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Spectroelectrochemistry of Morphine and Related Alkaloids and their Investigation by Fluorescence in a Gold Micromesh Cell

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A standard microflow cell containing a transparent gold micromesh electrode has been designed for *in situ* fluorescence monitoring of electrogenerated species by frontal illumination. Oxidative dimerisation of morphine to the fluorescent pseudomorphine proved to be a model fluorogenic reaction for study. The fluorescence calibration graph was linear over the concentration range 1×10^{-3}–1×10^{-6} M and the limit of detection was 5×10^{-7} M. The procedure, which is selective and free from interference from most of the opium alkaloids, enabled morphine to be assayed directly in papaveretum.

Keywords: *Spectroelectrochemistry*; *morphine*; *pseudomorphine*; *electrogeneration*; *fluorogenic reaction*

Spectroscopic techniques have been used extensively over the last decade for the *in situ* monitoring of electrogenerated species. Such studies have been mainly concerned with mechanistic aspects of electrode reactions and valuable information has been obtained by coupling electrochemical experiments with optical and electron spin resonance methods.[1–3] Little attention has, however, been devoted to analytical applications of the spectroelectrochemical technique although the possibility was suggested some time ago.[4]

Recently, absorptiometric monitoring at electrode surfaces has been shown to have potential for the trace determination of metal ions and certain organic compounds.[5–8]

A fluorescence-coupled electrochemical experiment may offer the additional analytical advantages of increased sensitivity and selectivity. To investigate this, a microvolume fluorescence cell incorporating gold micromesh as the working electrode was designed for the *in situ* fluorescence measurement of electrogenerated species. Gold micromesh was chosen as it exhibits favourable electrochemical characteristics[9] and was convenient for cell fabrication. The electrode material has been employed by a number of workers, notably Murray and co-workers[10–12] and others,[13,14] for spectroelectrochemical thin-layer studies. For application the possibility of electrochemically initiating and concurrently monitoring the course of fluorogenic reactions was considered. The fluorogenic reaction, whereby a weakly or non-fluorescent compound is converted by an appropriate chemical reaction (for example, a redox reaction) into a highly fluorescent species, is employed for trace determinations in the biological and medical fields. The texts by Guilbault[15] and White and Argauer[16] provide comprehensive lists of such reactions. Morphine, a powerful but addictive analgesic, is a good example of a potential fluorogen, undergoing oxidation to the highly fluorescent dimer, pseudomorphine, when treated with alkaline potassium hexacyanoferrate(III) solution. The reaction has attracted the attention of analysts in the pharmaceutical,[17] clinical[18,19] and forensic fields.[20]

Oxidative dimerisation of morphine, initiated by electrolysis at 0.25 V *versus* S.C.E., was performed successfully in the gold micromesh cell and preliminary experiments were concerned with establishing the optimum conditions for the formation and stability of pseudomorphine and determining the performance characteristics of the cell. Once interference studies had been evaluated, the potential of the cell for semi-automatic assay was demonstrated by determining morphine in the presence of other opium alkaloids.

Experimental

Construction of Cell

A piece of transparent (45% transmission) gold micromesh (1000 lines per inch; Buckbee Mears, Minneapolis, USA), the working electrode, was inserted between the two halves of a standard microflow cell (Hellma Ltd., Southend; Cell No. 136 in Suprasil; path length 0.5 mm) and the unit was made leakproof by sealing with silicone rubber sealant. The cell volume was approximately 120 μl. Electrical connection to the gold micromesh was made with a thin brass strip that was permanently attached to the cell. The mesh is extremely fragile and care must be exercised when handling. A platinum tube (internal diameter 1 mm) sealed to the cell outlet served as the counter electrode. Sample was delivered to the cell with a single-channel peristaltic pump (Perpex 10200) and tubing (Technicon 116–0522–11). A saturated calomel electrode (S.C.E.) placed in the downstream reservoir completed the three-electrode cell. A schematic diagram of the cell and associated equipment is shown in Fig. 1.

