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Chemiluminescence detection of heroin in illicit drug samples

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ABSTRACT

Heroin (3,6-diacetylmorphine) and several important extraction and synthesis impurities (morphine, 6-monoacetylmorphine, codeine and 6-acetylcodeine) were determined in illicit drug samples, using high performance liquid chromatography with 'parallel segmented flow', which enabled the simultaneous use of three complementary modes of detection (UV-absorbance, tris(2,2′-bipyridine)ruthenium (III) chemiluminescence and permanganate chemiluminescence). This rapid and sensitive approach for the analysis of street heroin was used to explore the chemistry of a proposed heroin screening test that is based on the relative response with these two chemiluminescence reagents using flow injection analysis. Although heroin was the major constituent of the six drug samples (between 16% and 67% by mass), the synthetic by-product 6-acetylcodeine (2.5–8.3%) made a greater contribution to the total $[Ru(bipy)_3]^{3+}$ chemiluminescence response of the screening test. The signal with permanganate was primarily due to the presence of 6-monoacetylmorphine (0.9–29%), and was therefore indicative of the degree of sample degradation during clandestine manufacture or poor storage conditions prior to the drug seizure. In the second part of the screening test, the sample is treated with sodium hydroxide, which results in a large increase in the signal with permanganate, due to the rapid hydrolysis of heroin to 6-monoacetylmorphine. As the emission of these two reagents with morphinan-alkaloids and their derivatives largely depends on the substituent at the O^3 position, the slower hydrolysis of 6-monoacetylmorphine to morphine, and 6-acetylcodeine to codeine, did not have a major impact on the characteristic pattern of responses in the screening test.

1. Introduction

The widespread illicit production and trafficking of heroin (3,6-diacetylmorphine) has created the need to identify and/or quantify the drug (and its precursors and derivatives) in suspected drug samples seized by law enforcement officials. Screening tests are often used to obtain rapid preliminary identification of drugs prior to confirmatory testing with more time-consuming and expensive instrumental methods [1]. The most commonly used screening method for opiates and their derivatives involves mixing samples with Mandelin, Marquis or Mecke reagent(s) and observing the resultant colour change. Our research group has demonstrated an alternative test for heroin [2,3] that exploits the complementary selectivity of two well-known chemiluminescence reagents: tris (2,2′-bipyridine)ruthenium(III) ($[Ru(bipy)_3]^{3+}$) [4] and potassium permanganate [5,6], using flow injection analysis [2] or sequential injection analysis [3] methodology. The chemical basis for the test involves two stages: in the first, the presence of heroin is indicated by a strong signal with $[Ru(bipy)_3]^{3+}$. The sample is then treated with a sodium hydroxide solution to hydrolyse the heroin to 6-monoacetylmorphine (6-MAM) and/or morphine, which produce strong signals with permanganate (Fig. S1, Electronic Supplementary information) [2,3]. Some common tertiary amines (such as codeine, strychnine and chloroquine) give false positives with the first reagent, but they do not produce the markedly increased response with the permanganate reagent after the hydrolysis procedure, thus providing a rapid and unambiguous (albeit qualitative) test for the target drug [2,3].

In our previously published screening test for heroin, the $[Ru(bipy)_3]^{3+}$ reagent was prepared by chemical oxidation of $[Ru(bipy)_3]^{2+}$, either with solid PbO_2 in acidic aqueous solution immediately prior to use [2,3], or with Cl_2 gas, in an initial procedure used to create $[Ru(bipy)_3](ClO_4)_3$, which was dried and then dissolved in acetonitrile [2]. The anhydrous reagent exhibited much greater stability and gave more intense and reproducible signals with heroin [2] and other species [7], but it

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was inconvenient to prepare and its use was hindered by batch-to-batch variation. To address this, we devised a simple procedure to prepare a stable anhydrous reagent, utilising PbO_2 to oxidise $[Ru(bipy)_3](ClO_4)_2$ in acetonitrile containing 0.05 M $HClO_4$ [7]. Furthermore, we have improved the stability and sensitivity of the permanganate reagent through an initial partial reduction of the oxidant with sodium thiosulfate [8].

Herein, we apply these enhanced chemiluminescence systems and UV-absorbance detection to HPLC for the rapid quantitative determination of heroin and related species in illicit drug samples seized by police. The detectors are coupled to a new HPLC column technology that has been referred to as parallel segmented flow (PSF) [9–11], in which greater separation efficiency is achieved because the radial central flow region is separated from the wall flow region, overcoming the limitations of HPLC associated with column bed heterogeneity. This column technology provides a suitable platform for multiplexed detection since the outlet fitting has multiple exit ports; in effect, the sample band can be divided into four portions and sent to separate, independent detectors [12]. The ratio of flow through any one of these exit ports can be controlled by the differential pressure at each port, providing simultaneous analysis in a way that until now has not been feasible using conventional approaches. We previously presented a preliminary exploration of PSF for multiplexed detection using the separation of six opium poppy alkaloids coupled with UV-absorbance and chemiluminescence detection as a model system [12]. In this study, we adapt the procedure for the analysis of illicit drug samples, which provides not only a rapid quantitative test of multiple opiates within these samples, but also the opportunity to further explore the fundamental parameters of the PSF approach, and examine the chemistry of the chemiluminescence screening test to understand the observed discrepancies between intensities obtained with each reagent using pure heroin standards and illicit drug samples.

