RESEARCH PAPERS

A SPECIFIC METHOD FOR THE DETERMINATION OF MORPHINE

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Introduction

Of the many colour reactions which morphine undergoes[1], only a few are applicable to its determination; the most frequently used and one included in the British Pharmacopœia 1953 is the reaction with nitrous acid. Even this reaction is not specific, however, and interference by other phenolic substances and by impurities accompanying morphine has frequently been reported[2]. The disadvantage of the lack of specificity has recently[3] been overcome in a novel manner by the successive reaction of morphine with a series of reagents: the first was iodic acid, which attacked morphine with liberation of iodine; secondly, ammonium carbonate was added which deepened the yellow-brown colour generated in the first stage; the product was then complexed with ferric chloride which produced a reddish violet colour (absorption maximum at 520 mμ) suitable for the absorptiometric and spectrophotometric determination of morphine[4]. This method has found use in toxicological investigations and has been applied to the determination of morphine in opium and opium preparations[5].

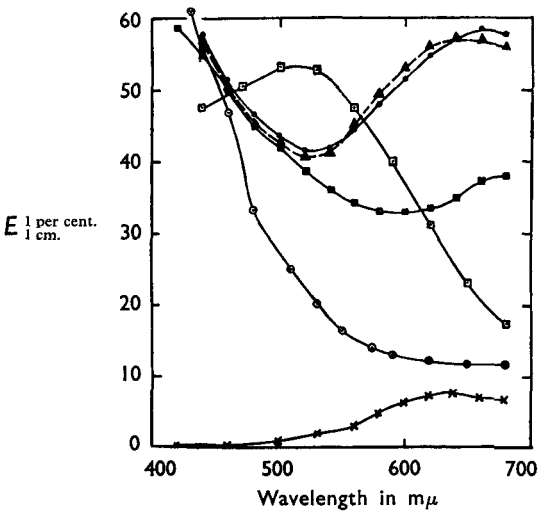


Fig. 1. Absorption curves of the iodic acid—ammonium bicarbonate—metal salt complexes of morphine.
—●—●— Nickel chloride.
—▲—▲— Cupric sulphate.
—▪—▪— Cobaltous chloride.
—⊡—⊡— Ferric chloride.
—⊙—⊙— Solution without metal ions.
—×—×— Morphine-ammonium bicarbonate-cupric sulphate solution (without iodic acid).

This series of reactions was highly specific, and of 2000 substances of pharmaceutical interest examined qualitatively none could be mistaken

for morphine[6], although some, notably apomorphine, physostigmine, and hyoscine gave interfering colours. The complex formation with ferric chloride suffered from some disadvantages, however: thus, the amount of ferric chloride added was critical because an excess gave rise to a gelatinous precipitate that interfered with colorimetry. Moreover, the absorption maximum near 520 mμ was subject to considerable interference by background absorption. These disdavantages were overcome[7] by substitution of nickel sulphate for ferric chloride, when a stable green complex (absorption maximum near 670 mμ) was slowly formed.

It was the purpose of the work reported here to adapt the method leading to the formation of the green complex for routine use, to examine the specificity of the reaction, and to apply it to the determination of morphine in solution, particularly in solutions of natural origin. In the course of this work, coloured complexes were obtained also with copper and cobalt salts (see Fig. 1), but these were less suitable than the nickel complex for routine spectrophotometry.

Development and Application of the Method

1. *Examination of Experimental Conditions*

For the development of the routine method, the experimental variables were investigated in order to find a convenient procedure that would yield the green complex with reproducible and maximum extinction at the absorption maximum. In this work, the effect on the absorption

Table I

Effect of varying the concentrations of iodic acid and of hydrochloric acid on the absorption intensity of the green complex

Reaction time at the first stage was 2 minutes and at second stage 90 minutes. The complexing reagent contained 8·0 per cent. w/v of ammonium bicarbonate, 1·0 N ammonium chloride, and 0·1 per cent. of nickel chloride in 0·20 N aqueous ammonia.

Concentration of Hydrochloric acid in medium — N	Concentration of Iodic acid per cent. w/v	Final *p*H	$E^{1\,\text{per cent.}}_{1\,\text{cm.}}$ at 530 mμ	$E^{1\,\text{per cent.}}_{1\,\text{cm.}}$ at 670 mμ
0·00	4·0	8·21	39·8	47·8
	6·0	8·14	41·6	53·0
0·02	1·0	—	22·6	29·3
	2·0	—	36·1	44·6
	4·0	8·13	42·8	55·9
	5·0	—	43·3	58·6
	6·0	—	42·8	59·6
0·05	1·0	—	31·8	41·2
	2·0	8·08	40·4	52·8
	4·0	8·04	42·8	58·5
	5·0	—	42·8	58·6
	6·0	8·01	42·1	59·0
0·10	1·0	—	38·8	51·0
	2·0	—	39·2	53·6
	4·0	7·95	38·5	53·2
	5·0	—	37·5	52·8
	6·0	—	36·8	51·9
0·25	1·0	—	27·2	34·4
	2·0	—	30·2	40·3
	5·0	—	22·2	25·8

intensity of varying, in turn, each of the experimental conditions was examined and the final selection of the standard routine procedure (detailed in the experimental section) was made on the basis of the results

TABLE II

EFFECT OF VARYING REACTION TIMES ON THE ABSORPTION INTENSITY OF THE GREEN COMPLEX

At the first stage 4·5 per cent. w/v of iodic acid was added to the morphine solution in 0·05 N hydrochloric acid. The standard complexing reagent was used (see Table I and experimental section).

