgesic medication synthesized from thebaine) and its metabolite noroxycodone in human plasma.[147] The reagent was electrochemically oxidized to the active ruthenium(III) state online prior

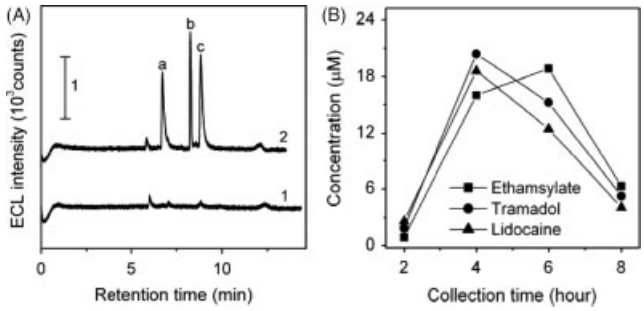

Figure 11. (A) CE-electrochemiluminescence electropherograms of: (1) blank urine, and (2) urine spiked with (a) 8×10^{-7} M ethamsylate, (b) 3×10^{-6} M tramadol, and (c) 2×10^{-6} M lidocaine. (B) Changes in the concentrations of the three analytes in patient urine after administration. Reprinted from Li and Ju.[65] (© Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

to merging with the post-column eluate (Figure 9). The lower limits of quantification for oxycodone and noroxycodone were 2×10^{-9} M and 3×10^{-9} M, respectively.

$Ru(bipy)_3^{2+}$ electrochemiluminescence detection has been used with electrophoretic separation to determine a range of opioids, including heroin,[136] codeine,[136,137] acetylcodeine,[137] thebaine,[137] pethidine,[66] methadone,[66] and tramadol (see Figures 5 and 10).[65,134,148,149] These procedures each involve 2–4 analytes (often including other pharmaceuticals such as lidocaine, ethamsylate, ofloxacin, and methylephedrine), separated in capillaries or on microfluidic platforms. Using capillaries, analytes were typically eluted within 10 min and limits of detection ranged from 8×10^{-9} M to 5×10^{-7} M.[65,66,134,137] The lowest limit of detection was reported for ethamsylate (a combination of 2,5-dihydroxybenzenesulfonic acid and diethylamine) and considering the strong response obtained for secondary and tertiary aliphatic amines and the known inhibition from phenols, it is likely that the peak for ethamsylate corresponds to the diethylamine component of the drug. Separations on microchips were much faster (<2 min), but the limits of detection in those studies were generally two orders of magnitude poorer[136,148,149] than those of the more conventional capillary systems. In all of these studies, the optimum pH for the electrochemiluminescence detection was reported to be in the range of 8.0 to 9.0, except for one procedure (the determination of pethidine and methadone), in which the optimum pH for the detection cell was found to be 6.5.[66] Opioids pethidine and fentanyl (Figure 10) have also been used as model analytes in the development of a microfabricated flow cell for $Ru(bipy)_3^{2+}$ chemiluminescence detection[150] and an electrochemiluminescence sensor,[67] for which limits of detection for the target analytes of 8×10^{-8} M and 9×10^{-9} M were reported.

Several of the CE procedures were applied to the analysis of urine spiked with the target analytes[65,136,137] or urine obtained from patients administered with the drugs[65,134] (Figure 11). Extraction[65,134] or 100-fold dilution[136,137] was used to minimize interference, particularly from the high ionic strength of the sample matrix. Although very few peaks other than the target analytes were observed in the chromatograms and electropherograms for plasma and urine samples in the studies described above, a wide range of pharmaceuticals contain suitable functionality for detection with $Ru(bipy)_3^{2+}$ chemiluminescence or electrochemiluminescence. Therefore, there may be many potential interferences in these sample matrices in situations in which the patient's intake

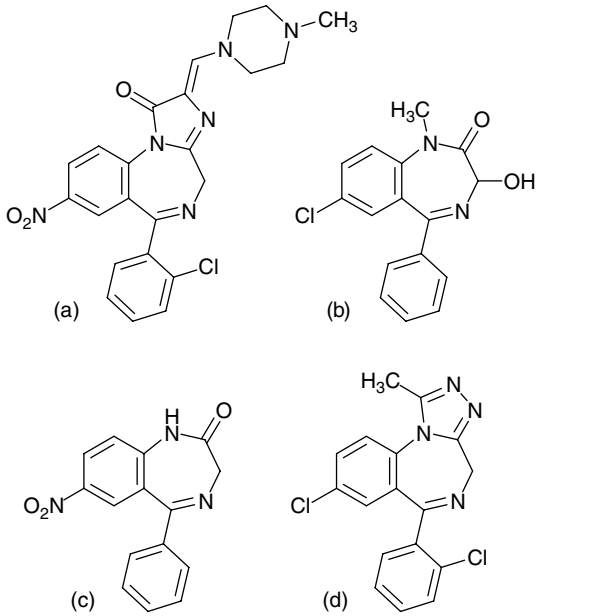

Figure 12. Benzodiazapines: (a) loprazolam, (b) temazepam, (c) nitrazepam, and (d) triazolam.

is not known. Nevertheless, this approach is certainly suitable for screening purposes, patient monitoring and pharmacokinetics studies (Figure 11). Moreover, the development of micro-total analytical systems incorporating electrophoretic separations opens new avenues for rapid screening of biological samples and other matrices for various pharmaceuticals and illicit drugs, before additional confirmatory testing.

