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FURTHER STUDIES OF BASIC DYE REAGENTS

by

Anne Willcox, M.Sc.

A Doctoral Thesis
Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the Loughborough University of Technology.

Supervisors: Dr. A.G. Fogg and Dr. D. Thorburn Burns

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I would also like to thank my colleagues, Mr A.S. Pathan for his advice on ion-selective electrodes and Mr S. Soleymanloo for the preparation of the sample of diphenyliodonium bisulphate.

My thanks are also due to the Loughborough University of Technology and in particular the Department of Chemistry for providing facilities and financial support for this work.

My stay in Loughborough would not have been the same without the presence of my many friends. To mention them all would be impossible, but I would particularly like to thank Miss Doris Hornsby and Bob and Margaret Ingram for many pleasant evenings spent at their houses, and also my fellow members of the 'Tea Club' for their cheerful company.

Finally, but by no means least, I would like to thank my parents, without whose help and encouragement this work could never have been undertaken.
Synopsis

The work consists of a study of four types of dye, with respect to their analytical uses: the Rhodamines, Methylene Blue and its related compounds, Brilliant Green and to a lesser extent Crystal Violet.

An extensive study, both literary and experimental has been performed on Methylene Blue and its 1,9 Dimethyl derivative, Taylor's Blue. A method for the determination of perchlorate with Taylor's Blue is proposed, by way of an illustration of its potential use.

The butyl ester of Rhodamine B was prepared and a comparative study of its analytical properties with the parent compound was performed. A tentative method for the determination of chromium using Butyl Rhodamine B is suggested. In the course of this work, a paper chromatographic method for the separation of Rhodamines and other red basic dyes was developed and published. This method is also applicable to column chromatography using cellulose.

Work was performed on the stabilities of various dye solutions, particularly with respect to the peculiarities of aqueous Brilliant Green solutions. An attempt was made to determine whether a series of onium compounds could be used as masking agents for previously developed Brilliant Green methods.

Adsorption phenomena of dyes, with regard to the amount of experimental error caused in photometric determinations using such dyes, have been studied for Methylene Blue derivatives, where the potential error is found to be significant and for Brilliant Green where the error was found to be negligible in comparison with other factors.
Finally, the determination of nitrate with Crystal Violet was investigated, both as a spectrophotometric method and as a possible ion-selective electrode.
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List of Dye Manufacturers and Suppliers

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Chapter One
Introduction

1.1 The History of the Dye Industry

The history of the dyeing industry is nearly as old as civilised man himself. Synthetic inorganic pigments have been found in mummy cloths taken from tombs of the ancient Egyptians and the ancient Mesopotamians have left us details of dyeing processes on stone tablets. Until the eighteenth century, plants were the main source of fabric dyes. For example, the only blue dye known until the sixteenth century was woad, derived from the madder plant, and much loved by the ancient Britons as a body cosmetic! It was still in use, in conjunction with indigo, also derived from a plant source, as a dye for the uniforms of policemen and postmen until the First World War. The only natural basic dye is berberine, a yellow dye derived from the barberry shrub (1).

The first synthetic organic dye, discovered by Woulfe in 1771, was picric acid, which was derived by treating natural indigo with nitric acid; it was regarded with little more than curiosity value at that time. It was not until 1856 that the synthetic dye industry got under way with the discovery of Mauve by Perkin. This was the first synthetic dye to be manufactured in any quantity.

In the succeeding years, many more dyes were discovered and the industry, centred mainly on the German firm, Badische Anilin und Soda Fabrik (B.A.S.F.) and the Swiss firms of Sandoz, Ciba and Geigy, went from strength to strength. The Rhodamines, Thiazines and Triphenyl-methane dyes were mostly discovered during the period 1870 to 1890 along with many other basic dyes (2).

The British dye industry was of little importance, until the First World War cut off supplies from foreign sources. It was at this
time that British Dyes Ltd., later to become part of I.C.I., was established and by 1938 was supplying 90% of the requirements of the home market.