Reaction time at first (iodic acid) stage minutes	Reaction time at second stage minutes	Final *p*H	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 530 mμ	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 670 mμ
	60	—	35·6	48·5
	90	—	36·2	50·0
0·5	120	8·08	36·6	51·3
	190	—	36·8	51·2
	300	8·09	36·4	50·1
	60	—	40·4	56·3
	90	—	42·7	59·1
2·0	120	8·08	43·1	59·4
	190	—	43·1	59·1
	300	8·08	42·0	58·0
	60	—	40·2	54·9
	90	—	41·7	57·6
5·0	120	8·06	42·1	58·5
	190	—	42·0	57·6
	300	8·11	41·5	55·9

TABLE III

EFFECT OF VARYING THE CONCENTRATION OF AMMONIUM BICARBONATE IN THE COMPLEXING REAGENT ON THE ABSORPTION INTENSITY OF THE GREEN COMPLEX

Reaction time at the first stage was 2 minutes and at the second stage 90 minutes. At the first stage 4·5 per cent. w/v of iodic acid was added to the morphine solution in 0·05 N hydrochloric acid. The complexing reagent contained, besides ammonium bicarbonate, 1 N ammonium chloride and 0·1 per cent. of nickel chloride in 0·20 N aqueous ammonia.

Concentration of ammonium bicarbonate per cent. w/v	Final *p*H	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 530 mμ	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 670 mμ
4·0	8·16*	39·2	50·9
8·0	8·06	42·4	58·6
12·0	8·04	41·6	57·6

* Final *p*H had to be increased from 7·7, which was well below the optimum, by addition of a few drops of concentrated ammonia solution, before the reaction mixture was adjusted to its final volume.

given in Tables I to VI. In particular, it was found that, in the reaction of iodic acid with morphine, the concentration both of iodic acid and of hydrochloric acid (Table I) and the time of reaction (Table II) were critical. For the subsequent stage, the most consistent results were obtained with a complexing reagent which comprised ammonium bicarbonate and ammonium chloride in aqueous ammonia solution, and in which the nickel salt was incorporated with advantage (*cf.* Tables III, IV and V). The *p*H of the final solution was found to be critical.

It also appeared that the final absorption intensity was higher when chloride (instead of sulphate) ions were present throughout (*cf.* Table VI). Colour development was complete after about 90 minutes (see Table II and Fig. 6).

TABLE IV

EFFECT OF VARIATION IN THE COMPOSITION OF THE COMPLEXING REAGENT ON THE ABSORPTION INTENSITY OF THE FINAL COMPLEX

Time of reaction was 2 minutes at the first and 90 minutes at the second stage. At the first stage, the concentration of iodic acid added was 4·5 per cent. w/v, and the strength of hydrochloric acid 0·05 N. The complexing reagent contained 8·0 per cent. w/v of ammonium bicarbonate and 0·1 per cent. nickel chloride, as well as ammonium chloride and ammonia.

Concentration of ammonia — N	Concentration of ammonium chloride — M	*p*H of reagent	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 670 mμ
0·10	—	8·10	46·9
0·15	—	8·25	47·4
0·20	—	8·35	46·6
0·25	—	8·45	45·7
0·30	—	8·50	44·5
0·20	0·25	8·40	55·0
0·10	1·00	7·90	56·0
0·15	1·00	8·00	58·7
0·20	1·00	8·10	58·7
0·25	1·00	8·15	58·7
0·30	1·00	8·30	57·0
0·30	1·60	8·25	59·8

The complexing reagent and reaction conditions finally selected gave reproducible results of high sensitivity (*cf.* Fig. 5). Moreover, accidental slight variations in the experimental procedure always led to lower results than were obtained by the normal procedure. Fresh solutions of the reagent had to be prepared each day because of its instability. The solid ammonium bicarbonate and the stock solution of ammonia from which the reagent was prepared also tended to be unstable. Another method of preparation of the complexing reagent from more stable materials was therefore examined. The reagent still contained the same concentrations of ammonium and carbonate ions as the original reagent but was prepared from sodium bicarbonate, ammonium chloride and nickel chloride in aqueous sodium hydroxide solution. By the standard reaction procedure, however, it gave a complex of rather lower absorption intensity (see

TABLE V

EFFECT OF VARYING THE CONCENTRATION OF NICKEL CHLORIDE IN THE COMPLEXING REAGENT ON THE ABSORPTION INTENSITY OF THE GREEN COMPLEX

The standard reagents and reaction conditions were used.

Concentration of nickel chloride per cent. w/v	Final *p*H	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 530 mμ	$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 670 mμ
0·05	8·04	44·4	59·0
0·10	8·00	43·1	59·3
0·20	8·00	42·9	59·1

Table VII). The use of the original reagent for the routine method was therefore continued.

2. *Specificity of the Method*

The specificity of the iodic acid—ammonium carbonate—nickel ion reaction has not hitherto received much attention, although interference by thebaine has been reported[8]; this could not, however, be substantiated in the present investigation, in the course of which two series of substances were submitted to the routine procedure developed. The first series comprised substances such as narceine, narcotine, papaverine, cryptopine,

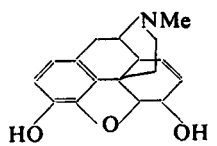
TABLE VI

EFFECT ON ABSORPTION INTENSITY OF SUBSTITUTION OF SULPHATE FOR CHLORIDE IONS IN THE REACTION MEDIUM

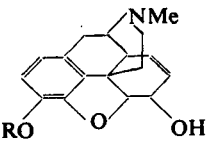
The standard reaction conditions were used.

