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TECHNICAL NOTE

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A Rapid Test for Heroin (3,6-Diacetylmorphine) Based on Two Chemiluminescence Reactions

ABSTRACT: A rapid method for screening drug seizure samples for 3,6-diacetylmorphine (heroin), which consists of a simple hydrolysis procedure and flow-injection analysis with two chemiluminescence reagents, is described. Before hydrolysis, 3,6-diacetylmorphine evokes an intense response with a tris(2,2′-bipyridyl)ruthenium(III) reagent (prepared by dissolving the perchlorate salt in acetonitrile), and a relatively weak chemiluminescence response with a second reagent: potassium permanganate in an aqueous acidic polyphosphate solution. However, the permanganate reagent is extremely sensitive toward the hydrolysis products of 3,6-diacetylmorphine (i.e., 6-monoacetylmorphine and morphine). Some compounds commonly found in drug laboratories may cause false positives with tris(2,2′-bipyridyl)ruthenium(III), but do not produce the markedly increased response with the permanganate reagent after the hydrolysis procedure. The combination of these two tests therefore provides an effective presumptive test for the presence of 3,6-diacetylmorphine, which we have verified with 14 samples obtained from a forensic science laboratory.

KEYWORDS: forensic science, forensic chemistry, chemiluminescence, drug screening, presumptive chemical test, spot test, heroin, 3,6-diacetylmorphine, morphine

Screening tests are useful for rapid preliminary identification of drug classes and selection of appropriate samples for analysis with confirmatory techniques such as GC-MS or HPLC-MS (1,2). Routine field tests for heroin, morphine, and other opiate derivatives involve mixing the suspect samples with either the Marquis reagent (formaldehyde in sulfuric acid) or Mecke's reagent (selenious acid in concentrated sulfuric acid) and a visual assessment of any resulting color changes (1,3,4). An additional test with nitric acid can be used to distinguish between heroin and morphine (2,4). Microcrystalline examinations have also been used as presumptive chemical tests, but require experience for adequate interpretation (1).

Chemiluminescence—the production of light from a chemical reaction—is an attractive option for screening tests, with many reagents offering exceedingly sensitive detection (5). This method of detection is well suited for the development of portable analytical instrumentation for at-scene applications, as (unlike absorbance or fluorescence detection) an external source of light and wavelength selection is not required. Prototype lab-on-a-chip devices with miniaturized photomultiplier tubes (PMTs) or photodiodes for chemiluminescence detection have recently been described (6–9). The development of new chemiluminescence detection systems will enable the full potential of this approach to be realized.

The most notable application of chemiluminescence that is currently used in forensic science is that of luminol and hydrogen peroxide for the visualization of blood at crime scenes (10). Potassium permanganate (in an aqueous acidic polyphosphate solution) has been used as a chemiluminescence reagent for the determination of morphine and other phenolic opiate alkaloids, using flow-injection analysis (11,12), HPLC (13,14), or capillary electrophoresis (15) methodology. In contrast, tris (2,2′-bipyridyl)ruthenium(III) (prepared by chemical or electrochemical oxidation of the more stable tris(2,2′-bipyridyl)ruthenium(II) complex (16)) produces a relatively intense emission with opiate alkaloids that do not possess phenolic functionality, such as codeine and thebaine (6,17,18); however, this reagent is insensitive toward morphine (19).

Heroin is a nonphenolic alkaloid derivative, but has been determined with both potassium permanganate (20,21) and tris (2,2′-bipyridyl)ruthenium(II) chemiluminescence (19,22). Recently, Zhuang and coworkers (23) reported the determination of heroin based on electrogenerated chemiluminescence of tris (2,2′-bipyridyl)ruthenium(II) immobilized in a zeolite Y-modified carbon paste electrode. However, a range of other compounds that elicit chemiluminescence with tris(2,2′-bipyridyl)ruthenium(III) (16) may interfere with this approach.

