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Improvements to Separation and Detection for Forensic Analysis of Illicit Substances

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This article gives an overview of our recent research into separation and detection of analytes of forensic interest. This work has been carried out in collaboration with local forensic service providers and is based on our previous studies of chemiluminescence detection, flow analysis and capillary electrophoresis as applied to process analytical chemistry for the pharmaceutical industry. Chemiluminescence has the potential to provide low limits of detection in combination with high selectivity, while capillary electrophoresis allows for rapid, highly efficient separations. Examples of recent forensic applications are presented and future directions are discussed.

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Within law enforcement agencies there is an increasing demand for rapid, selective and sensitive methods for the determination of illicit substances. The characterisation and quantification of illicit drugs and explosives in a variety of challenging matrices is an essential service provided by forensic science laboratories throughout Australia (Liddy & Pearson, 2000; Sims, 2000). In particular, the control of illicit drugs is of increasing social concern due to the significant increases in their availability and use in the last few years, and the associated crime and health problems (Australian Institute of Criminology, 2002; Victorian Institute of Forensic Medicine, 2002). The determination of trace explosives has also become an issue of high priority as a result of the rise in the profile of international terrorism. Over the last ten years our group at Deakin University has carried out research into improved methodologies for the demanding field of process analytical chemistry in the opiate pharmaceutical industry (Barnett, Bowser, Gerardi, & Smith, 1996; Barnett, Hinderson, & Lewis, 1998; Barnett, Hinderson, & Lewis, 2000; Barnett, Hinderson, Lewis & Purcell, 1998; Barnett, Rolfe, Bowser & Paton, 1993; Lewis, Francis, Lim, Jenkins, & Wang, 2000). The analytical challenges we have faced in this work are of a similar nature to those of a forensic scientist. Recognising this, we have recently entered into collaboration with the Victoria Forensic Science Centre and South Australia Forensic Science to research into new methods for forensic analysis of illicit substances. This article gives an overview of some of our recent studies in this area.

CAPILLARY ELECTROPHORESIS WITH SHORT END INJECTION

Many forensic samples are complex mixtures derived from biological extracts or debris associated with the scene of an explosion. Traditionally separation and identification of drugs and explosives has been carried out using gas chromatography (GC) and high performance liquid

chromatography (HPLC) often coupled with Mass Spectrometry (MS) (Cole, 1998). However, there are some cases where the analyte in question behaves poorly under GC or HPLC analysis. This might be due to thermal instability, or at least high temperature reactivity, as is the case with the drugs LSD and psilocin (the active ingredient in "magic mushrooms"), the benzodiazepines, and the explosives PETN, RDX and HMX, during GC analysis (Cole, 1998; Sims, 2000). Alternatively, the analyte might be too volatile to observe under "normal" GC operations; for example methylamine derived from methylammonium nitrate, a common component of commercial explosives (Sims, 2000). Capillary electrophoresis is an alternative separation technique which has been under investigation in our laboratories for its application to forensic analysis.

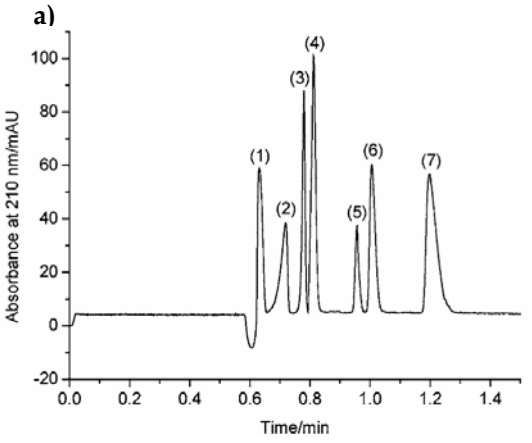
The term capillary electrophoresis (CE) describes a family of related techniques in which separations are carried out in narrow bore capillaries under the influence of an electric field (Altria, 1991). The separations obtained by CE are highly efficient, rapid and may be applied to both charged and neutral species. Our studies on this group of techniques have concentrated on improving the analysis of heroin drug seizures.