Acid in initial solution 0·05 N	Ammonium salt 1·0 M	Nickel salt 0·1 per cent. w/v	Final *p*H	$E^{1\%}_{1\,cm.}$ at 530 mμ	$E^{1\%}_{1\,cm.}$ at 670 mμ
sulphuric	chloride	chloride	7·92	40·4	51·4
		sulphate	7·92	40·4	51·4
	sulphate	chloride	8·00	44·5	54·1
		sulphate	8·00	44·5	54·1
hydrochloric	chloride	chloride	8·00	42·4	59·0
		sulphate	8·00	42·4	59·0
	sulphate	chloride	8·00	46·5	59·3
		sulphate	8·00	46·5	59·3

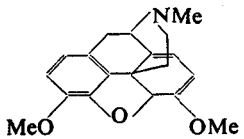
and meconic acid, occurring with morphine in vegetable material; none of these gave a coloured complex. The second series of substances comprised bases closely related to morphine. The natural products codeine, neopine, and thebaine gave no colour when treated by the standard procedure. The other phenolic ethers of morphine examined, namely dihydrocodeine, dihydrocodeinone, ethylmorphine, and morpholinoethylmorphine were also inert, as was diacetylmorphine.



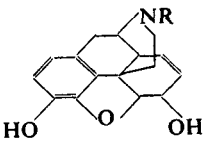
(I) Morphine



(II) Phenolic ethers: R = Me; Codeine
R = Et; Ethylmorphine



(III) Thebaine



(IV) R = H; Normorphine
R = $CH_2.CH:CH_2$; *N*-Allylnormorphine

In contrast, all morphine alkaloids and derived bases with the free phenolic hydroxyl group which were examined reacted with iodic acid and all except pseudomorphine (see below) gave a greenish colour after complex-formation with the ammonium bicarbonate—nickel chloride reagent.

TABLE VII

COMPARISON OF "STANDARD" REAGENT WITH A REAGENT PREPARED FROM MORE STABLE STOCK SOLUTIONS

Reaction conditions were otherwise identical.

Reagent	Composition of reagent			$E^{1\text{ per cent.}}_{1\text{ cm.}}$ at 670 mμ		
"Standard"	ammonium bicarbonate 8 per cent. w/v	ammonium chloride 1 M	ammonium hydroxide 0·20 M	58·2	58·4	58·7
"Stable"	sodium bicarbonate 8·4 per cent. w/v	ammonium chloride 2·2 M	sodium hydroxide 0·20 M	57·0	57·1	

The actual colour, i.e., the shape of the absorption curve and the position and intensity of the absorption maximum in the 600 to 700 mμ region, depended on the way in which the morphine molecule had been modified (see Figs. 2 and 3). The substituent at the nitrogen atom seemed to have little effect on complex formation and normorphine and *N*-allylnormorphine gave complexes with maxima at 670 mμ; in contrast, modification of the αβ-unsaturated alcohol system of morphine, by hydrogenation or etherification or both, caused a shift of the maximum to shorter wavelengths or even the complete disappearance of a well-defined maximum. Absence of this system and of the oxygen bridge as in *N*-methylmorphinan and in 3-hydroxy-*N*-methylmorphinan, resulted in inertness in the colour reaction.

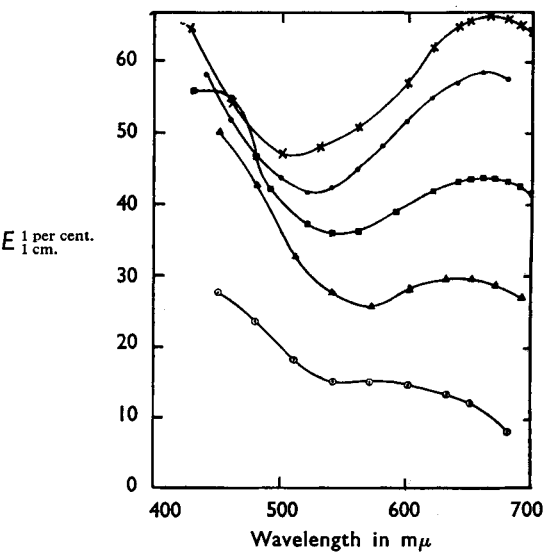


FIG. 2. Absorption curves of the iodic acid—ammonium bicarbonate—nickel chloride complexes of morphine derivatives.

—×—×— *N*-Allylnormorphine.
—●—●— Morphine.
—▪—▪— Normorphine.
—▲—▲— Dihydromorphine.
—⊙—⊙— Dihydromorphinone.

Apomorphine, the heterocyclic system of which differs from that of the morphine alkaloids, gave an orange colour with iodic acid which

on addition of ammonium bicarbonate immediately changed to blue-green: a green precipitate was gradually deposited. This sequence was unaffected by the presence of nickel.