Tranquilizers

In 1989, Andrews and Townshend screened seven benzodiazepines (temazepam, flunitrazepam, lormetazepam, alprazolam, loprazolam, lorazepam, and triazolam; Figure 12) with 15 oxidizing and reducing agents (using FIA) and found that only the reaction of loprazolam with acidic potassium permanganate produced measurable chemiluminescence.[81,151] However, the limit of detection (7×10^{-6} M) was insufficient for forensic or clinical analysis. The authors suggested that it could be used to measure loprazolam in pharmaceutical preparations, but they found that the results using FIA did not agree with the value stated by the manufacturer and verified using spectroscopic measurements.[81] Although the reagent conditions were optimized, species such as sodium polyphosphates (that have subsequently been found to provide significant enhancement with other analytes) were not tested.

Unlike the other benzodiazepines tested, loprazolam has an imidazole with a keto group and a piperazine ring (Figure 12a). Several other piperazine compounds, including cinnarizine,[152] perphenazine,[153] and naftopidil[154] have been detected with permanganate chemiluminescence, which suggests that this attribute of loprazolam is responsible for the emission with that reagent. On the other hand, ofloxacin (an *N*-methyl-piperazine pharmaceutical) does not evoke significant chemiluminescence with permanganate.[71] It does, however, produce a relatively intense emission with $Ru(bipy)_3^{3+}$; this reagent (not tested by

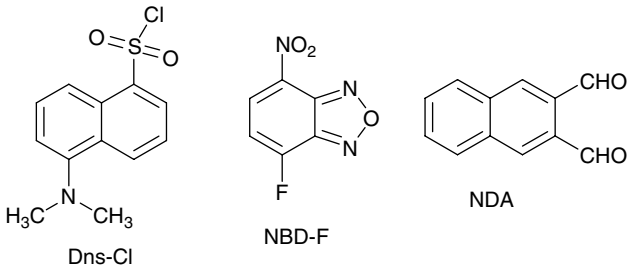

Figure 13. Derivatizing agents for the detection of amines with peroxyoxalate chemiluminescence: dansyl chloride (Dns-Cl), 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), and naphthalene-2,3-dicarbaldehyde (NDA).

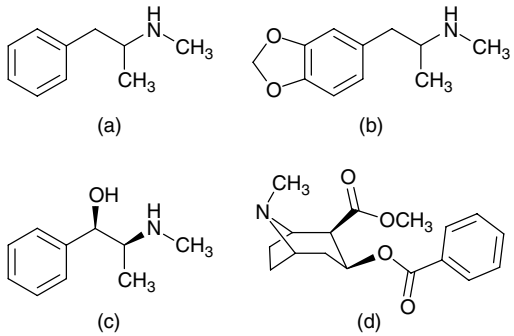

Figure 14. Stimulants: (a) methamphetamine, (b) 3,4-methylenedioxymethamphetamine (MDMA), (c) ephedrine, and (d) cocaine.

Andrews and Townshend) could perhaps provide a more sensitive means to detect loprazolam.

An FIA procedure for nitrazepam (and other nitro-compounds) incorporating online photodegradation and subsequent chemiluminescence detection with luminol and cobalt(II) has been reported.[155] The limit of detection for the nitro-benzodiazepine was 4×10^{-7} M. Although not applied to real samples containing nitrazepam, the approach was used to determine chloramphenicol in pharmaceutical formulations.[155]

Stimulants

The chemiluminescence or electrochemiluminescence detection of stimulants has predominantly been achieved in two ways: (1) derivatization with fluorophores that are efficiently excited with peroxyoxalate reagents; and (2) reaction with $Ru(bipy)_3^{2+}$ (after oxidation of the reagent). The main area of application has been the analysis of urine and hair samples from illicit drug users.

Hayakawa *et al*. examined three derivatizing agents (dansyl chloride (Dns-Cl), 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) and naphthalene-2,3-dicarbaldehyde (NDA); Figure 13) for the detection of methamphetamine (Figure 14a) and eight related amines with peroxyoxalate chemiluminescence.[156] Separation was performed on a reverse-phase (ODS) column (~20 min) with an acetonitrile-water mobile phase containing 1 mM imidazole (pH 7.0). The post-column eluate merged with a reagent containing TCPO (Figure 3) and H_2O_2 in acetonitrile, prior to entering a reaction coil and then a chemiluminescence detector (Figure 15a). Dns-Cl was found to be the most suitable derivatizing agent for the simultaneous determination of both primary and secondary amines, providing a limit of detection of methamphetamine at

