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Determination of morphine, oripavine and pseudomorphine using capillary electrophoresis with acidic potassium permanganate chemiluminescence detection†

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A simple and robust capillary electrophoresis chemiluminescence detection system for the determination of morphine, oripavine and pseudomorphine is described, based upon the reaction of these analytes with acidic potassium permanganate in the presence of sodium polyphosphate. The reagent solution was contained in a quartz detection cell which also held both the capillary and the anode. The resultant chemiluminescence was monitored directly using a photomultiplier tube mounted flush against the base of the detection cell. To ensure that no migration of the permanganate anion occurred, the anode was placed at the detector end whilst the electroosmotic flow was reversed by the addition of hexadimethrine bromide (0.001% m/v) to the electrolyte. The three analytes were separated counter to the electroosmotic flow *via* their interaction with α-cyclodextrin. The methodology realised detection limits (3 × S/N) of 2.5×10^{-7} M for both morphine and oripavine and 5×10^{-7} M for pseudomorphine. The relative standard deviations of the migration times and the peak heights for the three analytes ranged from 0.6 up to 0.8% and from 1.5 up to 2.1%, respectively.

Introduction

Since 1991, there has been considerable interest in the application of chemiluminescence detection to capillary electrophoresis, with the publication of 36 papers employing the majority of the analytically useful reactions. These have included, peroxyoxalate,[1–12] luminol,[13–28] acridinium,[29,30] firefly luciferase[21] and tris(2,2′-bipyridyl)ruthenium(III).[31–35] However, Lee and Whang[36] have reported the only methodology where aqueous acidic potassium permanganate was used to elicit chemiluminescence by reaction with serotonin, dopamine, norepinephrine and catechol. This approach[36] necessitated the fabrication of a cellulose acetate-coated porous polymer joint in the capillary (near the detector) to isolate the high voltage in order to prevent migration of the permanganate anion towards the anode. Using this device the electroosmotic flow acted as a pump to propel the analytes along the length of capillary (2 cm) after the joint. Consequently, the effluent from the capillary merged with a flowing stream of reagent and the resultant chemiluminescence realised detection limits, for the four analytes, of 1×10^{-4} M.

Morphine, oripavine and pseudomorphine are alkaloids (see Fig. 1) which are extracted from commercially grown opium poppies (*Papaver somniferum*) and both flow analysis[37–39] and high performance liquid chromatography,[40] with acidic potassium permanganate chemiluminescence detection, have been used to monitor these analytes in a variety of process streams. Notwithstanding this success, it was decided to investigate the feasibility of coupling this type of chemiluminescence detection to capillary electrophoresis with a view to eliminating the concomitant interferences sometimes observed with flow analysis whilst reducing the time taken per analysis. Recently, Bjørnsdottir and Hansen[41,42] reported a capillary electrophoresis procedure for the determination of six *Papaver somniferum* alkaloids present in opium and pharmaceutical preparations. This methodology[41,42] employed an aqueous electrolyte containing cyclodextrins and UV detection at 214 nm, which realised a detection limit of 1×10^{-6} M for morphine.

This paper describes some preliminary results on the determination of morphine, oripavine and pseudomorphine using a modified version of the separation previously described by Bjørnsdottir and Hansen[41,42] coupled with a simple and robust acidic potassium permanganate chemiluminescence detection system.

Experimental

Instrumentation

Capillary electrophoresis was performed on a Waters Quanta 4000 system (Millipore, Bedford, MA, USA). Untreated fused silica capillaries (SGE, Ringwood, Victoria, Australia) were of 75 μm id and 360 μm od with a total length of 32 cm. For the

