

# THE PHOTOMETRIC DETERMINATION OF QUATERNARY AMMONIUM SALTS AND OF CERTAIN AMINES BY COMPOUND FORMATION WITH INDICATORS

## PART I. QUATERNARY AMMONIUM SALTS

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Received July 12, 1954

### INTRODUCTION

METHODS available for the determination of quaternary ammonium salts have been reviewed by Dubois<sup>1,2</sup> and by others<sup>3,4</sup>. The now well-known reineckate, ferricyanide and indicator extraction methods have been adopted by standardising bodies such as the Association of Official and Agricultural Chemists of U.S.A. and the various pharmacopœia authorities. In the British Pharmacopœia the ferricyanide method is specified for cetrimide and the reineckate method for decamethonium iodide. The indicator extraction method has the advantages of high sensitivity, rapidity and partial selectivity and appeared to be of potential value for application to pharmaceutical preparations. Considerable attention has been given to this method in the literature, but very varying conditions have been laid down by different workers (Table I).

TABLE I  
EXTRACTION METHOD—DIFFERENT CONDITIONS USED BY DIFFERENT WORKERS

Reference	Indicator	pH	Solvent	Method of clarifying	Applicability
7	Bromophenol blue	11.5	Ethylene dichloride	Anhydrous sodium sulphate	$R_1R_2R_3R_4NX$ where $R = C_6H_5CH_2-$ or $C_6H_5$ , or derivative thereof
8	„	„	Benzene	Centrifuging	„ „
9	Bromocresol purple	7.0	Chloroform	„	Tetraethylammonium bromide
10	Bromophenol blue	—	Ethylene dichloride	„	Hexamethonium and tetraethylammonium bromides
11	Bromothymol blue	8.4	Chloroform	Filter paper	Quaternary ammonium and amine salts
12	„	7.6	„	Centrifuging	Hexamethonium bromide and decamethonium iodide

### REVIEW OF THE LITERATURE

Methods for the determination of amine and quaternary ammonium salts involving the formation of salts or complexes with indicators are of 4 types. The first and most widely applicable type involves extraction and is based on Prudhomme's<sup>5</sup> discovery in 1938 that alkaloids form chloroform-soluble complexes with acid dyes such as eosin, the reaction being

quantitative.<sup>6</sup> Auerbach<sup>7</sup> in 1943 described a method using bromophenol blue which is applicable to many quarternary ammonium salts, but not to amines. In this method, as first described by Auerbach,<sup>7</sup> a buffered solution of the quaternary ammonium compound and bromophenol blue, is extracted with ethylene dichloride to give a coloured solution of the complex which is clarified by a drying agent or by centrifuging<sup>8</sup> before final measurement of its optical density. Other workers since have varied indicator, *pH*, concentrations of reagents, solvent and method of clarifying (Table I) but the reasons underlying the selection of working conditions are not always clear.

Extraction methods employing different conditions have been described for tertiary amines<sup>11,13,14,15</sup>, alkaloids<sup>16,17</sup>, long chain surface active<sup>18,19</sup> and other high molecular weight quaternary ammonium compounds<sup>11,20</sup> as well as for short chain salts such as tetraethylammonium bromide<sup>9,10</sup> and hexamethonium bromide<sup>10,11,12</sup>. No methods have been described for gallamine triethiodide and pentapyrrolidonium salts.

Two types of method have been based on Hartley's<sup>21</sup> observations of the effects of surface active quaternary ammonium salts such as cetylpyridinium bromide and cetrimide on various indicators, the colour of sulphonphthaleins for example being "not only displaced to the alkaline side, but also qualitatively changed." Hartley and Runnicles<sup>22</sup> in 1938 used this effect to determine the concentration of cetylpyridinium ion in a solution by titration with sodium cetylsulphate using bromophenol blue as indicator. Methods published since<sup>23,24,25,26,27</sup> use other indicators but with one exception both reactants are surface active agents. Carkhuff and Boyd<sup>27</sup>, however, apply their method to a tertiary amine and claim that it is suitable for the determination of most quaternary ammonium salts and certain types of amine. A variation of the method involves titration of surface active agent with a suitable indicator<sup>28</sup>. The second of the two types of method based on Hartley's observations was provided by Colichman<sup>29</sup>, who in 1947 applied the indicator displacement effect to the direct photometric determination of quaternary ammonium salts by using an excess of bromophenol blue and measuring the colour of the aqueous solution. He applied the method to two surface-active compounds and implied that it was applicable to quaternary ammonium salts in general. Other workers<sup>4,30</sup> using bromocresol purple applied similar methods to cetylpyridinium chloride and they also suggested a wider application.

The fourth type of method is applicable only to organic solvent solutions of bases and consists in adding a solution of indicator in either aqueous buffer<sup>31,32,33,34</sup>, in which case shaking is necessary, or in organic solvent<sup>35</sup>, to a solution of the base in a halogenated solvent, followed by photometric measurement of colour produced. This method, which was first described by Brodie and Udenfriend<sup>31</sup>, is based on observations by LaMer and Downes<sup>36</sup> in 1933, supplemented later by Griffiths<sup>37</sup> and reaffirmed by others<sup>38,39</sup>, that addition of bases to organic solvent solutions of indicators results in changes of colour proportional to the concentration of base.

