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A screening test for heroin based on sequential injection analysis with dual-reagent chemiluminescence detection

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ABSTRACT

A sequential injection analysis procedure with dual-reagent chemiluminescence detection was applied to the screening of street drug seizure samples for the presence of heroin. The chemiluminescence reagents (acidic potassium permanganate and tris(2,2′-bipyridine)ruthenium(III)) were aspirated from either side of a sample aliquot that was sufficiently large to prevent interdispersion of the reagent zones, and therefore two different chemical reactions could be performed simultaneously at either end of the sample zone. The presence of heroin in seizure samples was indicated by a strong response with the tris(2,2′-bipyridine)ruthenium(III) reagent and confirmed by a significant increase in the response with the permanganate reagent when the sample was treated with sodium hydroxide to hydrolyse the heroin to morphine. Nicomorphine (a morphine-derived pharmaceutical) was synthesised and tested under the same conditions. The responses with the permanganate reagent were similar to those for heroin, which supports the proposed chemical basis for the test. However, the responses with tris(2,2′-bipyridine)ruthenium(III) were far lower for nicomorphine than heroin (approximately 5-fold for the samples that had not been hydrolysed).

1. Introduction

Since its inception in 1990 [1], sequential injection analysis has proven to be a highly useful and flexible protocol for manipulating a wide range of samples and reagents for chemical analysis [2,3]. The power of this approach stems from the configuration and computer control of the individual liquid handling apparatus (pump and multi-position valve), which allows the precise 'stacking' of a defined series of zones (that each contain a liquid, gas or suspended particles) within a narrow-bore conduit. This instrumental arrangement and control enables the use of complex chemical operations that would be difficult to achieve using traditional flow analysis techniques [4]. However, most procedures based on sequential injection analysis involve the detection of a single analyte within a sample and use reaction chemistry employing only one or two reagents [2,3].

Among several innovative approaches to extend sequential injection analysis to the detection of more than one compound per analytical cycle [5], Cerdà and co-workers described the aspiration of two reagents either side of a sample zone that was sufficiently large to prevent interdispersion of the reagents [6]. This 'sandwich' technique enabled two disparate chemical reactions to be performed simultaneously, resulting in two time-resolved signals when the reaction products were propelled through the detector. Although initially applied to the simultaneous determination of iron(II) and nitrite based on colour-forming reactions with *o*-phenanthroline and the Griess reagent [6], this approach has been adopted for other applications, including the determination of phosphate and silicate using vanadomolybdate and ammonium molybdate reagents [7], and the determination of nitrite and nitrate by sandwiching the sample between two zones of the Griess reagent and drawing half the total solution over a reduction column [8].

We have examined the sandwich technique using sequential injection analysis for dual-reagent chemiluminescence detection, which exploits the contrasting selectivity of two highly sensitive reagents to determine two concomitant species in a single aliquot of sample (Fig. 1). Furthermore, we have used this approach to combine two operations of a proposed qualitative screening test for heroin into a single manifold that is better suited for the development of miniaturised analytical devices.

Screening tests are vital for the rapid preliminary identification of drugs and selection of appropriate samples for analysis with confirmatory techniques such as GC–MS or HPLC–MS [9,10]. Current

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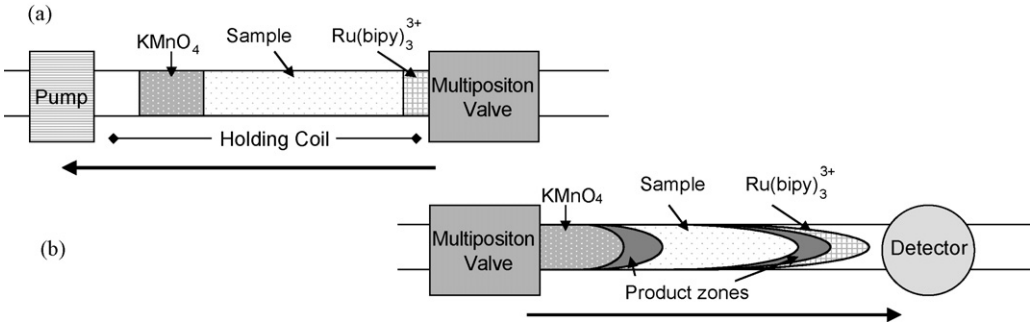

Fig. 1. (a) Stacking sample and reagents in the holding coil by operating the pump in the reverse direction; (b) dispersion of sample and reagent zones upon propulsion of the, stack, toward the detector.

tests for heroin, morphine, and other opiate derivatives involve a visual assessment of colour changes when samples are mixed with the Marquis reagent or Mecke's reagent [9,11,12]. An additional test with nitric acid can be used to distinguish between heroin and morphine [10,12]. Microcrystalline examinations have also been used as presumptive chemical tests, but require experience for adequate interpretation [9].