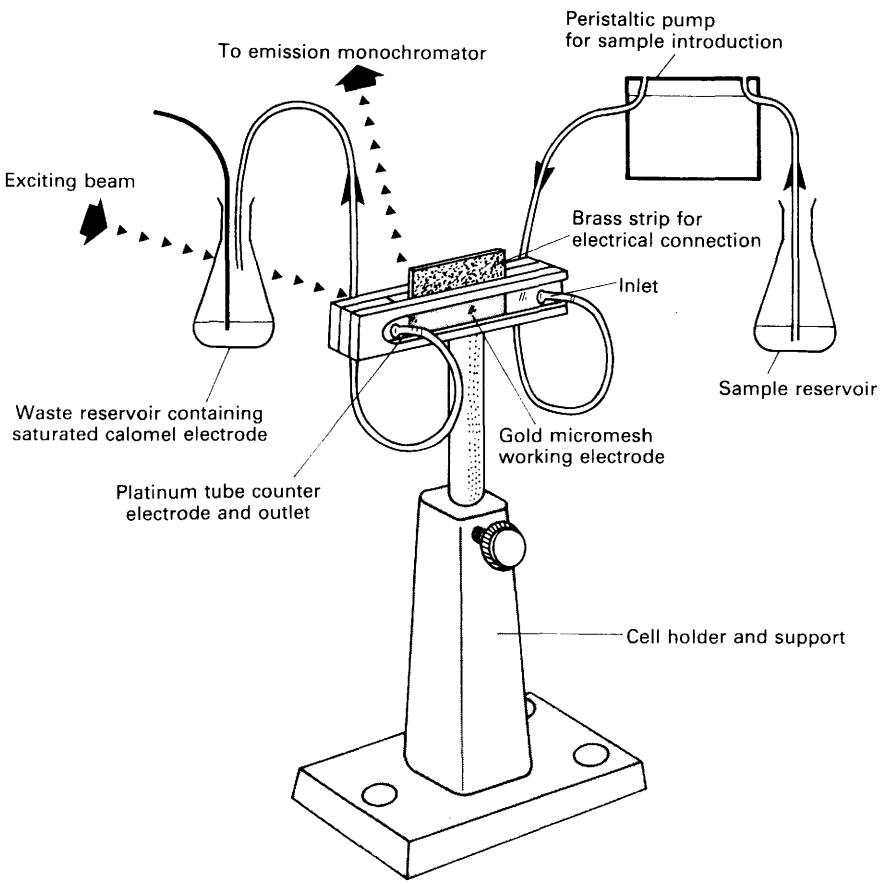


Fig. 1. Gold micromesh cell and associated equipment.

Equipment

Fluorescence measurements, which were uncorrected for grating/detector response characteristics, were obtained with a Farrand spectrofluorimeter, which utilised a 150-W xenon arc source, two modified Czerny–Turner-type monochromators (relative aperture $f/3.5$; dispersion 10 nm mm^{-1}), an RCA IP 28 photomultiplier and a Honeywell Electronik 15

recorder. Slits providing a band pass of 20 nm were used on both monochromators unless stated otherwise. The method of frontal illumination was used, with the gold micromesh cell being positioned at an angle approximately 30° to the excitation beam. Corning filters, 7–54 (maximum transmission 330 nm) and 3–73 (maximum transmission 550 nm), were fitted to the excitation and emission monochromators, respectively, to reduce scattered exciting light. Stray light was minimised by enclosing the gold micromesh cell within a light-tight box constructed from expanded polystyrene and black cotton cloth.

A PAR 174A polarographic analyser was employed for controlled potential electrolysis during fluorescence measurements. The instrument was also used in conjunction with a Metrohm cell (EA 875–20) and a Servoscribe two-channel recorder for obtaining the voltammetric curves of alkaloid solutions. A gold micro-wire and a platinum wire were used as the working and counter electrodes, respectively, and a saturated calomel electrode served as the reference.

Materials

Morphine hydrochloride, heroin hydrochloride, codeine phosphate, Papaveretum Tablet (BPC) and Omnopon injection ampoule (Roche) were of pharmaceutical grade. Pseudomorphine, narcotine hydrochloride, cotarnine hydrochloride, sinomenine hydrochloride, narceine and laudanosine were of normal reagent quality.

