

## TECHNICAL NOTE

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# Preliminary Investigations into Tris(2,2'-bipyridyl) Ruthenium (III) as a Chemiluminescent Reagent for the Detection of 3,6-Diacetylmorphine (Heroin) on Surfaces

**ABSTRACT:** The use of tris(2,2'-bipyridyl) ruthenium (III) as a chemiluminescent spray reagent spot-test for heroin is discussed. Two forms of the reagent are investigated an aqueous and an anhydrous where both were found to give vastly different results. The aqueous reagent giving slow, low intensity chemiluminescence whilst the anhydrous reagent gave a fast, bright response in the presence of 3,6-diacetylmorphine. The anhydrous reagent is less sensitive the slow, intensity response is characteristic of only two opiates tested 3,6-diacetylmorphine and 3-monoacetylmorphine.

**KEYWORDS:** forensic science, forensic chemistry, chemiluminescence, presumptive chemical test, heroin, 3,6-diacetylmorphine

Rapid identification of a suspicious material as an illicit substance at a crime scene can provide useful information in the early stages of a criminal investigation (1). When illicit drugs are seized a number of preliminary screening tests may be carried out; these tests are generally nonspecific and further testing is required to confirm the specific identity and to quantify the drug. Current screening methods for heroin during a drug seizure include the Marquis color test (2% formaldehyde in sulfuric acid). This reagent turns purple in the presence of heroin, morphine and most opium derivatives (1). Microcrystalline tests are also used where a chemical reagent is added to a small quantity of the drug on a microscope slide, resulting in the precipitation of the drug. Identification can be difficult as diluents and additives within a drug sample can alter the morphology of the crystals (1).

Chemiluminescence is the production of light from a chemical reaction (2). Chemical reactions involve changes in the energy levels of the reactants compared with products; where excess energy generated in a reaction is usually lost through collisional vibrations (heat). Chemiluminescence reactions lose a portion of this excess energy through the emission of photons, ranging from the near ultraviolet to the near infrared (3,4). These reactions are best known in forensic science, through the use of luminol and hydrogen peroxide for visualization of blood at crime scenes (5,6).

Research by our group at Deakin has established that chemiluminescent reactions provide valuable detection systems for flow analysis, liquid chromatography, and capillary electrophoresis (7–14). Over the last decade, we have successfully applied these technologies to the demanding field of process analytical chemistry in the

opiate pharmaceutical industry (7,8,14). In our previous studies, we utilized the reduction of tris(2,2'-bipyridyl)ruthenium (III) ( $\text{Ru}(\text{bipy})_3^{3+}$ ) to detect the opiate alkaloid codeine (Fig. 1) (8,10).

Chemiluminescence from tris(2,2'-bipyridyl) ruthenium (III) was first observed in 1966 by Hercules and Lytle (15). Since then, the reaction has been used to determine a wide range of analytes (16). Heroin (3,6-diacetylmorphine) has previously been shown to produce chemiluminescence upon reaction with the tris(2,2'-bipyridyl)ruthenium(III) complex (17).

Emerging methods reported recently in the literature for the analysis of drugs on surfaces such as banknotes focus on instrumental methods namely mass spectrometry (18–21). Infrared spectrometry is valuable technique but requires a relatively pure sample (22).

This paper describes preliminary studies on the utility of tris(2,2'-bipyridyl)ruthenium(III) chemiluminescence for the detection of heroin on surfaces, in particular polymer banknotes. The chemiluminescence reaction is studied using stopped-flow instrumentation (23) to gain an insight into the reaction kinetics of 3,6-diacetylmorphine and the tris(2,2'-bipyridyl)ruthenium(III) reagent in an aqueous and anhydrous environment as well as in the presence of some heroin cutting agents. The reagent is dispensed as a spray over suspect articles and a flash of orange light observed when heroin is present.

## Materials and Methods

### *Tris(2,2'-bipyridyl) Ruthenium*

Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate (Strem Chemicals, Newbury, MA) was prepared in aqueous sulfuric acid (Ajax, Sydney, NSW, Australia) and oxidized by shaking with lead dioxide (Ajax).

The anhydrous tris(2,2'-bipyridyl) ruthenium(III) perchlorate salt was prepared using the method previously reported by Barnett and co-workers (24). Chlorine was generated via the addition of

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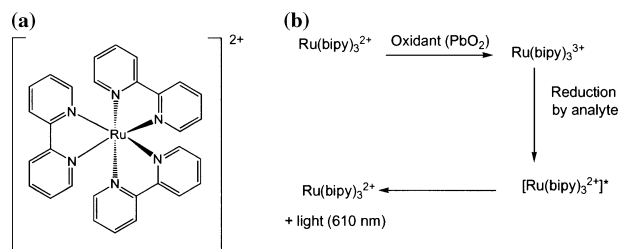


FIG. 1—(a) Chemical structure of  $\text{Ru}(\text{bipy})_3^{2+}$ . (b) Reaction pathway for the generation of chemiluminescence from  $\text{Ru}(\text{bipy})_3^{3+}$ .

hydrochloric acid (BDH; Kilsyth, Vic., Australia) to calcium hypochlorite (Hy-Chlor; Super Shock Granular Pool Chlorine, Gordon, NSW, Australia). Acetonitrile (Ajax) was dried over a 4 Å molecular sieve (BDH, Poole, UK).