After 1945, the demand for a greater variety of dyes grew with the development of the newer synthetic fabrics. Brand names such as Astrazon, Deorlene, Maxilon, Sevron and Synacrid appeared, but in most cases the structure of such dyes was not revealed (3).

1.2 Dyes and their Usage

Apart from their obvious application in the textile industry, there are a multitude of other uses to which dyes may be applicable, either because of their colouring properties or because of incidental properties, such as anti-bacterial activity.

Pigments, dyes which are insoluble in water, are used for the colouring of paints, plastics, inks and other materials where an opaque colouring is needed. Such dyes are either prepared as insoluble, such as the phthalocyanine dyes, or are insolubilised adaptations of normal textile dyes, called lakes. Oil- and wax-soluble dyes such as the Indolines are used in the manufacture of polishes, candles and the ink for ball-point pens.

Military applications of dyes include camouflage colouring, coloured smokes, and water dyes for the easier location of personnel who have been forced into the sea by an aircrash or other sea disaster. Fluorescent dyes were used during the last war for the night-marking of streets during the blackout. They are also used as tracers for the detection of leaks in drains and the plotting of the courses of subterranean water channels.

In addition to their uses in colour films, various dyes are used in photography for the prevention of halos and in retouching procedures.

Many processed foods lose some of their natural colouring during
canning or drying and require the addition of artificial colours in order to make them look appetising. A strictly-controlled list of food colours exists which sets out a range of reputedly safe and suitable dyes for this purpose. Some of these are natural and some are synthetic but all are manufactured to a very high degree of purity (4).

In the medicinal field, dyes find application as tracers and bactericides and in the past have been used in particular cases, as specific medicines for the treatment of such diseases as malaria and sleeping sickness. Triphenylmethane dyes, such as Crystal Violet are still used as skin bactericides for the treatment of such afflictions as Athlete's foot. Many dyes have also found application as biological stains, and specific grades of dye are manufactured for this purpose (5).

In the analytical field, dyes are used in a number of ways: as indicators, as titrimetric reagents, for spot tests, as extractants and chromogenic reagents in solvent extraction procedures and a few miscellaneous uses.

Many dyes find application as pH indicators. They are used either singly or as mixtures, which exhibit an easily detectable colour change over a given pH range. For example, such well-known dyes as Methyl Red and Phenolphthalein are used in this way. Other dyes, such as Methylene Blue, which exhibit a colour change on reduction are used as indicators in redox reactions (6).

In the titrimetric field, dyes have largely fallen out of use, except as indicators. In earlier years, a standard analytical method for the determination of dyes was the titration of a known acid dye with a basic dye of unknown composition and vica versa (7). This was abandoned because of problems of impurity in both types of dye, and because of difficulties which occurred due to the solubility of the product over the years, various spot-tests have been developed using
For example, in 1927 Eegriwe proposed a spot-test for antimony and tungsten using Rhodamine B (8). There are numerous other spot-tests using dyes, which have been detailed by Welcher (9) and by Feigl and Anger (10). Temperature sensitive dyes have also been used for the observation of temperature in machinery and household items such as heated hair rollers.

Solvent extraction methods, using basic dyes will be reviewed in more detail later in this chapter.

1.3 Classification of Dyes with Particular Reference to the Analytical Use of Basic Dyes

Dyes may be categorised in a number of different ways: by their origin, their dyeing properties and by their chemistry. It is the group of dyes, known loosely as the basic dyes, with which the present work is concerned. These were the earliest-known organic synthetic dyes, and they are characterised by exceptional brilliance, high tinctorial strength but a low fastness to light.

There are seven categories of traditional basic dyes, which will be illustrated in turn, and examples of the structures and analytical uses indicated. Some of the more recently developed dyes will also be mentioned. The use of basic dyes in analytical chemistry was reviewed by Fogg, Burgess and Thorburn Burns in 1971 (11), so specific references will only be given for more recent work, or to points omitted in the earlier review.