Reagent

Sodium pyrophosphate solution, 0.1 M, *pH* 8.5. An 11.15-g amount of sodium pyrophosphate (AnalaR grade) was dissolved in distilled water (250 cm^3) and adjusted to pH 8.5 (or the appropriate pH) with a small volume of sulphuric acid (1 M). This solution, freshly prepared each day, was used throughout unless stated otherwise.

Preparation of Alkaloid Solutions

Stock alkaloid solutions (typically 1×10^{-3} M) were prepared by dissolving the required amount of alkaloid in dilute sulphuric acid. Aliquots of the appropriate stock solution were then diluted to 25 cm^3 with sodium pyrophosphate background electrolyte.

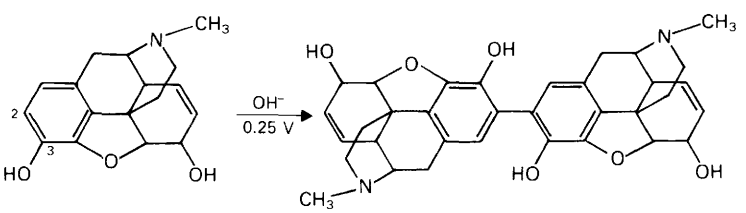
Operating Procedure

The sample solution was delivered to the cell by a peristaltic pump. With a stationary solution, a potential, sufficient to effect oxidation, was applied to the gold micromesh electrode and the resulting fluorescence with respect to time was monitored at the appropriate wavelength combination. For pseudomorphine, the excitation and emission maxima were 320 and 442 nm, respectively, and the fluorescence response curve went through a maximum and decayed to the background level. The procedure could be repeated by returning the electrode potential to 0 V and introducing fresh sample. Blank solution was generally passed through the cell for 10 min between different sample runs. This cleaning cycle was required for consistent results and was particularly important when examining solutions of differing pH.

Results and Discussion

Development of Method

Morphine undergoes oxidative dimerisation in alkaline solution to the highly fluorescent pseudomorphine (2,2′-bimorphine).[21]



The electrode reaction involves oxidation of the ionised phenolic hydroxy group and subsequent coupling of phenolate radicals at the C-2 position. As pseudomorphine also contains phenolic hydroxy groups, further oxidation is likely. Chemical oxidation studies by Burkhalter *et al.*[18] indicated that the optimum pH for fluorescence measurement of pseudomorphine is 8.5. To determine the optimum conditions in this study, the effects of the working electrode potential and pH on the fluorescence of electro-generated pseudomorphine were investigated. Prior to this the fluorescence spectra of pseudomorphine were obtained.

Fluorescence spectra

The excitation and emission spectra of the fluorescence signal were recorded *in situ* during the controlled potential oxidation (0.2 V *versus* S.C.E.) of morphine hydrochloride (2×10^{-4} M) in sodium pyrophosphate solution. The spectra, which were obtained with a band pass of 5 nm on both monochromators and without filters (7–54, 3–72), are shown in Fig. 2. They reveal the characteristic excitation (three peaks at 250, 280 and 320 nm) and emission pattern (one peak at 422 nm) for pseudomorphine previously reported by Burkhalter *et al.*[18] The spectra recorded in the presence of the complementary filters were distorted but the excitation and emission maxima remained the same. Subsequent fluorescence measurement was performed at the wavelength combination $\lambda_{ex.}$ 320 nm and $\lambda_{em.}$ 442 nm.

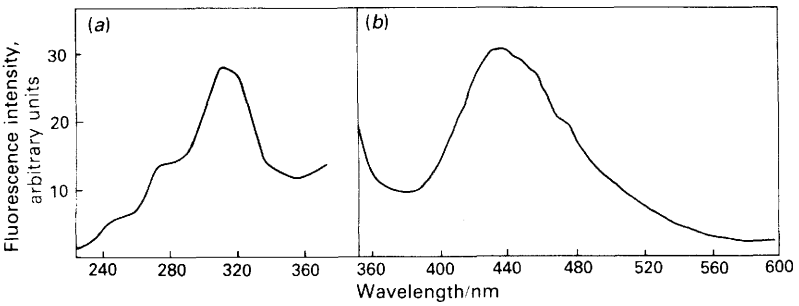


Fig. 2. Fluorescence excitation (*a*) and emission (*b*) spectra of electrogenerated pseudomorphine. Morphine hydrochloride (2×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential, 0.20 V *vs.* S.C.E.; excitation wavelength, 320 nm; emission wavelength, 442 nm; and band pass, 5 nm for both monochromators.