The titration and direct photometric methods appeared to have such considerable advantages as regards simplicity and rapidity that it was

clearly desirable to determine their range of applicability. If this were found to be limited then it was decided to elucidate the principles underlying the indicator extraction method sufficiently to facilitate selection of optimum conditions for determination of particular compounds either alone or in the presence of other substances.

## EXPERIMENTAL

### 1. *Titration Method*

Titration of a 0.1 per cent. solution of cetylpyridinium chloride with a 0.12 per cent. solution of sodium lauryl sulphate using methyl yellow, in the presence of chloroform, as indicator under the conditions laid down by Carkhuff and Boyd<sup>27</sup> gave a sharp end-point. With cetrimide the end-point was less sharp and with *p*-nitrophenoxymethylbenzyl-dimethylammonium chloride a diffuse and very premature end-point was obtained. With salts of hexamethonium, pentapyrrolidinium and gallamine the chloroform layer became coloured after the addition of as little as 3 per cent. of the expected volume of titrant.

### 2. *Direct Photometric Method*

Following Colichman's method<sup>29</sup>, addition of 0.5 mg. of cetylpyridinium chloride to a 0.0016 per cent. solution of bromophenol blue buffered with sodium carbonate gave an increase in optical density of 0.28 at a wavelength of 645  $m\mu$ , which is the  $\lambda_{min}$  for the indicator alone. At the  $\lambda_{max}$  of 585  $m\mu$ , which was not used by Colichman, a decrease in optical density of 0.49 occurred, but the reagent blank was large. With cetrimide a small increase in optical density was obtained at 645  $m\mu$  and no increase with gallamine; at the  $\lambda_{max}$  small decreases were obtained with both. Using bromothymol blue the corresponding changes in optical density were even smaller.

### 3. *Extraction Method*

Simple preliminary tests having indicated that chloroform was a better solvent for the complex than ethylene dichloride, a systematic study of conditions was commenced.

#### (a) *Choice of Indicator*

Working with cetrimide, pentapyrrolidinium and pentamethonium, tests were made in nearly neutral, slightly acid and slightly alkaline solutions with more than 40 anionic indicators to see whether a chloroform-soluble complex was formed. Indicators tried included halogenated and unhalogenated sulphonphthaleins, phthaleins, fluoresceins and azo-dyes. Extractable complexes formed most readily with cetrimide and least readily with pentamethonium, cetrimide giving extractable complexes with 28 indicators but pentamethonium with only 4, bromothymol blue, phenyl- $\alpha$ -naphthylamineazobenzene-*p*-sulphonic acid, dibutylaminoazobenzene-*p*-sulphonic acid<sup>40</sup> ("butyl orange"), and tetrachlorotetraiodofluorescein, most colour being obtained with bromothymol blue. Furthermore, as expected from Gottlieb's<sup>11</sup> results, complexes formed more readily in neutral or slightly acid solution than in alkaline solution.

(b) *Choice of Solvent*

Working with dimethylaminotoluene methiodide and bromothymol blue at pH 9.2, extraction of complex with one portion of chloroform being incomplete, single extractions were made with 24 solvents including halogenated and non-halogenated aliphatic, alicyclic and benzenoid hydrocarbons, alcohols, esters and nitrated hydrocarbons such as nitrobenzene, the latter being chosen because of its high dielectric constant. Alcohols and esters gave a large reagent blank, whilst unhalogenated hydrocarbons extracted no complex. The best solvents were nitrobenzene, acetylene tetrachloride, chloroform and methylene dichloride, the extinction values obtained being 0.33, 0.206, 0.123 and 0.118 respectively. Nitrobenzene, however, was rejected because of toxic hazards and tendency to form persistent emulsions. Using acetylene tetrachloride a solvent phase was obtained which could not be clarified with glass wool and hence chloroform was chosen for further work. Solubilities of the complexes of 3 quaternaries in certain solvents are given in Table II.

TABLE II  
SOLUBILITIES OF QUATERNARY AMMONIUM COMPLEXES OF BROMOTHYMOLO BLUE

Quaternary ammonium cation	Complex in 100 ml. of solution				
	Chloroform, g.	Ethylene dichloride, g.	Acetylene tetrachloride, g.	Carbon tetrachloride, g.	Benzene, g.
Cetyltrimethylammonium ..	More than 60	More than 50.0	—	4.6	40.0
Gallamine . . . . .	43	20.0	38	Insoluble	0.01
Hexamethonium .. . . .	34	0.14	14	„	0.04

(c) *Phase Volume Ratio*

To avoid emulsions it was necessary to have a volume ratio of the aqueous phase to the chloroform of not greater than 1.5 to 1. To obviate the measurement of small volumes of reagents and of sample the aqueous phase was fixed at about 25 ml. and the solvent phase at 20 ml.