As depicted in Fig. 2a, the proposed screening test for heroin in street seizure samples is based on the reaction of heroin and its hydrolysis products with two chemiluminescence reagents: tris(2,2′-bipyridine)ruthenium(III) [13] and acidic potassium permanganate [14], which have both previously been used in sensitive procedures for the determination of opiate alkaloids [13,14]. Many non-phenolic morphinan-type alkaloids (and semi-synthetic derivatives) that contain tertiary amine functionality, such as codeine, thebaine and heroin, evoke an intense chemiluminescence emission with tris(2,2′-bipyridine)ruthenium(III), but a very weak emission with acidic potassium permanganate [15–18]. However, the relative intensities are reversed for certain phenolic analogues such as morphine and 6-monoacetylmorphine (the hydrolysis products of heroin; Fig. 2a) [15–18]. The characteristic response for heroin and hydrolysed heroin samples with the two chemiluminescence reagents using flow injection analysis methodology is shown in Fig. 2b [19]. Some other tertiary amines (such as codeine, strychnine and chloroquine) cause false positives with tris(2,2′-bipyridine)ruthenium(III), but do not produce the markedly increased response with the permanganate reagent after the hydrolysis procedure. Therefore, the combination of the two reagents provides an unambiguous test for heroin [19].

In addition to adapting the screening test for sequential injection analysis methodology using the sandwich technique for multi-component analysis, we have synthesised nicomorphine (a morphine-derived pharmaceutical) and examined the relative response for this compound, to further explore the proposed chemical basis for the heroin test.

2. Experimental

2.1. General instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Jeol JNM-EX 400 MHz FT-NMR spectrometer, with samples dissolved in *d*-chloroform ($CDCl_3$) and referenced against tetramethylsilane ($(CH_3)_4Si$) at 0.00 ppm. Complete characterisation of nicomorphine was performed using 2D NMR techniques. Proton peaks were recorded as follows: chemical shift δ (ppm) (integral, multiplicity (s = singlet, d = doublet, and m = multiplet), coupling constant J = Hz, assignment). The CH_2 protons were designated as ax = axial, eq = equatorial, qa = quasi-axial (α) and qe = quasi-equatorial (β). Mass spectra were recorded with a 6210 MSD TOF mass spectrometer (Agilent Technologies, Blackburn, Victoria, Australia) using the following conditions: drying gas, nitrogen (7 ml/min, 350 °C); nebulizer gas, nitrogen (16 psi); capillary voltage, 4.0 kV; vaporizer temperature, 350 °C; cone voltage, 60 V. The melting point of nico-

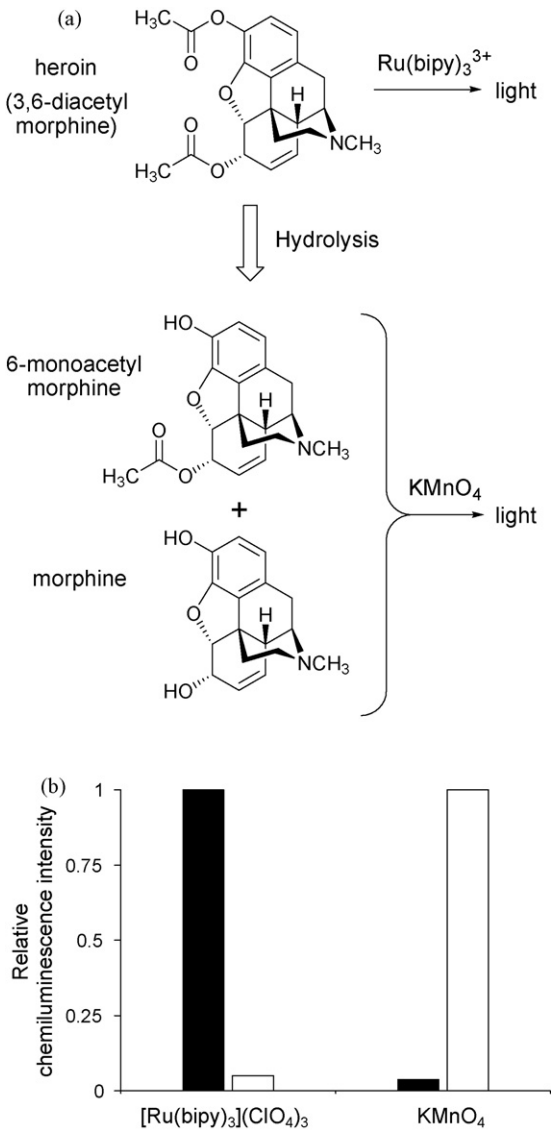

Fig. 2. (a) Concept for a rapid screening test for heroin; (b) chemiluminescence response for a non-hydrolyzed (black columns) and hydrolyzed (white columns) heroin standard with an anhydrous tris(2,2′-bipyridine)ruthenium(III) perchlorate reagent and a potassium permanganate reagent, using flow injection analysis methodology [19]. Signals were normalized for each reagent.