Effect of electrode potential

Morphine hydrochloride (8×10^{-5} M) in sodium pyrophosphate solution (0.1 M, pH 8.5) was oxidised in the gold micromesh cell at various electrode potentials. The results, summarised in Table I, indicate the effect of the electrode potential on the formation and stability

TABLE I

EFFECT OF ELECTRODE POTENTIAL ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (8×10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); excitation wavelength 320 nm; emission wavelength 442 nm.

Electrode potential/ V *vs.* S.C.E.	Maximum fluorescence intensity, arbitrary units	$Time_{MF}$*/min
0.15	—	—
0.20	—	—
0.25	40	9.8
0.30	35	6.2
0.40	22	3.2
0.50	18	2.4
0.60	16	2.2

* MF = maximum fluorescence.

of pseudomorphine. For potentials less than that required for oxidation, for example 0.15 V *versus* S.C.E., pseudomorphine was not formed and consequently fluorescence was not generated. At potentials up to 0.2 V *versus* S.C.E., the fluorescence developed slowly and did not reach a maximum in an acceptable time. For increasingly positive potentials, the rate of formation and removal of pseudomorphine increased, with the result that the fluorescence maxima progressively decreased. The fluorescence behaviour indicated the increasing rate of oxidation of the phenolic hydroxy groups of morphine and pseudomorphine with increasing electrode potential, the oxidation product of the latter compound being non-fluorescent. Maximum fluorescence (at pH 8.5) was obtained with an applied potential of 0.25 V *versus* S.C.E.

Effect of pH

Morphine hydrochloride solutions (8×10^{-5} M) containing sodium pyrophosphate (0.1 M) and covering the pH range 6.0–10.2 were individually oxidised at 0.3 V *versus* S.C.E. and the fluorescence response was recorded. The rate of fluorescence development and decay increased as the solution became more alkaline. The results are summarised in Table II. Maximum fluorescence was obtained for the pH 7.7 solution. Fluorescence was not recorded for solutions of pH 6.0 and 6.4 and, for the solution of pH 7.2, the time to reach maximum fluorescence was considerable. These results indicate that the oxidation of morphine and pseudomorphine was pH dependent and a subsequent study of the voltammetric behaviour of the two compounds with respect to pH confirmed this. For solutions in the pH range 7.5–8.5, and with oxidation at 0.25 V *versus* S.C.E., differences in the fluorescence maxima were less pronounced. Maximum fluorescence was still recorded for the pH 7.7 solution, but the time taken to reach the maximum was considerable.

Summarising, to obtain maximum fluorescence for electrogenerated pseudomorphine an oxidation potential of 0.25 V *versus* S.C.E. with a solution pH of about 7.7 should be chosen. If the development time is unacceptable then a faster fluorescence response can be obtained at the expense of sensitivity by increasing either (or both) parameter(s). In subsequent experiments solutions of pH 8.5 were used.

TABLE II

EFFECT OF pH ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (8×10^{-5} M) in sodium pyrophosphate solution (0.1 M); electrode potential 0.3 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

pH	Maximum fluorescence intensity, arbitrary units	$Time_{MF}$/min
6.0	—	—
6.4	—	—
7.2	40	17.7
7.7	53	7.5
8.0	41	3.7
8.6	28	2.6
9.1	20	2.3
9.6	16	1.9
10.2	12	1.4

Efficiency of Electrochemical Conversion and Comparison with Potassium Hexacyanoferrate(III) Oxidation