(d) *Effect of Time and Technique*

The complex appeared to form rapidly<sup>4</sup> in the aqueous phase and not slowly as believed by Marshal and Rogers<sup>16</sup>. Phase equilibrium was complete after 2 minutes shaking. Separation of the phases was complete after 2 minutes, but the upper portion of the chloroform phase was more intensely coloured than the lower. This resulted from the "foam" (chloroform dispersed in aqueous phase) hanging from the upper aqueous phase into the chloroform, complex being adsorbed at the large interfacial surface as in the case of indicators alone<sup>34</sup>. Reproducibility was obtained by swirling the chloroform to mix before running it off.

(e) *Method of Clarifying Chloroform Solution of Complex*

Different types of filter paper tried removed up to 20 per cent. of colour from the chloroform solution for one paper, additional colour being removed with each filtration through a fresh paper; Munktell No. 0 filter

papers used by Gottlieb<sup>11</sup> were not available at this stage. Sodium sulphate, used initially by Auerbach<sup>7</sup> and later by Wilson<sup>41</sup> removed complex at the rate of about 12 per cent. of colour for each 1 g., the amount increasing with time of contact. Several other substances such as silica<sup>42</sup>, celite and calcium oxide were also found unsatisfactory. However 0.06 g. of glass wool clarified 25 ml. of moist chloroform solution of complex in 10 minutes, no loss of colour occurring on standing for a further 15 minutes or on adding another 0.04 g.; even with 0.2 g. only a 5 per cent. loss of colour resulted. A slight fall in optical density during actual clarification was attributed to removal of the traces of indicator present in the dispersed water droplets since the glass wool acquired a greenish-blue colour removable by alcohol or chloroform B.P., but not by alcohol-free chloroform or by ethylene dichloride.

#### (f) Stability of Chloroform Solution of Complex

Use of a simple chloroform solution of the complex for optical measurements suffered from certain disadvantages such as adsorption of the complex on to the glass walls of flasks and cells and instability of colour arising from removal of alkali from the glass. A solution stable for at least one hour and free from adsorption effects was obtained by the addition of an ethanolic solution of boric acid containing a small but necessary amount of water; the concentration of boric acid and water chosen gave maximum sensitivity and small variations had no effect. Stronger acids such as hydrochloric lowered sensitivity whilst sulphonphthaleins tend to fade in alkaline solution<sup>43</sup>.

#### (g) Purity of Reagents

Some batches of both bromophenol blue and bromothymol blue failed to pass the purity test given by Wilson<sup>44</sup> and were purified as described by him. However, both original and purified indicators although differing appreciably in colour gave identical results in photometric work and the amount

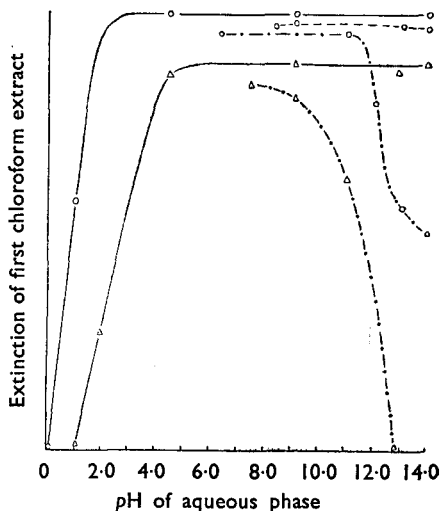


FIG. 1. Effect of *pH* of aqueous phase on the extraction of complex into chloroform using three different indicators.

— methyl orange  
 - - - bromophenol blue  
 - · - · - bromothymol blue

○

△ 2:4-dichlorophenoxyethyltriethylammonium chloride

N.B.—Extinction values were determined at different wavelengths and in solvents of different composition for each indicator. Arbitrary scales for the respective series of ordinates were chosen in order to obtain a clear diagram.

of impurity was probably small. With the exception of trichloroethylene solvents were not specially purified; trichloroethylene was freed from amines present as stabilisers<sup>45</sup> by extraction with acid. Quaternary ammonium salts were recrystallised from ethanol, ethanol-ether or ethanol-benzene<sup>46</sup> mixtures.