The analysis of heroin and associated opiates in drug seizure samples is of great importance for forensic investigations. As seized heroin is never pure, knowledge of the numerous manufacturing by-products, adulterants and diluents can also help to establish chemical links between samples from different seizures. The information obtained from a seized drug sample may be used for evidential purposes or for criminal intelligence to identify drug trafficking patterns and distribution. Our primary aim has been to reduce analysis time, thus allowing a higher analytical frequency (faster turn around time) and more rapid provision of information concerning seizure composition. Ideally, this should be achieved using conventional instrumentation, widely available in forensic laboratories.

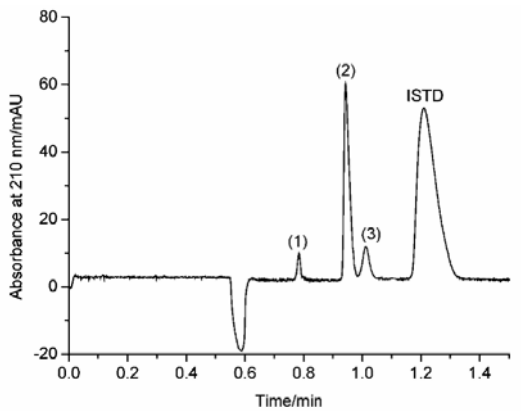
The main way to reduce analysis time in capillary electrophoresis is to apply a higher voltage or increased field strength (voltage/total length). This approach is limited due to joule heating (Altria, 1991). Problems arising from joule heating can result in high current production, generating temperature gradients and changes in solution viscosity, which result in degraded separations. Prolonged applications of a very high voltage across a buffer solution can also cause electrolysis of the solution, altering its pH and affecting the reproducibility of the system (Kelly, Altria, & Clark, 1997). While it is possible to reduce analysis time through the use of shorter capillaries, this approach is limited by the physical constraints of commercial instruments, where the minimum capillary length is fixed, typically around 30 cm (Altria, 1991).

In CE, injection is conventionally performed at the anode with detection at the cathode end of the capillary. If the normal polarity of the electrodes are reversed, such that the anode is at the detector (short) end of the capillary, samples and standards can then be introduced at the detector end, thus yielding a far shorter effective capillary length (Altria, Kelly & Clark, 1996).This technique of short-end injection allows for the reduction in analysis time, voltage applied, increase in sensitivity and also, a decrease in buffer depletion effects (Altria, Kelly & Clark, 1996). An increase in sensitivity is also generally observed due to diffusion processes being reduced, therefore minimizing band broadening and generating sharper peaks (Altria, Kelly & Clark, 1996). We have developed a capillary electrophoresis procedure based on short end injection for the determination of opiates and in heroin seizures using micellar electrokinetic chromatography (Anastos, Lewis, Barnett, Pearson & Kirkbride, 2005). This hybrid of capillary electrophoresis and chromatography allows the separation of neutral species through the addition of a surfactant. As can be seen from Figure 1 we were able to separate the opiate components of heroin seizures within 1.5 minutes, in addition when applied to the quantification of heroin in seizures the results obtained were in good agreement with a standard GC method (see Table 1). Moving from the opiate components of heroin seizures to other species, we have recently developed a short end injection capillary electrophoresis method for carbohydrates (Anastos Barnett, Lewis, Pearson and Kirkbride, 2005).

Figure 1: ***Separation of Opiate Components of Heroin***



b)



**Note: Electropherograms showing (a) the separation of a 0.1 mg/ml test mixture containing (1) caffeine, (2) paracetamol, (3) morphine, (4) codeine, (5) heroin, (6) acetylcodeine, and (7) N,N – dimethyl-5-methoxytryptamine and (b) the separation of a seized heroin sample, (1) morphine, (2) heroin, (3) acetylcodeine, ISTD = internal standard (N,N – dimethyl-5-methoxytryptamine). Conditions for both separations: UV absorbance at 210 nm, uncoated fused silica capillary 50 cm x 50 mm I.D. x 360 mm O.D., effective separation length 8 cm, background electrolyte: 15 mM sodium borate, 25 mM sodium dodecylsulfate, 15 % (v/v) acetonitrile, pH 9.5, 25 °C, -25 kV, hydrodynamic injection: 2 seconds at -50 mbar (Anastos, Lewis, Barnett, Pearson & Kirkbride, 2005a).*

Table 1: ***Quantification of Heroin in Heroin Seizure Samples***

Sample	GC Results (% diamorphine by mass)	MEKC Results (% diamorphine by mass)
1	11.9	11.9
2	51.6	54.1
3	41.1	43.1
4	59.4	57.1
5	81.9	81.4
6	79.7	78.9