1.3.1 The Diphenylmethane Dyes

There are only two important commercial dyes in this category, Auramine O (Basic Yellow 2, C.I.41000) and Auramine G (Basic Yellow 3, C.I.41005). Neither of these dyes has apparently been used in the analytical field.
1.3.2 Triarylmethane Dyes

A. The Malachite Green Series

This series of dyes have the triphenylmethane structure and contain two amino or substituted amino groups. Important members of this series of dyes are Brilliant Green (Basic Green 1, C.I.42040) and Malachite Green (Basic Green 4, C.I.42000).

Both these dyes have been used in solvent extraction methods for a wide range of ions including, antimony, thallium, gallium, tantalum, rhenium, perchlorate, boron, gold and silver (11). Ramanauskas and Zhilenaite (12) have recently published a method for the determination of tellurium using various triphenylmethane dyes and two separate authors have investigated the use of Malachite Green and Brilliant Green for the determination of tin by extraction of a dye – halotin complex into benzene (13,14). Ramanauskas et al. have also devoted some attention to the use of Brilliant Green and Malachite Green for the determination of chlorite (15) and hypochlorite (16). The unknown ions were reacted with iodine in acid solution, liberating the tri-iodide ion,
which was extracted into benzene as the dye – tri-iodide complex.

Altmann et al. used Malachite Green to determine phosphates in water (17). The method is based on the measurement of the absorbance at 623nm., which is the peak wavelength of the Malachite Green – phosphomolybdate.

B. The Rosaniline Series

This series of dyes have the triphenylmethane structure and contain three amino or substituted amino groups. These dyes include Crystal Violet (Basic Violet 3, C.I.42555) shown in figure 35 later in this thesis, Methyl Violet (Basic Violet 1, C.I.42535) and Fuchsin (Basic Violet 14, C.I.42510). The addition of a further N-alkyl group produces a purple colour in contrast to the greens of the Malachite Green series.

These dyes have been used for the determination of antimony, thallium, gallium, tantalum, rhenium, perchlorate, boron, silver, chromium, nitrate, osmium and tin (11). Recently a series of papers have been published on the determination of phosphorus in various matrices using Crystal Violet (18,19,20). Ramanauskas et al. have published a further series of papers using these dyes for the determination of iodide and sulphide (21,22). Methyl Green also belongs to this class of dye. It contains three methylated amino groups, but one of them is trimethylated, so blocking resonance and creating the green colour.
It has been used principally by Lebedeva et al. for the determination of mercury by the extraction of a dye–metal halide complex into benzene (23, 24).

C. Other Triarylmethane Dyes

The main group of these dyes resembles the triphenylmethane dyes, except that one benzene ring has been replaced by a naphthalene group. This is the Victoria Blue series of dyes. Victoria Blue B (Basic Blue 26, C.I.44045) has been used for the determination of mercury by Pilipenko et al. (25) and Victoria Blue 4R (Basic Blue 8, C.I.42563) has been used for the determination of tellurium by the extraction of a dye–bromotellurate (26) and for the determination of perrhenate (27).

1.3.3 Xanthene Dyes

All these dyes are based on xanthene, and contain amino groups, meta with respect to the oxygen bridge. These dyes are discussed in detail in chapters three and four.

\[
\begin{align*}
\text{EtHN} &\quad \text{O} &\quad \text{EtHN} \\
\text{Me} &\quad \text{Me} &\quad \text{COOEt} \\
\text{Cl}^- &\quad &\quad &\quad \\
\end{align*}
\]

Rhodamine 6G

1.3.4 Acridine Dyes

These dyes are based on the parent compound acridine, which usually has an amino group or substituted amino group in the para position with respect to the methane carbon atom. An example of this type of dye is Acridine Orange R (Basic Orange 14, C.I.46005).

No basic dyes of this type have so far found application in analysis.
1.3.5 Azine Dyes

The Azine dyes are derivatives of the parent compound, dibenzopyrazine. It is to this class of dye that Mauve, the first commercial dye, synthesised by Perkin belongs. An example of this group which has found use in analysis is Safranine T (Basic Red 2, C.I.50240), which has been used for the determination of phosphate by the extraction of a phosphomolybdate (28).