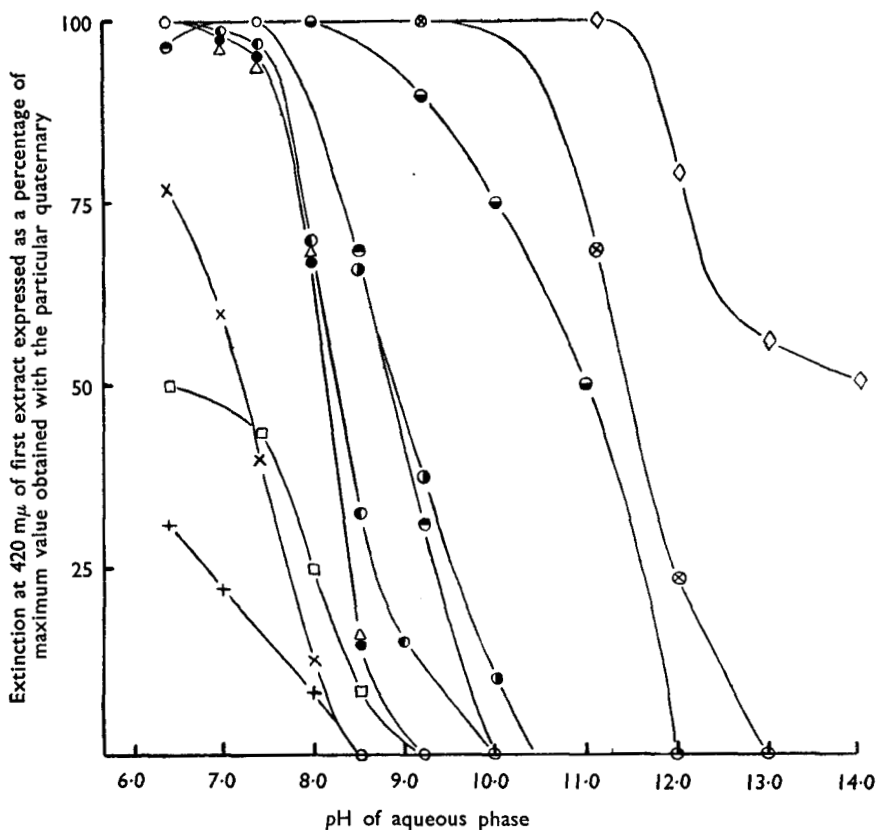


FIG. 2. Effect of *pH* of aqueous phase on the extraction of complex into chloroform using bromothymol blue and different quaternary ammonium salts.

- + pyridine methiodide
- tetraethylammonium hydrogen tartrate
- × hexamethonium bromide
- Δ decamethonium iodide
- pentapyrrolidinium bitartrate
- ⊙ phenyltrimethylammonium chloride
- ⊙ gallamine triethiodide
- ⊙ diethylaminotoluene methiodide
- ⊙  $\gamma$ -phenoxypropyltriethylammonium iodide
- ⊗ *p*-nitrophenoxymethylbenzyl dimethylammonium chloride
- ◇ cetrimide
- is used where several lines pass through the same point and where the points lie on the base line.

(h) *Effect of pH*

The effect of variation of the *pH* of the aqueous phase on the extraction of the complex into chloroform was studied to a limited extent with different indicators (Fig. 1) and in greater detail with bromothymol blue (Fig. 2). McIlvaine standard buffers<sup>47</sup> containing varying ratios of citric acid and sodium phosphate were used for *pH* values between 6.4 and 8; hydrochloric and boric acids were used to obtain low *pH* values and solutions of sodium bicarbonate, borax, sodium carbonate and sodium hydroxide for the higher *pH* values. Extinction values (2 cm.) for the reagent blank (3 extractions) rose, with decrease of *pH*, from 0.008 at *pH* 7.6 to 0.32 at *pH* 5.8 using chloroform, and were rather less using ethylene dichloride.

(i) *Effect of Excess of Bromothymol Blue*

Using chloroform at *pH* 7.4 increasing the amount of indicator from 1 ml. to 5 ml. of 0.1 per cent. raised the reagent blank from 0.01 to 0.16. With quaternaries giving calibration curves of theoretical slope (Fig. 5) complete extraction of complex was obtained in the presence of an excess of indicator varying between 10 and 40 per cent. (Table III). Other compounds required a much greater and varying excess, the hexamethonium complex not being completely extracted even with 100 per cent. excess.

TABLE III  
EXTENT OF LINEARITY OF CALIBRATION CURVES FOR QUATERNARY AMMONIUM COMPOUNDS

Compound	Extent of linearity, per cent.
Gallamine triethiodide	80
Pentapyrrolidinium bitartrate	60
2:4-Dichlorophenoxyethyltriethylammonium chloride	Greater than 70
Decamethonium iodide	70
3:5-Dimethyl-4-chlorophenoxyethyl-dimethylbenzylammonium chloride	85
Dimethylbenzyl- <i>p</i> -nitrophenoxymethylammonium chloride	90

(j) *General Method using Bromothymol Blue*

*Apparatus.* All apparatus, including separator stopcocks, must be completely free from grease.

*Reagents*

*Bromothymol Blue.* If this does not pass the purity test given by Wilson<sup>44</sup>, purify as he describes but using a buffer of *pH* 7.5.

*Bromothymol Blue Solution.* Dissolve 0.15 g. of bromothymol blue and 0.15 g. of anhydrous sodium carbonate in water and dilute to 100 ml.

*Buffer pH 7.5*<sup>47</sup>. Dilute 7.5 ml. of freshly prepared citric acid solution 0.1 M (2.10 per cent.) to 100 ml. with sodium phosphate solution 0.2 M (7.16 per cent. Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O).

*Boric Acid in Ethanol.* Dissolve 5.0 g. of boric acid in dehydrated ethanol, add 20.0 ml. of water and dilute with dehydrated ethanol to 250 ml.