The efficiency of electrochemical dimerisation was determined by comparing the maximum fluorescence of electrogenerated pseudomorphine with the fluorescence of standard pseudomorphine solutions. The molar concentrations of the morphine and pseudomorphine solutions were in the ratio 2:1, as two molecules of morphine are required for dimerisation. The efficiency was also compared with the chemical oxidation method of Burkhalter *et al.*[18]

Procedure

The maximum fluorescence of pseudomorphine was recorded in the gold micromesh cell for three situations. First, morphine (2×10^{-5} M) in pH 8.5 sodium pyrophosphate solution

(0.1 M) was oxidised at 0.25 V *versus* S.C.E. and the fluorescence response was recorded. Next, morphine (2×10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) was oxidised by potassium hexacyanoferrate(III) (6×10^{-5} M) - potassium hexacyanoferrate(II) (3.7×10^{-6} M) solution according to the procedure of Burkhalter *et al.*[18] and the fluorescence at zero applied potential was recorded. Finally, the fluorescence of standard pseudomorphine solution (10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) was recorded for zero applied potential. The fluorescence results and the appropriate percentage conversion values are presented in Table III. It can be seen that electrochemical dimerisation is comparable to the chemical oxidation method of Burkhalter *et al.*[18]

In both instances the efficiency of morphine dimerisation was about 55%. Dimerisation efficiency via chemical oxidation has, however, been improved by Takemori.[19]

TABLE III

EFFICIENCY OF ELECTROCHEMICAL AND CHEMICAL OXIDATION

Alkaloids in pH 8.5 sodium pyrophosphate solution (0.1 M); excitation wavelength 320 nm; emission wavelength 442 nm.

Parameter	Pseudomorphine (10^{-5} M)	Morphine (2×10^{-5} M)	
		Chemical	Electrochemical
Maximum fluorescence intensity of pseudomorphine, arbitrary units	27.5	15.5	15.2
Conversion, %	—	56.4	55.3

Calibration and Limit of Detection

The fluorescence calibration graph was obtained for morphine hydrochloride solutions covering the concentration range 1×10^{-2}–1×10^{-6} M. Measurements recorded for oxidation at 0.25 V *versus* S.C.E. in pH 8.5 solutions were generally repeated at least once and an average value taken. The calibration graph showed an extremely good fit of the experimental data, linearity being observed over a 1000-fold range. Fluorescence measurements were not extended beyond 1×10^{-3} M because precipitation of morphine occurred in the pH 8.5 media. Fluorescence was, however, measured for morphine concentrations up to 3×10^{-3} M in pH 11 media. The calibration graph still exhibited a wide linear analytical range, but the onset of self-absorption, which became noticeable at concentrations above 1×10^{-3} M, brought a departure from linearity. Decreased sensitivity in the pH 11 media was observed, as expected. The limit of detection, based on a limiting signal twice the standard deviation of the background noise level, was calculated to be 5×10^{-7} M morphine (as hydrochloride). The detection limit for morphine (free alkaloid) based on the cell volume of 120 μl was 19 ng.

Interference Studies

It might be expected that the morphine dimerisation reaction could offer a degree of specificity and provide an interference-free route for morphine. Unfortunately, a large number of phenolic compounds undergo this reaction. Burkhalter *et al.*[18] reported that the phenolic alkaloids nor-morphine, allylmorphine, dihydromorphine and monoacetylmorphine yielded fluorescence characteristics identical with those of pseudomorphine and that they would interfere in the determination of morphine. Non-phenolic compounds including codeine and its derivatives and heroin (diacetylmorphine) did not yield fluorescence products similar to pseudomorphine and it was stated that such compounds would not cause interference. Essentially the same results were reported by Deys[22] for the anodic voltammetric determination of morphine in acidic solution: those alkaloids bearing a phenolic hydroxy group (pseudomorphine, dihydromorphine, nalorphine, apomorphine) yielded oxidative waves similar to morphine, causing interference, whereas compounds containing alkylated hydroxy groups (codeine, narcotine, narceine, thebaine, papaverine) were not electroactive and did not interfere. With the results of Burkhalter *et al.* and Deys providing the basis for interference studies, a selection of opium alkaloids that were readily available—codeine, narceine, narcotine, papaverine, cotarnine, laudanosine and sinomenine (phenolic alkaloid)—were examined for possible interference (papaverine, apomorphine and thebaine

were considered, but were insoluble at pH 8.5). Heroin, a synthetic derivative of morphine, was also investigated. Experiments were first concerned with establishing the electroactivity of each alkaloid and whether it exhibited oxidative fluorescence before conducting interference studies. Possible interference mechanisms considered were spectral, from either natural or oxidative fluorescence (not necessarily phenolic coupling), and chemical, whereby the morphine dimerisation reaction is inhibited.