**Note: MEKC Conditions as for Figure 1, GC Conditions: HP 5890, FID, 12m x 0.2 mm x 0.33 ?m HP Ultra-1 capillary column, oven temperature: 260 °C, injector temperature: 250 °C, detector temperature: 300 °C. (Anastos, Lewis, Barnett, Pearson & Kirkbride, 2005a).*

LIQUID PHASE CHEMILUMINESCENCE

The analytes of interest in forensic samples are likely to be present at low levels so any detection techniques should be selective and sensitive. A key area of research in analytical chemistry at Deakin University has been application of liquid phase chemiluminescence reactions to chemical measurement.

The term "chemiluminescence" was first coined by Eilhardt Weidemann in 1888, and refers to the emission of light from a chemical reaction (Garcia-Campana & Baeyens, 2001). While the generation of light by living creatures (bioluminescence) had been observed from ancient times, the first report of artificial chemiluminescence, was not until 1669. In that year the German physician, Hennig Brandt, hoping to make his fortune from alchemy, isolated a substance from urine which glowed continuously in the dark. The substance, then termed "phosphorus mirabilis", but better known now as white phosphorus, was extensively studied by Sir Robert Boyle who published his results in two pamphlets; *The Aerial Noctiluca* (London, 1680) and *The Icy Noctiluca* (London, 1681). In the former Boyle suggested some practical applications of "phosphorus mirabilis" including the possibility of using the emission of light from phosphorus as a "guide knowable at a good distance off in spite of tempestuous winds and greatest showers, and this in the darkest night"(Emsley, 2000).

Chemiluminescence from synthetic organic compounds was first observed in 1877 by Bronsilaus Radziszewski during the preparation of lophine (2,4,5-triphenylimidazole) from hydrobenzamide. Since that time many other chemiluminescent compounds have been synthesized, most notably luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), lucigenin (N,N'-dimethyl-9,9'-diacridinium nitrate) and pyrogallol (1,2,3-trihydroxybenzene). Of particular interest was the oxidation of certain diaryl oxalates in the presence of a fluorescent sensitiser, the so-called "peroxyoxalate reaction" initially observed by Chandross in 1963. Rauhut and coworkers subsequently developed this type of reaction which now forms the basis of Cyalume chemical light sticks.

A chemiluminescent reaction in its simplest form it can be represented by;

$$A + B \rightarrow [I]^* \rightarrow \text{PRODUCTS} + \text{LIGHT}$$

Where A and B are reactants and [I]* is an excited state intermediate. This is termed "direct chemiluminescence", a well known example is the luminol reaction. In certain cases where the excited state is an inefficient emitter, its energy may be passed on to another species (a sensitiser, F) for light emission to be observed. This is called "indirect chemiluminescence", an example of this is the peroxyoxalate (light stick) reaction;

$$A + B \rightarrow [I]^* + F \rightarrow [F]^* \rightarrow F + \text{LIGHT}$$

The light emitted from the chemiluminescent reactions has differing degrees of intensity, lifetime and wavelength with the latter parameter covering the spectrum from near

ultraviolet, through the visible and into the near infrared. Chemiluminescent reactions which have found analytical application often produce light in the visible region and some of these have been summarised in Table 2.

Table 2: ***Properties of Liquid-Phase Chemiluminescence Reactions***

Reaction	Colour (λ_{max})	Quantum yield*
Oxidation of luminol in aqueous alkali	blue (425 nm)	0.01
Oxidation of luminol in dimethyl sulfoxide	green-yellow (500 nm)	0.05
Oxidation of lucigenin in alkaline hydrogen peroxide	blue-green (440 nm)	0.016
Oxidation of lophine in alcoholic sodium hydroxide	yellow (525 nm)	-
Peroxyoxalate reaction	sensitiser dependant	0.05 - 0.5
Reduction of tris(2,2'-bipyridyl)ruthenium (III) by certain amines, opiate alkaloids and oxalate	orange (610 nm)	-
Oxidation of certain opiate alkaloids by acidic potassium permanganate in the presence of polyphosphates	red (680 nm)	-
ATP-dependant oxidation of D-luciferin with firefly luciferase		
pH 8.6	green-yellow (560 nm)	0.88
pH 7.0	red (615 nm)	

Note: The intensity of emission of a reaction is dependant on the quantum yield. The quantum yield is a measure of the efficiency of the chemiluminescence reaction. Quantum yields vary from 10^{-15} (ultra weak chemiluminescence) to nearly 1 (bioluminescent processes).