*Procedure*

To 20 ml. of chloroform B.P. in a separator add 5.0 ml. of solution containing the equivalent of up to 2 micromols of univalent quaternary ammonium salt, 1.0 ml. of bromothymol blue solution and 20.0 ml. of buffer. Shake vigorously for 2 minutes, allow to separate, slowly invert the separator to mix the chloroform phase and allow to stand for 2 minutes.

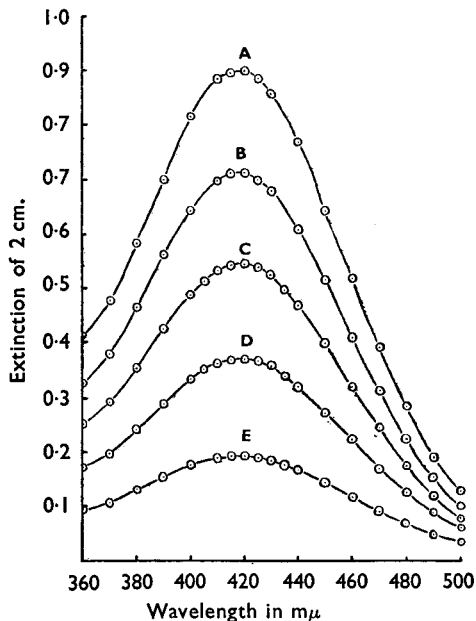


FIG. 3. Absorption spectra of solutions of bromothymol blue in chloroform containing 25 per cent. v/v of solution of boric acid in ethanol. A, 2.43 micromols; B, 1.94 micromols; C, 1.46 micromols; D, 0.97 micromols; E, 0.48 micromols.

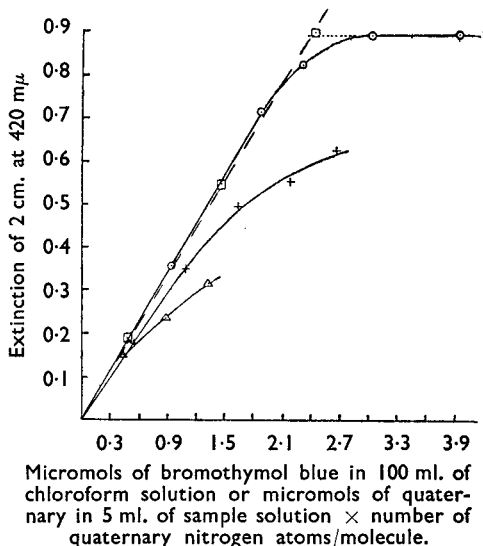


FIG. 4. Calibration curves for bromothymol blue, gallamine triethiodide and hexamethonium bromide.

—□— bromothymol blue in chloroform containing 25 per cent. v/v of solution of boric acid in ethanol  
 ○ bromothymol blue in chloroform containing 25 per cent. v/v of solution of boric acid in ethanol by proposed general method  
 Δ gallamine triethiodide by proposed general method  
 + hexamethonium bromide by proposed general method but at pH 6.8.

Run off the chloroform into a stoppered flask containing 0.4 g. of glass wool and repeat the extractions and separations with 2 further portions of chloroform. Shake the bulked chloroform for 1 minute and allow to stand for 5 minutes. Decant the chloroform through a small plug of glass wool into a 100 ml. graduated flask containing 25.0 ml. of solution of boric acid in ethanol. Wash the flask and filter with successive small quantities of ethylene dichloride or ethanol-free chloroform, adding the washings to the main portion of filtrate and finally diluting to 100 ml. Measure the extinction of 2 cm. at 420  $m\mu$  against chloroform, and read off the corresponding amount of quaternary ammonium salt from a calibration curve.



## (k) Calibration Curves

Solutions in chloroform (containing ethanol and boric acid) of the gallamine complex and of bromothymol blue, having the same concentration of indicator gave identical spectra. Absorption spectra for solutions covering a range of concentrations of indicator are shown in Figure 3. The extinction values at  $420\text{ m}\mu$  are shown plotted in Figure 4. The effect of variation in path length was small, extinction values for 1 cm., 2 cm., and 4 cm. being 0.179, 0.361 and 0.75. Calibration curves were linear from the origin for a varying range of extinction values, flattening off at a value which was a varying fraction of the extinction of 0.89 which corresponded to the total available bromothymol blue (Table III). The curves for gallamine triethiodide and hexamethonium bromide (Fig. 4) illustrate the types obtained with compounds of high and low molecular weight respectively. In the case of hexamethonium bromide the slope was affected by change of  $pH$ , being greatest at  $pH$  6.8. These curves were plotted from values corrected for the reagent blank on the basis that this is greatest in the absence of quaternary and zero at the extinction (0.89) corresponding to complete extraction of bromothymol blue.

