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Review

Chemiluminescence detection of opium poppy (*Papaver somniferum*) alkaloids

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ABSTRACT

A review with 98 references. The determination of the opium poppy (*Papaver somniferum*) alkaloids and their semi-synthetic derivatives has important applications in industrial process monitoring, clinical analysis and forensic science. Liquid-phase chemiluminescence reagents such as tris(2,2′-bipyridyl)ruthenium(II) and acidic potassium permanganate exhibit remarkable sensitivity and complementary selectivity for many *P. somniferum* alkaloids, which has been exploited in the development of a range of analytical procedures using flow analysis, high-performance liquid chromatography, capillary electrophoresis and microfluidic instrumentation.

Contents

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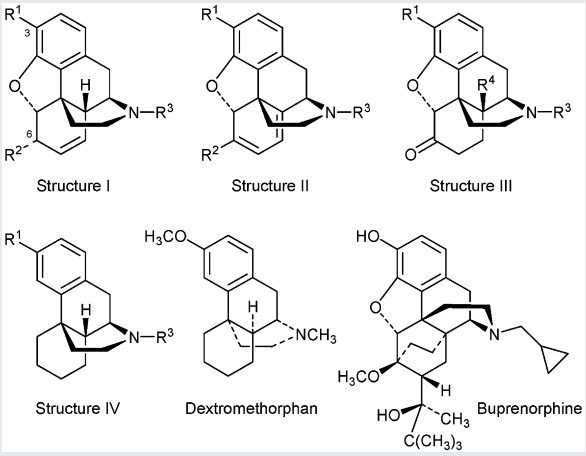
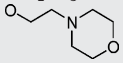
1. Introduction

Medicinal use of the opium poppy (*Papaver somniferum*) and opium – the alkaloid-rich latex exuded from surface incisions in the unripe seed heads – predates written history, but the isolation of morphine was not described until the early nineteenth century [1]. Many *P. somniferum* alkaloids are now known; the most significant in terms of their quantity within the plant are morphine, codeine, thebaine, noscapine, and papaverine [1]. Opiate alkaloids and their semi-synthetic derivatives (such as oxycodone, hydrocodone and pholcodine) are used extensively in medicine, and hundreds of tonnes of these compounds are produced by the pharmaceutical industry [2]. Accurate means to determine the *P. somniferum* alkaloids are therefore required for samples such as raw plant materials (to establish or monitor alkaloid abundance in different crops), industrial process streams (to optimise the extraction yields and reduce waste) and pharmaceutical formulations (for quality control and regulatory requirements). Furthermore, the misuse of opiate alkaloids, particularly the illegal trafficking and abuse of heroin (3,6-diacetylmorphine), has created the need to detect these substances on surfaces and in suspected illicit drug seizures, and identify and/or quantify the parent compounds and their metabolites in biological fluids and hair samples. General methodology for the determination of *P. somniferum* alkaloids has been discussed in previous reviews [3–7]; the determination of single or multiple analytes in complex sample matrices most commonly involves GC with mass spectrometric detection, or either HPLC or CE with UV-absorbance, fluorescence, electrochemical or mass spectrometric detection. Chemiluminescence (the emission of light from a chemical reaction) is an alternative mode of detection that provides high sensitivity using relatively simple instrumentation [8–11]. Chemiluminescence has been used to determine a wide range of *P. somniferum* alkaloids; many of their chemical structures are shown in Tables 1 and 2. It should be noted that these tables include derivatives and analogues that do not naturally occur in *P. somniferum*. The IUPAC numbering of relevant carbon atoms in Structure I (Table 1) has been shown to clarify the structure of some simple derivatives such as 6-monoacetylmorphine and 3-methoxycodeine, which were not included in the table.

Table 1
Selected morphinan alkaloids and their semi-synthetic derivatives



Structure I	R^1	R^2	R^3
Morphine	OH	OH	CH_3
Codeine	OCH_3	OH	CH_3
Normorphine	OH	OH	H
Nalorphine	OH	OH	$H_2C{=}CH_2$
Ethylmorphine	OCH_2CH_3	OH	CH_3
Pholcodine		OH	CH_3
Heroin			CH_3
Structure II	R^1	R^2	R^3
Oripavine	OH	OCH_3	CH_3
Thebaine	OCH_3	OCH_3	CH_3
Structure III	R^1	R^3	R^4
Naloxone	OH	$H_2C{=}CH_2$	OH
Naltrexone	OH		OH
Hydrocodone	OCH_3	CH_3	H
Oxycodone	OCH_3	CH_3	OH
Noroxycodone	OCH_3	H	OH
Structure IV	R^1	R^3	
Levallorphan	OH	$H_2C{=}CH_2$	
Norlevorphanol	OH	H	

2. Chemiluminescence reagents

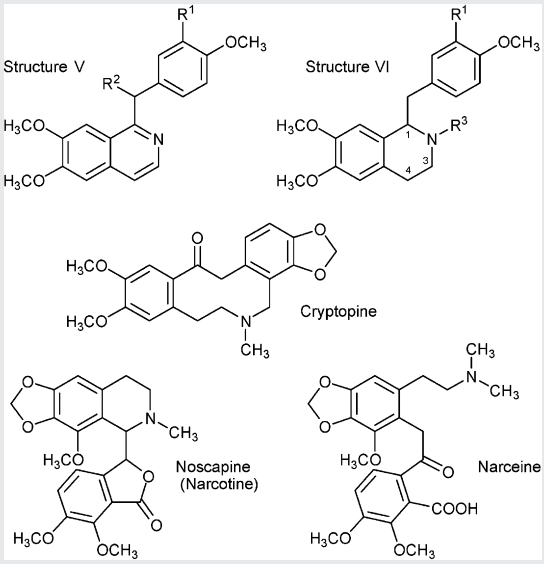
2.1. Potassium permanganate

Morphine was one of the first organic compounds to be detected with acidic potassium permanganate chemiluminescence [11,12], and although many other compounds have since been examined, very few can be detected at the exceedingly low concentrations reported for morphine and selected other *P. somniferum* alkaloids. The characteristic red emission from these reactions has been attributed to the production of an excited manganese(II) species and in corrected chemiluminescence spectra, the wavelength of maximum intensity is 734 ± 5 nm [13,14]. Polyphosphates and polyphosphoric acids are commonly used to enhance the chemiluminescence from reactions with acidic potassium permanganate. Interestingly, these enhancers shift the wavelength of maximum intensity to 689 ± 5 nm [13]. Polyphosphates have been employed extensively in the determination of *P. somniferum* alkaloids, but formic acid and formaldehyde, which have been shown to enhance the chemiluminescence with other analytes [11], have very rarely been used in the detection of these alkaloids.

Abbott et al. [15] and Barnett et al. [16] compared the chemiluminescence intensity from a range of *P. somniferum* alkaloids and other narcotic analgesics with acidic potassium permanganate, using flow injection analysis (FIA) methodology (Table 3). Compounds with a morphinan backbone, phenolic OH group at carbon-3 and furan bridge between C4 and C5 (Table 1; Structures I, II and III: R^1 = OH, and buprenorphine) were found to evoke a far more intense emission than all other compounds under investigation. For example, morphine and codeine differ only by their hydroxy and methoxy groups at carbon-3, but the response for codeine was only 2% of the response for morphine. The response for papaverine (a benzylisoquinoline alkaloid also found in *P. somniferum*) was 0.3%. Analgesics that shared little common structure with morphine, such as methadone, pethidine and fentanyl (not shown in table), gave a response of less than 0.1% [15].

However, a different relationship between analyte structure and chemiluminescence intensity was observed when *P. somniferum* alkaloids were treated with acidic potassium permanganate and sodium sulfite. Zhang and co-workers used these reagents to determine papaverine [17] and noscapine [18] (see Table 2) and found that morphine, codeine and heroin did not interfere at concentrations two orders of magnitude higher than that of the analytes.

Table 2
Selected benzylisoquinoline alkaloids and related species



Structure V	R^1	R^2
Papaverine	OCH_3	H
Papaveraldine	OCH_3	=O
Structure VI	**R^1**	**R^3**
Laudanosine[a]	OCH_3	CH_3
Laudanidine[b]	OH	CH_3

[a] S(+) configuration at carbon-1.
[b] R(−) configuration at carbon-1.