1.3.6 Oxazine Dyes

These dyes, which are mainly blue are characterised by the oxazine chromophore, a benzene ring with two para carbon atoms replaced by a nitrogen and an oxygen atom. Of these dyes, Nile Blue A (Basic Blue 12, C.I.51180) and Capri Blue (C.I.51000) have been found to be useful to the analyst. Capri Blue was recommended as a reagent for boron by Skaar (29) and Nile Blue has been used for the determination of nitrate (30), tantalum (31), rhenium (32) and boron (33).
1.3.7 *Thiazine Dyes*

These are similar to the oxazine dyes, except that in the chromophoric group, a sulphur atom replaces the oxygen. This group consists of Methylene Blue and related compounds such as the Azure dyes. It is reviewed in detail in chapter two.

1.3.8 *Antipyrine Dyes*

In the last few years, the Russian workers have examined the Antipyrine dyes as analytical reagents. The use of these dyes and their derivatives has been reviewed recently by Akimov and Busev (34,35).

Antipyrine itself for example, has been used to determine copper, silver, magnesium, calcium, strontium, barium, zinc, cadmium, mercury, aluminium, scandium, indium, tin, lead, titanium, zirconium, thorium, bismuth, vanadium, chromium, uranium, manganese, iron, cobalt, nickel and palladium. This would seem to be a suitable area of study for future workers, as these dyes do not appear to have received any attention in the West. Although the versatility of Antipyrine would seem to
indicate that it is not very selective, this may not be true for all its many derivatives.

1.3.9 The Newer Cationic Dyes

In recent years, many dyes have been developed for use with synthetic fabrics. Because of the understandable reluctance of the manufacturers to divulge the structure of many of these dyes, they have been largely ignored as potential reagents. However, an example of the use of one of them, is the extraction and determination of indium (III) bromide with Astrazon Pink FG (Basic Red 13) into a benzene - methyl ethyl ketone mixture, by Popa, Patroescu and Costache (36).

1.4 Nomenclature relating to Dyes

Because of language problems, and variation in trade names, difficulties sometimes occur in the identification of dyes. In this country, the British Dyers and Colourists have produced a standard Colour Index, in which each dye is given a systematic name and a number which usually relates to its chemical composition where this is known (37). For example, Brilliant Green has the systematic name Basic Green 1 and the number C.I.42040. In this way dyes which are the same, but are manufactured by different companies and have different trade names can be identified.

Fogg, Burgess and Thorburn Burns (11) noted that in papers translated from Russian, a different nomenclature is used for xanthene dyes which could cause confusion. The dye known in these translations as Rhodamine S, for example, is known to us as Rhodamine B (C.I.45170), whilst the dye known as Rhodamine S (C.I.45050) in this country has a completely different structure.
1.5 Colour and the Measurement of Absorbance

The phenomenon of colour, is caused by the selective absorbance of certain wavelengths of white light, resulting in the transmitted or reflected light being deficient in certain components causing an object to appear coloured to the eye. Thus the wavelength at which the absorbance of the solution is measured will be that "colour" in which the solution appears to be lacking. Table one shows the relationship of the colour absorbed to the complimentary colour seen by the eye.

<table>
<thead>
<tr>
<th>Wavelength (nm.)</th>
<th>Colour Absorbed</th>
<th>Complimentary Colour seen</th>
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<tr>
<td>400-435</td>
<td>Violet</td>
<td>Yellow-green</td>
</tr>
<tr>
<td>435-480</td>
<td>Blue</td>
<td>Yellow</td>
</tr>
<tr>
<td>480-490</td>
<td>Green-blue</td>
<td>Orange</td>
</tr>
<tr>
<td>490-500</td>
<td>Blue-green</td>
<td>Red</td>
</tr>
<tr>
<td>500-560</td>
<td>Green</td>
<td>Purple</td>
</tr>
<tr>
<td>560-580</td>
<td>Yellow-green</td>
<td>Violet</td>
</tr>
<tr>
<td>580-595</td>
<td>Yellow</td>
<td>Blue</td>
</tr>
<tr>
<td>595-605</td>
<td>Orange</td>
<td>Green-blue</td>
</tr>
<tr>
<td>605-750</td>
<td>Red</td>
<td>Blue-green</td>
</tr>
</tbody>
</table>

Within the dye molecule, certain groups called chromophores are associated with colour. These are such groups as C=S, C=N, N=N, N=O, NO₂. Usually, the more unsaturated the molecule, the more the absorbance wavelength moves from the ultra-violet region to the infra-red region.