Voltammetric behaviour

The current - potential graphs for each alkaloid (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) were recorded in a Metrohm cell during the cyclic sweep (0 to 0.75 V *versus* S.C.E. at 20 mV s^{-1}) of the gold wire working electrode. Oxidative waves were recorded for morphine, sinomenine and heroin solutions but codeine, narceine, cotarnine were electroinactive. The scan for laudanosine indicated possible electroactivity and a further sweep from 0.5 to 1.25 V *versus* S.C.E. confirmed this. The voltammograms for sinomenine, morphine, codeine and heroin are presented in Fig. 3 and a summary of the electrochemical behaviour of the alkaloids investigated is given in Table IV. Of the compounds investigated, sinomenine is the only one to possess a phenolic hydroxy group and, as shown in Fig. 3(*b*), an oxidative wave similar to that for morphine was recorded. The oxidative wave for heroin [solid line, Fig. 3(*d*)] was indicative of phenolic oxidation. Heroin, however, undergoes alkaline hydrolysis[23] to monoacetylmorphine and thus the characteristic phenolic oxidative wave was observed.

On the basis of this study it would be expected that sinomenine and heroin would cause interference either by scavenging morphine free radicals and lowering the dimerisation yield of pseudomorphine and/or by undergoing self-coupling to give a fluorescent product. It

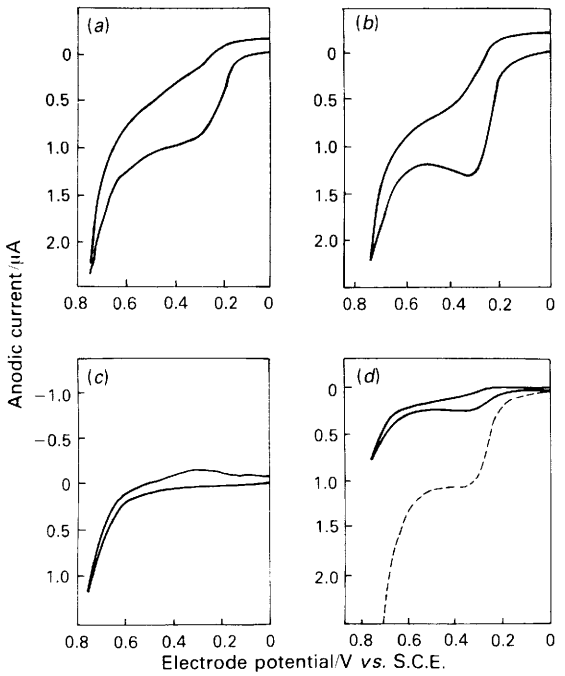


Fig. 3. Voltammetric curves for (*a*) sinomenine, (*b*) morphine, (*c*) codeine and (*d*) heroin. Alkaloid (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); working electrode, gold wire; and scan rate 20 mV s^{-1}. For broken line [in (*d*)] response sensitivity was increased by a factor of 5.

TABLE IV

VOLTAMMETRIC CHARACTERISTICS OF ALKALOIDS

Alkaloids (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M). Cyclic sweep, 0 to 0.75 to 0 V *vs.* S.C.E. at 20 mV s^{-1}, except for laudanosine (0.5 to 1.25 to 0.5 V *vs.* S.C.E.); working electrode, gold wire.