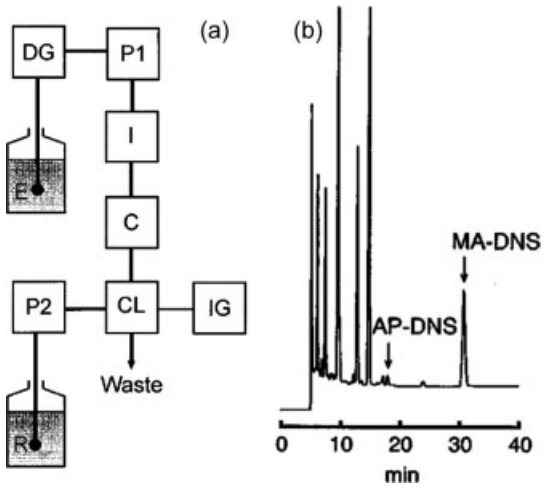

Figure 15. (a) Manifold used by Hayakawa *et al*. for the determination of methamphetamine and its derivatives (after derivatization with dansyl chloride) using HPLC with peroxyoxalate chemiluminescence detection. (b) Chromatogram for a hair sample from a methamphetamine user. (E = mobile phase, DG = online degasser, P = pump, I = autoinjector, C = separation column, R = reagent solution, CL = chemiluminescence detector, IG = intergrator, AP-DNS = dansylated amphetamine, MA-DNS = dansylated methamphetamine). Reprinted from Takayama *et al*.[159] (© John Wiley & Sons. Reproduced with permission).

2×10^{-10} M. However, NDA provided lower limits of detection (~1×10^{-11} M) for the primary amines.

The procedure with Dns-Cl derivatization was applied to the determination of methamphetamine, amphetamine and piperidine in urine (after extraction into diethyl ether).[157] Interference from side-reaction products such as Dns-OH precluded the determination of early eluting derivatives, but this problem was alleviated with a longer separation (50 min), gradient elution (acetonitrile-imidazole buffer) and the addition of THF to the mobile phase.[158] The modified procedure (incorporating chloroform-isopropanol (3 : 1) extraction) was successfully applied to the determination of methamphetamine, amphetamine, norephedrine, *p*-hydroxymethamphetamine, *p*-hydroxyamphetamine and an internal standard (β-phenylethylamine) in urine samples obtained from methamphetamine users. Hydroxyamphetamine glucuronides were also quantified by introducing a preliminary enzymatic hydrolysis step.[158] Hayakawa *et al*. later adapted this procedure for the determination of methamphetamine and amphetamine in a single human hair (Figure 15b).[159] Examination of hair samples from many methamphetamine users revealed that the concentration of methamphetamine and amphetamine was generally lower in white hairs than in black hairs,[160] and that permanent wave, dye and decolourant treatments can interfere with the determination.[53]

The pre-column derivatization with Dns-Cl described in each of these studies involved incubation in a carbonate buffer (pH 9.0) for 1 h at 45 °C,[53,156–160] but others have reported that 10 min at 70 °C is more appropriate.[161] Moreover, sample clean-up and derivatization can be performed in C_{18} solid phase extraction supports, either off-line,[162] or online within a short column accessed *via* a 6-port switching valve,[161] which – coupled with reverse-phase HPLC separation and post-column peroxyoxalate chemiluminescence detection – gave limits of detection of approximately 7×10^{-9} M for amphetamine and methamphetamine.

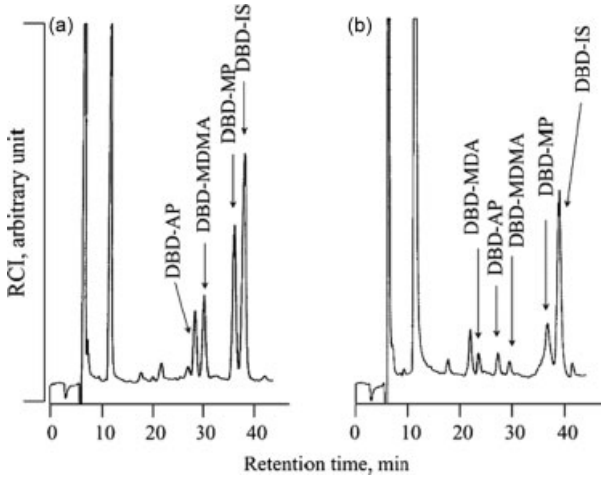

Figure 16. Chromatograms for the determination of amphetamines in hair samples from two drug users. Sample preparation included digestion, extraction (n-heptane) and derivatization with DBD-F. Reverse-phase (C18) separation with post-column peroxyoxalate chemiluminescence detection (CPPO and H_2O_2 in acetonitrile). AP = amphetamine; MDMA = 3,4-methylenedioxymethamphetamine; MP = methamphetamine; IS = internal standard (1-methyl-3-phenylpropylamine). Reprinted from Nakamura *et al*.[57] with kind permission from Springer Science + Business Media: Analytical and Bioanalytical Chemistry. (© Springer-Verlag 2006).