Table 3
Relative chemiluminescence signal for *P. Somniferum* alkaloids and related species with acidic potassium permanganate

Compound	Relative signal
Dihydromorphine	104
Buprenorphine	104
Normorphine	101
Nalorphine	100
Morphine	**100**
Morphine-*N*-oxide	99
6-Monoacetylmorphine	97
Oripavine[a]	71
Naloxone	59
Pseudomorphine[a]	29
Benzylmorphine	8.2
Ethylmorphine	3.3
Norcodeine	2.7
Phenazocine	2.2
Pentazocine	2.2
Codeine	**2.0**
Pholcodeine	1.9
Levallorphan	1.7
Dihydrocodeine	1.4
Morphine-3-glucuronide	0.8
Norlevorphanol	0.7
Thebacon	0.7
3,6-Diacetylmorphine	0.6
Hydrocodone	0.6
Thebaine	0.5
Papaverine	0.3
Oxycodone	0.2

[a] These compounds were compared to morphine in a separate study [16] under conditions that were not identical to main study (0.5 mg/mL analyte with 0.6 mM $KMnO_4$ in 0.1 M polyphosphoric acid, adjusted to pH 1.2 with HCl [15]).

Table 4
Relative chemiluminescence signal for various *P. somniferum* alkaloids with tris(2,2′-bipyridyl)ruthenium(III)

Compound	Relative signal
Codeine	**100**
6-Methoxycodeine	98
2,2′-Biscodeine (codeine dimer)	23
Noscapine[a]	16
Thebaine	15
Narcotine[a]	11
Narceine	2
Laudanosine	0.6
Papaverine	0.5
Papaveraldine	0.2
Cryptoine	0.1
Morphine-*N*-oxide	0.05
Oripavine	<0.01
Laudanidine	<0.01
Morphine	**<0.01**
Pseudomorphine	<0.01

[a] Although listed as two separate entries in table, these two names refer to the same compound (perhaps in this case from two different batches or sources).

Spectroscopic evidence presented by Zhang et al. [17,18], and the fact that a similar emission was observed from the reaction between papaverine, cerium(IV) and sulfite [19], point to an alternative light-producing pathway.

2.2. Tris(2,2′-bipyridyl)ruthenium(III)

Many compounds react with tris(2,2′-bipyridyl)ruthenium(III) (Fig. 1a), but only certain species evoke the characteristic orange chemiluminescence ($\lambda_{max} \sim 610$–620 nm) from this reagent [10,20]. Analytical investigations have largely focused on the oxalate ion and a variety of amines. As a general rule, the emission intensity from the reaction with amines is in the order: tertiary > secondary > primary; but subtle differences in chemical structure can have a dramatic effect [10,20]. Barnett and coworkers measured the response from a range of *P. somniferum* alkaloids with tris(2,2′-bipyridyl)ruthenium(III) in acetate buffer at pH 5.8 (Table 4) [21]. The greatest chemiluminescence intensity was obtained with compounds such as codeine, 6-methoxycodeine and thebaine, which possess a tertiary amine within a morphinan backbone, and a methoxy group at carbon-3 (Table 1; Structures I and II: $R^1 = OCH_3$) [21]. Alkaloid derivatives such as heroin, oxycodone and 10-oxocodeine have also been found to evoke an intense emission with tris(2,2′-bipyridyl)ruthenium(III) [22,23] and therefore species in Table 1 that evoke an intense emission with this reagent can be broadened to those where R^1 is not a hydroxyl group, and R^3 is a methyl or larger alkyl group.

Derivatives with aromatic or quaternary amines, such as codeine-*N*-oxide and codeine-*N*-methyliodide, did not evoke the intense emission observed for closely related species with tertiary aliphatic amines [22]. At pH 5.8, a reasonable response was obtained with noscapine, but most of the benzylisoquinoline alkaloids and related species shown in Table 2 gave a response that was less than 1% of that for codeine [21]. In stark contrast to the selectivity of acidic potassium permanganate, phenolic compounds such as morphine, oripavine, and laudanidine gave a relatively poor response: less than 0.01% of that observed for codeine [21]. Greenway et al. reported similar differences in emission intensity for codeine and morphine using electrogenerated chemiluminescence with tris(2,2′-bipyridyl)ruthenium(II) [24].

At higher pH (between 7 and 12), an intense emission can be observed with compounds that gave a moderate or poor response below pH 6, including morphine, pseudomorphine, laudanosine,

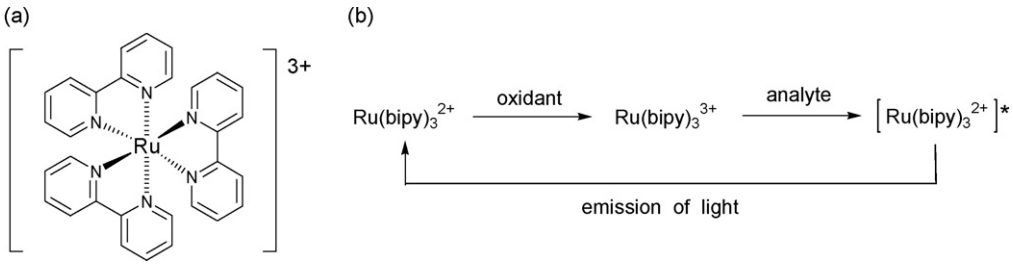


Fig. 1. (a) Chemical structure and (b) preparation, reaction and regeneration of the tris(2,2′-bipyridyl)ruthenium(III) reagent.

narceine and noscapine [22], but analysis at high pH is complicated by the oxidation of water by the reagent [25].

Tris(2,2′-bipyridyl)ruthenium(III) is only moderately stable in acidic aqueous solutions [25] and therefore it is normally produced immediately prior to use by chemical or electrochemical oxidation of the corresponding ruthenium(II) complex (Fig. 1b). Subsequent reaction with a suitable reducing agent (analyte) produces the ruthenium(II) complex in an electronically excited state (a short-lived $d\pi^*$ triplet), that can emit a photon of light to return to the ground state. If the reagent is immobilised [26–28], it can be isolated from the spent analyte solution and re-oxidised to the active ruthenium(III) form (Fig. 1b). Cerium(IV) and lead dioxide are the two most commonly used chemical oxidants for the generation of tris(2,2′-bipyridyl)ruthenium(III) [26,27]. Cerium(IV) has been used for on-line oxidation of the reagent (both solution-phase [29–31] and immobilised [27,32]). In contrast, solid lead dioxide is used prior to the analytical procedure and the excess oxidant is filtered off. A re-circulating reagent system, where the ruthenium chelate is continually aspirated from (and returned to) a beaker containing lead dioxide, has been used to minimise reagent variation [25,33]. The anhydrous perchlorate salt of tris(2,2′-bipyridyl)ruthenium(III) is temporally stable as a solid or dissolved in dry acetonitrile [34], and has been shown to be a useful alternative for chemiluminescence detection [34–36].

2.3. Other reagents

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is one of the most commonly used liquid-phase chemiluminescence reagents, which has been applied to the determination of oxidants, transition metal ions and complexes (which catalyse the oxidation of luminol with hydrogen peroxide), and a wide variety of compounds that either enhance or inhibit the production of light [9]. This approach has been used to determine alkaloids such as morphine [37,38], codeine [37–39] and heroin [40].

Soluble manganese(IV) is a relatively new chemiluminescence reagent [41,42], which can be prepared by reducing potassium permanganate with sodium formate and dissolving the solid manganese dioxide product in 3 M orthophosphoric acid. The spectral distribution for reactions with soluble manganese(IV) ($\lambda_{max} = 730 \pm 5$ nm) [42] is similar to that of acidic potassium permanganate [manganese(VII)] chemiluminescence, and there is substantial evidence to support a manganese(II) species as the common emitter [13]. Although the light-producing reaction pathways of these two reagents are related, they do not exhibit the same selectivity. For example, limits of detection for morphine, codeine and papaverine are vastly different with permanganate [15,43], but are all within one order of magnitude with soluble manganese(IV) [42]. Although this reagent is not as sensitive as acidic potassium permanganate for phenolic morphinan alkaloids, it could provide a more universal chemiluminescence detection system for HPLC [42].