CHEMILUMINESCENCE AND CHEMICAL ANALYSIS

Chemiluminescence is well known as the basis for detection in the gas phase, particularly in the form of nitrogen oxide detectors and selective gas chromatography detectors for sulphur and phosphorus. However, over the past twenty years liquid phase chemiluminescence has gained wider acceptance, especially in the areas of liquid chromatography and flow analysis detection (Garcia-Campana & Baeyens, 2001). Chemiluminescence is an attractive proposition for detection in both laboratory based and process chemical analysis for the following reasons;

· The potential for excellent limits of detection due to the absence of source noise and light scatter.

· High selectivity as a result of the limited number of available reactions.

· Robust and inexpensive instrumentation for use in static and flow through modes.

Undoubtedly it is the high sensitivity of chemiluminescence detection, with detection limits often being orders of magnitude lower than those obtainable by conventional absorbance and fluorometric measurement that has prompted research in this area. Examples of the detection limits that have been achieved by our group and others using chemiluminescence are presented in Table 3. The selectivity of chemiluminescence detection, which can be manipulated to suit a particular analytical requirement, can also be extremely advantageous (Garcia-Campana & Baeyens, 2001).

Table 3: ***Examples of Detection Limits Achievable with Liquid-Phase Chemiluminescence Detection***

Analyte	Detection Limit		Reference
	mol dm^{-3}	g mL^{-1}	
Heroin	4.5×10^{-8}	1.6×10^{-8}	20*
Morphine	5×10^{-10}	1.4×10^{-10}	7*
Codeine	5×10^{-9}	1.5×10^{-9}	6*

Note: References: 20 = Greenway, Knight, and Kinight, 1995, 7 = Barnett, Hindson and Lewis, 1998a, 6 = Barnett, Bowser, Gerardi, and Smith, 1996.

The most successful analytical application area for liquid phase chemiluminescence to date has been the biomedical field. Particular application areas have been as a means of detection in immunoassay and for the direct analysis of various substances of clinical interest by bioluminescence. Chemiluminescence has been used in forensic science, through the well-known luminol test for bloodstains. The chemiluminescence emission observed when a reagent containing luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione) and hydrogen peroxide is sprayed on a bloodstain has been widely utilised by forensic scientists worldwide in investigations involving violent crime (Laux, 1998).

The instrumentation required for monitoring liquid phase chemiluminescence reactions is relatively simple and inexpensive. The most important component of the

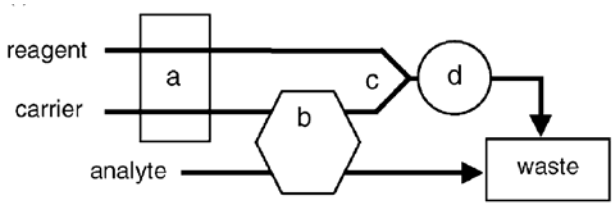
instrument is the light detector, which traditionally has been the photo-multiplier tube (PMT). Solid state devices for the detection of light, e.g. the photodiode and the charge coupled device (CCD) are more robust than PMT's. Photo diodes are inexpensive, small, rugged and have low power requirements (<15 V DC). Red and blue/UV sensitive photo diodes are available and with careful instrument design they can give sensitivity comparable to PMTs. CCDs have found application for imaging, particularly in astronomy, and their use for spectroscopy is increasing.

Batch luminometers are widely available with various degrees of automation. In a typical instrument a fixed volume of reagent is injected into a tube containing the sample. This type of instrument is well suited to monitoring reactions that are selective and have a high quantum yield and/or a long lifetime e.g. bioluminescence reactions. However, many of the most analytically useful chemiluminescence reactions are too fast to obtain precise results using batch methods. For these reactions other approaches to liquid handling are required.

Flow Analytical Techniques

One well-established sample handling technique for laboratory analysis is flow injection (FI) (Martinez-Calatayud, 1996). This technique has been defined as the injection of a liquid sample (10-200 μL) into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported to the detector via conduits constructed of PTFE tubing (0.3-0.8 mm internal diameter). The sample is modified by reaction with reagents merging with the main carrier stream. The response at the detector is in the form of a peak, the dimensions of which are directly related to analyte concentration. A schematic diagram of a typical FI instrument (manifold) is shown in Figure 2.