In a similar method described by Nakashima *et al*.,[163] methamphetamine and its metabolites were derivatized off-line (25 min, 80 °C) with 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F), separated on a reverse-phase column with gradient elution (65 min), and detected by peroxyoxalate chemiluminescence using TDPO (Figure 3) and H_2O_2. Limits of detection for the six target analytes ranged from 1×10^{-9} M to 7×10^{-9} M (injected onto column). Urine samples from 14 methamphetamine users were analyzed and the results were in good agreement with comparison data for methamphetamine and amphetamine obtained with GC.[163] The same research group subsequently determined 3,4-methylenedioxymethamphetamine (MDMA; Figure 14b) and related compounds (3,4-methylenedioxyamphetamine (MDA), methamphetamine and amphetamine) in hair, with a procedure that involved alkaline digestion, *n*-heptane extraction, derivatization with DBD-F, separation on a semi-micro column (40 min), and chemiluminescence detection with CPPO (Figure 3) and H_2O_2.[54] Limits of detection were 0.03, 0.16, 0.02 and 0.15 ng mg^{-1} hair (3×10^{-10} M, 2×10^{-9} M, 3×10^{-10} M and 2×10^{-9} M injected onto column), respectively. Hair samples from patients being treated in a chemical dependency unit were analyzed (Figure 16). The application of this procedure to explore the detection window of MDMA and methamphetamine in hair root and hair shaft samples was recently presented by Nakashima at the XIV International Symposium on Luminescence Spectrometry (13–16 July 2010, Prague, Czech Republic).

During the development of the peroxyoxalate chemiluminescence detection of amphetamines, an alternative approach, based on derivatization with *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI; Figure 1c), was investigated and applied to the determination of methamphetamine in serum[164] and then methamphetamine and amphetamine in urine.[165] The limits of detection (1×10^{-9} M methamphetamine and 5×10^{-9} M amphetamine, injected onto column) were poorer than those for some of the peroxyoxalate systems, and significant interference from large early eluting peaks was observed.

Many of the forensically important stimulants are non-phenolic secondary or tertiary amines and therefore the $Ru(bipy)_3^{2+}$ reagent is an obvious candidate for chemiluminescence or electrochemiluminescence detection of these molecules. An FIA procedure for methamphetamine based on electrochemiluminescence detection with $Ru(bipy)_3^{2+}$ immobilized on a glassy carbon electrode provided a limit of detection of 2×10^{-7} M.[72] Without a separation step, however, this approach was limited to analyses involving very simple sample matrices. The electrochemiluminescence response for MDMA with $Ru(bipy)_3^{2+}$ is poor compared to that for methamphetamine, and the response for its primary-amine metabolite MDA is even poorer; however, they are more electroactive at a platinum electrode.[73] CE (10 min separation) with simultaneous electrochemical and electrochemiluminescence detection was used to determine these three compounds in urine, after extraction with ethyl acetate. The limit of electrochemiluminescence detection for methamphetamine was 3×10^{-8} M.[73]

The detection of ephedrine (Figure 14c), pseudoephedrine and methylephedrine based on chemiluminescence or electrochemiluminescence reactions with $Ru(bipy)_3^{2+}$ has been demonstrated.[137,166,167] Suliman *et al*. found that the chemiluminescence intensity for ephedrine was enhanced by over an order of magnitude by derivatization with formaldehyde, which they attributed to greater substitution at the amine. The reported limit of detection for their SIA procedure was only 2×10^{-7} M,[166] but this was sufficient for the suggested application involving the analysis of pharmaceutical preparations.

A CE-electrochemiluminescence procedure for the determination of methylephedrine, thebaine, codeine and acetylcodeine has recently been developed.[137] The addition of an ionic liquid to the electrophoretic buffer was found to improve resolution and electrochemiluminescence intensity, and under the optimized conditions, the limit of detection for methylephedrine (a tertiary amine analogue of ephedrine) was 2×10^{-8} M. The potential of the procedure for urine analysis was demonstrated with samples spiked with the target analytes.[137] The same research group have published a CE-electrochemiluminescence procedure for the determination of methylephedrine and pseudoephedrine.[167] Interestingly, although the optimum conditions for the two analytes were very similar, superior limits of detection were obtained for the secondary amine (9×10^{-9} M) than the *N*-methyl tertiary amine (2×10^{-8} M). The procedure was applied to spiked urine samples and then used to monitor the concentration of pseudoephedrine in the urine of two patients administered with the drug.[167]

Numerous phenolic amines with structures closely related to ephedrine and methamphetamine, such as tyramine, synephrine, etilefrine, prenalterol, and salbutamol, have been detected with acidic potassium permanganate chemiluminescence,[168–170] which, as discussed in the above section on *Opioids*, is a somewhat complementary reagent to $Ru(bipy)_3^{2+}$.