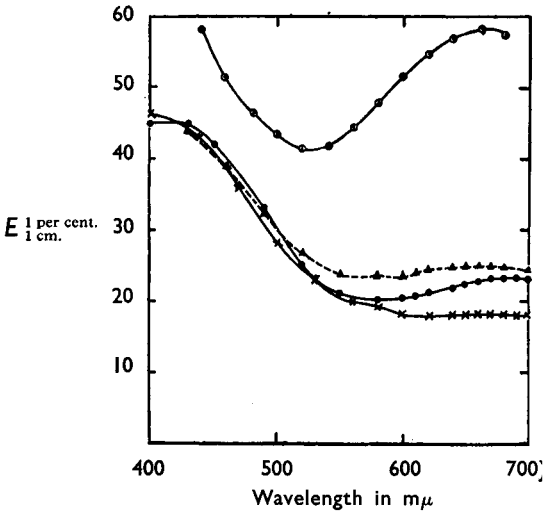


FIG. 3. Absorption curve of the iodic acid—ammonium bicarbonate—nickel chloride complexes of alcoholic ethers of morphine.
—⊙—⊙— Morphine.
—▲—▲— Heterocodeine.
—●—●— Heteroethylmorphine.
—×—×— Dihydroheterocodeine.

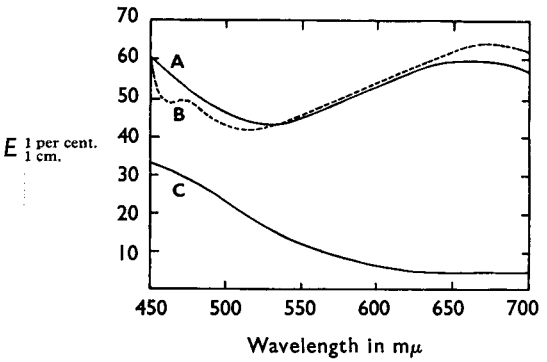


FIG. 4. Absorption curve of the iodic acid—ammonium bicarbonate—nickel chloride reaction product.
A. Morphine complex (on Unicam S.P. 350 spectrophotometer).
B. Morphine complex (on Unicam S.P. 500 spectrophotometer and Hilger Medium Quartz spectrograph).
C. Pseudomorphine product (on Unicam S.P. 350 spectrophotometer).

Pseudomorphine. As discussed above, the naturally occurring contaminants of morphine were inert under the standard conditions for the determination of morphine. Serious interference was encountered only with bases having the full morphine skeleton and a free phenolic hydroxyl group: of these, only the bimolecular pseudomorphine occurs with morphine in natural material. Pseudomorphine, however, does not give a green complex, but the brown colour of the iodic acid reaction product, which is deepended by addition of ammonium bicarbonate and unaffected by nickel (Fig. 4); this colour may be regarded as background absorption. Although the intensity of the absorption in the 500 to 700 mμ region is not high, it was thought of interest to examine the effect of interference by "pseudomorphine - like absorption" on the results of determinations of morphine, and a method of correcting for such background absorption was therefore devised.

It was assumed that the Beer-Lambert laws were obeyed. The wavelengths for extinction measurements were chosen at the maximum and minimum (670 and 530 mμ, respectively) of the absorption curve of the complex, where variation of extinction with wavelength was least. The extinction due to morphine in the mixture was denoted by K and that due to pseudomorphine by k; the observed total extinction at any wavelength, λ,

$$E_2 = K + k$$

The ratios of the extinctions at the two wavelengths chosen were denoted by r. Thus the observed ratio $r_o = \frac{E_{670}}{E_{530}}$,

$$\text{for pure morphine } r_m = \frac{K_{670}}{K_{530}}$$

$$\text{for pseudomorphine } r_p = \frac{k_{670}}{k_{530}}$$

$$\text{Then } E_{670} = K_{670} + r_p.k_{530}$$

$$\text{and } E_{530} = \frac{K_{670}}{r_m} + k_{530}$$

$$\text{By substitution } E_{670} = K_{670} + r_p E_{530} - \frac{r_p K_{670}}{r_m}$$

$$\text{Therefore } K_{670} = \frac{r_m\,(E_{670} - r_p E_{530})}{r_m - r_p}$$

$$\text{or} \quad \frac{K_{670}}{E_{670}} = \frac{r_m\,(1 - r_p E_{530}/E_{670})}{r_m - r_p} = \frac{r_m\,(1 - r_p/r_o)}{r_m - r_p}$$

Thus the corrected extinction value for morphine (K_{670}) was determined from the observed extinction (E_{670}) and from the ratio (r_o) of the observed extinction at the maximum and minimum of the absorption curve, the ratios r_m and r_p for pure morphine and pseudomorphine, respectively, having been previously determined from absorption curves.

Values for r_o and for K_{670}/E_{670} calculated are collected in Table VIII, and the effect of applying the correction to three synthetic mixtures of morphine and pseudomorphine is shown in Table IX.

From the results tabulated, it was deduced that interference by pseudomorphine or other substances producing similar background absorption was appreciable only when the amount of impurity exceeded the amount of morphine present. The method of correction adopted then produced results that tended to be somewhat low. The inaccuracy in this method of correction was probably due to the non-applicability of Beer's law to the pseudomorphine absorption and consequent variation in the ratio r_p.