Methyltriphenylphosphonium permanganate was examined as an alternative to potassium permanganate for the determination of morphine in non-aqueous process samples [44]. It was thought that this reagent may be more available to react with the analyte through the formation of an ion-pair in the water-immiscible solvent, but similar analytical performance to that of the potassium salt was observed.

Rezaei and co-workers used tris(1,10-phenanthroline) ruthenium(II) to determine noscapine (using on-line chemical oxidation of the reagent to the ruthenium(III) state) [45]. They stated that the reagent provided greater sensitivity than tris(2,2′-bipyridyl)ruthenium(II) (citing previous work involving other analytes) but the two reagents were not directly compared for the determination of noscapine [45]. Michel and co-workers compared the electrochemiluminescence of codeine with four different ruthenium(II) complexes and found that bis(2,2′-bipyridyl)(1,10-phenanthroline)ruthenium(II) produced a more intense signal (2.5-fold greater) than tris(2,2′-bipyridyl)ruthenium(II) [46]. However, this closely related complex is not commercially available and is therefore less practical for routine application.

Papadopoulos and co-workers reported the determination of papaverine and other aza-aromatics using 'photostorage chemiluminescence' [47]. The analyte was dissolved in DMF and irradiated with a xenon lamp until the characteristic bands in the ultraviolet region disappeared and the light-producing reaction was then initiated by adding a strong base. A linear range for papaverine of 5×10^{-7} to 1×10^{-4} M was reported.

Gas-phase chemiluminescence detectors have been applied to the determination of opiate alkaloids [48,49], and chemiluminescence has been used to study the microbicidal oxidative function of human neutrophils after clinical doses of pharmaceuticals, including morphine [50], but a detailed discussion of these approaches is outside the scope of this review.

3. Instrumental approaches and applications

Analytical procedures for the determination of *P. somniferum* alkaloids based on chemiluminescence detection with potassium permanganate, tris(2,2′-bipyridyl)ruthenium(III) and other reagents are shown in Tables 5–7, respectively. Each table is divided into various instrumental approaches and then arranged in chronological order of publication. The following discussion is focussed on procedures that were applied to real samples.

3.1. Flow analysis (FIA and related approaches)

Potassium permanganate and tris(2,2′-bipyridyl)ruthenium(III) react rapidly with the analytes of interest, and therefore reproducible mixing at (or immediately prior to) the point of detection is essential for analytical applications. Chemiluminescence detectors for flow analysis are often custom built and generally consist of a flat reaction coil placed against a photomultiplier tube in light-

Table 5
Detection of *P. somniferum* alkaloids with acidic potassium permanganate chemiluminescence

Approach	Enhancer	Analytes	Limit of detection	Sample(s)	Reference
FIA	Polyphosphoric acid	Morphine	1×10^{-10} M	Not applied	[15,51]
FIA	Polyphosphoric acid	Buprenorphine	1×10^{-8} M	Tablets	[90]
FIA	–	Morphine	7×10^{-7} M	Not applied	[91]
FIA	Tetraphosphoric acid	Morphine	5×10^{-8} M	Process streams	[16]
FIA	Polyphosphoric acid	Codeine	3×10^{-7} M	Not applied	[43]
FIA	Polyphosphate	Morphine	1×10^{-10} M	Not applied	[52]
FIA	Sulfite	Papaverine	1×10^{-7} M	Injections and tablets	[17]
FIA	Sulfite	Noscapine	8×10^{-9} M	Synthetic samples	[18]
FIA[a]	Sulfite, polyphosphoric acid	Morphine	7×10^{-9} M	Urine	[55]
FIA	Polyphosphate	Heroin (after hydrolysis to morphine)	Not stated	Drug seizure samples	[35]
SIA	Hexametaphosphate	Morphine	1×10^{-8} M	Process streams	[54]
SIA	Hexametaphosphate	Morphine	1×10^{-6} M	Non-aqueous process streams	[44]
SIA	Polyphosphate	Morphine	5×10^{-11} M	Not applied	[92]
[b]	Tetraphosphoric acid	Morphine (and urinary metabolite)	1×10^{-8} M	Urine	[93]
Batch	–	Naltrexone	6×10^{-9} M	Tablets and capsules	[94]
SFA[c]	Polyphosphate	Morphine	2×10^{-8} M	Process samples	[95]
SFA	–	Naloxone	5×10^{-7} M	Pharmaceuticals	[53]
SFA	Formaldehyde	Morphine	1×10^{-8} M	Not applied	[96]
		Naloxone	1×10^{-7} M		
HPLC	Polyphosphoric acid	Morphine	9×10^{-8} M	Blood and urine	[62]
HPLC	Polyphosphoric acid	Codeine	3×10^{-6} M[d]	Opium and urine	[63]
		Heroin	3×10^{-7} M		
		3-Monoacetylmorphine	3×10^{-8} M		
		Morphine	4×10^{-10} M		
HPLC	Polyphosphoric acid, hexametaphosphate	Monoacetylmorphine	5×10^{-8} M	Not applied	[64]
		Morphine	4×10^{-9} M		
HPLC[e]	Polyphosphate	Codeine	5×10^{-7} M	Industrial process samples	[67]
		Morphine	1×10^{-6} M		
		Oripavine	3×10^{-6} M		
		Thebaine	2×10^{-6} M		
HPLC	Polyphosphate	Morphine	1×10^{-10} M	Industrial process samples	[56]
		Oripavine	5×10^{-10} M		
HPLC	Polyphosphate	Morphine	3×10^{-9} M	Not applied	[97]
		Oripavine	3×10^{-9} M		
		Pseudomorphine	1×10^{-8} M		
CE	Polyphosphoric acid, β-cyclodextrin	Heroin	115 fmol[f]	Not applied	[77]
		6-Monoacetylmorphine	66 fmol		
		Morphine	23 fmol		
CE	Polyphosphate	Morphine	3×10^{-7} M	Industrial process samples	[78]
		Oripavine	3×10^{-7} M		
		Pseudomorphine	5×10^{-7} M		

[a] Incorporating a molecular imprinted polymer glass column.
[b] Papers published in a language other than English and the required information was not included in the English language abstract.
[c] SFA: stopped-flow analysis.
[d] Corrected limits of detection (see [11]).
[e] Dual chemiluminescence reagent (permanganate combined with $Ru(bipy)_3^{2+}$).
[f] Injection volume not stated.

tight housing (Fig. 2). In most of the flow analysis systems that have been used for the determination of opiate alkaloids, there is no physical separation of the target analyte from the sample matrix. Accurate measurement therefore depends upon: (i) the inherent selectivity of the particular chemiluminescence reagent under the selected conditions; (ii) sufficiently low concentrations of interfering species in the sample.

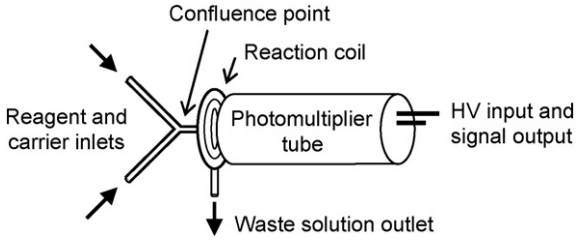


Fig. 2. Components of a chemiluminescence detector for flow analysis.