#### Drug Standard and Seizure Samples

Morphine and codeine were obtained from GlaxoSmithKline (Port Fairy, Vic., Australia). 3-monoacetylmorphine, 6-monoacetylmorphine, acetylcodeine and drug seizure samples were provided by the Victoria Police Forensic Services Laboratory. 3,6-diacetylmorphine was synthesized from morphine (25). Opiates were applied to the standard surface (Whatman 1 Qualitative filter paper) either as solutions (1 mL of 1000 ppm in methanol) then dried under a small commercial heater or as solids where a small amount of opiate (~5 mg) was rubbed into the surface using a spatula. Filter papers were secured on a vertical surface in a fume cupboard and tris(2,2'-bipyridyl) ruthenium(III) solutions were delivered from a hand operated plant sprayer. Lead dioxide was filtered on-line through filter paper (Whatman 1 Qualitative) as the solution was delivered.

#### Stopped Flow Chemiluminescence Analysis

Kinetics experiments were performed using a purpose built stopped flow instrument. Control of the syringe pump (World Precision Instruments WPI210iw; World Precision Instruments, Melbourne, Vic., Australia) and data acquisition was achieved using a desktop computer (Pentium 133 MHz, 32 Mb RAM; Posicom, Geelong, Vic., Australia) equipped with a data acquisition board (LabPC 1200, National Instruments, Ringwood, Vic., Australia) running software written using LabVIEW<sup>®</sup> version 6.0 (National Instruments). Detection was accomplished using a custom built flow-through luminometer featuring a glass spiral flow cell (80  $\mu\text{L}$ ; Embell Scientific, Murwillimbah, NSW, Australia) with integral Y-piece (Embell Scientific).

## Results

Preliminary investigations using codeine as the model compound and the aqueous ruthenium solution found three main factors affecting the response:

- 1 Reagent concentration.
- 2 Spray distance.
- 3 Amount and type of spraying.

Acid concentration was also investigated but found to have little effect on the response, 0.05 M  $\text{H}_2\text{SO}_4$  was chosen because, in past work, it has been shown to have reasonable temporal stability and minimal effect on the chemiluminescence response (26).

The difference in response for reagent concentration was minimal between 2 and 5 mM, so as to minimize reagent waste a concentration 2 mM was chosen. Spray distance was found to be optimal around 50 cm: too close resulted in flooding of the surface and poor response; too far and insufficient reagent was found to reach the surface to elucidate a visible response. The amount and type of spraying had a significant effect on the intensity of chemiluminescence observed. Excessive spraying or large 'jet-like' spraying does not give an easily visible response. The best response is observed from slow spraying when a very fine dense mist is applied that blankets the area of interest.

Using a stopped flow instrument, the reaction kinetics were investigated for both the aqueous and anhydrous ruthenium reagent and also possible effects of diluents present in a seizure sample. None of the diluents investigated individually (paracetamol, caffeine, sucrose, chloroquine, and quinine) elucidated a chemiluminescence response with tris(2,2'-bipyridyl)ruthenium(III). The chemiluminescence response observed for 3,6-diacetylmorphine was not affected by the presence of these diluents.

As can be seen in Fig. 2, the reaction profile is vastly different between the aqueous and anhydrous environments, with the light being produced at a lower intensity for a longer period of time under aqueous conditions. This is a result of the slower rate of reaction under these conditions. The reaction rate of the aqueous reaction can be increased, with a resultant increase in the maximum intensity viewed, by reducing the concentration of sulfuric acid in the ruthenium reagent and by preparing the heroin sample without acid. However, the use of acid cannot be avoided as acid is required to stabilize the tris(2,2'-bipyridyl) ruthenium as it oxidizes water (26).

All heroin seizure samples gave strongly visible chemiluminescence, with the anhydrous perchlorate salt reagent of tris(2,2'-bipyridyl) ruthenium(III) ( $\text{Ru}(\text{bipy})_3(\text{ClO}_4)_3$ ) (2 mM in acetonitrile), whilst the aqueous tris(2,2'-bipyridyl) ruthenium(III) ( $\text{Ru}(\text{bipy})_3\text{Cl}_2$ ) (in 0.05 M  $\text{H}_2\text{SO}_4$ , oxidised by  $\text{PbO}_2$ ) resulted in a faintly visible response (Table 1), consistent with the stopped-flow experiments. The only drug seizure that did not give visible chemiluminescence was an MDMA (ecstasy) sample. The spray reagent test is quick and easy to perform, requiring no sample preparation and just a darkened room or a light tight housing, simply spray a suspect surface with the reagent and observe the chemiluminescence response. Several surfaces were investigated including polymer bank notes and the laboratory bench; neither exhibiting a blank response nor

