THE USE OF TETRAPHENYLBORON FOR THE DETERMINATION AND CHARACTERISATION OF ORGANIC BASES IN PHARMACEUTICAL PREPARATIONS

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A method has been developed for the assay of basic nitrogen compounds by precipitation at pH 3·7 with sodium tetraphenylboron; excess reagent is then determined by back titration with a quaternary ammonium salt. Melting-points of the organic tetraphenylboron salts may be used for the identification of the bases concerned. The method has been applied to the determination of 15 compounds in a variety of pharmaceutical preparations. It compares well in accuracy and speed with existing methods.

Sodium tetraphenylboron, well known as a reagent for potassium, has also been used for the identification and determination of organic bases. Schultz and Mayer (1952) suggested a gravimetric method whilst volumetric procedures involving argentometric determination of organic tetraphenylboron salts have been described by Keller and Weiss (1957) and by Rüdorff and Zannier (1952 and 1954). In our hands these methods proved unsuitable for application to pharmaceutical preparations containing small amounts of bases. The alkalimetric micromethod of Flaschka, Holasek and Amin (1954) is suitable, but involves destruction of the organic tetraphenylborate, which would otherwise be useful for characterisation purposes. Schall (1957) described an indirect volumetric method for the determination of potassium in which precipitation with sodium tetraphenylboron is carried out at pH 12, excess of the reagent then being titrated with a quaternary ammonium salt at the same pH. This principle has been developed to give a method suitable for the determination and characterisation of organic bases in pharmaceutical preparations.

Experimental

Cetylpyridinium chloride (CPC) was chosen as the quaternary ammonium titrant and preliminary work was carried out with 0·01M solutions. Difficulties in preparing these accurately, due to excessive frothing, were overcome by dissolving the CPC in a little ethanol before diluting to volume with water. 0·01M tetraphenylboron (TPB) was prepared using the technique described by Cluley (1955), adjusting the pH to 8·0–9·0 for maximum stability (Cooper, 1957). Of the various indicators in the aminoazo, sulphophthalein and fluorescein groups, bromophenol blue gave the best end-point in acid solution and was sufficiently sharp to permit the use of 0·005M CPC. The optimum pH for this titration is 3·7, the observed "middle tint" of the indicator, although variations between 4·1 and 2·6 can be tolerated. A volume of 0·5 ml. of indicator is necessary for a clear colour change; it must be accurately measured since it introduces a blank of about 0·1 ml. of titrant.

In general, precipitation of organic tetraphenylborates is carried out between pH 2 and 6 and between 20° and 70°. pH 3·7 and 20° were chosen since these conditions applied to the subsequent titrations. Under these conditions semi-colloidal precipitates which are difficult to filter are obtained. Schultz and Goerner (1953) overcame this difficulty by adding aluminium chloride, but this could not be used here because the quaternary ammonium tetraphenylborate formed during the titration tended to coagulate, to absorb the indicator, and to cause a marked deterioration in the end-point. Since hydrophobic sols are less susceptible to coagulation by monovalent than by trivalent ions, sodium chloride was used instead. At a concentration of 1 per cent w/v of the total precipitation volume this allowed ready filtration of the organic tetraphenylborates without affecting the subsequent titration. Under the conditions described below, complete precipitation of all the compounds

TABLE I

APPLICATION TO OFFICIAL SUBSTANCES

Compound	Per cent w/w compound found by		Approximate[1] melting-points of tetraphenyl-boron salts °C
	Proposed method	Alternative method	
Atropine	100·3; 100·3	100·2[1]	160
Atropine sulphate	98·3; 98·2	98·3[1]	160
Homatropine hydrobromide	100·7; 100·8	99·9[1]	160
Atropine methonitrate	100·2; 100·0	99·9[1]; 100·1[2]	*—
Hyoscine hydrobromide	99·2; 99·1	—	104
Lachesine hydrochloride	99·8; 99·6	99·1[1]	164
Physostigmine salicylate	98·2; 98·5	—	109
Pilocarpine nitrate	99·2; 99·2	—	85
Cocaine hydrochloride	99·7; 99·8	99·7[2]	99
Lobeline hydrochloride	99·4; 101·0	—	93
Morphine sulphate	98·8; 98·8	98·6[1]	*—
Codeine phosphate	99·7; 100·0	99·3[1]	*—
Methadone hydrochloride	100·6; 100·8	100·2[1]	83
Pethidine hydrochloride	99·7; 99·7	100·3[1]	155
Procaine hydrochloride	100·9; 101·6	100·5[1]	145

* Decomposed with charring at about 170°–180°.