Morphine can be detected at concentrations as low as 1×10^{-10} M using acidic potassium permanganate in a simple two-line FIA instrument [15,51,52]. Alwarthan and Townshend used this approach to determine buprenorphine [15], and Murillo Pulgarín et al. used a related stopped-flow system to determine naloxone [53], in pharmaceutical preparations. In both cases, no sample pre-treatment was required, other than dissolution of the tablets [15,53]. Barnett and co-workers applied this simple configuration to the determination of morphine in 'rich extract' process liquors and, in spite of the complexity of the sample matrix, the results were in good agreement with those obtained using a validated liquid chromatographic method [16]. Interference from non-phenolic alkaloids, such as codeine, thebaine and papaverine was negligible

Table 6
Detection of *P. somniferum* alkaloids with tris(2,2′-bipyridyl)ruthenium(III) chemiluminescence

Approach	Method of $Ru(bipy)_3^{2+}$ oxidation	Analytes	Limits of detection	Sample(s)	Reference
FIA	Electrochemical	Codeine Dextromethorphan Heroin Morphine	2×10^{-8} M 4×10^{-8} M 5×10^{-8} M 1×10^{-5} M	Not applied	[24]
FIA	Chemical (lead dioxide), in on-line solid phase reactor	Codeine	5×10^{-9} M	Industrial process samples	[21]
FIA	Chemical (lead dioxide), in re-circulating system	Codeine	Not stated	Not applied	[25]
FIA	Chemical (chlorine), during preparation of $[Ru(bipy)_3](ClO_4)_3$	Codeine	5×10^{-9} M	Not applied	[34]
FIA	Electrochemical	Codeine Morphine	[a]	[a]	[98]
FIA	Chemical (cerium(IV)) oxidation of immobilised reagent	Codeine	3×10^{-7} M	Not applied	[27]
FIA	Electrochemical oxidation of immobilised reagent	Heroin	1×10^{-6} M	Not applied	[28]
FIA	Chemical (chlorine), during preparation of $[Ru(bipy)_3](ClO_4)_3$	Heroin	Not stated	Heroin seizure samples	[35]
FIA	Electrochemical oxidation of immobilised reagent	Codeine Morphine	5×10^{-9} M 3×10^{-8} M	Drugs seized from illegal suppliers	[58]
SIA	Chemical (cerium(IV)) oxidation of immobilised reagent	Codeine	1×10^{-8} M	Not applied	[27]
SIA	Chemical (cerium(IV)) oxidation of immobilised reagent	Codeine Thebaine	5×10^{-10} M 5×10^{-9} M	Not applied	[32]
HPLC	Photochemical ($S_2O_8^{2-}$)	Dihydrocodeine	[a]	Cough syrup	[65]
HPLC	Chemical (permanganate), dual chemiluminescence reagent	Codeine Morphine Oripavine Thebaine	5×10^{-7} M 1×10^{-6} M 3×10^{-6} M 2×10^{-6} M	Industrial process samples	[67]
HPLC	Electrochemical	Oxycodone Noroxycodone	2×10^{-9} M 9×10^{-9} M	Dog plasma and dog urine	[23]
HPLC	Electrochemical	Oxycodone Noroxycodone	2×10^{-9} M 3×10^{-9} M	Human plasma	[66]
HPLC	Chemical (lead dioxide)	Codeine Thebaine	5×10^{-10} M 1×10^{-9} M	Industrial process samples	[56]
HPLC	Chemical (lead dioxide)	Codeine Ethylmorphine Thebaine	5×10^{-9} M 5×10^{-9} M 5×10^{-9} M	Not applied	[97]
CE	Chemical (lead dioxide)	Codeine 6-Methoxycodeine Thebaine	5×10^{-8} M 5×10^{-8} M 1×10^{-7} M	Not applied	[79]
CE	Electrochemical	Heroin	5×10^{-8} M	Contaminated banknotes	[80]
MD[b]	Electrochemical oxidation of $Ru(bipy)_3^{2+}$ or $[Ru(bipy)_2(phen)]^{2+}$	Codeine	1×10^{-7} M (batch) 5×10^{-6} M (FIA)	Pharmaceutical products	[46]
MD	Electrochemical	Codeine	1×10^{-4} M	Pharmaceutical products	[86]
MD	Electrochemical oxidation of immobilised reagent	Codeine	2×10^{-5} M (batch)	Pharmaceutical products	[87]
MD	Chemical (lead dioxide)	Codeine	8×10^{-7} M	Not applied	[88]

[a] Papers published in languages other than English and the required information was not included in the English language abstract.
[b] MD: microfabricated device.

due to the selectivity of the permanganate reagent and, although a comparable signal is obtained from oripavine and pseudomorphine (Table 3), these species were present at concentrations 100 times less than that of morphine [16]. Morphine has been determined in aqueous [54] and non-aqueous [44] process streams using sequential injection analysis (SIA) with permanganate chemiluminescence detection. Samples were collected from numerous points along the process line and for some samples there was a significant (up to 40%) difference between the SIA chemiluminescence and conventional HPLC results, which was attributed to matrix effects associated with pH and suspended solids [54].

He and co-workers reported a flow system incorporating a glass column packed with a molecular-imprinted polymer for the determination of morphine in the urine of heroin abusers [55]. The sample was pumped through the column, followed by a wash solution to remove interfering species, and then a mixture of the wash solution and the acidic permanganate reagent to initiate the chemiluminescence reaction and, finally, the column was flushed with water. The use of the column increased the tolerance ratio of interfering species (e.g. epinephrine, ascorbic acid and codeine) to morphine by approximately two orders of magnitude. Interestingly, He and co-workers added sodium sulfite to the wash solution to minimise oxidation of the polymer over time [55]; this reducing agent has been used in conjunction with acidic permanganate or cerium(IV) by other researchers to determine non-phenolic alkaloids (papaverine [17,19] and noscapine [18]) and it was reported that morphine did not interfere, even at concentrations two orders of magnitude greater than the target analytes [17,18]. The flow analysis manifold used to mix the sample with permanganate and sulfite reagents was more complex than those generally required for acidic potassium permanganate chemiluminescence. The authors described the determination of papaverine in pharmaceutical preparations [17,19] and compound liquorice tablets [17], and percentage recoveries in spiked urine and serum

Table 7
Detection of *P. somniferum* alkaloids with other chemiluminescence reagents

Reagent	Approach	Analytes	Limit of detection	Sample(s)	Reference
Luminol, hydrogen peroxide and Reinecke salt	FIA	Codeine	2×10^{-6} M	Pharmaceutical preparations	[37,38]
		Morphine	2×10^{-7} M		
		Sinomenine	2×10^{-7} M		
Luminol and hydrogen peroxide	[a]	Heroin	3×10^{-9} M	[a]	[40]
Luminol and potassium ferricyanide	FIA	Codeine	1×10^{-7} M	Codeine tablets	[39]
Cerium(IV) and sulfite	FIA	Papaverine	9×10^{-8} M	Pharmaceutical preparations and biological fluids	[19]
Strong base after irradiation with a xenon lamp	Batch	Papaverine	Not stated	Not applied	[47]
Soluble manganese(IV)	FIA	Codeine	5×10^{-8} M	Not applied	[41]
		Morphine	8×10^{-8} M		
Soluble manganese(IV)	FIA (HPLC demonstrated)	Codeine	1×10^{-8} M	Not applied	[42]
		Heroin	1×10^{-6} M		
		Morphine	5×10^{-8} M		
		Oripavine	5×10^{-9} M		
		Papaverine	1×10^{-9} M		
		Pseudomorphine	1×10^{-9} M		
		Thebaine	5×10^{-9} M		
Tris(1,10-phenantholine) ruthenium(II) and cerium(IV)	FIA	Noscapine	7×10^{-8} M	Cough syrup	[45]

[a] Paper published in a language other than English and the required information was not included in the English language abstract.

extracts [19], but it was not mentioned whether a response was obtained from other species in the biological samples before the samples were spiked with papaverine.

A limit of detection of 3×10^{-7} M has been reported for codeine with an acidic potassium permanganate reagent using FIA methodology [43], but the determination of codeine in process liquors with this reagent is hindered by the overwhelming response from morphine. However, tris(2,2′-bipyridyl)ruthenium(III) is far more selective towards non-phenolic morphinan alkaloids (Table 4) and detection limits for codeine as low as 5×10^{-10} M have been reported [32]. This chemistry has been applied to the determination of codeine in process liquors using FIA methodology [21]. In that particular study, a solid-phase reactor containing lead dioxide was used to oxidise tris(2,2′-bipyridyl)ruthenium(II) on-line, instead of the more conventional oxidation prior to injection. Samples were collected from two different points along the process line. The results for the FIA-chemiluminescence and conventional HPLC procedures were in good agreement for one sample, but the standard additions method was required to reach agreement for the other sample [21]. As with the determination of morphine in process liquors with acidic potassium permanganate [54], this highlights the susceptibility of FIA and SIA methodology to matrix effects, in spite of the fact that the high sensitivity of the reagents allows sample dilution of between 100- and 100,000-fold [16,21,54]. Furthermore, the concentrations of morphine, codeine and other alkaloids vary widely at different points of the extraction and methylation processes [56] and therefore physical separation of sample components is often required for an accurate determination of opiate alkaloids in industrial process samples.