Alkaloid	Oxidative wave	$E_{\frac{1}{2}}$/V *vs.* S.C.E.
Morphine	Yes	0.24
Sinomenine	Yes	0.21
Heroin*	Yes	0.27
Laudanosine	Yes	—†
Codeine	No	—
Narceine	No	—
Cotarnine	No	—
Narcotine	No	—

* After hydrolysis to monoacetylmorphine.
† Difficult to estimate.

cannot be predicted at this stage whether oxidation of laudanosine would interfere either chemically or spectrally. The remaining electroinactive alkaloids should not interfere (neglecting the natural fluorescence of the alkaloid).

Fluorescence studies

Solutions of each alkaloid (3×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) were examined individually in the gold micromesh cell during oxidation at various positive applied potentials. Only the heroin and laudanosine solutions exhibited fluorescence (excitation, 320 nm; emission, 442 nm) during electrolysis. The emission spectrum for the heroin product was identical with that of pseudomorphine, confirming the findings of Burkhalter *et al.*[18] When the heroin solution was prepared immediately before examination only a small signal was recorded on electrolysis, indicating that hydrolysis to monoacetylmorphine was not significant. The laudanosine product exhibited sensitive fluorescence, the excitation and emission maxima being 361 and 470 nm, respectively. The oxidation product was not identified. The remaining alkaloid solutions did not exhibit fluorescence.

The possible interference of each alkaloid on the fluorescence of electrogenerated pseudomorphine was next investigated in a systematic manner. Morphine solutions (3×10^{-5} M) containing the particular alkaloid (1) at the same concentration (3×10^{-5} M), (2) in a 10-fold excess (3×10^{-4} M) and (3) in a 100-fold excess (3×10^{-3} M), all in pH 8.5 sodium pyrophosphate solution (0.1 M), were oxidised at 0.25 V *versus* S.C.E. and the maximum fluorescence was recorded. This procedure was carried out for sinomenine, heroin, laudanosine, narceine, cotarnine and narcotine; for codeine, concentrations 1000-fold greater (3×10^{-2} M) were examined. The fluorescence results are summarised in Table V.

The electroinactive alkaloids, codeine narceine and narcotine, caused no interference at any of the concentration levels investigated but cotarnine interfered at the 3×10^{-3} M level when its natural fluorescence became significant. The remaining electroactive alkaloids, sinomenine heroin and laudanosine caused interference. For sinomenine at the 3×10^{-5} M level, the fluorescence signal was reduced to 33% of the original value whereas morphine solutions containing 3×10^{-4} and 3×10^{-3} M sinomenine did not exhibit fluorescence on electrolysis. Laudanosine and heroin solutions individually caused serious positive interference (increased fluorescence) at concentrations 3×10^{-4} M and higher. Interference was not observed at the 3×10^{-5} M level.

The results of this study are in good agreement with the predictions made on the basis of the voltammetric behaviour of the alkaloids. Alkaloids containing a phenolic hydroxy group undergo phenolic oxidation and decrease the efficiency of pseudomorphine formation by scavenging the morphine free radical species. This interference mechanism was exhibited by sinomenine. Spectral interference from non-phenolic alkaloids which become fluorescent on oxidation is also possible; this was demonstrated for laudanosine. The high specificity of the dimerisation reaction in the presence of electroinactive alkaloids (codeine, narcotine, cotarnine and narceine) was evident, however, and the result for codeine was particularly important.

TABLE V

INTERFERENCE OF ALKALOIDS ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (3×10^{-5} M) and alkaloid in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential 0.25 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

Alkaloid	Oxidative fluorescence	Interference results: alkaloid concentration/M*			
		3×10^{-5}	3×10^{-4}	3×10^{-3}	3×10^{-2}
Morphine	Yes				
Sinomenine	No	×	×	×	
Heroin†	Yes	—	×	×	
Laudanosine	Yes	—	×	×	
Codeine	No	—	—	—	—
Narceine	No	—	—	—	
Cotarnine	No	—	—	—	
Narcotine	No	—	—	—	

* × represents interference; — represents no interference.
† After hydrolysis to monoacetylmorphine.