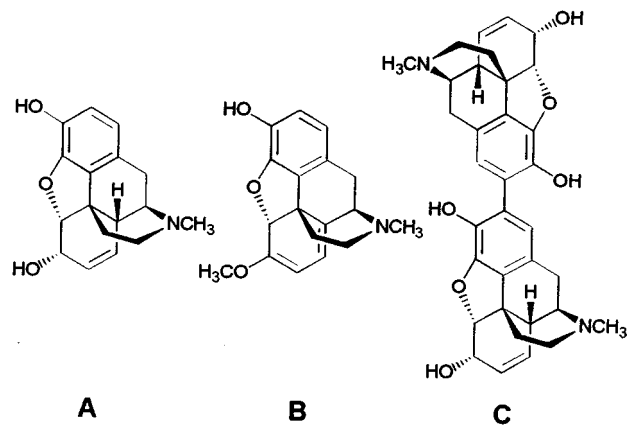

Fig. 1 Structures of (A) morphine, (B) oripavine and (C) pseudomorphine.

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experiments using UV detection at 214 nm, the effective length to the detector was 24 cm. A cuvette (1 × 1 × 4 cm, Spectrosil quartz, Buck Scientific, Melbourne, Victoria, Australia) was employed as the chemiluminescence detection cell (see Fig. 2). Using this cell, no modifications to the existing electrode mount of the Waters Quanta 4000 were required and it allowed the use of the vacuum purge system which was not possible with the previously reported[34] instrument configuration. The detection cell was aligned with the vacuum seal of the electrode mount with the capillary end approximately 1 mm from the bottom of the cell. Two reagent lines (Teflon tubing) were inserted through the vacuum seal and the inlet and outlet lines were positioned at the bottom of the cell and 1 cm from the top the cell, respectively. A Gilson Minipuls 3 peristaltic pump (John Morris Scientific, Melbourne, Victoria, Australia) was employed to fill the cell with reagent prior to each analysis.

Chemiluminescence was monitored by mounting the detection cell flush against the end window of a photomultiplier tube (R9798SB, Thorn EMI, Ruislip, Middlesex, UK) operated at 1100 V provided by a stable power supply (PM28BN, Thorn EMI). The signal from the photomultiplier tube was connected to a transimpedance amplifier (Type A1, Thorn EMI) and the output recorded using a chart recorder (Type 3066, Yokogawa Hokushin Electric, Tokyo, Japan).

Reagents

All reagents were of analytical-reagent grade unless stated otherwise and solutions were prepared with de-ionised water from a Milli-Q system (Millipore). Sodium polyphosphate (96%), 6-aminocaproic acid (98%) and hexadimethrine bromide were purchased from Aldrich (Milwaukee, WI, USA), glacial acetic acid and sodium hydroxide from BDH (Poole, Dorset, UK), α-cyclodextrin from Tokyo Kasei Kogyo (Tokyo, Japan) and potassium permanganate and concentrated sulfuric acid from Ajax Chemicals (Sydney, NSW, Australia). Morphine, oripavine and pseudomorphine standards were obtained from Glaxo Wellcome Australia (Port Fairy, Victoria, Australia).

Procedures

Sample solutions were introduced on to the capillary using gravity injection with a height displacement of 9.8 cm for 10 s.

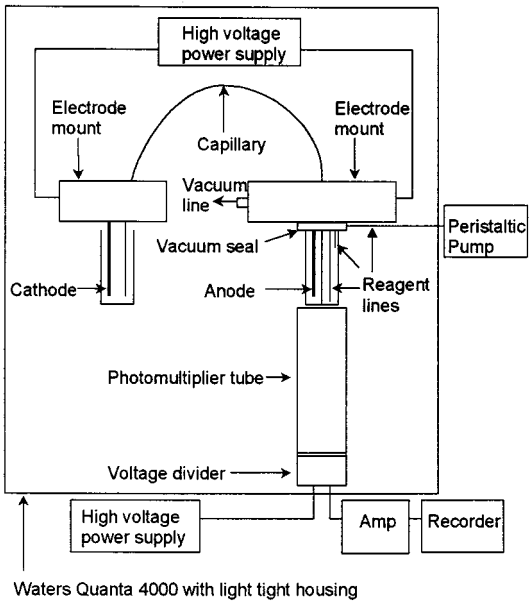

Fig. 2 Schematic diagram of the capillary electrophoresis instrumentation coupled to the chemiluminescence detection system.