[1] Official method of the British Pharmacopoeia.
[2] Non-aqueous titration.

listed in Table I takes place within 5 min., with the formation of 1 : 1 complexes. It has been found convenient to add the sodium chloride with the standard solution of TPB. The amount of TPB in excess can be varied between 46 and 120 per cent without affecting the accuracy. The procedure is as follows.

METHOD

Reagents

Bromophenol blue solution of the British Pharmacopoeia, Appendix 2B.

Concentrated buffer solution pH 3·7. Dissolve anhydrous sodium acetate (analytical reagent grade) (10 g.) in distilled water (approximately 300 ml.); add bromophenol blue solution (1 ml.) and sufficient glacial acetic acid (35 to 40 ml.) until the indicator changes from blue to a pure green. Dilute to 500 ml. with distilled water.

Dilute buffer solution pH 3·7. Dilute concentrated buffer solution with an equal volume of distilled water.

0·005M CPC. Dissolve cetylpyridinium chloride (1·80 g.) in 95 per cent ethanol (10 ml.) and dilute to 1 litre with distilled water. Store in an amber bottle.

TABLE II

APPLICATION TO AQUEOUS EYE-DROP PREPARATIONS

Eye-drops	Volume of sample (ml.)	Dilution to* (ml.)	Per cent w/v compound	
			Present	Found by proposed method
Atropine sulphate B.P.C.	4	20	0·98	1·00; 0·98 0·98; 0·98 0·98
Homatropine B.P.C.	3	20	1·99	2·01; 2·00
Cocaine B.P.C.	3	20	1·99	1·99; 1·98
Pilocarpine B.P.C.	4	20	1·00	0·99; 0·99
Atropine methonitrate B.N.F. ..	4	20	1·00	1·00; 1·00
Physostigmine B.P.C.	5	10†	0·49	0·50; 0·51 0·50
Lachesine B.P.C.	4	20	0·98	0·98; 0·98
Hyoscine B.P.C.	5	10†	0·246	0·241; 0·247

* With dilute buffer solution except those marked †, where concentrated buffer solution is used.

0·01M TPB. Dissolve sodium tetraphenylboron (3·42 g.) in distilled water (50 ml.), add moist aluminium hydroxide gel (0·5 g.) and shake for 20 min. Dilute to 300 ml. with distilled water; dissolve sodium chloride (16·6 g.) in this solution and stand for 30 min. Filter clear,* under suction, through two thicknesses of No. 42 Whatman filter paper. After washing the filter, dilute the filtrate to 1 litre with distilled water and adjust the pH to 8·0 to 9·0 with 0·1N sodium hydroxide using narrow range Universal pH papers. Store in an amber bottle.

TABLE III

APPLICATION TO INJECTION SOLUTIONS

Injection solutions	Volume of sample (ml.)	*dilution to** (ml.)	Compound	
			Present	Found by proposed method
Lobeline hydrochloride B.P.C. 3 mg./ml.	5	10†	2·96 mg./ml.	2·99 mg./ml.
Methadone B.P. 1·00 per cent w/v ..	4	20	1·01 per cent w/v	1·01 per cent w/v
Morphine sulphate B.P. 32·4 mg./ml. ..	2	20	32·6 mg./ml.	32·7 mg./ml.
21·6 mg./ml. ..	3	20	21·5 mg./ml.	21·4 mg./ml.
Pethidine B.P. 5·0 per cent. w/v .. .	1	20	5·03 per cent w/v	5·02 per cent w/v
Procaine and Adrenaline B.P.	2	20	2·03 per cent w/v (Procaine hydrochloride)	2·06 per cent w/v

* With dilute buffer solution except those marked †, where concentrated buffer solution is used.