In this paper, we have used flow-injection analysis methodology to demonstrate a rapid and selective test for the presence of heroin in drug seizures, based on the rapid hydrolysis of heroin and the relative sensitivity of tris(2,2′-bipyridyl)ruthenium(III) and permanganate chemiluminescence for heroin and its hydrolysis products, as summarized in Fig. 1.

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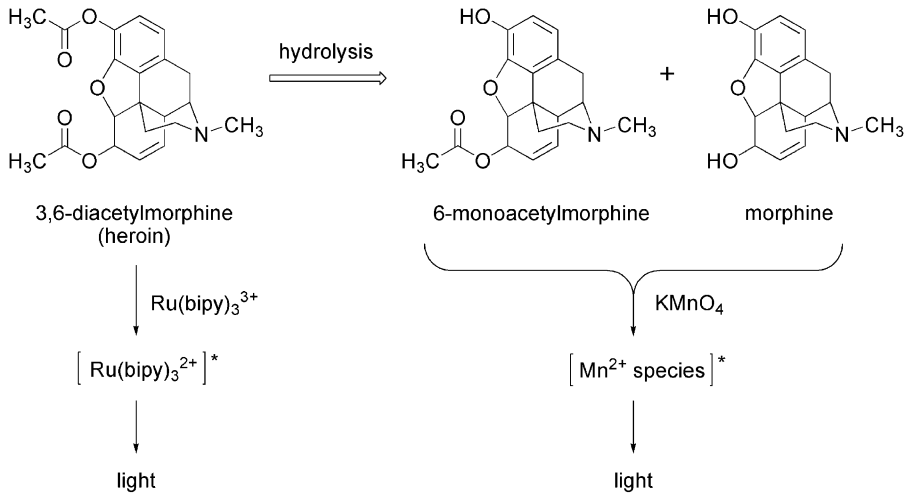

FIG. 1—*Two-stage test for heroin.*

Materials and Methods

The extent of heroin hydrolysis under different chemical conditions was examined using a Hewlett Packard 1100 LC system (Agilent Technologies, Forest Hill, VIC, Australia) that consisted of a quaternary pump, solvent degasser system, autosampler, UV absorbance detector, HP Vectra X Series 4 data analysis workstation, and Chemstation software. Samples were separated with a monolithic column (Chromolith SpeedROD RP-18e, 50 × 4.6 mm i.d.; Merck, Germany) using the solvent gradient described in Table 1, a flow rate of 3 mL/min, and an injection volume of 20 μL. The components were detected by absorbance at 280 nm. Mobile phases were filtered through a 0.45 μm membrane. Retention times for morphine, 6-monoacetylmorphine, and 3,6-diacetylmorphine were 0.7, 2.2, and 3.7 min, respectively.

Solutions of drugs, cutting agents, and seizure samples were combined with the chemiluminescence reagents, both with and without prior hydrolysis, using flow-injection analysis manifolds (Figs. 2*a* and *b*) that incorporated a custom-built chemiluminescence detector encased in a light-tight housing. The detector contained a T-piece and coiled PTFE flow-cell positioned in front of a PMT (Electron Tubes Model 98285B, ETP, Ermington, NSW, Australia) that was operated at 900 V, provided by a stable power supply (Electron Tubes Model PMZOD, ETP) via a voltage divider (Electron Tubes Model C611, ETP). The output from the PMT was documented with a chart recorder (YEW Type 3066, Yokogawa Hokushin Electric, Tokyo, Japan). Reactant solutions were propelled to the detector through 0.8 mm i.d. PTFE tubing using a peristaltic pump (Gilson Minipuls 3, John Morris Scientific, Chatswood, NSW, Australia) with silicone pump tubing (1.02 mm i.d.; Pro-tech Group, Coolum Beach, QLD, Australia), either continuously or when injected into a carrier stream using a six-port injection valve (Model E60-220, Valco Instruments, SGE, Ringwood, VIC, Australia) with a 20 μL injection loop.