Agg and co-workers reported a rapid method for the detection of heroin in drug seizure samples, based on a simple hydrolysis procedure and FIA or SIA with two chemiluminescence reagents [35,57]. The concept behind this test is depicted in Fig. 3. Before hydrolysis, heroin evokes an intense response from tris(2,2′-bipyridyl)ruthenium(III) perchlorate and a relatively weak response with potassium permanganate. However, the reverse was observed with the hydrolysis products: 6-monoacetylmorphine and morphine. Some tertiary amines (such as codeine, strychnine and chloroquine) caused false positives with tris(2,2′-bipyridyl)ruthenium(III), but they did not produce the markedly increased response with the permanganate reagent after the hydrolysis procedure, and therefore these species

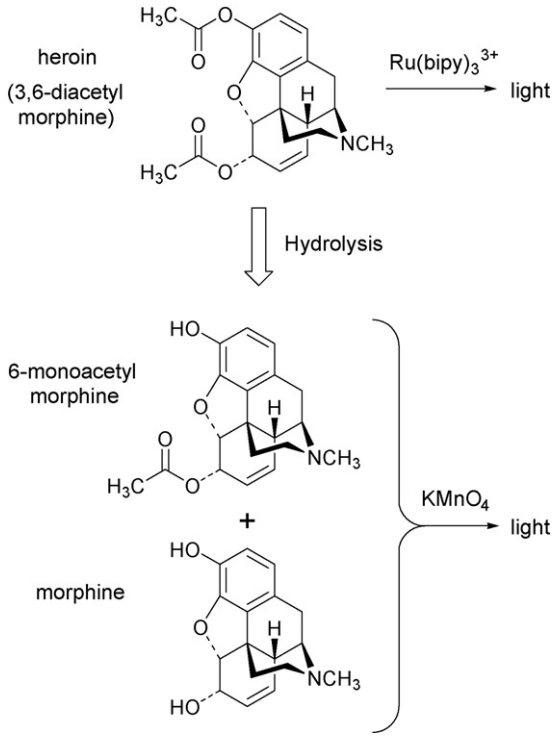


Fig. 3. Concept for a rapid screening test for heroin. Reprinted from Ref. [57], Copyright (2008), with permission from Elsevier.

did not interfere in the overall procedure [35,57]. Agg and co-workers have also described a spray reagent containing tris(2,2′-bipyridyl)ruthenium(III) for the detection of heroin on surfaces [36]. Qiu and co-workers described an electrochemiluminescence flow through detector containing tris(2,2′-bipyridyl)ruthenium(II) immobilised in a film of organically modified silicates on the electrode surface, which they used to establish the purity of drugs (codeine and morphine) seized from illegal suppliers [58]. The purity of three samples of each drug (between 81.5 and 97.2%) established using the proposed procedure was in good agreement with the results obtained using GC-MS.

Luminol has been used to detect certain transition metal ions at concentrations as low as 10^{-11} M [9], but the sensitivity of luminol systems for enhancers and inhibitors is much poorer [59–61]. Detection limits for opiate alkaloids using this approach are generally poorer than those obtained with potassium permanganate and tris(2,2′-bipyridyl)ruthenium(III), and the selectivity has not been as thoroughly explored. Nevertheless, Li [37] and Feng et al. [39] have determined several alkaloids in pharmaceutical preparations.

3.2. High-performance liquid chromatography

Chemiluminescence detectors designed for FIA can be effectively coupled to HPLC by replacing the carrier line in the T-piece with the outlet line from the column (or after the UV-absorbance detector). Unlike FIA, this approach allows many opiate alkaloids to be detected without interference from the sample matrix. The main complication is that the optimum conditions for separation may not match those for detection.

Soon after Abbott and co-workers reported the determination of morphine with acidic potassium permanganate using FIA [15,51], they applied this method of detection to the determination of morphine in urine and blood using HPLC [62]. An examination of common HPLC mobile phase solvents revealed that acetonitrile completely quenched the chemiluminescence. THF did not quench, but dissolved the PVC tubing that they used to connect the T-piece to the manifold tubing. Alcohols caused some loss of signal, particularly at high concentrations. Therefore, a mobile phase consisting of 12.5% methanol and 87.5% polyphosphoric acid (0.01 M aqueous solution) was selected. Samples were pre-treated by solid–liquid extraction and *N*-ethylnormorphine was used as an internal standard. Chemiluminescence was found to be more selective and sensitive than UV-absorbance under identical separation conditions [62].

Zhu and co-workers used a similar approach to determine morphine, heroin, 3-monoacetylmorphine and codeine in raw opium and the urine from drug addicts [63], but the detection limits for the three non-phenolic derivatives were between two and four orders of magnitude poorer than that for morphine. Amiott and Andrews modified the procedure reported by Abbott and co-workers and determined morphine, 6-monoacetylmorphine and an internal standard (nalorphine; a commercially available morphine derivative) [64].

Tris(2,2′-bipyridyl)ruthenium(III) has also been used for post-column chemiluminescence detection of opiate alkaloids and their derivatives [23,65–67]. Gemba and co-workers determined oxycodone (a synthetic opiate agonist) and its *N*-demethylated metabolite, noroxycodone, in dog urine [23] and human and dog plasma [23,66] using reversed-phase HPLC after solid-phase extraction. The tris(2,2′-bipyridyl)ruthenium(II) reagent was electrochemically oxidised to the ruthenium(III) state, on-line, prior to merging with the column eluent [66]. In spite of the different degree of amine substitution (see Table 1: Structure III), similar detection limits were reported for the two analytes [66]. Chiba and co-workers used narceine (see Table 2) as an internal standard for the determination of yohimbine (an alkaloid from other plants) in serum by ion-pair HPLC with tris(2,2′-bipyridyl)ruthenium(III) chemiluminescence detection [68].

Costin and co-workers recently determined opiate alkaloids in industrial process samples using monolithic column chromatography with chemiluminescence detection [56]. Compared to conventional packed columns, highly porous monolithic columns (such as the Chromolith SpeedROD) allow high flow rates to be applied at low pressure and without a significant decrease in separation efficiency. This reduces the time required for separation and can improve sensitivity though reduced band broadening and flow-rates that are closer to the optimum conditions for light-production within coiled flow-through detection cells [69,70]. Costin and co-workers determined codeine and thebaine within 2 min using a flow rate of 3 mL/min, solvent gradient of acetonitrile in an aqueous solution of trifluoroacetic acid, and tris(2,2′-bipyridyl)ruthenium(III) chemiluminescence detection [56]. Morphine and oripavine could also be determined in 2 min, using a solvent gradient of methanol in an aqueous solution of trifluoroacetic acid, and acidic potassium permanganate chemiluminescence. These two procedures provided limits of detection for the four opiate alkaloids that were similar to the best values obtained with FIA and SIA methodology. Samples were taken from various points in the process line and diluted between 250 and 125,000 times to operate within linear calibration ranges. The results were in good agreement with those obtained using the GlaxoSmithKline standard procedure, based on ion-pairing HPLC with UV-absorbance detection [56].

This approach could also be useful in forensic entomotoxicology. Gunn and co-workers developed a HPLC procedure incorporating a monolithic column and permanganate chemiluminescence detection for the determination of morphine in the larvae of the Australian blow fly (*Calliphora stygia*) [71].

As described above, both acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(III) are highly selective towards distinct groups of *P. somniferum* alkaloids, which often provides an advantage over other modes of detection. However, in some instances, the determination of analytes from both groups is required. Two methods to determine a broader range of *P. somniferum* alkaloids with chemiluminescence detection have been reported [42,67].

In the first [67], a dual function chemiluminescence reagent was prepared on-line by combining solutions of tris(2,2′-bipyridyl)ruthenium(II) and acidic potassium permanganate before merging with the column eluent immediately prior to the flow-through detector. In this system, the permanganate solution also conveniently served the role of oxidising the other reagent to the ruthenium(III) state. Morphine, codeine, oripavine and thebaine were separated with ion-paring HPLC and detection limits for the four analytes were within one order of magnitude. The dual reagent was far less sensitive than tris(2,2′-bipyridyl)ruthenium(II) and acidic potassium permanganate individually (for codeine and thebaine and for morphine and oripavine, respectively) but still superior to UV-absorbance detection for these compounds in industrial process samples [67].