The applied voltage was −20 kV and the capillary was rinsed with sodium hydroxide (0.1 M) for 30 s and then with the separation buffer for 30 s prior to each analysis by application of vacuum (12 mmHg). After completion of this purging routine and sample injection, the peristaltic pump was switched on to provide a constant flow of reagent to the cell during the analysis.

The separation buffer consisted of 50 mM 6-aminocaproic acid prepared in 0.001% m/v hexadimethrine bromide (diluted from a 0.1% m/v stock solution). The pH of this solution was adjusted to 4.5 with dilute acetic acid (1 + 4). Once the pH had been adjusted, this solution was used to dissolve the appropriate amount of α-cyclodextrin. The chemiluminescence reagent was prepared by dissolving the desired quantity of potassium permanganate and sodium polyphosphate followed by adjustment to the required pH with concentrated sulfuric acid. The process liquor was diluted with water (1 + 49) prior to analysis.

Resolution (R_s) was calculated using the equation $R_s = 2[(t_2 - t_1)]/W_1 + W_2$, where t is the migration time in minutes, W is the baseline peak width in minutes and the subscripts 1 and 2 refer to morphine and oripavine, respectively.

Results and discussion

In a recent paper from our laboratory, we reported a methodology which utilised capillary electrophoresis with tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection for the determination of codeine, *O*-methylcodeine and thebaine.[34] The direct transfer of this technology, for use with the acidic potassium permanganate detection chemistry, was problematic owing to the migration of the reagent anion towards the anode (the injection end) as previously noted by Lee and Whang.[36] However, reversing the polarity of the electrodes (such that the anode was at the detector end) in conjunction with changing the direction of the electroosmotic flow, *via* the addition of a suitable modifier to the electrolyte, should theoretically prevent the migration of the permanganate anion. Several research groups[43–46] have shown that hexadimethrine bromide, when present at low concentrations (~ 0.001% m/v) in the electrolyte leads to the reversal of the electroosmotic flow in a highly reproducible fashion (< 0.5% RSD). Using this approach, we were able to separate morphine, oripavine and pseudomorphine as cations, counter to the electroosmotic flow, without migration of the permanganate anion, thus alleviating the need for tedious modifications to the capillary.[36]

Bjørnsdottir and Hansen[42] reported the electrophoretic separation of six opiate alkaloids using the following conditions: 64 cm × 50 μm id fused silica capillary, an applied voltage of 30 kV with a 50 mM 6-aminocaproic acid buffer adjusted to pH 4.0 containing heptakis(2,6-di-*O*-methyl)-β-cyclodextrin. In our preliminary experiments, based upon this study,[42] we utilised a similar electrolyte (including hexadimethrine bromide at 0.001% m/v) with a 32 cm × 75 μm id fused silica capillary and an applied voltage of −20 kV, which resulted in currents ranging from 90 to 120 μA. Adjustment of the electrolyte to pH 4.5 reduced the current to an acceptable level (48 μA). The lower current minimised the undesirable effects which Joule heating can have on the separation.[47] The unavailability of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin led us to explore the feasibility of separating our three analytes with and without α-cyclodextrin. In the absence of α-cyclodextrin, pseudomorphine was separated from morphine and oripavine, but the latter two analytes were not resolved from each other. The inability to separate morphine from oripavine is not surprising considering the structural similarities of these two compounds (see Fig. 1). Furthermore, this result is consistent with that reported by Bjørnsdottir and Hansen,[41] who showed

that without any cyclodextrins present, the separation of six *Papaver somniferum* alkaloids yielded only two peaks. Subsequently, the effect of the addition of α-cyclodextrin to the electrolyte over the concentration range 30–80 mM was examined with complete separation of the three analytes being achieved at 80 mM.