Two different forms of the tris(2,2′-bipyridyl)ruthenium(III) reagent (1×10^{-3} M) were evaluated. The first was an acidic aqueous solution of tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate (Strem Chemicals, Newbury, MA) oxidized by adding 0.5% m/v lead dioxide (Ajax, Sydney, NSW, Australia). The excess solid oxidant was removed with a 0.45 μm filter as the solution was drawn into the syringe for injection into the flow-injection manifold. The second reagent was prepared by dissolving the perchlorate salt of tris(2,2′-bipyridyl)ruthenium(III) (24) in HPLC grade acetonitrile that had been dried over calcium hydride and distilled before use. Preliminary oxidation of this reagent was not required. To conserve the tris(2,2′-bipyridyl)ruthenium(III) solutions during flow-injection analysis experiments, the reagents were manually loaded into the 20 μL injection loop and injected into the carrier stream, which merged at the T-piece with a continuously flowing sample stream (Fig. 2*a*).

The permanganate reagent (1×10^{-3} M, Ajax, Australia) contained 1% w/v sodium polyphosphate (Sigma-Aldrich, Castle Hill, NSW, Australia) and was adjusted to pH 2.5 with concentrated sulfuric acid (Ajax, Australia). To prevent degradation of this reagent, the volumetric flask was stored away from direct light. In the flow-injection analysis manifold, the permanganate reagent was pumped continuously and the samples were loaded into the

TABLE 1—*HPLC flow program for the determination of 3,6-diacetylmorphine and its hydrolysis products.*

Time (min)	% Solvent A	% Solvent B
0.0	95	5
2.0	89	11
4.0	78	22
8.0	45	55

Solvent A, aqueous solution of trifluoroacetic acid (pH 2.5); solvent B, methanol.

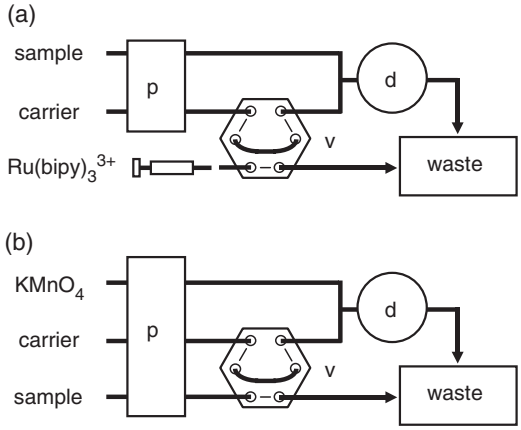

FIG. 2—*Flow-injection analysis manifold for (a) tris(2,2′-bipyridyl)ruthenium(III) chemiluminescence and (b) permanganate chemiluminescence. p, peristaltic pump; d, chemiluminescence detector; v, 6-port injection valve.*

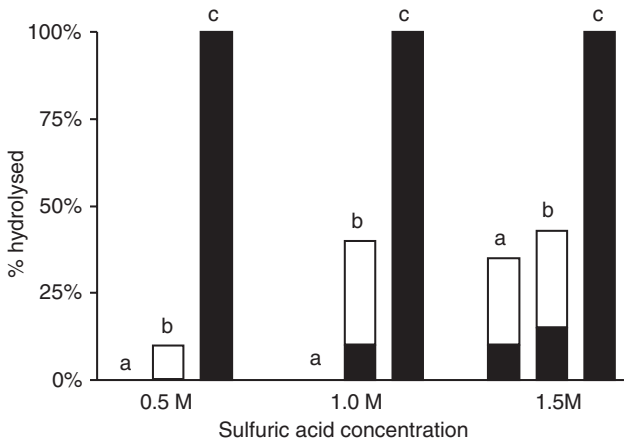

FIG. 3—*Hydrolysis of 3,6-diacetylmorphine to 6-monoacetylmorphine (white columns) and morphine (black columns), in a sulfuric acid solution at (a) 0 min, (b) 5 min, and (c) 60 min after dissolution.*