In addition, dyes also have groups known as auxochromes which
modify the colour and improve the affinity of the dye for the substrate. Such groups include the halogens, alkyl groups, and hydroxyl groups. If the shift in the absorbance wavelength caused by the auxochrome is towards a longer wavelength, it is known as a bathochromic shift, and the converse is known as a hypsochromic shift.

The measurement of light absorbed by solutions is governed by two laws. The first of these, a universal law, is known as **Lambert’s Law**. It states that when monochromatic light is passed through a transparent medium, the rate of decrease in intensity with the thickness of the medium is proportional to the intensity of the light.

The second law, which is only applicable to dilute solutions is the **Beer–Lambert Law**. This states that the light absorbed is proportional to the number of molecules of the absorbing substance (i.e. its concentration) through which the light passes. Thus if there is any interaction between the molecules in solution, such as dimerisation, the law will not hold. It may be expressed in the following form:

\[ A = \varepsilon c l \]

- **A** is the optical density
- **c** is the concentration in moles per litre
- **l** is the path length in centimetres
- **\( \varepsilon \)** is the molar extinction coefficient

In the case of an ideal solvent extraction basic dye method, the value of the molar extinction coefficient should be as near to 100,000 \(1\,\text{mol}^{-1}\text{cm}^{-1}\) as possible. This is the approximate theoretical maximum value as calculated by Braude (38) from considerations of the cross-sectional area of the absorbing electron systems.
1.6 **Solvent Extraction Procedures**

The object of using a solvent extraction procedure in these basic dye determinations is to provide a means of separating the complex of interest from the surplus dye reagent and unwanted matrix material. This is achieved by a suitable choice of organic solvent, and careful control of the conditions in the aqueous phase.

The anion to be determined is allowed to react with the basic dye in the aqueous phase to form an ion-associate which is soluble in the organic phase. In an ideal case, this ion-associate is then extracted completely into the organic phase in one extraction, and the unreacted dye is left behind in the aqueous phase. Thus the distribution coefficient of the former must be very high, and that of the latter must be very low.

The solvent of choice is said to depend mainly on the dye used (39). For triphenylmethane dyes, benzene is said to produce the best results, while for xanthene dyes, a mixture of benzene with either ether or acetone is said to be the most satisfactory. As a general tendency, a solvent with a high dielectric constant such as chlorobenzene will extract a complex better than one of low dielectric constant such as benzene. However, this tendency unfortunately applies just as much to the unreacted dye as it does to the complex of interest.

1.7 **Interfering Ions**

Although the basic dyes generally give very sensitive analytical methods, their selectivity is not always very good. Some selectivity can be achieved by the choice of medium (the type of buffer or acid used) in addition to the choice of dye. In general, an increase in the molecular weight of the dye cation will increase the solubility of the ion-associate in the organic phase but decrease the selectivity.
Triphenylmethane dyes are also said to be more selective than the xanthene dyes (40).

In some cases, selectivity within one group of the periodic table may be achieved by varying the oxidation states of the ions. For example, gallium, thallium and indium only react with Rhodamine dyes in the III oxidation state, but because of the varying stability of this oxidation state within the group, selectivity can be achieved by varying the type of oxidant used. Thus gallium, which is stable as gallium (III) but not as gallium (I), can be determined in the presence of thallium, which is stable as thallium (I) but less so as thallium (III), by suppressing the thallium in the single oxidation state by the addition of titanium (III) chloride.

It is to be regretted that many authors neglect to mention the effect of other ions on their determinations, or which is even worse, only mention those ions which do not interfere.

1.8 The Present Work

The research undertaken in this study, is a continuation of the work done by Burgess (40). His main field of study was the triphenylmethane dyes, which resulted in the development of several improved methods, for the determination of such ions as perchlorate, perrhenate, antimony and thallium.