For the high molecular weight compounds the slopes of the calibration curves, expressed as extinction/concentration (mg./100 ml.), were directly proportional to the number of quaternary nitrogen atoms per molecule and inversely proportional to the molecular weight (Fig. 5), the line in this figure showing the linear relationship which holds when complete extraction of complex is obtained.

Calibration curves set up by different workers at different times using different reagents agreed to within 1 per cent. Fuller information regarding the precision of the method when applied to pharmaceutical preparations will be given in a later paper.

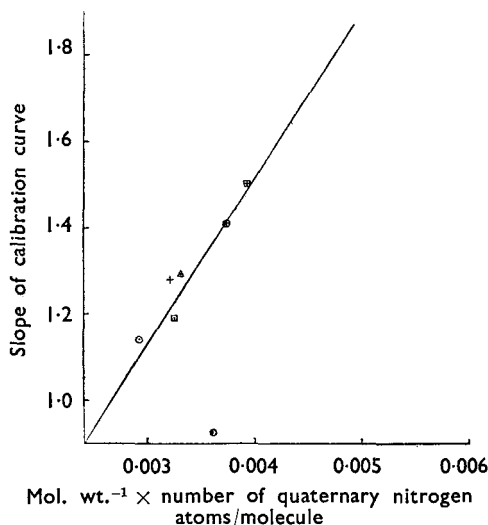


FIG. 5. Relationship between slope of calibration curve, expressed as extinction of 2 cm. at  $420\text{ m}\mu$  for 1 mg. in 5 ml. of sample solution, and reciprocal of molecular weight  $\times$  number of quaternary nitrogen atoms per molecule.

- $\Delta$  gallamine triethiodide
- $\oplus$  pentapyrrolidinium bitartrate
- $\square$  decamethonium iodide
- $\bullet$  hexamethonium bromide
- $\odot$  tetraethylammonium bromide
- $\circ$  3:5-dimethyl-4-chlorphenoxy-methyl-dimethylbenzylammonium chloride
- $\square$  dimethylbenzyl-*p*-nitrophenoxy-methylammonium chloride
- +
- 2:4-dichlorphenoxy-methyltriethylammonium chloride

(1) *Composition of Complex*

The composition of bromothymol blue complexes was determined in 3 different ways. (1) Assuming that for the linear portion of the calibration curve complete extraction of quaternary compound is taking place, then at each point the molar ratio of indicator to quaternary compound can be calculated. For gallamine, for example, the ratio is 3 to 1 and since there are 3 quaternary nitrogen atoms per molecule then one molecule of indicator is associated with each nitrogen atom. For methyl orange and 2:4-dichlorophenoxyethyltriethylammonium chloride a ratio of unity was also obtained. Similar results were obtained with the other relatively high molecular weight quaternary ammonium compounds. (2) In the case

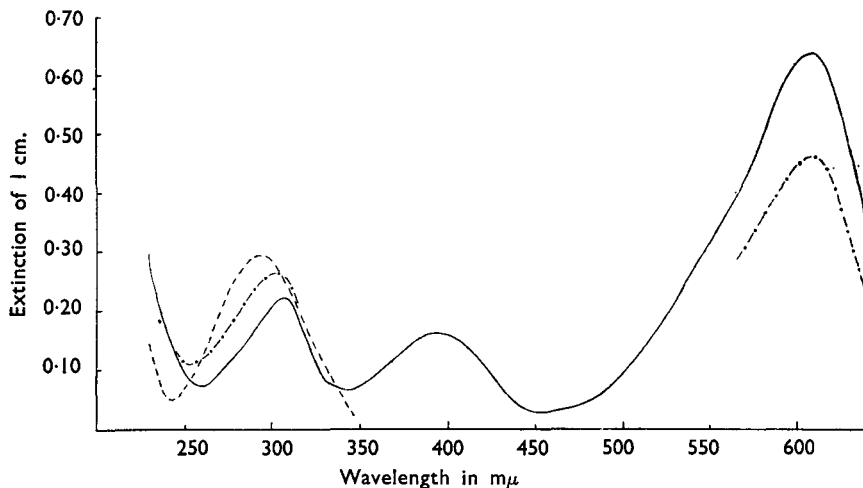


FIG. 6. Determination of composition of bromothymol complex of *p*-nitrophenoxymethylbenzyltrimethylammonium chloride. Absorption spectra of about 0.001 per cent. solutions in 0.1 N sodium hydroxide of

— bromothymol blue  
 - - - *p*-nitrophenoxymethylbenzyltrimethylammonium chloride  
 - · - · complex.

of the *p*-nitrophenoxymethylbenzyltrimethylammonium complex the composition of the chloroform extract was determined by extracting the chloroform with 0.1N sodium hydroxide and making extinction measurements at wavelengths of 295  $m\mu$ , 306  $m\mu$  and 610  $m\mu$  (Fig. 6). The amount of bromothymol blue was calculated from the extinction value at 610  $m\mu$  and the amount of quaternary from the remaining two values by the two-colour method. The ratio obtained was 1 to 1. (3) The hexamethonium complex was isolated, as described later, its indicator content calculated from the extinction at 420  $m\mu$  of its chloroform solution, and its quaternary content determined by the general method described in section (j). The ratio found was again unity.