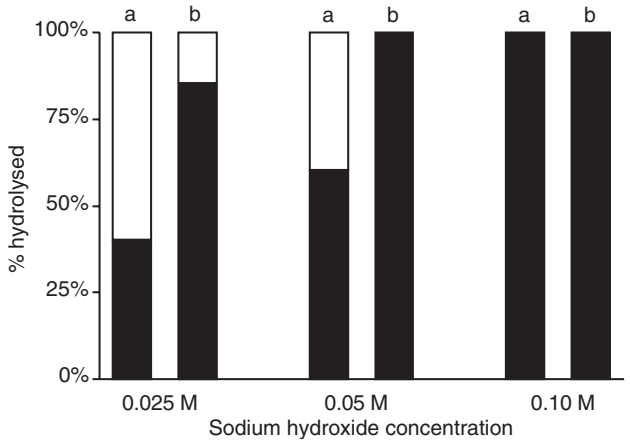

FIG. 4—*Hydrolysis of 3,6-diacetylmorphine to 6-monoacetylmorphine (white columns) and morphine (black columns), in a sodium hydroxide solution at (a) 0 min, and (b) 5 min after dissolution.*

20 μL sample loop and injected into an aqueous carrier stream (Fig. 2*b*).

The Victoria Police Forensic Services Laboratory provided seizure samples. Pure opiate alkaloids were obtained from Glaxo-SmithKline (Port Fairy, VIC, Australia). The 3,6-diacetylmorphine was synthesized from morphine (25) and fully characterized using NMR. Shifts for ^{1}H NMR in p.p.m.: (H-1) 6.55, (H-2) 6.75, (H-5) 5.10, (H-6) 5.12, (H-7) 5.41, (H-8) 5.60, (H-9) 3.38, (H-10) 3.03, 2.33, (H-14) 2.79, (H-15) 1.87, 2.07, (H-16) 2.60, 2.35, (N-CH_3) 2.43, (3-acetyl) 2.23, (6-acetyl) 2.20. Shifts for ^{13}C NMR in p.p.m.: (C-1) 119.4, (C-2) 122.1, (C-3) 131.4, (C-4) 149.5, (C-5) 88.5, (C-6) 68.1, (C-7) 129.3, (C-8) 128.7, (C-9) 59.1, (C-10) 20.9, (C-11) 132.6, (C-12) 131.4, (C-13) 42.7, (C-14) 40.4, (C-15) 35, (C-16) 46.6, (N-CH_3) 43.0, (3-acetyl) 168.5, 20.72, (6-acetyl) 170.5, 20.70.

Results and Discussion

Optimization of the Hydrolysis Procedure

The hydrolysis of 3,6-diacetylmorphine to 6-monoacetylmorphine and then morphine is strongly dependent on the pH and the temperature of the solution (25). In a weakly acidic solution, 3,6-diacetylmorphine is quite stable (half-life greater than 2 weeks (25)) and, therefore, in this study the stock solution was prepared in 1% (v/v) acetic acid. In alkaline and strongly acidic solutions, hydrolysis is rapid. To establish suitable conditions for the hydrolysis of samples in the proposed test, we examined the extent of 3,6-diacetylmorphine hydrolysis with monolithic column HPLC. Separation and detection (absorbance at 280 nm) of the three components were completed within 4 min. At sulfuric acid concentrations of between 0.5 and 1.5 M, less than 50% of the 3,6-diacetylmorphine was hydrolyzed after mixing for 5 min (Fig. 3). However, when a 0.1 M sodium hydroxide solution was used, complete conversion to morphine was observed (Fig. 4), even when the HPLC procedure was initiated immediately after the solid was dissolved. The incompatibility between this alkaline hydrolysis solution and the optimum conditions for the chemiluminescence reactions was overcome by using 0.05% (v/v) acetic acid for further dilution.