This study has concentrated on an investigation into the thiazine dyes, particularly Methylene Blue and Taylor's Blue and the Rhodamine dyes, particularly Butyl Rhodamine B. Work has also been undertaken on the use of onium compounds as possible masking agents in basic dye systems. In addition, some of the more practical problems, which arise during analyses such as the effect of adsorption and the stability characteristics of reagents have been investigated. Finally, a short study was undertaken on the use of Crystal Violet for the determination of nitrate as both a spectrophotometric reagent and in an ion-selective electrode.
Chapter Two
The Use of Methylene Blue and Taylor's Blue in Analytical Chemistry

2.1 Introduction

Of all the basic dye analytical reagents, Methylene Blue (Basic Blue 8, C.I.52015) has probably found widest application. It was first produced by Caro in 1876. Two grades of the dye are in common use; the commercial dye, which consists of a double chloride of Methylene Blue and zinc and the medicinal grade of dye, which is the pure dye hydrochloride. It is this latter grade which is used by the analyst.

At the beginning of this century, Methylene Blue was in common use as a fabric dye, but like all traditional basic dyes, it has now largely been superceded by newer dyes which have been specially designed for use on modern synthetic fabrics. However, it still finds a limited use in the dyeing of mordanted cottons and silks because of its brightness, and also in the colouring of fats, waxes and oils.

In the medicinal field it has found application as an external paint for skin diseases, as an antimalarial agent, as an analgesic and as a weak antiseptic, but once again it has largely been superceded and is now only used for renal function tests as it is excreted unchanged by the kidneys. As a biological stain it still finds wide application. It is a selective stain, and is commonly used for the staining of pathogenic organisms such as tubercular and cholera bacilli. It is a standard reagent for the tuberculin testing of milk.

In the analytical field it is used both as a reagent and in conjunction with other dyes, as an indicator. An example of the latter is its use in conjunction with Varnish Scarlet C for the determination of calcium by titration with E.D.T.A. (41).
THIAZINE DYES

(Figure 1)

Methylene Blue
C.I. 52015

1,9 Dimethylmethylene Blue
(Taylor's Blue)
2.2 Analysis of Methylene Blue

Because the purity of a dye used for analysis is important, the main methods which have been used to analyse Methylene Blue will be discussed.

(1) Degree of Hydration

Although the British Pharmacopoeia quotes Methylene Blue as having two molecules of water attached, the exact degree of hydration is open to dispute. From one to five molecules of water have been quoted in various parts of the literature, but Wales and Nelson (42) claim that definite hydrates are not formed. The determination of the water content of Methylene Blue is difficult, firstly because the dye molecule itself decomposes easily on heating and secondly because part of the water seems to be very strongly bound to the dye. Attack claimed that at 105°C all the water was not expelled, but by 110°C the dye molecule started to decompose (43). On the other hand, Wales and Nelson claim that heating the sample to 110°C removes the water without sample decomposition. Maurina and Deahl also used a standard sample which was heated to 110°C for 18 hours in their work (44).

(2) Elemental Analysis

In this technique, one element present in the dye is determined and hence the total molecular weight is calculated; a method of analysis used by Maurina and Deahl (44). Sulphur is determined by fusion of the dye with a sodium – potassium carbonate mixture, oxidation of the sulphur to sulphate and finally precipitation of the sulphate with a barium salt. Nitrogen is determined by the standard Kjeldahl – Gunning technique. Chloride cannot be precipitated directly with silver nitrate but if it is first displaced from the dye by addition of perchlorate, a quantitative silver precipitation can be carried out.

These methods are convenient means of determining the purity of
dyes, especially in an organic laboratory which is equipped for such routine determinations. However, if any nitrogen-, sulphur-, or chloride-containing impurity is present, a false result will automatically be obtained.

(3) Gravimetric methods of Analysis

Such traditional methods are often popular, easily performed and believed to be reliable. However, basic dye salts are notoriously water-soluble to a greater or lesser extent.