That other ratios can exist was shown as follows. (1) The chloroform solution of the cetrimide-bromothymol blue complex obtained at pH 13 (Fig. 1) was equilibrated with a buffer solution at pH 8.4 containing

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indicator. The extinction rose to that obtained normally at pH 8.4, the value being actually doubled, showing that the original ratio of indicator to quaternary was 1 : 2. (2) Using "butyl orange" a ratio of 4 : 1 was obtained with pentapyrrolidinium and with gallamine.

Complexes were obtained in the solid state in two ways. (1) Complexes of bromothymol blue with gallamine, hexamethonium and pentapyrrolidinium were obtained as orange-red to red-brown powders by evaporating chloroform solutions, obtained by extraction, to dryness. (2) Addition of the appropriate amount of bromothymol blue to a solution of quaternary hydroxide in methanol and evaporation to dryness gave the complexes of gallamine and hexamethonium as green powders. The ratio of indicator to quaternary compound was the same in both forms of complex.

(m) *Application of General Method in the Presence of Interfering Substances*

Possible interference by various substances in the concentrations likely to be encountered in pharmaceutical preparations was investigated. Sodium sulphite (5 mg.), chlorbutanol (0.06 mg.), dextrose (0.1 g.), lactose (0.1 g.), and polyvinylpyrrolidone (2.7 mg.) had no effect but sodium lauryl sulphate (0.2 mg.) lowered the extinction from 0.35 to 0.30. The effect of inorganic salts was to increase the amounts of both free indicator and complex extracted, the effect on the reagent blank being greatest at low pH values (Table IV). Series of tests using different inorganic salts

TABLE IV  
EFFECT OF SODIUM CHLORIDE ON EXTRACTION OF INDICATOR ALONE AND OF INDICATOR-QUATERNARY COMPLEX

Compound extracted •	Sodium chloride, g.	Extinction of first extract	
		pH 7.4	pH 8.5
Bromothymol blue alone	—	0.01	0
	0.2	0.035	0
	1.0	0.68	0
	2.0	1.07	0.035
	4.0	Greater than 1.07	1.1
Pentapyrrolidinium complex	0	—	0.096
	1.5	—	0.148

showed that those yielding doubly charged ions such as sulphate had a greater effect, the effect being actually related to the ionic strength of the solution.

DISCUSSION

*Titration and Direct Photometric Methods*

The failure of the titration method of Carkhuff and Boyd<sup>27</sup> when applied to hexamethonium, pentapyrrolidinium and gallamine may be attributed to the relatively greater stability of the complex formed with methyl yellow in chloroform solution compared with that formed with sodium lauryl sulphate in aqueous solution. Barr *et al.*<sup>25</sup> obtained divergent results with dodecyltriethylammonium bromide using methylene blue, whilst Epton<sup>24</sup> obtained quantitative results with cetylpyridinium bromide only when the

carbon chain length of the alkyl sulphate was greater than eight. Alkyl sulphates with even greater chain length may be necessary with the lower molecular weight quaternaries. Different workers have used different indicators and different *pH* conditions, but the theoretical principles underlying the method do not appear to have been worked out. According to Hartley and Runnicles<sup>22</sup> end-points may be obtained with bromophenol blue in the aqueous phase at concentrations well below the critical micelle concentration (C.M.C.) for the cetylpyridinium ion and hence the undoubtedly higher C.M.C. values for the lower molecular weight quaternaries are probably not the reason for failure of the method. Systematic studies of this potentially valuable method are in progress.

According to Colichman<sup>46</sup> the colour change of bromophenol blue in the presence of long chain cations results from electrostatic interaction between the quaternary cations and the indicator anions; conductivity results obtained were in accordance with the complete formation of non-conducting ion-pairs even in highly dilute solutions. Interfacial tension studies<sup>48</sup> also indicated ion-pair formation in aqueous solution followed by micellisation. According to Lewis *et al.*<sup>49,50</sup> ion-pair formation in itself does not alter the spectral absorption of associated ions and Colichman<sup>51</sup> in a later spectral study concluded that the ion-pairs were at least partially in the form of micelles even below the C.M.C. for the quaternary. Failure to obtain a colour change with the lower molecular weight quaternary compounds may be attributed to absence of micelles, bromothymol blue being less useful than bromophenol blue for the high molecular weight quaternaries, as was found also in interfacial tension titrations, because the C.M.C. for the metathesis compound is higher<sup>48</sup>.

### *Extraction Method*

*Applicability.* The general method described in section (j) is applicable to all except the low molecular weight quaternaries such as tetramethylammonium and choline salts. However, it cannot be regarded as entirely satisfactory for hexamethonium and tetraethylammonium salts since calibration curves of theoretical slope are not obtained owing to incomplete extraction of complex. Almost complete extraction of hexamethonium has been obtained using acetylene tetrachloride at *pH* 6.6 and it is hoped that a method incorporating these and other modifications will be more suitable for all low molecular weight quaternaries. Although calibration curves of theoretical slope are obtained with the remaining compounds a separate calibration curve for each is necessary on account of the varying extent of linearity.