In the second method to broaden this mode of detection, the column eluent was merged with a formaldehyde solution and then a soluble manganese(IV) reagent [42]. Six opiate alkaloids were separated within 4 min using monolithic column HPLC. As with the dual reagent, analytes that produced a strong response with tris(2,2′-bipyridyl)ruthenium(III) (codeine and thebaine) and with acidic potassium permanganate (morphine, oripavine and pseudomorphine) both produced a reasonable response with soluble manganese(IV), but in this case papaverine also gave a comparable signal. The limit of detection for all six alkaloids was approxi-

mately 5×10^{-7} M, when an injection volume of 2 μL was used [42].

3.3. Capillary electrophoresis

Capillary electrophoresis (CE) can provide excellent resolution within short analysis times and is a useful alternative to HPLC for the separation of complex mixtures, particularly biological materials, as relatively small sample volumes can be analysed [72–74]. As discussed by Hindson and co-workers [75], many CE procedures for the determination of opiate alkaloids in process monitoring, pharmaceutical and forensic science applications have been reported. Most of these have incorporated UV-absorbance detection, which has limited sensitivity due to the small internal diameter of the separation capillary [72,73]. Chemiluminescence detection is a low-cost option to increase the sensitivity and selectivity, but the capillary end inside the buffer reservoir complicates the addition of reagents and the optimal conditions for separation are not always compatible with those for the chemiluminescence detection. Consequently, much of the research into CE with chemiluminescence detection has focussed on the development of suitable detectors [72,73,76].

Nevertheless, opiate alkaloids have been determined using CE with acidic potassium permanganate [77,78] and tris(2,2′-bipyridyl)ruthenium(III) [79,80] as chemiluminescence reagents. Barnett and co-workers determined codeine, 6-methoxycodeine and thebaine in a standard mixture using tris(2,2′-bipyridyl)ruthenium(III) chemiluminescence detection at the capillary end [79]. In this system, the capillary and electrode were inserted through a rubber seal into a glass vial that was positioned on top of a photomultiplier tube (Fig. 4). The vial was filled with reagent solution prior to each analysis. Separation was complete within 14 min and the detection limits were between 5×10^{-8} and 1×10^{-7} M. The direct transfer of this technology to the determination of morphine, oripavine and pseudomorphine with acidic potassium permanganate was problematic, due to migration of the reagent anion into the separation capillary [78]. However, this was overcome by reversing the polarity of the electrodes. Hexadiamethrine bromide was added to the electrolyte to direct the electroosmotic flow towards the anode (and detector), and α-cyclodextrin was added to improve the separation efficiency. In addition, the use of a flowing reagent increased signal intensity and reduced peak width. The three analytes were resolved within 5 min, with detection limits from 2.5×10^{-7} to 5×10^{-7} M. In a preliminary evaluation with a process liquor, relatively small peaks for oripavine and pseudomorphine were detected on either side of the dominant peak for morphine [78].

Cheng and co-workers developed a sheath-flow chemiluminescence detector for CE [81], which they applied to the determination of morphine, 6-monoacetylmorphine and heroin with acidic potassium permanganate [77]. After removing the last 10 cm of coating from the separation capillary (50 μm i.d.), it was inserted into a larger (530 μm i.d.) 'reaction' capillary. A third capillary was used to deliver the chemiluminescence reagent (by gravity). The capillaries and grounding electrode were fixed in place within a four-way Plexiglass joint and a photomultiplier tube was placed against a 1-cm detection window burnt into the reaction capillary. β-Cyclodextrins were added to the separation buffer to enhance separation efficiency and the three analytes were resolved within 9 min.

3.4. Miniaturised devices

Miniaturised chemical analysis involves the quantitation of low concentrations of analytes in exceedingly small volumes of solution and therefore highly sensitive and selective modes of detection such as laser-induced fluorescence, chemiluminescence and electrochemistry are often more suitable than UV-absorbance [82,83]. Chemiluminescence is an attractive option because (unlike other spectroscopic modes of detection) an external light source is not required and therefore the instrumentation is far simpler [76,83]. Electrochemiluminescence detectors for microfluidic devices have also been developed [84].

The chemiluminescence or electrochemiluminescence detection of codeine with tris(2,2′-bipyridyl)ruthenium(II) has been used to demonstrate the viability of several miniaturised analytical approaches. Researchers at the University of Neuchâtel in Switzerland developed a miniaturised electrochemiluminescence detector, where electrode transducer and photodetector were incorporated on a 5 × 6 mm silicon chip [46,85–87]. The performance of the device was initially evaluated using tris(2,2′-bipyridyl)ruthenium(II) with tripropylamine [85], but it was later adapted for use as a flow-though detection cell for FIA (Fig. 5) and the electrochemiluminescence from a range of ruthenium(II) complexes with codeine was examined, and applied to the analysis of two pharmaceutical preparations [46]. This group also immobilised

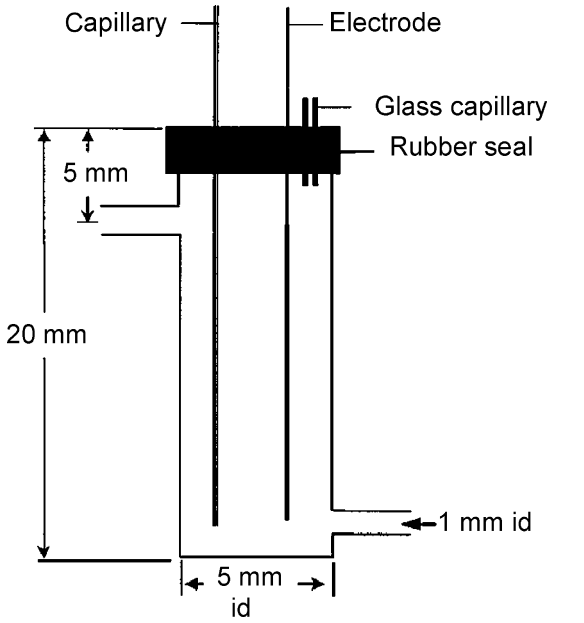


Fig. 4. A glass cell for chemiluminescence detection after separation using capillary electrophoresis. Reprinted from Ref. [79], Copyright (1998), with permission from The Royal Society of Chemistry.

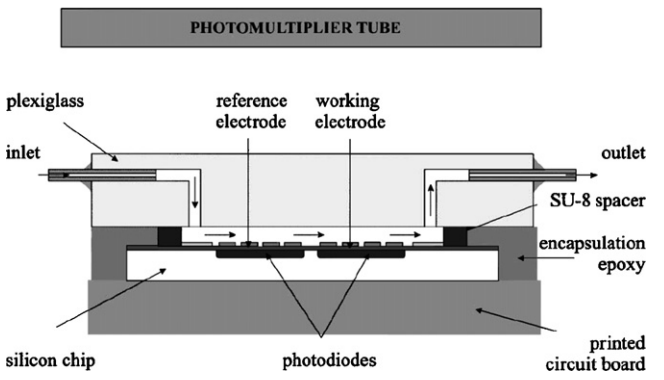


Fig. 5. A flow-through electrochemiluminescence detector for FIA. Reprinted from Ref. [46], Copyright (1999), with permission from Elsevier.

tris(2,2′-bipyridyl)ruthenium(II) at the surface of these sensors, by encapsulating the complex within a sol–gel matrix and grinding it to a powder for entrapment in a polyhydroxyethyl methacrylate membrane [87]. This system was less sensitive than the previous similar approach with solution phase reagents, but was also applied to the determination of codeine in a pharmaceutical preparation. It was reported that the electrochemiluminescence response for codeine was reasonably consistent for 7 days, but decreased rapidly after that period of time [87].

Greenway and co-workers oxidised codeine with tris(2,2′-bipyridyl)ruthenium(III) within a microfluidic device (20 mm × 25 mm chip) constructed by etching channels (200 μL width, 100 μL depth) into borosilicate glass [88]. Three solution reservoirs were drilled into a glass cover plate and were aligned to the ends of the T-shaped channel design. A fourth pre-drilled hole in the cover plate was used to position the end of a fibre optic cable above the emission point, to transfer the light to a small photomultiplier tube. The solutions were propelled using electroosmotic flow when a voltage was applied. The tris(2,2′-bipyridyl)ruthenium(III) was generated off-line by oxidising the ruthenium(II) complex with lead dioxide.