3. *The Determination of Morphine in Opium and Poppy Capsule*

Morphine in opium is generally determined gravimetrically or volumetrically and the extracts obtained therefore have to be submitted

TABLE VIII

CORRECTION FOR BACKGROUND ABSORPTION DUE TO PSEUDOMORPHINE (AND SIMILAR SUBSTANCES)

Ratio r_0 represents the ratio of the extinction at 670 mμ to that at 530 mμ. Ratio K_{670}/E_{670} represents the ratio of the corrected extinction (due to morphine) to the observed extinction at 670 mμ: this is the correction factor.

r_0 (E_{670}/E_{530})	K_{670}/E_{670}	Fraction of total absorption due to morphine per cent.	Approximate content of pseudomorphine per cent.	Approximate error introduced by correction factor per cent.
1·400	1·000 ...	 100	Nil	
1·350	0·994			
1·300	0·987			
1·250	0·979			
1·200	0·971			
1·150	0·962			
1·100	0·952			
1·050	0·942 ...	 94	50	.±0·5
1·000	0·930			
0·950	0·917			
0·900	0·902			
0·850	0·884			
0·800	0·868 ...	 87	73	.±0·8
0·750	0·847			
0·600	0·765			
0·400	0·565			
0·365	0·501 ...	 50	94	.±5·5
0·300	0·353			

TABLE IX

DETERMINATION OF MORPHINE IN ADMIXTURE WITH PSEUDOMORPHINE

Composition of mixture		Percentage content of morphine	r_0 (E_{670})/(E_{530})	Morphine found mg./10 ml.	
Morphine mg./10 ml.	Pseudomorphine mg./10 ml.			Without correction	With correction
1·92	Nil	100	1·40	1·92	1·92
1·92	0·96	66·7	1·17	1·93	1·89
1·92	1·92	50	1·00	1·98	1·87
1·92	3·84	33·3	0·83	2·03	1·83
Nil	3·84	0	0·21	—	—

to extensive and time-consuming purification. The specificity of the iodic acid—ammonium bicarbonate—nickel chloride reaction, however, permitted the determination of morphine in the crude extract obtained by slurrying opium with calcium hydroxide and water; adjustment of the *p*H and dilution were the only additional steps before the colour reaction. Some results obtained on 4 random samples of opium are collected in Table X, which also shows the effect of applying the correction for "pseudomorphine-like

TABLE X

DETERMINATION OF MORPHINE IN OPIUM

Sample	Morphine content		
	Present method		Modified B.P. method* per cent.
	Uncorrected per cent.	Corrected per cent.	
1	11·15	10·75	10·79
2	11·3	11·05	10·70
3	10·95	10·70	10·64
4	11·3	11·05	10·87

* Results obtained by Mr. D. R. Wood

colour." The results obtained by the present method, especially when thus corrected, were in satisfactory agreement with the results obtained by a modification of the B.P. method of assay.

Whilst opium contains about 10 per cent. of morphine, the morphine content of poppy capsules is generally between 0·1 per cent. and 0·7 per cent. and the extraction and purification procedures are correspondingly more difficult[9]. Again, a great saving in time and labour was possible by application of the iodic acid-ammonium bicarbonate—nickel chloride reaction, since morphine could be determined in comparatively crude solution. The results obtained were reproducible (see Table XI) and the amount of solid morphine isolated in the laboratory from a bulk sample of poppy capsule agreed satisfactorily with the morphine content indicated by analysis.

TABLE XI

DETERMINATION OF MORPHINE IN POPPY CAPSULE

REPRODUCIBILITY OF RESULTS

Sample	Morphine content per cent.		
	Unicam S.P. 350 Spectrophotometer Operator A	E.E.L. Absorptiometer*	
		Operator B	Operator C
1	0·24	0·28	0·30
2	0·095	0·095	0·090
3	0·235	0·225	0·235
4	0·23	0·21	0·215
5	0·055	0·055	0·050
6	0·125	0·115	0·120
7	0·10	0·10	0·10

* Analyses by the control department of Messrs. T. & H. Smith, Ltd.

EXPERIMENTAL

Apparatus. Except where otherwise indicated, a Unicam S.P. 350 diffraction grating spectrophotometer was used with 1-cm. absorption cells. The band-width was ca. 30 mμ.

1. *Method*

Reagents. All inorganic reagents were of analytical reagent grade or B.P. quality. The organic bases used complied with B.P. specifications or were purified by the usual methods.

Ammonium bicarbonate (B.P. quality) was stored in stoppered bottles in a desiccator over silica gel.

Morphine was purified by two crystallisations as the hydrogen tartrate and then converted into the hydrochloride.

Boiled-out distilled water was used throughout.

Stock solutions. *A.* 4·5 per cent. w/v iodic acid.
B. 21·4 per cent. w/v ammonium chloride (i.e., 4 M).
C. 1·0 N aqueous ammonia solution.
D. 1·0 per cent. w/v nickel chloride.

Ammonium bicarbonate—nickel chloride reagent. Ammonium bicarbonate (8·0 g.) was shaken with a mixture of stock solutions *B* (25 ml.), *C* (20 ml.), and *D* (10 ml.). On dilution to 100 ml. a clear solution was obtained which was used only on the day of preparation.

Calibration curve. The calibration curve (Fig. 5) was constructed by use of solutions containing known amounts of pure morphine (4·0 to 40·0 mg. of anhydrous base per 100 ml.) in 0·05 N hydrochloric acid. 10-ml. aliquots of solution in a 25-ml. graduated flask were treated with aqueous iodic acid (2 ml. of stock solution *A*) for exactly 2 minutes; then the ammonium bicarbonate—nickel chloride solution (10 ml.) was added and the mixture diluted to 25 ml. Extinction readings were taken at the absorption maximum (670 mμ) after 90 minutes, when colour development was complete (see Fig. 6).