Our previous experience with acidic potassium permanganate detection chemistry[37–39,48,49] has shown that performing a series of univariate searches, with respect to certain parameters, may increase the chemiluminescence intensity. The variables investigated in this study were the concentrations of potassium permanganate and sodium polyphosphate and the pH. The effect of potassium permanganate concentration over the range 0.01 to 1 mM on the chemiluminescence emission is shown in Fig. 3(a). Not only did the emission intensity increase with increasing potassium permanganate concentrations up to 0.5 mM but a significant improvement in apparent resolution was also observed. The resolution between oripavine and morphine was calculated, using the equation given earlier, to be 0.9 and 1.5 at 0.075 and 0.75 mM potassium permanganate, respectively. This result was probably due to more rapid kinetics of the chemiluminescent reaction as the ratio of reagent to analyte was increased, thereby reducing band broadening as a consequence of dilution. The concentration of sodium polyphosphate had a marked effect on the sensitivity [see Fig. 3(b)], particularly in the region from 0.0025 up to 0.075% m/v, where a steep rise in signal intensity occurred. Increasing the concentration of sodium polyphosphate, from 0.25 up to 1% m/v, produced only small changes in the chemiluminescence response for the three analytes. Although the mechanism of this detection chemistry has, as yet, not been elucidated, the sodium polyphosphate clearly plays an essential role in the production of analytically useful chemiluminescence under these and similar conditions.[37–39,48,49] The chemiluminescence response was also affected by the reaction pH [see Fig. 3(c)], fortuitously with the three analytes each exhibiting maximum emission intensity at approximately pH 1.9. Based on these observations, we chose the chemiluminescence reagent conditions 0.5 mM potassium permanganate and 0.5% m/v sodium polyphosphate at pH 1.9 for all further experiments.

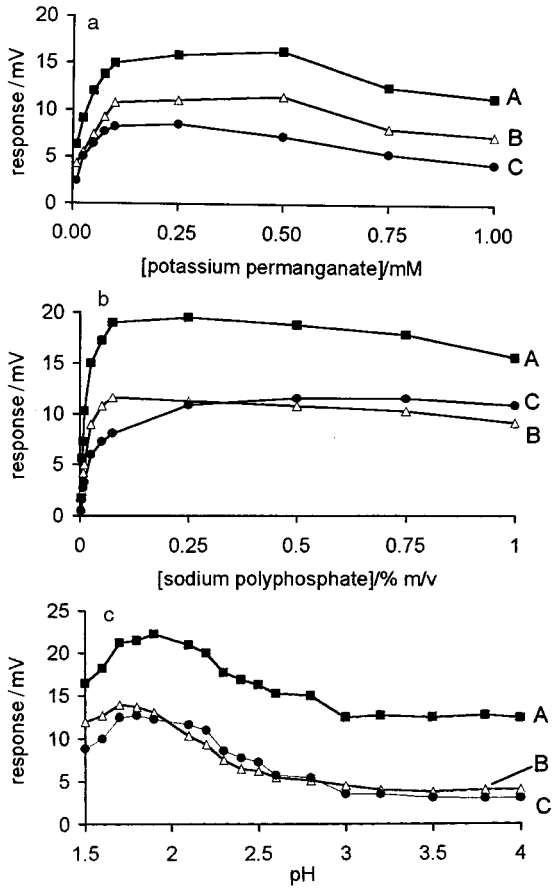

Fig. 3 (a) Effect of potassium permanganate concentration on the chemiluminescence response of (A) morphine, (B) oripavine and (C) pseudomorphine (1×10^{-5} M). The sodium polyphosphate concentration was 0.1% m/v, adjusted to pH 2.0 with concentrated sulfuric acid. The electrolyte consisted of 50 mM 6-aminocaproic acid, pH 4.5, 0.001% m/v hexadimethrine bromide with 80 mM α-cyclodextrin added. Other conditions were applied voltage –20 kV, injection time 10 s, current 49–56 μA, 20 °C, capillary 32 cm × 75 μm id. (b) Effect of sodium polyphosphate concentration on the chemiluminescence response for (A) morphine, (B) oripavine and (C) pseudomorphine (1×10^{-5} M). The potassium permanganate concentration was 0.5 mM, each solution contained the appropriate concentration of sodium polyphosphate and was adjusted to pH 2.0 with concentrated sulfuric acid. All other conditions as in (a). (c) Effect of pH on the chemiluminescence response for (A) morphine, (B) oripavine and (C) pseudomorphine (1×10^{-5} M). The potassium permanganate concentration was 0.5 mM and the sodium polyphosphate concentration was 0.5% m/v, adjusted to the required pH with concentrated sulfuric acid. All other conditions as in (a).