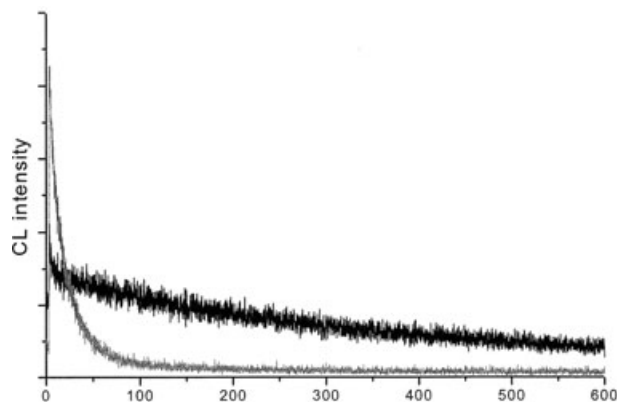


FIG. 2—Stopped-flow chemiluminescence profile of 3,6-diacetylmorphine and ruthenium tris(2,2'-bipyridyl) ruthenium (III) in aqueous (black) and anhydrous (grey) conditions.

TABLE 1—Observations of the chemiluminescence response for both the aqueous and anhydrous tris(2,2'-bipyridyl) ruthenium (III) reagent.

Analyte	Ru(bipy) <sub>3</sub> Cl <sub>2</sub> (0.05 M H <sub>2</sub> SO <sub>4</sub> )	Ru(bipy) <sub>3</sub> (ClO <sub>4</sub> ) <sub>3</sub> (in acetonitrile)
Codeine (solid)	Strongly visible Fast reaction	Strongly visible Fast reaction
Morphine (solid)	Not visible	Not visible
3,6-Diacetylmorphine (solid)	Not visible	Strongly visible Fast reaction
3,6-Diacetylmorphine (MeOH solution)	Faintly visible Slow reaction	Strongly visible Fast reaction
Acetylcodeine (solid)	Strongly visible Fast reaction	Strongly visible Fast reaction
Acetylcodeine (MeOH solution)	Strongly visible Fast reaction	Strongly visible Fast reaction
6-Monoacetylmorphine (MeOH solution)	Not visible	Not visible
3-Monoacetylmorphine (MeOH solution)	Faintly visible Slow reaction	Strongly visible Fast reaction
Noscapine (solid)	Clearly visible Fast reaction	Clearly visible Fast reaction
Papaverine (solid)	Not visible	Not visible
Seizure 1 (MeOH solution)	Faintly visible Slow reaction	Strongly visible Fast reaction
Seizure 2 (MeOH solution)	Faintly visible Slow reaction	Strongly visible Fast reaction
Seizure 3 (MeOH solution)	Faintly visible Slow reaction	Strongly visible Fast reaction
MDMA seizure (MeOH solution)	Not visible	Not visible

affecting the intensity of the chemiluminescence response of the reaction.

The vast difference in the intensity of the chemiluminescence emission between the aqueous and anhydrous reagent was only seen for 3,6-diacetylmorphine and 3-monoacetylmorphine (Table 1). Whilst the anhydrous reagent gave an increase in sensitivity the aqueous reagent was more selective. The aqueous reagent being selective for 3,6-diacetylmorphine and 3-monoacetylmorphine (a by-product of heroin synthesis) over codeine, acetylcodeine and noscapine in that the reaction although not intensely bright can be identified by the slow long-lasting reaction with the aqueous reagent. Codeine, acetylcodeine and noscapine gave a fast bright response to both the aqueous and anhydrous reagents. Using the two reagents to test a seizure could discriminate between heroin and the other opiates which also give chemiluminescence with tris(2,2'-bipyridyl)ruthenium(III). No other alkaloids are known to exhibit this unusual chemiluminescence that is characteristic of 3,6-diacetylmorphine and 3-monoacetylmorphine.

From this work with both the aqueous and anhydrous reagent of tris(2,2'-bipyridyl)ruthenium(III) it is apparent that the detection limit for 3,6-diacetylmorphine to the naked eye of the anhydrous reagent is considerably better than that of the aqueous. The reaction with the anhydrous perchlorate salt gives a 'brighter' flash as it is a much faster reaction and the light is emitted over a much shorter length of time. The drawback of the anhydrous reagent is the use of a flammable solvent being sprayed as an aerosol, so any naked flame must be kept away when using this reagent. The use of a nonflammable solvent could be investigated, however, they can be expensive. The aqueous reagent requires the use of lead dioxide to oxidize the tris(2,2'-bipyridyl)ruthenium(II) to the 'active' tris(2,2'-bipyridyl)ruthenium(III) the lead dioxide is easily filtered within the hand held sprayer. However, the lead dioxide is toxic and needs to be disposed of in a safe manner.

Both reagents are suitable chemiluminescent reagents for the identification of 3,6-diacetylmorphine on surfaces with the

anhydrous being more sensitive because of its faster reaction with 3,6-diacetylmorphine. The aqueous reagent although not as sensitive was selective for 3,6-diacetylmorphine over codeine, acetylcodeine, and noscapine but 3-monoacetylmorphine also gave slow long-lasting chemiluminescence with the aqueous reagent. This should not be a problem since 3-monoacetylmorphine is a by-product in the synthesis of heroin.

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