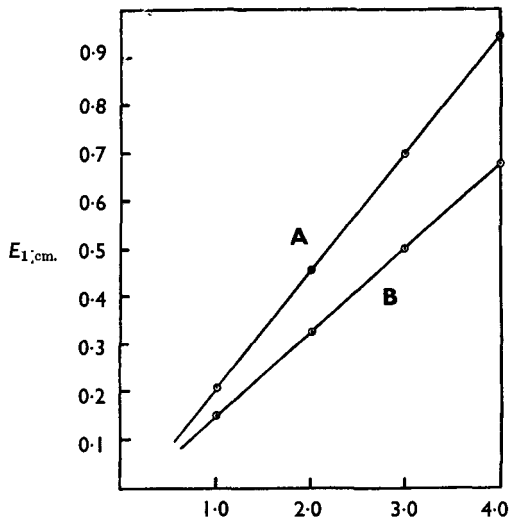


FIG. 5. Calibration curve for morphine (on the Unicam S.P. 350 spectrophotometer).
A. Extinction at 670 mμ (absorption maximum).
B. Extinction at 530 mμ (absorption minimum).

The "blank" solution consisted of a 10-ml. aliquot of the same morphine solution, to which was added, instead of iodic acid, 0·1 N hydrochloric acid (5 ml.) and then the complexing reagent (10 ml.).

To permit application of the correction for "pseudo - morphine - like background colour," similar calibration curves were constructed from extinction readings at the minimum (530 mμ) of the absorption curve, and for pseudomorphine from readings at 530 mμ and at 670 mμ.

For the work here reported the calibration curve for morphine at 670 mμ (Fig. 5) was fitted by the method of least squares to a series of results (collected in Table XII) obtained for 3 different samples of morphine at 5 dilutions. Room temperature during colour development was 20° ± 1·5° C.

Analysis of the results

x = Morphine content (mg. per 10 ml.) of aliquot

y = Extinction ($E_{1\,cm.}$).

Standard error of predicted x, S.E. (x), for y = 0·70 (i.e., in the optimum extinction range) = ± 0·021 (i.e., ± 0·7 per cent.) when x = 3·00. Therefore, 99 per cent. confidence limits for x (13 degrees of freedom, t = 3·01) = 3·00 ± 0·063 (i.e., ± 2·1 per cent.).

The results indicate that Beer's law applies, within the usual limits, over the range of concentrations examined.

Determination of morphine. The solution of unknown morphine content was first adjusted to *p*H 4 to 5 and then diluted until the morphine concentration was such that extinction readings were within the optimum range on the spectrophotometer (E = 0·3 to 0·7 for the Unicam SP 350); in the course of this dilution the hydrochloric acid concentration was adjusted to 0·05 N. One 10-ml. aliquot was then treated for 2 minutes exactly with iodic acid (2 ml. of stock solution *A*); the ammonium bicarbonate-nickel chloride reagent (10 ml.) was added, the solution diluted to 25 ml., and the extinction at 530 mμ and 670 mμ determined after 90 minutes. The "blank" was prepared from another 10-ml aliquot. The morphine content was then determined with the aid of the calibration curve at 670 mμ (Fig. 5), and corrected, if so desired, by application of the correction factors (see Table VIII)

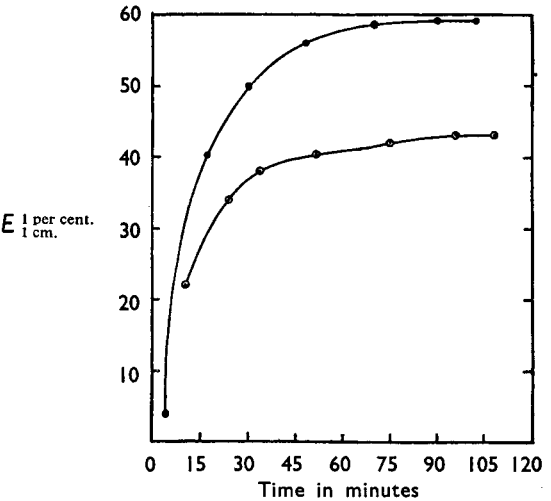


FIG. 6. Variation of extinction of the morphine iodic acid—ammonium bicarbonate—nickel chloride complex with time.
—●—●— Extinction at 670 mμ.
—⊙—⊙— Extinction at 530 mμ.

TABLE XII

DATA FOR CALIBRATION CURVE VARIATION OF EXTINCTION AT 670mμ WITH MORPHINE CONCENTRATION

Sample	Morphine in aliquot mg./10 ml.	Extinction (E_1 cm.)
(i)	0·420	0·07
(ii)	0·425	0·07
(iii)	0·440	0·08
(i)	0·835	0·17
(ii)	0·850	0·17
(iii)	0·880	0·18
(i)	1·670	0·37
(ii)	1·700	0·38
(iii)	1·755	0·40
(i)	2·500	0·58
(ii)	2·550	0·60
(iii)	2·635	0·60
(i)	3·340	0·78
(ii)	3·400	0·80
(iii)	3·510	0·83

Precautions. The following precautions were taken to ensure valid and reproducible results in the routine application of the method:

(i) The *p*H of the solution (containing the correct amount of hydrochloric acid) was measured before analysis. The *p*H should be below 1·6. In the presence of buffering impurities, however, the *p*H may be above 1·6 and the iodic acid reaction will then not be complete within 2 minutes. When necessary, therefore, the morphine was extracted into an organic solvent, such as chloroform-ethanol, re-extracted into acid, and thus freed from buffering impurities.

(ii) The *p*H of the green solution after analysis was measured. This *p*H should be in the optimum range of *p*H 8·00 ± 0·05.

(iii) A standard morphine sample was included in each batch of samples analysed to check that the instrument response and the reagent composition (final *p*H value) were satisfactory.