Figure 2: ***Schematic Diagram of a FI Manifold***



Note: Components: (a) peristaltic pump, (b) injection valve, (c) T-piece (mixing point), (d) detector, lines represent PTFE tubing (0.5 mm i.d.)

Automated flow injection systems have been applied to on-line process analysis in industrial and environmental situations with a great deal of success. FI is ideally suited to monitoring chemiluminescence reactions because of the rapid and reproducible mixing of sample and reagent that can be achieved in close proximity to the detector, which results in maximum sensitivity and reproducibility for weak and short-lived emissions.

We have used FI and its close relative, sequential injection analysis (SIA), to determine a wide range of analytes, from pharmaceuticals to neurotransmitters [5-10]. One key area of our research has been the development of methodologies for the determination of morphine and codeine in process streams. We utilised two chemiluminescence reactions with our FI systems, namely (i) the reduction of acidic potassium permanganate in the presence of polyphosphates and (ii) the reduction of tris(2,2´-bipyridyl)ruthenium (III) ($Ru(bipy)_3^{3+}$):

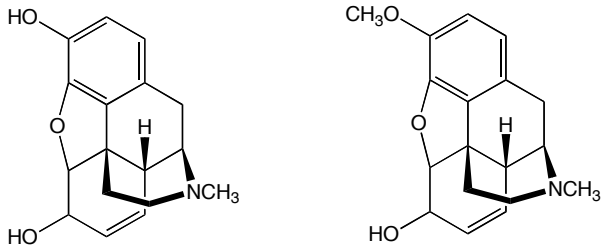
(i) $Analyte \xrightarrow{KMnO_4 / H^+ / polyphosphates} [manganese\ species]^* \rightarrow products + light\ (680\ nm)$

(ii) $Analyte \xrightarrow{Ru(bipy)_3^{3+}} [Ru(bipy)_3^{2+}]^* \rightarrow products + light\ (610\ nm)$

Where [manganese species]* and $[Ru(bipy)_3^{2+}]^*$ are excited species (see e.g., Barnett, Gerardi & Lewis, 1999; Hindson & Barnett, 2001).

The two main alkaloids of interest, morphine and codeine, differ markedly in the observed relative emission intensities. Morphine gives a bright flash of light upon reaction with acidic potassium permanganate while codeine elicits no chemiluminescent signal with this reagent. Codeine however gives a bright emission with the $(Ru(bipy)_3^{3+})$ complex while morphine gives only a barely measurable signal at high pH. This is remarkable considering the close structural similarity between these two compounds at a molecular level (see Figure 3).

Figure 3: ***Chemical Structures of Morphine and Codeine***



Morphine **Codeine**

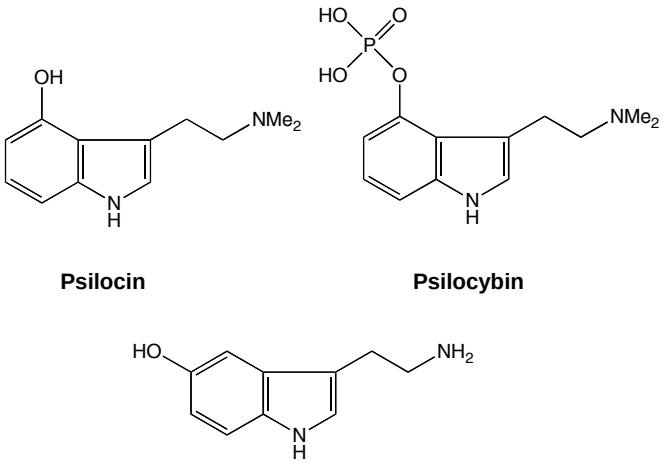
Note: Both molecules only differ in the top left hand corner of these structures (-OH group in morphine, -OCH_3 group in codeine)

Taking advantage of this serendipitous result we developed selective FI procedures with chemiluminescence detection for the determination of morphine and codeine

employing the respective chemiluminescence reagents discussed previously. In both cases the methodology was validated with actual process stream samples and good agreement was found between our new chemiluminescence assays and the standard liquid chromatographic methodology.