Both the CPC and TPB solutions deteriorate slightly on standing; they should be standardised at frequent intervals.

0·01M *Potassium chloride solution.* Dissolve analytical reagent grade potassium chloride (0·1491 g.), previously dried at 150° for 1 hr., in dilute buffer solution (200 ml.).

Sample Preparation

Pure compounds. Prepare an approximately 0·01M solution in dilute buffer solution.

Aqueous eye-drops and injection solutions. Dilute with buffer solution as directed in Tables II and III; use 10 ml. for the assay.

* Re-filter the first 20-30 ml. of filtrate if cloudy.

Tablets (containing lactose basis). Dissolve the powdered tablets, with gentle warming, and dilute to the volume given in Table IV; centrifuge if necessary; use 10 ml. for the assay.

Suppositories, eye-ointments and oily eye-drops. Dissolve the preparation, with warming, in the specified organic solvent and extract as directed in Table V. Filter each extract in turn through a small plug of cotton wool. Gently warm the combined extracts, to remove traces of organic solvent, and then dilute to volume with concentrated buffer solution; use 10 ml. for the assay.

TABLE IV

APPLICATION TO SIMPLE UNCOATED TABLETS

Tablets	Sample preparation		Compound found by	
	No. of tablets	Dilution to* (ml.)	Proposed method	Alternative method
Hypodermic tablets—				
Atropine sulphate 1 mg.	40	20	1·06 mg./tab.	1·03 mg./tab.[1]
Pilocarpine nitrate 0·65 mg. ..	6	20	0·63 mg./tab.	0·62 mg./tab.[1]
Morphine sulphate 16·2 mg. ..	5	25	15·7 mg./tab.	16·1 mg./tab.[2]
Ophthalmic tablets—				
Cocaine hydrochloride 5 mg. ..	14	20	5·05 mg./tab.	4·98 mg./tab.[2]
Simple tablets—				
Atropine sulphate B.P. 0·65 mg. ..	40	25	1. 0·64 mg./tab. 2. 0·67 mg./tab.	0·61 mg./tab.[3] 0·64 mg./tab.
Pethidine B.P. 25 mg.	2	25	23·7; 24·0 mg./tab.	23·9 mg./tab.[3]
Codeine phosphate B.P. 32·4 mg. ..	2	25	33·2 mg./tab.	32·8 mg./tab.[3]
16·2 mg. ..	4	25	17·0 mg./tab.	16·9 mg./tab.
Hyoscine B.P. 0·65 mg.	40	25	0·61 mg./tab.	0·61 mg./tab.[3]

* With dilute buffer solution.

[1] The method described for Atropine Sulphate Tablets B.P. 1958.
[2] U.V. Spectroscopy.
[3] Official method of the British Pharmacopoeia.

Procedure

Transfer the prepared solution (10 ml.) to a clean, dry beaker, add 0·01M sodium tetraphenylboron (15 ml.) accurately measured, while swirling the contents of the beaker, and allow to stand for 5 min. Filter through a dry sintered-glass funnel (porosity 4) under gentle suction into a dry flask. (Reserve the residue for identification purposes.) Transfer exactly 20 ml. of the filtrate to a 150 ml. flask, add bromophenol blue solution (0·5 ml.), accurately measured, and titrate with 0·005M cetylpyridinium chloride to a blue end-point ('a' ml.). To a further 15 ml. of sodium tetraphenylboron, add concentrated buffer solution (4 ml.) followed by bromophenol blue solution (0·5 ml.), accurately measured, and titrate as above to the same end-point ('b' ml.).

The difference in titres $(b - \frac{5a}{4})$ ml. is equivalent to the volume of 0·005M sodium tetraphenylboron precipitated by the organic base.