2. Materials and methods

2.1. Chemicals and reagents

Codeine, morphine, oripavine and thebaine were provided by GlaxoSmithKline (Vic., Australia). Heroin (3,6-diacetylmorphine), 6-MAM and 6-acetylcodeine were obtained from the National Measurement Institute (NSW, Australia). Seized drug samples were provided by the Australian Federal Police. All other chemicals were sourced as previously described [12]. Stock solutions of the opiate alkaloids (1 mM) were prepared in acidified deionised water. Heroin (1 mM) was prepared in 0.1% (v/v) acetic acid and diluted in 0.05% (v/v) acetic acid. Stock solutions of the drug samples were prepared by dissolving 15 mg of the solid material into 50 mL of 0.1% (v/v) acetic acid. The 'non-hydrolysed' samples were prepared by taking 1 mL of stock solution (heroin or the drug sample) and diluting to 100 mL (for HPLC experiments) or 200 mL (for FIA experiments) with a 0.05% (v/v) acetic acid solution. The 'hydrolysed' samples were prepared by mixing 1 mL of stock solution with 100 μL of sodium hydroxide (1.0 M) and then diluting the mixture to 100 mL or 200 mL with the 0.05% (v/v) acetic acid solution.

The permanganate reagent was prepared by dissolving 1.9 mM $KMnO_4$ in 1% (m/v) sodium polyphosphate and adjusting to pH 2.5 with H_2SO_4 and then adding 0.6 mM $Na_2S_2O_3$, using a small volume of a 0.1 M $Na_2S_2O_3$ solution [8]. The tris(2,2′-bipyridine) ruthenium(III) reagent was prepared as previously described [7]. This involved treating $[Ru(bipy)_3]Cl_2$ with $NaClO_4$ in aqueous solution to yield a bright orange $[Ru(bipy)_3](ClO_4)_2$ precipitate, which was collected by vacuum filtration, washed twice with ice water, and dried over P_4O_{10} for 24 h. The reagent was then prepared by oxidising the $[Ru(bipy)_3](ClO_4)_2$ crystals (1 mM) with PbO_2 (0.2 g/100 mL) in acetonitrile containing 0.05 M $HClO_4$, which was observed as a change in the colour of the solution from orange to blue-green. The excess solid oxidant left in the reagent was prevented from entering the chemiluminescence detector by a filter (consisting of a small Pasteur pipette packed tightly with glass wool) fitted to the end of the tubing in the reagent reservoir.

2.2. Flow injection analysis (FIA)

The FIA manifold was constructed as previously described [13], incorporating a GloCel chemiluminescence detector (Global FIA, WA, USA) with a Teflon dual-inlet serpentine-channel flow-cell [14,15] and an extended-range photomultiplier module (model P30A-05; ETP, NSW, Australia). All tubing entering and exiting the detector was black PTFE (0.76 mm i.d., Global FIA). The output signal from the detector was recorded with an e-corder 410 data acquisition system (eDAQ, NSW, Australia). The analytes (5×10^{-6} M) were injected (70 μL) into an aqueous carrier stream (adjusted to pH 2.5 with trifluoroacetic acid, 3.5 mL min^{-1}) that merged with the chemiluminescence reagent (3.5 mL min^{-1}) in the reaction channel of the flow-cell.

2.3. High performance liquid chromatography (HPLC)

Analyses were carried out on an Agilent Technologies 1260 series liquid chromatography system with an injection volume of 20 μL, flow rate of 2.5 mL min^{-1}, and gradient elution using deionised water adjusted to pH 2.5 with trifluoroacetic acid (solvent A) and methanol (solvent B) as follows: 0–2 min: 7–21% B, 2–10 min: 21% B, 10–12 min: 21–7% B, and 12–14 min: 7% B (unless otherwise stated).

2.4. Multiplexed detection

Separations were performed using a reversed-phase Hypersil GOLD chromatography column (100 mm × 4.6 mm i.d., 5 μm) with a parallel segmented flow end-fitting (ThermoFisher Scientific, Cheshire, UK). The column eluate (total flow rate: 2.5 mL min^{-1}) was divided in the following manner: 32% (0.8 mL min^{-1}) was directed to the first chemiluminescence detector (permanganate reagent) via peripheral port 1, 32% (0.8 mL min^{-1}) to the second chemiluminescence detector ($[Ru(bipy)_3]^{3+}$ reagent) via peripheral port 2, 24% (0.6 mL min^{-1}) to the UV-absorbance detector (280 nm) via peripheral port 3; and the remaining 12% (0.3 mL min^{-1}) to a collection vessel via the central port. For permanganate chemiluminescence, we used a GloCel detector with dual-inlet serpentine-channel flow-cell (fabricated from Teflon impregnated with glass microspheres [14,15]) and an extended-range photomultiplier module (model P30A-05; ETP). For $[Ru(bipy)_3]^{3+}$ chemiluminescence, we used a GloCel detector with dual-inlet serpentine flow-cell (Teflon) and 9282SB photomultiplier tube (ETP) powered by an external high-voltage power supply (PM20D, ETP) set at 0.9 kV. The reagents were pumped to the detectors at 1 mL min^{-1} using Dual Piston Pumps (Series 12 × 6, model D05PFD01; Scientific Systems, PA, USA).

2.5. Conventional detection

For comparison purposes, separations were also performed with a Hypersil GOLD column with a conventional outlet fitting, which was connected to each detector individually, using the same tubing and detectors as described above.