We have recently applied the two chemiluminescence reactions described above to the detection of psilocin and psilocybin (Anastos, Barnett, Lewis, Gathergood, Scammells, & Sims, 2005). These compounds are naturally occurring indoles (see Figure 4) which are found in several species of so-called "magic mushrooms". These compounds are structurally related to the neurotransmitter serotonin (see Figure 4). Psilocin blocks the release of the neurotransmitter thus giving rise to hallucinogenic effects. Both psilocin and psilocybin are controlled substances in many countries, with intentional intoxication from these compounds continuing to be an issue in the USA and Europe. Consequently, a simple, rapid and sensitive method for quantifying both psilocin and psilocybin in various matrices would be of interest to forensic scientists.

Figure 4: ***Chemical Structures of Psilocin, Psilocybin and Serotonin***



Psilocin **Psilocybin**

Serotonin

In previous studies we had found that acidic potassium permanganate elicited intense chemiluminescence upon reaction with serotonin (Barnett, et al., 1998a) and it was reasoned that this was also likely to occur with psilocin given their structural likeness (see Figure 4). Moreover, as tertiary amines have exhibited good analytical detectability with tris(2,2'-bipyridyl)ruthenium(III) (Barnett, Gerardi, & Lewis, 1999; Hinderson, & Barnett, 2001) it was thought that psilocybin ought to do likewise with this reagent.

Our experiments soon revealed that our reasoning had been correct with both psilocin and psilocybin elicited strong chemiluminescence with their respective reagents (Anastos, et al., 2005b). As can be seen from Table 4 the detection limits attained with chemiluminescence were more than competitive with those achieved with various other techniques.

Table 4: ***Detection Limits Reported in the Literature for the Determination of Psilocin and Psilocybin***

Technique	Psilocin	Psilocybin
	(mol L^{-1})	
Ion mobility spectrometry		1.4×10^{-4}
GC-MS	2.4×10^{-8}	
HPLC		
(i)UV absorption		
(ii) fluorescence		
(iii) electrochemical		
	(i) 3.9×10^{-6}	3.5×10^{-6}
	(ii) 9.7×10^{-6}	1.7×10^{-6}
	(iii) 3.7×10^{-8}	1.7×10^{-6}
Chemiluminescence (Continuous addition of reagent)	4.8×10^{-5}	
Flow Injection with Chemiluminescence Detection	**9×10^{-10}**	**3×10^{-10}**

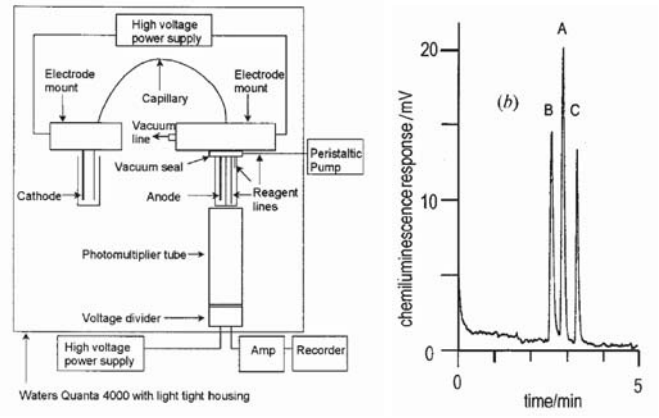
We applied our flow injection – chemiluminescence methodology to the analysis of dried samples of *Psilocybe subaeruginosa* and *Hypholoma aurantiaca*, and were able to detect both psilocin and psilocybin. This can only be considered a screening test as there may be other compounds present in the mushroom extract that could give chemiluminescence with our two reagents. Chemiluminescence can also be used as a detection system for separation techniques such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Indeed one of the first reports of the analytical use of the acidic potassium permanganate reaction was for detection of morphine in forensic samples after separation by HPLC (Abbott, Townshend & Gill, 1987). Due to the enhanced selectivity of chemiluminescence detection compared to conventional absorbance methods, chromatograms may be simpler to interpret and if only the components of interest give a response faster separations can be achieved. We have previously used HPLC with dual reagent chemiluminescence detection with the two reactions described

above for the determination of morphine and codeine in process samples (Lenehan, Barnett, Lewis & Essery, 2004). We are currently developing rapid HPLC separation for psilocin and psilocybin for which we will use a similar dual reagent approach.