The method is liable to interference from (a) anionic surface-active agents, proteins<sup>52</sup>, peptones and other substances which may react with the quaternary, (b) substances such as albumin<sup>53</sup> and polyvinylpyrrolidone which may react with the indicator and (c) substances such as ointment bases and salts which may affect the partition of the indicator or of the complex between the two phases. Anionic detergents may possibly be removed by extraction with chloroform as the methylene blue complex<sup>54</sup>. Although polyvinylpyrrolidone combines with certain dyes<sup>55</sup> particularly

brilliant vital red, a sulphonated azo-dye used in a method for its determination<sup>56</sup>, in the concentration tried it did not interfere with the extraction method. Modifications necessary in the presence of certain interfering substances will be given in a later paper.

*Selectivity.* The general method, like those of many other workers, is applicable to certain amines, but these may be readily separated from quaternary compounds by extraction from alkaline solution. It should be possible to differentiate quaternary compounds by taking advantage of such factors as (a) the greatly differing solubility of the complexes in different solvents, benzene for example being a poor solvent for complexes of gallamine and hexamethonium, but a good one for that of cetrimide, (b) differences in behaviour with different indicators, the lower molecular weight quaternary compounds not giving extractable complexes with bromophenol blue, and (c) the effect of *pH*, only the high molecular weight quaternaries being extracted at the higher *pH* values.

*Theoretical Aspects.* A considerable amount of work remains to be done before the theoretical basis of the method can be fully established. Probably the most important relationship to be further studied is that between the constitution of a quaternary compound or amine and the *pH* range over which it can be extracted. It seems probable that the reacting species in the case of amines is the cation  $RR'R''NH^+$  formed in acid solution by the addition of a proton. Preliminary experiments have shown that amine-indicator complexes are extractable over different *pH* ranges extending into the alkaline side where, however, complexes may not form, the amine itself being extracted and then causing partition of indicator between the two phases. The limited *pH* ranges over which the complexes of different quaternaries can be extracted may be accounted for by the formation of undissociated quaternary hydroxide in sufficiently alkaline solutions.

#### SUMMARY

1. Published methods for the determination of quaternary ammonium compounds by (a) titration with anionic surface-active agents, and (b) measuring the change in colour intensity produced in an indicator in aqueous solution, have been found inapplicable to compounds of molecular weight lower than that of cetrimide.

2. The indicator extraction method has been studied in some detail and the results of variations of conditions and the effects produced by interfering substances described and discussed.

3. A general indicator extraction method is proposed which is applicable to all quaternary compounds except those of very low molecular weight.

The authors wish to thank the Directors of Messrs. May and Baker, Ltd., for permission to publish this paper.

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## DISCUSSION

The paper was presented by MR. J. ISAACS.

DR. D. C. GARRATT (Nottingham) said the authors had not sufficiently stressed the purity of the indicators used for the reaction. He had been unable to obtain English bromophenol blue which was sufficiently pure for spectrophotometric work, but American material gave good results. The chemical method was just as quick as the spectrophotometric method.

## DETERMINATION OF QUATERNARY AMMONIUM SALTS. PART I

DR. G. E. FOSTER (Dartford) remarked that he had used this type of method for the determination of tubocurarine. There was some difficulty in the standardisation of cetrimide, as the substance supplied by different makers did not seem to be of uniform composition. Had the authors been able to detect any variation?

DR. W. MITCHELL (London) said that the authors had selected chloroform as a solvent in preference to nitrobenzene or acetylene tetrachloride, one reason being that nitrobenzene presented a toxic hazard. This was not mentioned as a reason for the rejection of acetylene tetrachloride, which was probably the most toxic of the chlorinated hydrocarbons.

MR. H. E. BROOKES (Nottingham) said that in certain formulations of cetrimide, due to inactivation of the quaternary compound, the microbiological determination did not agree with the chemical method, although the latter still gave the expected figure. Had the authors any information on this subject?

MR. ISAACS, in reply, referring to the purity of the indicators, said that they discontinued the use of bromophenol blue because bromothymol blue was found to be superior in many respects. They purified the bromothymol blue by Wilson's method, but towards the end of the work they checked 6 or 7 samples of the commercial indicator and found the unpurified material to be as satisfactory as the purified. It seemed that purification by Wilson's method might be unnecessary, but by this means one was certain of having satisfactory material. The general method could be applied to tubocurarine fairly well. It was true that cetrimide was not uniform, and for that reason in most of their quantitative work they had used quaternary compounds of known composition. Replying to Dr. Mitchell, he said that acetylene tetrachloride was possibly more toxic than nitrobenzene but, in view of the odour of the latter, workers preferred to face the greater dangers of the former. The general method did not apply too well to hexamethonium bromide, but using acetylene tetrachloride and an alteration in volume-phase ratios, quantitative extraction had been obtained and it seemed that this solvent should be used until something better was found.