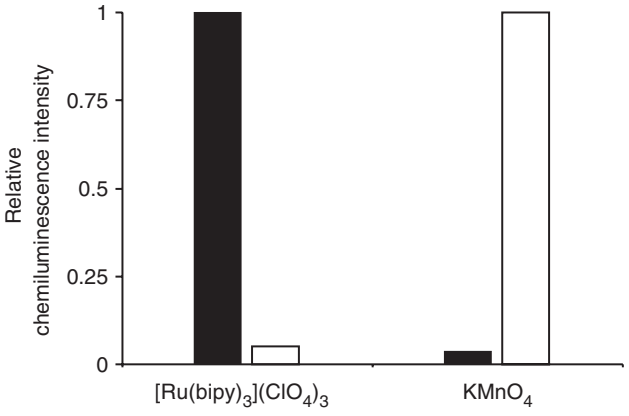

FIG. 5—*Chemiluminescence response for the nonhydrolyzed (black columns) and hydrolyzed (white columns) samples of pure 3,6-diacetylmorphine, with the anhydrous tris(2,2′-bipyridyl)ruthenium(III) perchlorate reagent, [Ru(bipy)$_3$](ClO$_4$)$_3$, and the potassium permanganate reagent, KMnO$_4$. Signals were normalized for each reagent.*

Chemiluminescence Reactions

Stock solutions were prepared by dissolving *c*. 15 mg of the solid in 100 mL of deionized water. In some cases, a small amount of acid was added to improve dissolution. Samples for analysis were prepared by diluting 1 mL of the stock solutions to 100 mL with 0.05% (v/v) acetic acid. "Hydrolyzed" samples for analysis were prepared by mixing 1 mL of the stock solution with 100 μL of 1.0 M sodium hydroxide and then diluting to 100 mL with 0.05% (v/v) acetic acid. Deionized water was used as the carrier solution in both flow-injection analysis manifolds (Fig. 2).

For standard solutions of 3,6-diacetylmorphine, a more intense and reproducible chemiluminescence response was obtained with tris(2,2′-bipyridyl)ruthenium(III) perchlorate (24) in dry acetonitrile compared with an acidic aqueous solution of tris(2,2′-bipyridyl)ruthenium(III) generated by chemical oxidation. Therefore, the perchlorate salt was used in all subsequent experiments.

After the 3,6-diacetylmorphine was hydrolyzed to morphine (via 6-monoactylmorphine), the response with the tris(2,2′-bipyridyl)ruthenium(III) reagent was considerably reduced (Fig. 5). In contrast, the response with the permanganate reagent was far greater after hydrolysis than before.

Responses for Common Cutting Agents

The response pattern for 3,6-diacetylmorphine (Fig. 5) is dependent on the sensitivity of the two chemiluminescence reagents for distinct functionality and the rapid chemical conversion of the target analyte under relatively mild conditions (Fig. 1). Nevertheless, we examined a series of compounds that may also be present in drug seizure samples (barbitone, caffeine, chloroquine, codeine, creatine, paracetamol, phenolphthalein, procaine, quinine, strychnine, and sucrose) for their potential to interfere with the analysis. Stock solutions were prepared at 1×10^{-3} M and the "nonhydrolyzed" and "hydrolyzed" samples for analysis were therefore 1×10^{-5} M.

The reaction of the anhydrous tris(2,2′-bipyridyl)ruthenium(III) reagent with nonhydrolyzed codeine, strychnine, and quinine samples produced the maximum instrument response (limited by the amplifier setting), which was also observed for the nonhydrolyzed heroin sample. However, unlike heroin, the maximum signal was also observed for the hydrolyzed samples of these three analytes. A relatively small signal (less than 2.5%) was observed from the nonhydrolyzed creatine and caffeine samples. A small signal (from 2% to 12% of the maximum response) was observed from most of the hydrolyzed samples.

Although many compounds elicit chemiluminescence when reacted with acidic potassium permanganate (26), morphine and other phenolic opiate alkaloids evoke a particularly intense emission, which has been exploited for a variety of analytical applications (11–15). Each of the compounds described above was tested with the permanganate reagent. Only paracetamol (prepared with and without the hydrolysis step) evoked an emission of sufficient intensity to be detected using the amplifier and chart recorder settings that were selected to measure the signal for heroin and its hydrolysis products. The response for paracetamol was around 1% of that observed for the hydrolyzed heroin sample.