Wang and co-workers constructed a miniaturised electrochemiluminescence detection cell for FIA and CE [89], which was applied to the determination of heroin and cocaine on bank notes, using tris(2,2′-bipyridyl)ruthenium(II) [80]. Cell channels were created by wet chemical etching two pieces of glass (20 mm × 15 mm × 1.7 mm) and drilling access holes. Solution and waste reservoirs, capillary guides and electrodes were glued in place and the two glass plates sealed together with epoxy resin. The cell was placed directly in front of the photomultiplier tube window. The target analytes were extracted from banknotes with an acetic acid solution. Heroin, cocaine and three unknown compounds from the banknotes were separated with baseline resolution in under 9 min [80].

4. Conclusions

Investigations into the chemiluminescence determination of *P. somniferum* alkaloids using flow-injection and sequential-injection analysis have revealed the remarkable sensitivity and selectivity of this mode of detection. Tris(2,2′-bipyridyl)ruthenium(III) is most suitable for the detection of non-phenolic alkaloids that possess tertiary amine functionality, such as codeine, 6-methoxycodeine, noscapine and thebaine; whereas acidic potassium permanganate provides the greatest limits of detection for phenolic morphinan species such as buprenorphine, morphine, naloxone and oripavine. Papaverine does not produce a relatively intense response with either of these reagents, but has been detected with permanganate and sulfite, and with manganese(IV) and formaldehyde. The application of these reagents for the determination of opiate alkaloids in complex samples normally requires a separation procedure to avoid interferences (particularly from structurally related alkaloids) and matrix effects. The use of monolithic columns has considerably reduced the time required for HPLC separation and improved limits of detection to levels that are equivalent to those obtained with FIA or SIA, and coupled with the inherent selectivity of the chemiluminescence reactions, this approach is far superior to conventional HPLC with UV-absorbance detection. CE has been less thoroughly explored, presumably due to the additional complexity of developing a suitable detector. Nevertheless, CE procedures with tris(2,2′-bipyridyl)ruthenium(III) and permanganate chemiluminescence detection have been developed and this separation approach may become more important with the increasing development of microfluidic devices.

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References

[1] L.D. Kapoor, Opium Poppy: Botany, Chemistry and Pharmacology, Haworth Press, New York, 1995.
[2] International Narcotics Control Board, Narcotic Drugs 2006 Report: Estimated World Requirements for 2007—Statistics for 2005, United Nations, New York, 2007.
[3] M. Espinosa Bosch, A. Ruiz Sánchez, F. Sánchez Rojas, C. Bosch Ojeda, J. Pharm. Biomed. Anal. 43 (2007) 799–815.
[4] J. Garrido, C. Delerue-Matos, F. Borges, T. Macedo, A.M. Oliveira-Brett, Anal. Lett. 37 (2004) 831–844.
[5] F. Tagliaro, D. Franchi, R. Dorizzi, M. Marigo, J. Chromatogr. 488 (1989) 215–228.
[6] F. Tagliaro, S. Turrina, P. Pisi, F.P. Smith, M. Marigo, J. Chromatogr. B: Biomed. Sci. Appl. 713 (1998) 27–49.
[7] D. Thieme, H. Sachs, Anal. Chim. Acta 492 (2003) 171–186.
[8] N.W. Barnett, P.S. Francis, in: P.J. Worsfold, A. Townshend, C.F. Poole (Eds.), Encyclopedia of Analytical Science, 2nd ed., Elsevier, Oxford, 2005, pp. 506–510.
[9] N.W. Barnett, P.S. Francis, in: P.J. Worsfold, A. Townshend, C.F. Poole (Eds.), Encyclopedia of Analytical Science, 2nd ed., Elsevier, Oxford, 2005, pp. 511–520.
[10] B.A. Gorman, P.S. Francis, N.W. Barnett, Analyst 131 (2006) 616–639.
[11] J.L. Adcock, P.S. Francis, N.W. Barnett, Anal. Chim. Acta 601 (2007) 36–67.
[12] B.J. Hindson, N.W. Barnett, Anal. Chim. Acta 445 (2001) 1–19.
[13] N.W. Barnett, B.J. Hindson, P. Jones, T.A. Smith, Anal. Chim. Acta 451 (2002) 181–188.
[14] J.L. Adcock, P.S. Francis, T.A. Smith, N.W. Barnett, Analyst 133 (2008) 49–51.
[15] R.W. Abbott, A. Townshend, R. Gill, Analyst 111 (1986) 635–640.
[16] N.W. Barnett, D.G. Rolfe, T.A. Bowser, T.W. Paton, Anal. Chim. Acta 282 (1993) 551–557.
[17] Y.-F. Zhuang, S.-C. Zhang, J.-S. Yu, H.-X. Ju, Anal. Bioanal. Chem. 375 (2003) 281–286.
[18] Y. Zhuang, X. Cai, J. Yu, H. Ju, J. Photochem. Photobiol. A 162 (2004) 457–462.
[19] S. Zhang, Y. Zhuang, H. Ju, Anal. Lett. 37 (2004) 143–155.
[20] R.D. Gerardi, N.W. Barnett, S.W. Lewis, Anal. Chim. Acta 378 (1999) 1–41.
[21] N.W. Barnett, T.A. Bowser, R.D. Gerardi, B. Smith, Anal. Chim. Acta 318 (1996) 309–317.
[22] N.W. Barnett, R.D. Gerardi, D.L. Hampson, R.A. Russell, Anal. Commun. 33 (1996) 255–260.
[23] Y. Gemba, H. Hashimoto, M. Konishi, J. Liquid Chromatogr. Relat. Technol. 27 (2004) 843–862.
[24] G.M. Greenway, A.W. Knight, P.J. Knight, Analyst 120 (1995) 2549–2552.
[25] R.D. Gerardi, N.W. Barnett, P. Jones, Anal. Chim. Acta 388 (1999) 1–10.
[26] G.M. Greenway, A. Greenwood, P. Watts, C. Wiles, Chem. Commun. (2006) 85–87.
[27] N.W. Barnett, R. Bos, H. Brand, P. Jones, K.F. Lim, S.D. Purcell, R.A. Russell, Analyst 127 (2002) 455–458.
[28] Y. Zhuang, D. Zhang, H. Ju, Analyst 130 (2005) 534–540.
[29] F.A. Aly, S.A. Al-Tamimi, A.A. Alwarthan, Talanta 53 (2001) 885–893.
[30] F.A. Aly, S.A. Al-Tamimi, A.A. Alwarthan, Anal. Sci. 17 (2001) 1257–1261.
[31] T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás, J. Fenoll, Anal. Chim. Acta 485 (2003) 63–72.
[32] B.A. Gorman, N.W. Barnett, R. Bos, Anal. Chim. Acta 541 (2005) 119–124.
[33] J.L. Adcock, N.W. Barnett, R.D. Gerardi, C.E. Lenehan, S.W. Lewis, Talanta 64 (2004) 534–537.
[34] N.W. Barnett, B.J. Hindson, S.W. Lewis, S.D. Purcell, P. Jones, Anal. Chim. Acta 421 (2000) 1–6.
[35] K.M. Agg, A.F. Craddock, R. Bos, P.S. Francis, S.W. Lewis, N.W. Barnett, J. Forensic Sci. 51 (2006) 1080–1084.
[36] K.M. Agg, N.W. Barnett, S.W. Lewis, J.R. Pearson, J. Forensic Sci. 52 (2007) 1111–1114.
[37] J. Li, Fenxi Shiyanshi 16 (1997) 41–44.
[38] J. Li, J. Lu, Ziran Kexueban 26 (1998) 60–62.
[39] N. Feng, Y.-H. He, J.-X. Du, J.-R. Lu, Fenxi Shiyanshi 24 (2005) 1–3.
[40] Y.-F. Sun, L.-C. Wang, X.-J. Huang, Z.-C. Wu, J.-H. Liu, Guangpu Shiyanshi 21 (2004) 663–667.
[41] N.W. Barnett, B.J. Hindson, S.W. Lewis, P. Jones, P.J. Worsfold, Analyst 126 (2001) 1636–1639.
[42] A.J. Brown, C.E. Lenehan, P.S. Francis, D.E. Dunstan, N.W. Barnett, Talanta 71 (2007) 1951–1957.
[43] T.J. Christie, R.H. Hanway, D.A. Paulls, A. Townshend, Anal. Proc. 32 (1995) 91–93.
[44] N.W. Barnett, C.E. Lenehan, S.W. Lewis, D.J. Tucker, K.M. Essery, Analyst 123 (1998) 601–605.
[45] B. Rezaei, A. Mokhtari, T. Khayamian, Ann. Chim. (Rome) 97 (2007) 605–614.
[46] P.E. Michel, G.C. Fiaccabrino, N.F. de Rooij, M. Koudelka-Hep, Anal. Chim. Acta 392 (1999) 95–103.
[47] K. Papadopoulos, T. Triantis, D. Dimotikali, J. Nikokavouras, Anal. Chim. Acta 423 (2000) 239–245.