Attack (43) suggested that the precipitation of Methylene Blue perchlorate from a neutral solution would be a reasonable method for the gravimetric determination of the dye, but noticed that hydrolysis of the complex tended to occur which produced low results. Maurina and Deahl (44) however, claimed that if the precipitate were washed with an ether–ethanol mixture (9 : 1) little hydrolysis occurred; this procedure was recommended by the U.S. Pharmacopoeia XIII (1970). Perhaps a sufficient comment on these methods is to remind the reader that a British explosives patent exists on basic dye perchlorates!! (45)

François and Seguin used a Methylene Blue picrate precipitation and claimed that satisfactory results could be obtained after a mere 10 ml washing with water (46). However, Maurina and Deahl showed that the results varied with the number of times the precipitate was washed, indicating a significant solubility of the picrate salt. These dye salts are also of doubtful stability.

In spite of the fact that Attack (43) had indicated that Methylene Blue dichromate underwent hydrolysis in water, Ferry (47) used it successfully as a gravimetric precipitate. After precipitation of the salt with 0.1 N potassium dichromate, the solid was filtered, washed with ice-cold water and dried at 110°C for 1 hour.

Probably the most reliable method is that of Mareš and Stejskal (48).
They used a 0.01 M solution of tungstosilicic acid to precipitate the dye as a Methylene Blue 12-tungstosilicate (3 : 1). This method was recommended by Burgess as being unlikely to precipitate impurities and was used successfully by him (40). The original method was also adapted for use as an amperometric titration method by Ogawa (49).

(4) Volumetric Methods

Undoubtedly the most widely used method of this type is the titration of the dye with titanium(III) chloride developed by Knecht (50) and based on the quantitative reduction of Methylene Blue to its leuco derivative. This procedure is experimentally inconvenient due to the need to exclude air during the course of the titration and because of the difficulties in the standardisation of the titanium(III) titrant. It is used as a standard method by which most new methods are evaluated and is the procedure recommended in the British Pharmacopoeia (1973).

Various versions of the iodine titration are also widely used. The official method of the Association of Official Agricultural Chemists consists of the titration of Methylene Blue with a solution of iodine in the presence of acetic acid which stabilises the precipitate formed, as a 1 : 5 complex (51). If sodium acetate is present, the complex ratio is 1 : 6 although Holmes found that to some extent the ratio was also affected by the amount of iodine in solution (52). Sabalitschka and Erdmann performed the titration in neutral solution, adding an excess of iodine solution and back titrating the excess iodine with thiosulphate (53).

The precipitation of Methylene Blue dichromate is also used as a volumetric method. Ferrey used the following method, determining the iodine in the final stage with thiosulphate in the usual way (47):

\[(\text{MB})\text{Cl} + \text{xS} \cdot \text{K}_2\text{Cr}_2\text{O}_7 = 2\text{KCl} + (\text{MB})_2\text{Cr}_2\text{O}_7 + \text{xS} \cdot \text{Cr}_2\text{O}_7^{2-}\]

\[\text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ + 6\text{I}^- = 2\text{Cr}^{3+} + 7\text{H}_2\text{O} + 3\text{I}_2\]

Davidson (54) determined the excess dichromate, remaining after
precipitation of the dye with Iron(II) ammonium sulphate. He also used
dichromate to determine an excess of Chromium(II) sulphate, which had been
used to reduce Methylene Blue to its leuco derivative.

Both the perchlorate precipitation method and the picric acid
precipitation method have been used volumetrically, but Maurina and
Deahl (44) claimed that neither had any advantage over the iodine method,
as particularly the latter had a very uncertain end-point.

Růžička and Ktoušek used a solution of ascorbic acid in 2N mineral
acid to reduce the dye to its leuco derivative. They used a platinum
electrode to follow the reaction and claimed that the method gave good
results (55).

Finally in this section, mention must be made of the technique of
titrating acid dyes with basic dyes. Both Crystal Scarlet (56) and
Naphthol Yellow S (7) have been used for this purpose, but it is felt that
whilst this is an interesting idea, it is of little practical use, because
acid dyes tend to be just as impure as basic ones. Therefore any
method of this type would have to be calibrated by a second method
for each new batch of acid dye reagent.