At the same time, determine the molarity of the cetylpyridinium chloride solution by pipetting 0·01M potassium chloride (10 ml.) into a clean, dry beaker and continuing as above from the words "add 0·01M sodium tetraphenylboron (15 ml.) . . . titrate with 0·005M CPC to a blue end-point" ('c' ml.).

TABLE V

APPLICATION TO MISCELLANEOUS PREPARATIONS

Preparation	Sample preparation				Compound found by	
	Quantity of preparation	Organic solvent	Extraction	Dilution to* (ml.)	Proposed method	Alternative method
Suppositories of morphine B.P.C. 16·2 mg.	4 suppositories	10 ml. light petroleum (b.p. 40°–60°)	1 × 10 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	25	16·1; 16·1 mg. suppository	15·0 mg. suppository[1]
Suppositories of morphine B.P.C. 64·8 mg.	1 suppository	10 ml. light petroleum (b.p. 40°–60°)	1 × 10 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	25	61·6; 60·3 mg. suppository	68·0 mg. suppository
Oily eye-drops of atropine 1 per cent w/v ..	5 g.	5 ml. solvent ether	1 × 5 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	20	1·00; 1·00 per cent w/v	1·00 per cent w/v[2]
Oily eye-drops of physostigmine 1 per cent w/v	5 g.	5 ml. solvent ether	1 × 5 ml. 2N acetic acid 3 × 5 ml. concentrated buffer	20	0·99; 1·00 per cent w/v	1·00 per cent w/v[2]
Atropine eye ointment 1 per cent w/w ..	8 g.	5 ml. solvent ether	1 × 15 ml. 2N acetic acid 2 × 10 ml. and 1 × 5 ml. concentrated buffer	50	1. 0·97; 0·98 per cent w/w	0·98 per cent w/w[3]
					2. 0·96; 0·96 per cent w/w	0·98 per cent w/w

* With concentrated buffer solution.

[1] Nitroso morphine method after extraction.
[2] Laboratory prepared.
[3] Official method of the British Pharmacopoeia.

The molarity of the CPC is then given by the relationship $M = \frac{10\,M'}{(b - \frac{5c}{4})}$ where M′ is the molarity of the potassium chloride solution.

The residue obtained in the above assay may be used for the determination of melting-point as follows.

Wash the residue with distilled water (5 portions of 20 ml.) and dry over phosphorus pentoxide at a pressure not exceeding 5 mm. Determine the melting-point by Method I, Appendix IVA of the British Pharmacopoeia.

Results and Discussion

The recommended method has been applied to the determination of 15 basic nitrogen compounds. The results together with those obtained by alternative procedures are given in Table I. This also lists melting-points of the tetraphenylborates which are of value for identification purposes. Since some organic tetraphenylboron salts are thermally unstable (Wendlandt and Dunham, 1958) the conditions of the British Pharmacopoeia for the determination of melting points must be closely followed. In a number of instances it is possible to carry out supplementary chemical identification tests on the residue; for example, the Vitali test can be applied directly to the atropine derivative.

Applications to aqueous eye-drop and injection solutions are given in Tables II and III. In the study of interfering substances it has been established that esters of *p*-hydroxybenzoic acid, phenol, chlorbutol, chlorocresol and chloroxylenol in concentrations at which these materials are used as fungistats, bactericides and bacteriostats do not interfere. Phenylmercuric nitrate (0·002 per cent w/v) causes a small positive error. The extent of this error will depend on the ratio of phenylmercuric nitrate to active ingredient present and will usually lie between 0·2 and 1 per cent.

Solutions prepared by treating up to 1 g. of lactose, liquid glucose, mannitol, stearic acid, starch, calcium stearate, talc and sucrose by the method recommended for tablets produce no interference. Gelatin and polyvinylpyrrolidone precipitate with TPB and thus invalidate direct application of the method. Table V shows the application of the method to a number of preparations containing oils and fats. Acid extracts, prepared as directed in the method, from Theobroma Oil B.P.C., Basis for Eye Ointment, B.P. and Castor Oil B.P. contained no interfering materials.

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