2. *Determination of morphine in opium*

Water (25 ml.) was added to opium (5 g.) in a deep evaporating basin. The mixture was warmed and agitated until pasty. Calcium hydroxide (2 g.) was added to the cool paste and the mixture stirred until apparently homogeneous. After suction filtration, the cake was pressed dry, re-slurried with water (25 ml.), and again filtered. Washing by re-slurrying was repeated 4 times more. The combined filtrates were adjusted to *p*H 4 to 5 with hydrochloric acid and diluted to 500 ml. A 20-ml. aliquot of the resulting solution was treated with 1 N hydrochloric acid (5 ml.) and diluted to 100 ml. This solution was analysed as follows:—

Two 10-ml. aliquots were pipetted into 25-ml. graduated flasks. To one (the "blank") was added 0·1 N hydrochloric acid (5 ml.) and to the other iodic acid (2 ml. of stock solution *A*). After 2 minutes the ammonium bicarbonate–nickel chloride reagent (10 ml.) was added to each solution and the volume adjusted to 25 ml. The absorption intensity was measured after 90 minutes both at the absorption maximum at 670 mμ and at the minimum at 530 mμ, and the morphine content determined by reference to the calibration curves. The correction for "pseudo-morphine-like" background absorption was applied in the usual manner. Results are recorded in Table X.

3. *Determination of morphine in poppy capsule*

Ground poppy capsules (5 g.) were triturated with 10 per cent. w/v aqueous sodium carbonate solution (5 ml.) and kept for 1 hour. The stirred mixture was then treated with 20 per cent. w/v aqueous sodium carbonate solution (5 ml.) and shaken for 1 hour with a solvent mixture (45 ml.) of equal volumes of benzene and *n*-butanol. The resulting mass was suction-filtered and washed with 4 quantities, each of 5 ml. of the solvent mixture. The combined filtrate and washings were extracted with 0·5 N sulphuric acid (20 ml.) and then with water (2 quantities, each of 10 ml.). The aqueous layers were filtered through cotton wool, mixed, adjusted to *p*H 4 to 5, treated with 0·5 N hydrochloric acid (5 ml.) and diluted to 50 ml. A 10-ml. aliquot of this diluted solution was analysed by the routine procedure (see above). Typical results obtained by this procedure are given in Table XI.

Isolation of morphine from bulk sample of poppy capsule

A sample (500 g.) of ground poppy capsules (containing 0·25 per cent. of morphine by the above method of analysis) was mixed with 10 per cent. w/v aqueous sodium carbonate (500 ml.) and kept for 1 hour with occasional stirring. 20 per cent. w/v aqueous sodium

carbonate solution (500 ml.) was then added, the mass was strongly agitated, and finally Soxhlet-extracted with a mixture of equal volumes of benzene and *n*-butanol for 16 hours. The solvent was removed and the solid further extracted for 8 hours. The organic extracts were separately exhausted with 0·2 N hydrochloric acid and samples of the acidic solutions were analysed: the first extract contained 1·25 g. of morphine by assay (i.e., all the morphine originally present in the capsules), the second extract none. The acidic solution (2300 ml.) containing the morphine was concentrated to small bulk (340 ml.) under water-pump vacuum; the concentrate was made alkaline with concentrated aqueous ammonia solution and extracted with a mixture of chloroform (340 ml.) and ethanol (340 ml.). Eleven further extractions were done with chloroform (340 ml.) and ethanol (160 ml.). The mixed organic extracts were evaporated to dryness under water-pump vacuum, the residue was dissolved in aqueous acid (20 ml.) and the solution adjusted to *p*H 9 when morphine was precipitated. The dry solid (2·535 g.) was analysed both by an extraction method concluding in a volumetric determination and by the iodic acid—ammonium bicarbonate—nickel chloride method: both analyses showed the morphine content of the solid to be 47 per cent. (i.e., the presence of 1·195 g. of morphine; 95·6 per cent. of the morphine content originally found for the capsules). The filtrate was analysed by the latter method which showed a morphine content of 32 mg. (2·6 per cent. of the content determined for the capsules).

4. *Reaction of other substances: specificity of the method*

The following substances were submitted to the iodic acid–ammonium bicarbonate–nickel chloride reaction under the standard conditions, and were inert:—narceine, narcotine, papaverine, cryptopine, codeine, neopine, thebaine, dihydrocodeine, dihydrocodeinone, ethylmorphine, morpholinoethylmorphine, diacetylmorphine, *N*-methylmorphinan, 3-hydroxy-*N*-methylmorphinan, and meconic acid. The following substances gave nickel complexes (absorption curves of which are recorded in Figs. 2 and 3) under the standard conditions: normorphine, *N*-allylnormorphine, dihydromorphine, dihydromorphinone, heterocodeine, heteroethylmorphine, and dihydroheterocodeine. Pseudomorphine and apomorphine reacted with iodic acid-ammonium bicarbonate, but the products were unaffected by nickel. Pseudomorphine with iodic acid, gave a brown colour that deepened on addition of ammonium bicarbonate (see Fig. 4 for absorption curve); apomorphine with iodic acid gave an orange colour that changed to blue-green on addition of ammonium bicarbonate: a greenish precipitate was gradually deposited.