In addition to the change in the apparent resolution with permanganate concentration mentioned previously, a flowing as opposed to a static reagent was also observed to have a profound effect on the separation (see Fig. 4). Contrary to what was expected, a static reagent yielded both poorer apparent resolution and sensitivity when compared with a flowing reagent. Under the static conditions, the reagent may be diluted or displaced from the tip of the capillary by the electrolyte. Consequently, dilution and dispersion of the analyte zones may occur prior to their chemiluminescent detection. In contrast, pumping fresh potassium permanganate into the detection cell resulted in a continuous supply of fresh reagent to the region surrounding the tip of the capillary and as such the analytes reacted immediately upon elution. As with our earlier observations, when using increased reagent concentrations, the explanation for this unexpected behaviour probably stems from the rapid kinetics of this detection chemistry, which are clearly faster than dilution (see Fig. 4).

Calibration functions were obtained for oripavine, morphine and pseudomorphine using a series of 12 mixed standard solutions over the concentration range 2.5×10^{-7}–1×10^{-4} M and the analytical figures of merit are summarised in Table 1. The responses observed for morphine, oripavine and pseudomorphine, over the entire concentration range, closely approximated linearity and this is reflected by the slopes of the log–log calibration functions (see Table 1). Although the detection

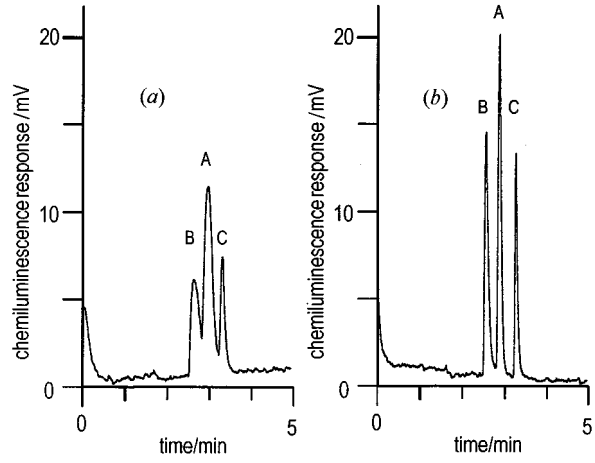

Fig. 4 Electropherograms showing the effect of reagent flow on the separation of (A) morphine, (B) oripavine and (C) pseudomorphine (1×10^{-5} M), using (a) a static reagent and (b) a flowing reagent. The potassium permanganate concentration was 0.5 mM, the sodium polyphosphate concentration was 0.5% m/v and the solution was adjusted to pH 1.9 with concentrated sulfuric acid. All other conditions as described in Fig. 3(a).

Table 1 Analytical figures of merit for morphine, oripavine, and pseudomorphine obtained using the conditions outlined in Fig. 4(b)

Analyte	Detection limit[a]/M	Calibration function[b]	Correlation coefficient (r^2)	Log–log calibration function[c]	Correlation coefficient (r^2)	Relative standard deviation (%)[d]	
						Migration time	Peak height
Morphine	2.5×10^{-7} (5×10^{-6})	$y = 2.08 \times 10^6 x$	0.9985	$y = 0.99x + 6.23$	0.9988	0.6	1.5
Oripavine	2.5×10^{-7} (5×10^{-6})	$y = 1.84 \times 10^6 x$	0.9910	$y = 1.04x + 6.38$	0.9963	0.7	1.5
Pseudomorphine	5×10^{-7} (5×10^{-6})	$y = 1.15 \times 10^6 x$	0.9976	$y = 0.97x + 5.95$	0.9978	0.8	2.1

[a] Calculated as 3 × S/N, values in parentheses were estimated using UV absorbance detection at 214 nm. [b] y = peak height/mV, x = concentration/M. [c] y = log (peak height/mV), x = log (concentration/M). [d] $n = 10$ at 2.5×10^{-5} M.