Cocaine possesses a tertiary amine (Figure 14d), but unlike codeine (Figure 5b), it does not elicit an intense chemiluminescence emission with $Ru(bipy)_3^{3+}$ in acidic solution.[171] However, reasonable limits of detection have been reported for the electrochemiluminescence reaction with the ruthenium complex in near-neutral or alkaline conditions.[69,132] Xu *et al*. described a CE procedure with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection (phosphate-acetate buffer at pH 7.2) for the determination of cocaine and heroin on banknotes (Figure 17).[132] On-column field-amplified sample stacking was used to increase the sensitivity; the

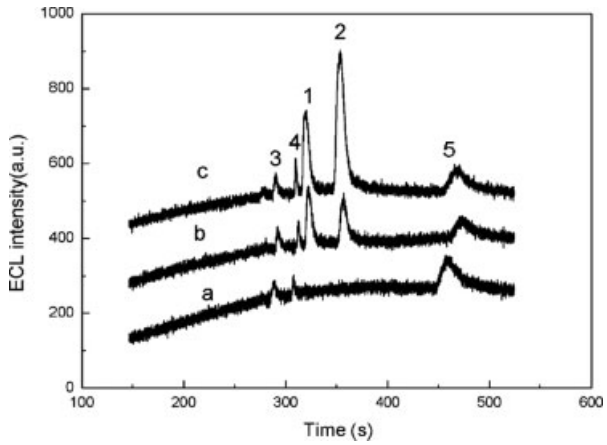

Figure 17. Electropherograms for (a) extract from the surface of 'clean' banknotes, (b) extract from banknotes contaminated with illicit drugs, and (c) clean extract spiked with 1.5×10^{-6} M of cocaine and heroin. Peak 1: cocaine, Peak 2: heroin, Peaks 3–5: unknown compounds extracted from the banknotes. Reprinted from Xu *et al*.[132] (© Elsevier, 2006. Reproduced with permission).

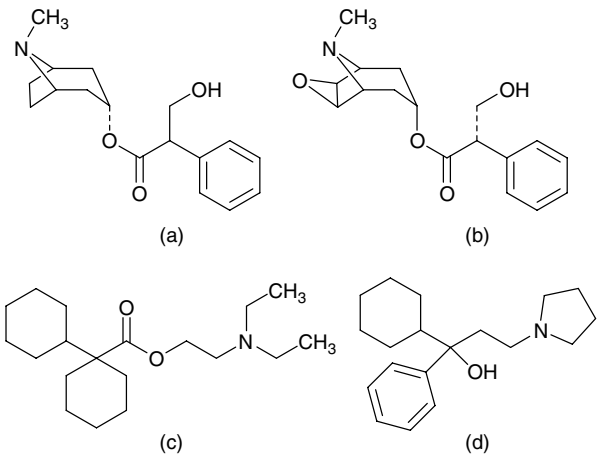

Figure 18. Anticholinergics: (a) atropine, (b) scopolamine, (c) dicyclomine, and (d) procyclidine.

limits of detection were 6×10^{-8} M cocaine and 5×10^{-8} M heroin. Cocaine and ecgonine were used as test analytes in the development of a microchip-based micellar electrokinetic chromatography (MEKC) procedure with an electrolyte containing a surfactant and an ionic liquid, coupled with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection (phosphate buffer at pH 8.5).[136] Compared to the previous procedure, the separation was much faster (completed in 100 s), but the limits of detection were orders of magnitude poorer (3×10^{-5} M cocaine and 1×10^{-5} M ecgonine).

Halucinogens

Various deliriants (anticholinergics) containing tertiary amine functionality, such as those shown in Figure 18, have been detected with $Ru(bipy)_3^{2+}$.[68,69,150,172–174] In 1995, Holeman and Danielson used microbore C_8 silica or polymeric reverse-phase columns to separate various combinations of atropine, scopolamine, cyclopentolate, cyclobenzaprene, procyclidine and dicyclomine

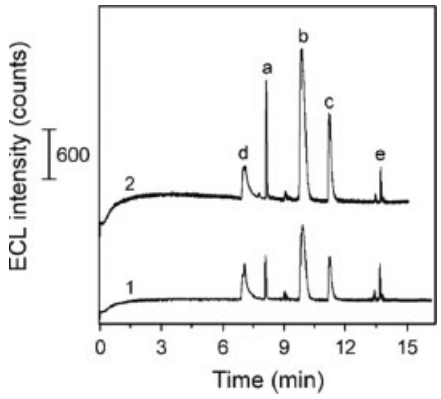

Figure 19. Electropherogram of (1) a *Flos daturae* extract, and (2) the extract spiked with 1.5×10^{-6} M anisodine (peak a), 1.5×10^{-6} M atropine (peak b), and 5×10^{-6} M scopolamine (peak c), using $Ru(bipy)_3^{2+}$ electrochemiluminescence detection. Peaks (d) and (e) are unknown compounds. Reprinted from Li *et al*.[173] (© Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission).

prior to chemiluminescence detection with $Ru(bipy)_3^{3+}$ generated by electrochemical oxidation of the ruthenium(II) complex within the reagent reservoir.[68] The low flow-rates utilized in this approach accentuated the need to initiate the relatively fast chemiluminescence reactions with this reagent close to the point of detection, and therefore a custom-made flow cell was developed for that purpose. Limits of detection ranged from 3×10^{-7} M (procyclidine) to 3×10^{-6} M (atropine).