Assay of Papaveretum Tablet and Omnopon Injection Ampoule

The official assay method for morphine in pharmaceutical preparations based on opium requires a preliminary extraction procedure prior to titrimetry.[24] An extraction procedure is also required in the recently recommended gas - liquid chromatographic methods.[25] To test the suitability of the gold micromesh technique for the direct determination of morphine, papaveretum, an important preparation based on the principal opium alkaloids, was selected. Papaveretum consists of the hydrochloride salts of morphine (47.5–52.5% *m/m*), naroctine (16–22% *m/m*), codeine (2.5–5% *m/m*) and papaverine (2.5–7% *m/m*) and is obtained in the form of a tablet [10 mg of papaveretum (BPC)] or injection ampoule (Omnopon, 20 mg ml^{-1} of papaveretum; Roche).

Procedure

One papaveretum tablet weighing 63.4 mg was powdered in a mortar. An accurately weighed amount (58.6 mg) was transferred into a calibrated flask (10 cm^3) containing dilute sulphuric acid (0.1 M) and left overnight to allow extraction of the alkaloids. A few drops of sodium hydroxide solution (1 M) were added to precipitate papaverine and also some narcotine. The cloudy solution was filtered (Whatman No. 52 paper) and diluted to 10 cm^3, giving stock solution P. Omnopon injection solution (1 cm^3) was similarly treated, giving stock solution O.

Stock morphine solution (1500 $\mu g\ cm^{-3}$) was prepared and suitable aliquots were diluted with pH 8.5 sodium pyrophosphate solution (0.1 M) to 25 cm^3, giving standard solutions with concentrations ranging from 3 to 30 $\mu g\ cm^{-3}$. Aliquots (500 μl) of the stock P and O solutions were treated likewise. The fluorescence of the standard and unknown solutions was measured in the usual manner at 0.25 V *versus* S.C.E. and the results are presented in Table VI. The unknown concentrations, 9.6 $\mu g\ cm^{-3}$ (solution P) and 19.8 $\mu g\ cm^{-3}$ (solution O) are also included in the table and were read from the calibration graph defined by the equation $y = 3.75x + 6.25$. The result of the assay is shown in Table VII and confirms that the procedure is accurate and interference free.

In a separate experiment, the relative standard deviations (ten determinations on the same solution) at the 6, 30 and 60 $\mu g\ cm^{-3}$ levels were 2.56, 1.20 and 0.74%, respectively.

Conclusions

The gold micromesh fluorescence cell has been demonstrated to be an attractive analytical tool, providing a novel approach for the semi-automatic determination of morphine at trace levels. Electrogeneration of pseudomorphine was reproducible and permitted the direct assay of morphine in pharmaceutical preparations based on papaveretum. The sensitivity

TABLE VI

ASSAY OF PAPAVERETUM TABLET BPC AND OMNOPON INJECTION AMPOULE: FLUORESCENCE RESULTS

Solutions in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential 0.25 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

Morphine concentration/ μg cm^{-3}	Maximum fluorescence intensity, arbitrary units
3.0	10.2,10.3,10.2
6.0	26.8,26.6,26.7
12.0	51.0,50.6,50.8
18.0	75.9,74.3,75.1
24.0	95.4,93.6,94.5
30.0	117.0,115.8,116.4
Unknown solution P—	
9.6	42.6,41.9,42.2
Unknown solution O—	
19.8	81.0,80.4,80.6

of the procedure was comparable to that of the chemical oxidation method of Burkhalter *et al.*[18] A sensitive fluorescence response was obtained for hydrolysed heroin solutions and the above conclusions would apply for heroin. Initial studies have been promising and future work should be directed at assessing the suitability of the cell for routine determinations of morphine (and heroin) in biological samples. The possibility of utilising the gold micromesh cell as a general-purpose fluorogenic unit should also be considered. Compounds examined successfully so far in addition to morphine, monoacetylmorphine and laudanosine include reserpine, thioguanine and homovanillic acid.

TABLE VII

MORPHINE ASSAY IN PAPAVERETUM TABLET BPC AND OMNOPON INJECTION AMPOULE

Sample	Morphine concentration: Specified	Found
Papaveretum Tablet BPC (10 mg)	47.5–52.5% *m/m*	52.0% *m/m*
Omnopon injection ampoule (20 mg cm^{-3}) ..	10 mg cm^{-3}	9.9 mg cm^{-3}

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