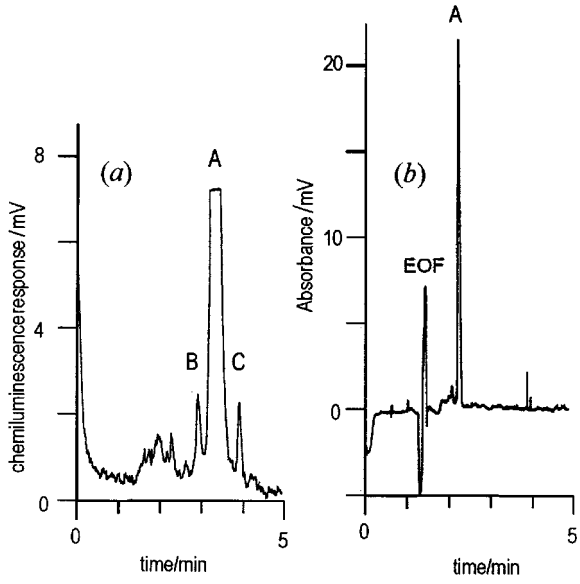

Fig. 5 Electropherograms showing the separation of (A) morphine, (B) oripavine and (C) pseudomorphine in a dilute process liquor (1 + 49) using (a) chemiluminescence detection and (b) UV absorbance detection at 214 nm. All other conditions as in Fig. 4(b).

limits attained were superior to those achieved by Lee and Whang,[36] such a direct comparison of performance would not be strictly valid owing to the different nature of the analytes. Likewise, it is similarly inappropriate to compare the peak height precision achieved during the present study (see Table 1) with the peak area precision reported by Lee and Whang[36] (relative standard deviations from 4.9 up to 8.3%). However, it is our contention that as we achieved superior precision values at a lower analyte concentration, using peak height, then our methodology would appear to be more reproducible than that described by Lee and Whang.[36] As with our earlier report,[34] using tris(2,2′-bipyridyl)ruthenium(III), the satisfactory precision attained during the current investigation probably reflected the simple and robust nature of the detection device. It should be borne in mind that the results described here are of a preliminary nature, as a thorough optimisation of the detector configuration has not yet been completed. Notwithstanding this, the approach described above appears to offer certain advantages for the determination of these and other analytes which elicit analytically useful chemiluminescence upon reaction with acidic potassium permanganate.[48–50] Specifically, the determination of these three analytes in process liquors. As can be seen from Fig. 5, the UV absorbance electropherogram exhibited inferior sensitivity for oripavine and pseudomorphine compared with that achieved with chemiluminescence, as would be expected from the UV absorbance detection limits shown in Table 1. As can also be seen from Fig. 5(a), the analysis time was 4 min, which compared favourably with a run time of 10.2 min using the current Glaxo Wellcome standard HPLC methodology.[40] Furthermore, the major possible interferences (oripavine and pseudomorphine) with respect to flow analytical methodology are clearly separated from morphine [Fig. 5(a)]. Whereas in certain process samples the morphine to oripavine and pseudomorphine ratio is high [see Fig 5(a)], in other liquors this ratio is significantly lower, hence the contribution of the interferences is much larger with respect to flow analysis. It is also evident from Fig. 5 that a peak corresponding to the electroosmotic flow (1.4 min) was observed in the UV absorbance electropherogram, but, such a peak was not visible when chemiluminescence detection was employed. In order to monitor the electroosmotic flow when using this type of detection, a suitable neutral marker species which also elicits chemiluminescence upon reaction with acidic potassium permanganate would be required. Upon completion of the optimisation, the methodology described above will be validated against the current standard HPLC method,[40] and these results will be reported in due course.

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