More recently, the electrochemiluminescence detection of atropine (Figure 18a) and homoatropine with $Ru(bipy)_3^{2+}$ in a borate buffer at pH 10 was demonstrated using a simple flow system with thin-layer flow cell.[69] Limits of detection were 2×10^{-7} M and 3×10^{-7} M, respectively. A lower limit for atropine (1×10^{-9} M) was obtained using FIA with chemiluminescence detection using $Ru(bipy)_3^{2+}$ and cerium(IV).[150] This reaction was also performed in a microfluidic reactor with only a small loss in sensitivity (L.O.D. 4×10^{-9} M atropine). In these cases, the analyte was prepared in borate buffer at pH 10, but the cerium(IV) solution contained 0.02 M sulfuric acid and the ruthenium(II) reagent was buffered at pH 5, and therefore the final reaction pH is unclear.[150]

Several CE procedures with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection have been used to determine atropine, scopolamine (Figure 18b) and anisodamine in solanaceae plant (*Flos daturae*) extracts.[172–174] In the first of these methods, atropine and scopolamine were separated within 11 min in a 50 cm (25 μm i.d.) capillary using a phosphate buffer at pH 8.48 with end-column electrochemiluminescence detection (phosphate buffer pH 7.48).[172] Similar to the difference in sensitivity for methamphetamine (Figure 14a) and MDMA (Figure 14b) with this reagent, the detection limit of scopolamine was significantly poorer than that of atropine (1×10^{-6} M and 5×10^{-8} M, respectively). In the second procedure, β-cyclodextrin was added to the running buffer, which improved peak shape and resolution of the three analytes (Figure 19).[173] The separation time was 15 min and detection was performed in a phosphate buffer at pH 7. The limits of detection were 1×10^{-8} M anisodamine, 2×10^{-8} M atropine and 2×10^{-7} M scopolamine. The most recent method involved a faster 6 min separation in an 18 cm (25 μm i.d.) capillary, using non-aqueous running and detection buffers (which minimized electrode fouling) and dual electrochemical and electrochemiluminescence detection.[174] However, the limits of detection

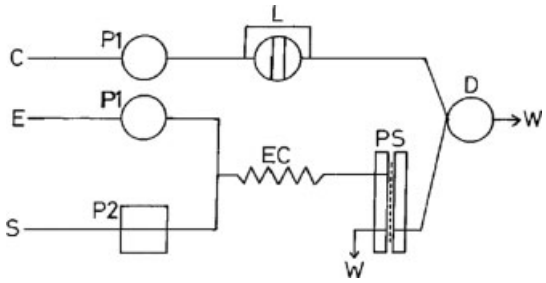

Figure 20. FIA manifold for the determination of atropine with luminol chemiluminescence detection. C: dichloromethane, E: dichloromethane, S: acidic aqueous solution containing the sample and $AuCl_4^-$, P: pump, L: luminol in reversed micellar solution, EC: liquid-liquid extraction coil, PS: phase separator, D: detector (spiral flow cell), W: waste. Reprinted Fujiwara *et al.*[175] (© American Chemical Society, 2000. Reproduced with permission).

(5×10^{-6} M anisodamine, 5×10^{-7} M atropine and 5×10^{-5} M scopolamine) were poorer than those previously reported.

Atropine has been detected with several other chemiluminescence reagents.[92,106,175] Limits of detection for atropine with cerium(IV) and sodium sulfite[106] and with soluble manganese(IV) and formaldehyde,[92] using simple FIA methodology, are 1×10^{-6} M and 5×10^{-6} M, respectively. However, many other analytes can be detected with these reagent systems; some at much lower concentrations.[92,109] An alternative FIA approach involved online ion-pair complex formation between atropinium and tetrachloroaurate(III) ($AuCl_4^-$), extraction into dichloromethane, phase separation, and chemiluminescence reaction with luminol in reversed micellar solution (Figure 20).[175] The limit of detection for atropine was 4×10^{-9} M. The method was applied to synthetic urine containing 100 ng mL^{-1} (4×10^{-7} M) atropine (using the standard additions technique) and pharmaceutical preparations containing atropine or scopolamine. The complexation and extraction impart significant selectivity, and approaches to eliminate interferences from sodium ions, cysteine and related tropane alkaloids were described. However, without chromatographic or electrophoretic separation, this approach is susceptible to unexpected interference from other pharmaceuticals, for which there is no obvious indicator (such as retention time or peak shape). This approach has since been adopted to determine amino- and nitro-aromatics,[176] and a related method with off-line complexation and extraction has been used to determine chlorpromazine in pharmaceutical preparations and biological fluids.[177]

Monforte *et al*. reported that cannabinoids inhibit the chemiluminescence reaction of luminol and hydrogen peroxide catalysed by haemoglobin,[178] and although many flow-analysis procedures based on inhibition or enhancement of luminol systems have since been developed,[45] this approach to determine cannabinoids has not been explored.