As has been mentioned above, one of the main driving forces behind research into chemiluminescence detection has been the potential for very low detection limits. With this in mind a number of research groups have attempted to develop chemiluminescence detection systems for CE (Garcia-Campana & Baeyens, 2001). The main problem has been with the coupling of the separation capillary to a post-capillary reactor in order to mix the capillary effluent with chemiluminescence reagents immediately prior to detection. We have developed a chemiluminescence detection system for CE that has proven to be robust and in combination with the previously described acidic potassium permanganate and $Ru(bipy)_3^{3+}$ reactions has been applied to the determination of low levels of opiate alkaloids in process liquors (see Figure 5 from Barnett, Hindson & Lewis, 2000).

Figure 5: ***(a) Schematic diagram of the capillary electrophoresis instrumentation coupled to the chemiluminescence detection system (b) Electropherogram of opiate separation using acidic potassium permanganate chemiluminescence, (A) morphine, (B) oripavine (C) pseudomorphine***

(a)



As would be expected, the chemiluminescence detection was more sensitive than conventional UV-absorbance, with the detection limits for morphine, oripavine and pseudomorphine being an order of magnitude lower using the acidic potassium permanganate reaction.

THE FUTURE

Our studies into the application of liquid phase chemiluminescence detection to forensic analysis are at an early stage. Our initial studies have concentrated on the opiate drugs of abuse; we are now beginning to move beyond that field to investigate other potential target analytes. In addition to new chemistries we are also investigating novel approaches to carrying out chemiluminescence analyses, particularly in the field. We have recently established that the $Ru(bipy)_3^{3+}$ can be used as a spray reagent for the detection of heroin on polymer banknotes (Agg, Barnett, Lewis, Lyne, & Pearson, 2005). It was observed that different heroin seizures when tested with the spray reagent exhibited different characteristics of light emission in terms of brightness and length, this effect is due to the presence of other species (Barnett, Gerardi, Hampson & Russell, 1996). Heroin is highly variable containing different proportions of a number of other *papaver* alkaloids including 6-O-monoacetyl morphine, acetyl codeine and noscapine. The proportions of each of these contaminants can aid in identification of a particular source of heroin (Cole, 1998; Liddy & Pearson, 2000; Seigel, 1988) . Each one of these opiates gives chemiluminescence with either acidic potassium permanganate or $Ru(bipy)_3^{3+}$, however the exact reagent conditions (such as reaction pH), which give a detectable emission, vary for each opiate. It can be envisaged that a testing protocol could be developed that enables the determination of the proportions of different opiates within a heroin seizure to give an approximate profile or chemical "fingerprint". This information would be available to the investigator at the scene of the seizure in short order. It should be noted that this in no way would replace the more detailed analysis that would be obtained from the laboratory for use in court, it would however furnish the investigator with useful intelligence at an early stage in a criminal investigation.

Looking further into the future, one area where chemiluminescence has an opportunity to make an impact, is as a detection system for the so-called "lab-on-a- chip" devices (Baker, 1999; Fletcher & Haswell, 1999). Industrial and academic interest in this area is high, as evidenced by the establishment of a British consortium of industry and universities to investigate miniature analytical and synthetic systems and the launch of a journal in 2001 devoted to this area of scientific research (Lab on Chip, published by the Royal Society of Chemistry). A key area that needs to be addressed is the method of detection. "Lab-on-a-chip" devices have extremely small detection volumes and suffer from the same detectability issues as CE. Already several workers in this field have shown that chemiluminescence is a viable detection tech-

nique for these devices, for example Gillian Greenway and co-workers at the University of Hull successfully determined codeine with a micro-flow device by reaction with $Ru(bipy)_3^{3+}$ (Greenway, Nelstrop & Port, 2000). It should also be noted that capillary electrophoretic separations have also been carried out on chip devices (Baker, 1999). The effective length of the capillary length in our short-end injection work was no more than 8 cm, this size is compatible with miniaturised lab on chip devices. While this would lead to higher throughput in a laboratory situation, there is also the possibility of portable instrumentation that would allow detailed analysis at or near the crime scene. The National Institute of Forensic Sciences in Australia has identified that the rapid at scene detection of illicit drugs or explosives is of paramount importance in the fight against organised crime and terrorism (Kirkbride, 2001). In a recent review by one of the world's foremost exponents of lab on chip technology the author notes "microfluidic devices offer great promise for transporting the forensic laboratory to the sample source" (Wang, 2004).

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