[48] S. Laks, A. Pelander, E. Vuori, E. Ali-Tolppa, E. Sippola, I. Ojanperä, Anal. Chem. 76 (2004) 7375–7379.
[49] S. Lane, B. Boughtflower, I. Mutton, C. Paterson, D. Farrant, N. Taylor, Z. Blaxill, C. Carmody, P. Borman, Anal. Chem. 77 (2005) 4354–4365.
[50] I.W.C. White, A.W. Gelb, H.R. Wexler, C.R. Stiller, P.A. Keown, Can. Anaesth. Soc. J. 30 (1983) 506–511.
[51] R.W. Abbott, A. Townshend, Anal. Proc. 23 (1986) 25–26.
[52] N.W. Barnett, B.J. Hindson, S.W. Lewis, Anal. Chim. Acta 362 (1998) 131–139.
[53] J.A. Murillo Pulgarín, L.F. García Bermejo, J.A. Rubio Aranda, Anal. Chim. Acta 517 (2004) 111–117.
[54] N.W. Barnett, S.W. Lewis, D.J. Tucker, Fresenius J. Anal. Chem. 355 (1996) 591–595.
[55] Y. He, J. Lu, M. Liu, J. Du, F. Nie, J. Anal. Toxicol. 29 (2005) 528–532.
[56] J.W. Costin, S.W. Lewis, S.D. Purcell, L.R. Waddell, P.S. Francis, N.W. Barnett, Anal. Chim. Acta 597 (2007) 19–23.
[57] L.A. Hill, C.E. Lenehan, P.S. Francis, J.L. Adcock, M.E. Gange, F.M. Pfeffer, N.W. Barnett, Talanta 76 (2008) 674–679.
[58] B. Qiu, X. Chen, H.-L. Chen, G.-N. Chen, Luminescence 22 (2007) 189–194.
[59] A. Navas Díaz, F. García Sánchez, J.A. González García, J. Biolumin. Chemilumin. 10 (1995) 175–184.
[60] A. Navas Díaz, J.A. González García, Anal. Chem. 66 (1994) 988–993.
[61] N.W. Barnett, P.S. Francis, S.W. Lewis, K.F. Lim, Anal. Commun. 36 (1999) 131–134.
[62] R.W. Abbott, A. Townshend, R. Gill, Analyst 112 (1987) 397–406.
[63] L. Zhu, M. Feng, X. Wan, J. Lu, Fenxi Huaxue 24 (1996) 1295–1297.
[64] E. Amiott, A.R.J. Andrews, J. Liquid Chromatogr. Relat. Technol. 20 (1997) 311–325.
[65] T. Shinozaki, N. Kunezaki, S. Yamazaki, T. Tanimura, Kuromatogurafi 13 (1992) 275–276.
[66] Y. Gemba, M. Konishi, T. Sakata, Y. Okabayashi, J. Liquid Chromatogr. Relat. Technol. 27 (2004) 1611–1626.
[67] C.E. Lenehan, N.W. Barnett, S.W. Lewis, K.M. Essery, Aust. J. Chem. 57 (2004) 1001–1004.
[68] R. Chiba, M. Shinriki, Y. Ishii, A. Tanaka, Anal. Sci. 14 (1998) 975–978.
[69] P.S. Francis, J.L. Adcock, J.W. Costin, K.M. Agg, Anal. Biochem. 336 (2005) 141–143.
[70] J.L. Adcock, N.W. Barnett, J.W. Costin, P.S. Francis, S.W. Lewis, Talanta 67 (2005) 585–589.
[71] J.A. Gunn, C. Shelley, S.W. Lewis, T. Toop, M. Archer, J. Anal. Toxicol. 30 (2006) 519–523.
[72] K. Tsukagoshi, Sci. Eng. Rev. Doshisha Univ. 45 (2005) 168–186.
[73] X.-J. Huang, Z.-L. Fang, Anal. Chim. Acta 414 (2000) 1–14.
[74] X. Huang, J. Ren, Trends Anal. Chem. 25 (2006) 155–166.
[75] B.J. Hindson, P.S. Francis, S.D. Purcell, N.W. Barnett, J. Pharm. Biomed. Anal. 43 (2007) 1164–1168.
[76] S. Kulmala, J. Suomi, Anal. Chim. Acta 500 (2003) 21–69.
[77] Z. Gong, Y. Zhang, H. Zhang, J. Cheng, J. Chromatogr. A 855 (1999) 329–335.
[78] N.W. Barnett, B.J. Hindson, S.W. Lewis, Analyst 125 (2000) 91–95.
[79] N.W. Barnett, B.J. Hindson, S.W. Lewis, S.D. Purcell, Anal. Commun. 35 (1998) 321–324.
[80] Y. Xu, Y. Gao, H. Wei, Y. Du, E. Wang, J. Chromatogr. A 1115 (2006) 260–266.
[81] B. Huang, J.-J. Li, L. Zhang, J.-K. Cheng, Anal. Chem. 68 (1996) 2366–2369.
[82] M. Krishnan, V. Namasivayam, R. Lin, R. Pal, M.A. Burns, Curr. Opin. Biotechnol. 12 (2001) 92–98.
[83] A. Roda, P. Pasini, M. Mirasoli, E. Michelini, M. Guardigli, Trends Biotechnol. 22 (2004) 295–303.
[84] J. Yan, X. Yang, E. Wang, Anal. Bioanal. Chem. 381 (2005) 48–50.
[85] G.C. Fiaccabrino, N.F. de Rooij, M. Koudelka-Hep, Anal. Chim. Acta 359 (1998) 263–267.
[86] E. L'Hostis, P.E. Michel, G.C. Fiaccabrino, D.J. Strike, N.F. de Rooij, M. Koudelka-Hep, Sens. Actuators B 64 (2000) 156–162.
[87] P.E. Michel, P.D. van der Wal, G.C. Fiaccabrino, N.F. de Rooij, M. Koudelka-Hep, Electroanalysis 11 (1999) 1361–1367.
[88] G.M. Greenway, L.J. Nelstrop, S.N. Port, Anal. Chim. Acta 405 (2000) 43–50.
[89] J. Liu, J. Yan, X. Yang, E. Wang, Anal. Chem. 75 (2003) 3637–3642.
[90] A.A. Alwarthan, A. Townshend, Anal. Chim. Acta 185 (1986) 329–333.
[91] Y.B. Tsaplev, Russ. J. Phys. Chem. (Engl. Transl.) 65 (1991) 420–422.
[92] C.E. Lenehan, N.W. Barnett, S.W. Lewis, J. Autom. Methods Manage. Chem. 24 (2002) 99–103.
[93] W. Chang, B. Zhang, N. Lu, Y. Ci, Fenxi Shiyanshi 14 (1995) 1–5.
[94] A. Campiglio, Analyst 123 (1998) 1053–1056.
[95] S.W. Lewis, P.S. Francis, K.F. Lim, G.E. Jenkins, X.D. Wang, Analyst 125 (2000) 1869–1874.
[96] J.A. Murillo Pulgarín, L.F. García Bermejo, M.N. Sánchez García, Anal. Chim. Acta 602 (2007) 66–74.
[97] J.L. Adcock, P.S. Francis, K.M. Agg, G.D. Marshall, N.W. Barnett, Anal. Chim. Acta 600 (2007) 136–141.
[98] X. Chen, M. Li, Z. Li, C. Yi, X. Wang, B. Wang, Fenxi Huaxue 30 (2002) 513–517.