As in the case of deliriants, many compounds with dissociative or psychedelic properties (and their metabolites) possess tertiary or secondary amines (Figure 21), and could therefore potentially be detected at very low concentrations with $Ru(bipy)_3^{2+}$.[6] Chiba *et al*. developed a HPLC procedure with reverse-phase separation and $Ru(bipy)_3^{2+}$ chemiluminescence detection for phencyclidine (PCP; Figure 21a), its hydroxylated metabolites (Figure 21b), and their glucuronide conjugates (after acid hydrolysis).[70] The chemiluminescence reagent was electrochemically oxidized to the ruthenium(III) state online prior to mixing with the column

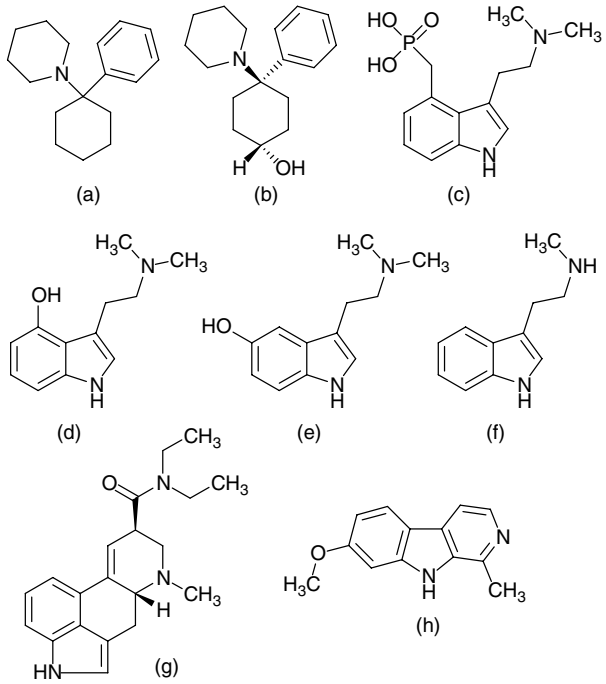

Figure 21. Dissociatives, psychedelics and related compounds: (a) phencyclidine, (b) *trans*-4-phenyl-4-piperidinocyclohexanol, (c) psilocybin, (d) psilocin, (e) bufotenine, (f) *N*-methyltryptamine, (g) lysergic acid diethylamide, and (h) harmine.

eluate.[179] Limits of detection ranged from 3×10^{-8} M to 6×10^{-8} M. The procedure was successfully applied to urine from rats administered with PCP; sample preparation involved solid-phase extraction.[70]

Using simple FIA systems, limits of detection of 4×10^{-8} M for the morphinan dissociative hallucinogen dextromethorphan (Figure 5c) with $Ru(bipy)_3^{2+}$ electrochemiluminescence detection,[60] and 3×10^{-10} M for the psychedelic prodrug psilocybin (Figure 21c) with $Ru(bipy)_3^{3+}$ (generated by off-line oxidation with lead dioxide), were obtained.[180] Due to the phenolic functionality of the active metabolite psilocin (Figure 21d), acidic potassium permanganate is a more suitable chemiluminescence reagent for this compound, and a limit of detection at 9×10^{-10} M psilocin has been reported. These FIA procedures were used to determine psilocybin and psilocin in hallucinogenic mushrooms, but the results were not validated.[180] The dual $Ru(bipy)_3^{2+}$/permanganate reagent developed for the simultaneous determination of phenolic and non-phenolic opiate alkaloids (Figure 8)[140] was applied to the post-column detection of psilocybin and psilocin.[181] Baseline resolution of the two analytes and an internal standard, 4-hydroxyindole, was obtained within five minutes on a C_{12} column. The limits of detection (4×10^{-9} M psilocybin and 1×10^{-8} M psilocin) were poorer than those using FIA with individual chemiluminescence reagents,[180] but still superior to those obtained with post-column UV absorbance detection (2×10^{-7} M psilocybin and 5×10^{-7} M psilocin) and those of previously published methodology.[181] Methanolic extracts from three hallucinogenic mushroom species were analyzed and good agreement was obtained between the UV absorbance and chemiluminescence modes of detection.

Using FIA methodology, Polo Martí *et al*., examined chemiluminescence reactions between the ergot alkaloid ergotamine and various oxidants in acidic and alkaline solution and found

that potassium hexacyanoferrate(III) in alkaline solution gave the greatest signal intensities.[98] Further improvements were made by adding dioxane to the sample and increasing the reaction temperature to 70 °C. The limit of detection was reported to be 1×10^{-12} M. The optimized procedure was also applied to methylergonovine and dihydroergocristine. Calculations based on molecular connectivity suggested that various other ergot alkaloids, including lysergic acid diethylamide (Figure 21g), would also be chemiluminescent.[98]

Cepas *et al*. have demonstrated peroxyoxalate chemiluminescence detection of β-carboline alkaloids[182] and dansylated methyltryptamines[55] with either TCPO or DNPO (Figure 3) and H_2O_2. Solutions were combined using the continuous-addition-of-reagent (CAR) technique,[183] where the aryl oxalate reagent was added to a reaction vessel containing the analyte and hydrogen peroxide, which minimizes the detrimental background emission from the reagents. The DNPO reagent provided more sensitive detection of the β-carboline alkaloids than TCPO; limits of detection with DNPO/H_2O_2 were 1×10^{-9} M harmalol, 1×10^{-9} M harmaline, 2×10^{-7} M harmane, 4×10^{-7} M harmol and 4×10^{-7} M harmine (Figure 21h).[182] Under the conditions optimized for these analytes (that did not require derivatization), 100-fold concentrations of the methyltryptamines did not interfere.[182] Limits of detection for the methyltryptamines that were derivatized with dansyl chloride and reacted with TCPO/H_2O_2 were 7×10^{-7} M *N*-methyltryptamine (Figure 21f), 9×10^{-7} M 5-methyltryptamine, 1×10^{-6} M bufotenin (Figure 21e), 4×10^{-6} M α-methyltryptamine and 1×10^{-5} M psilocin (Figure 21d).[55] These concentrations represent the sample before the derivatization procedure and extraction with chloroform. The limit of detection for dansylated psilocin added to the reaction vessel was 15 ng.