Response for Real Samples

We examined the response from fourteen seizure samples that contained between 10% and 85% 3,6-diacetylmorphine, diluents including glucose, sucrose, lactose, mannitol, caffeine and/or paracetamol, and other alkaloids that remained from the extraction of morphine and subsequent synthesis of heroin. Stock solutions were prepared by dissolving between 3 and 15 mg of the solid (depending on availability) in 100 mL of deionized water. Samples for analysis were prepared as described above.

The nonhydrolyzed and hydrolyzed samples produced the maximum instrument response with the anhydrous tris(2,2′-bipyridyl) ruthenium(III) reagent in all cases, except for sample number 10, which produced a relative response of 30% without hydrolysis, and 17% with hydrolysis. This anomaly was attributed to a combination of a relatively low 3,6-diacetylmorphine concentration and small mass used for analysis due to limited availability. The predominant cause of the intense signal from each of the seizure samples after hydrolysis, which was not observed with pure 3,6-diacetylmorphine, was assumed to be either native codeine extracted from the opium or 6-acetylcodeine (formed during heroin synthesis (27)), which may be converted to codeine during the hydrolysis step.

Although the response for 3,6-diacetylmorphine and interferents such as codeine with tris(2,2′-bipyridyl)ruthenium(III) was indistinguishable, only 3,6-diacetylmorphine produced a significant increase in permanganate chemiluminescence signal after hydrolysation. As shown in Fig. 6, this change was observed for

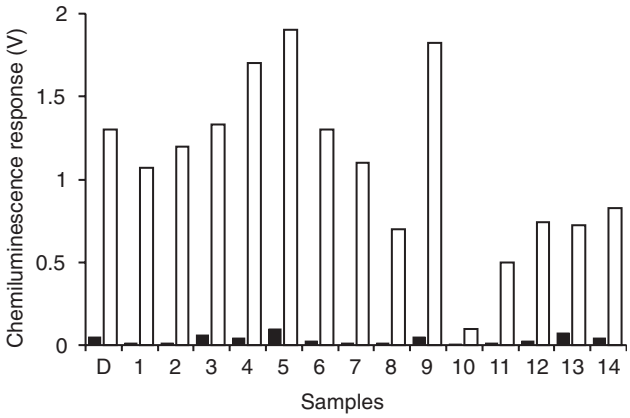

FIG. 6—*Chemiluminescence response for the nonhydrolyzed (black columns) and hydrolyzed (white columns) solutions of pure 3,6-diacetylmorphine (D) and heroin seizure samples (1–14), with the potassium permanganate reagent.*

all 14 seizure samples. Although there was a wide range of absolute intensities, thirteen of the nonhydrolyzed samples yielded a chemiluminescence signal that was between 1.1% and 5.3% of that observed for the corresponding hydrolyzed sample, which was similar to the response observed for pure 3,6-diacetylmorphine (3.8%). Nonhydrolyzed sample number 13 yielded 9.7% of the response of its corresponding hydrolyzed sample.

These preliminary results suggest that the combination of the two chemiluminescence tests and a simple hydrolysation step provides a sound chemical basis for an effective method for screening suspected heroin seizure samples. Unlike morphine and other phenolic opiate alkaloids, 3,6-diacetylmorphine produces an intense response with tris(2,2′-bipyridyl)ruthenium(III) (prepared by dissolving the perchlorate salt in acetonitrile). Furthermore, the hydrolysis procedure rapidly removes the acetyl groups from 3,6-diacetylmorphine to produce 6-monoacetylmorphine and morphine, both of which give a relatively intense response with acidic potassium permanganate. The hydrolysis procedure may also hydrolyze 6-acetylcodeine to codeine, but neither of these species evokes an intense chemiluminescence response with acidic potassium permanganate. This detection system is ideal for miniaturized devices as the flow-manifold is simple and the response is rapid and intense. The immobilization and regeneration of tris(2,2′-bipyridyl)ruthenium(III) for the detection of heroin have been demonstrated (23), and the other chemiluminescence reagent, acidic potassium permanganate, is inexpensive and simple to prepare.

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