5. *Other metal complexes of the morphine-iodic acid reaction product*

In these experiments stock solution *D* (1·0 per cent. w/v nickel chloride) was replaced by (i) 0·5 per cent. w/v cupric sulphate; (ii) 1·0 per cent. w/v cobaltous chloride. The standard procedure was used for the formation of the metal complexes. The iron complex was obtained as

follows: a solution (10 ml.) of morphine in 0·05 N hydrochloric acid was treated first with iodic acid (2 ml. of stock solution *A*) for 2 minutes and then with the ammonium bicarbonate reagent (in absence of stock solution *D*). Then 6 drops of 1·0 per cent. w/v ferric chloride solution were added and the solution was diluted to 25 ml. The "blank" was prepared analogously, the iodic acid solution being replaced by 0·1 N hydrochloric acid (5 ml.). The absorption curves of the complexes are reproduced in Figure 1.

6. *Miscellaneous experiments exploring the reaction mechanism.*

(i) The iodine liberated in the reaction of iodic acid with morphine was rapidly extracted into carbon tetrachloride before addition of the ammonium bicarbonate-nickel chloride reagent. The green complex was formed as usual with the usual absorption intensity.

(ii) Sodium salts were substituted for ammonium salts in all the reagents. No green complex was formed, even when the *p*H of the final solution was adjusted to 8·00.

(iii) Ammonium hydrogen phosphate was substituted for ammonium bicarbonate in the reagent solution and the final *p*H was adjusted to 8·00. A green complex was formed, the absorption intensity of which at the absorption maximum (670 mμ) was much lower than usual ($E_{1\,\text{cm.}}^{1\,\text{per cent.}}$ was 42·0, as compared with 58·7).

Discussion and Conclusions

The iodic acid-ammonium bicarbonate-nickel chloride method for the determination of morphine has proved satisfactory in routine operation when the critical factors, namely the time of reaction (particularly at the first stage), the hydrochloric acid concentration and *p*H of the solutions analysed, and the composition of the reagent were closely controlled. The great advantages of the method were its specificity and the low absorption intensity of any background colour in the region of maximum absorption of the stable green complex which compensated amply for the additional time required for the colour development of the nickel complex (as compared with that of the iron complex). The sensitivity of the method permitted determination of about 0·75 mg. of morphine in 10 ml. of solution; for about 3 mg. of morphine in 10 ml. of solution the accuracy was within $\pm$ 2 per cent.

The method has been applied successfully to solutions containing relatively pure morphine and also to crude extracts of vegetable origin. It can obviously be applied to other problems, such as the limit test for morphine in pharmaceutical materials (e.g., in codeine or morpholinoethylmorphine) or the decomposition of morphine in solution.

The mechanism of the reaction has not yet been elucidated. It has been suggested[3] that an *o*-diphenol may be formed by the action of iodic acid with morphine which forms a complex with ferric chloride under alkaline conditions. For the nickel complex, the *p*H range within which the absorption intensity is at its highest has been found to be very narrow: the intensity falls off rapidly at higher *p*H values, whilst the

complex dissociates completely in acidic solution. The reagent solution must therefore be a good buffer. Moreover, the experimental results obtained in the present work clearly indicate that the presence of ammonium ions is essential and that of carbonate ions is advantageous for complex formation. In this connection it is important that nickel, copper and cobalt which give complexes with absorption maxima in the same spectral region (650 to 670 mμ), also form ammines, and it is probable that the nature of the complexes derived from these metals differs from that of the iron complex for the formation of which ammonium ions are not essential.

The structural features of the morphine molecule affecting complex formation are the phenolic hydroxyl group and the $\alpha\beta$-unsaturated alcohol system (in ring *C*, Formula I). Changes at the nitrogen atom do not affect complex formation greatly. Little evidence on the effect of the oxygen bridge is available; it is of interest, however, that 3-hydroxy-*N*-methylmorphinan (in which this bridge and the unsaturated alcohol system are both absent) does not form the usual complex.

When the free phenolic hydroxyl group is blocked, either by etherification or by esterification, the molecule no longer reacts with iodic acid and no coloured complex is formed in the later stages. Etherification of the alcoholic hydroxyl group does not, of course, inhibit the iodic acid reaction and the fact that modification of the $\alpha\beta$-unsaturated alcohol system of morphine greatly affects the absorption curve of the final complex may probably be best explained in terms of the steric factors involved in complex formation. In morphine (*cis*-fused at C_{13} to C_{14}) the alicyclic ring (ring *C* in Formula I) is at a sharp angle to the rest of the molecule and the alcoholic hydroxyl group is close to the other oxygen atoms in space[10]. In dihydromorphine, where two extra hydrogen atoms are accommodated and the ethylenic linkage is saturated, the alicyclic ring (*C*) is, in consequence, somewhat distorted and complex formation appears to be affected by this change. Interference in complex formation by etherification of the alcoholic hydroxyl group can be explained in terms of the greater volume of the methoxy and ethoxy groups as compared with that of the hydroxy group; the bulkier groups may prevent a sufficiently close approach of the complexing centres to form the normal stable complex. This interpretation is supported by the fact that interference in heteroethylmorphine (ethoxy group) is greater than in heterocodeine (methoxy group) (see also Fig. 3).

Summary

1. The iodic acid-ammonium-carbonate-nickel salt reaction has been adapted for the routine spectrophotometric determination of morphine; the method is sensitive down to about 0·005 per cent. of morphine in solution and 0·03 per cent. of morphine can be determined with an accuracy within $\pm$ 2 per cent.

2. Of the bases occurring with morphine in nature only pseudomorphine interfered weakly and the method has been used for the rapid determination of morphine in opium and poppy capsule.

3. The factors affecting the formation of the green complex are briefly discussed.

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