These procedures were applied to the determination of harmaline in spiked plasma,[182] and psilocybin in mushrooms,[55] but as with much of the previously discussed FIA methodology, they are vulnerable to interference (although preliminary extraction steps may improve selectivity). Nevertheless, the FIA and CAR procedures often demonstrate a sound chemiluminescence-based approach to detect an important class of analyte, which can be readily coupled with HPLC or CE separations for the analysis of complex samples. In the case of the peroxyoxalate chemiluminescence detection of β-carboline alkaloids,[182] Cepas *et al*. subsequently applied this approach to the HPLC determination of these alkaloids in *Heliconiini* butterflies (Figure 22).[184] Unlike the conventional approach to chemiluminescence detection in HPLC,[185] the column eluate was continuously merged with the reagents in a cuvette within a spectrofluorimeter (with its light source turned off), to prevent reactions occurring prior to the detection zone. Limits of detection for the six analytes were between 5- and 517-fold better than those obtained using HPLC with fluorescence detection.[184]

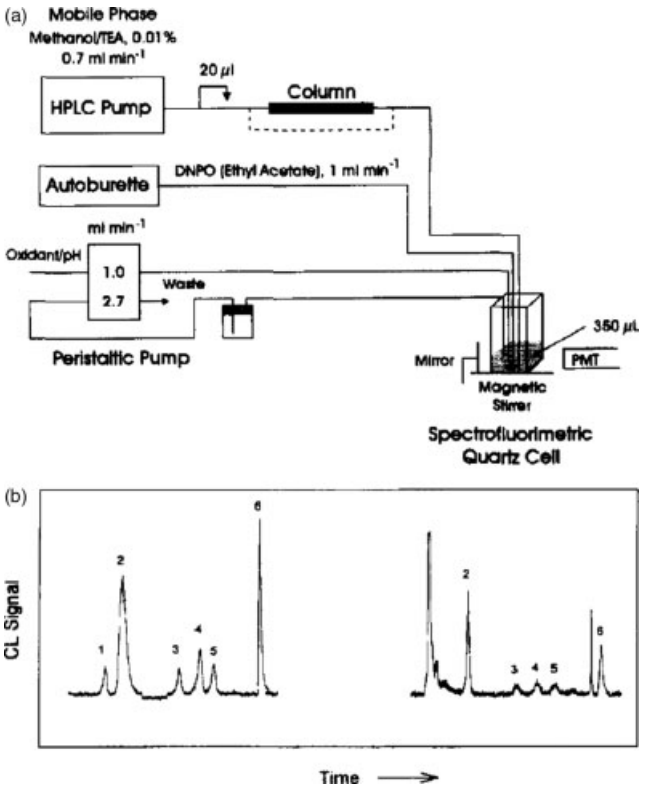

Figure 22. (a) Flow manifold used by Cepas *et al*. for the HPLC determination of β-carboline alkaloids with peroxyoxalate (DNPO/H2O2) chemiluminescence detection. (b) Chromatogram A: standard mixture, and B: extract from *Heliconiini eratohydara* butterflies. Peaks: 1 = harmol, 2 = harmalol, 3 = norharmane, 4 = harmane, 5 = harmine and 6 = harmaline. Reprinted from Cepas *et al*.[184] (© Elsevier, 1996. Reproduced with permission).

Concluding Remarks

Flow analysis procedures with chemiluminescence or electrochemiluminescence detection have been reported for a wide range of controlled drugs. These procedures have often provided better selectivity and significantly greater sensitivity than established methodology, using relatively simple and inexpensive instrumentation. In particular, HPLC with preliminary off-line derivatization and post-column peroxyoxalate chemiluminescence detection has provided an excellent means to detect illicit primary and secondary amines (and phenols) in complex sample matrices. Tris(2,2′-bipyridine)ruthenium(II) has emerged as the preeminent (electro)chemiluminescence reagent for the detection of tertiary and secondary amines, and acidic potassium permanganate has been found to be effective for various phenols and related compounds. The chemiluminescent reactions of analytes with other oxidants has significant potential but at present is only poorly understood. The rapid rates of many of these reactions are well suited to FIA, HPLC, and CE, and further improvements to sensitivity could be derived from the development of new chemiluminescence detectors that maximise mixing efficiency and the proportion of emitted light that is transferred to the photodetector. Perhaps most importantly, a greater understanding of the mechanism of the light-producing pathways and the relationships between analyte structure and chemiluminescence intensity is required for the true potential of chemiluminescence detection to be realized.

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