(5) Miscellaneous Analytical Methods

Mares and Stejskal (48) determined Methylene Blue amperometrically
with a dropping mercury electrode by titration with tungstosilicic acid
at 0.65 V against a saturated calomel electrode. The method was used
to determine Methylene Blue in the presence of glucose, as found in
Coloxyd S.P.O.F.A. injections.

Chromatographic methods for the determination of Thiazine dyes
are numerous and varied. By their very nature the determinations are
mainly qualitative. A recent review suggests a mixture of ethanol –
chloroform – acetic acid (85 : 10 : 5) on silica gel G as being a
useful medium (57)
The choice of an analytical method for the determination of this dye will largely depend on the facilities available and the required degree of accuracy and precision. For many purposes, a simple chromatographic test will suffice to indicate the presence of foreign coloured matter. Elemental analyses may also be convenient as various commercial laboratories specialise in this type of analysis and the sample can be simply sent along to them. However, the difficulties which may arise in the interpretation of the results has already been pointed out. Most of the volumetric and gravimetric methods seem to have some disadvantage such as solubility of the product, hydrolysis of the product, uncertain formulation of the complex etc. It seems that as Burgess (40) suggested, either the gravimetric version or the polarographic version of the tungstosilicic acid method is potentially the most useful.

2.3 The Spectrum of Methylene Blue

Methylene Blue is characterised by a major peak at 660 nm, with a shoulder peak at 610 nm. Lillie (5) tentatively ascribed the peak at 610 nm to an oxidation product of Methylene Blue, but most other authors such as Davidson (17) have ascribed it to the dimeric form of the dye.

Kirsten and Patel (58) showed that the addition of pyridine, a molecule of similar structure to the centre portion of the dye molecule, caused the shoulder at 610 nm to disappear. They postulated that a pyridine – Methylene Blue complex was formed, thus diluting the remaining dye molecules so that dimerisation did not occur.

A spectrophotometric method for the determination of Methylene Blue was proposed by Robertson (59) whereby Methylene Blue stock solutions were standardised against aqueous solutions of methyl thionine chloride which had been precipitated by dichromate. The necessary dilution of the unknown solution was determined by measuring its absorbance at 665 nm. In view of the dimerisation effect, this would seem to be an unreliable method. However, Stoutjesdik and Visser (60) stated that
dimerisation did not occur very readily in ethanolic solutions, so perhaps under these conditions a more reliable method could be developed.

2.4 Introduction to the Analytical Uses of Methylene Blue

Methylene Blue has numerous uses as an analytical reagent. Unlike many basic dyes, it is very light-fast; it is stable in acid solution and possesses comparatively stable leuco derivatives. However, compared with the triphenylmethane dyes it is not very selective and because of the drying problems which have been discussed previously, the concentration of any reagent solution is somewhat uncertain. Because the determination of inorganic ions is of prime interest, this literature survey concentrates on this area but some examples of organic and pharmaceutical determinations are given at the end.

Analyses using Methylene Blue fall roughly into four categories: those involving the formation of a neutral salt which may or may not be extracted into an organic solvent; those based on the reduction of the blue form of the dye to a leuco derivative; those based on the oxidation of the leuco derivative and a few miscellaneous methods.

Most attention will be given to the elements of group IIIb (Boron, Gallium, Indium and Thallium) and consequently this will be discussed first to illustrate the principles and problems which arise. Most of these determinations are solvent extractions.

2.5 Solvent Extraction Methods, based on the extraction of a Neutral
Methylene Blue - Anion complex into an Organic Solvent.

2.5.1 Methylene Blue as a Reagent for Boron

Boron is an element for which there is a serious lack of a good analytical method. According to Goward and Wiederkher (61), quinalizarin, curcumin and carminic acid all give molar absorptivities of about $2,000 \text{ l.mol}^{-1}\text{cm}^{-1}$ while Methylene Blue methods give values of about $75,000 \text{ l.mol}^{-1}\text{cm}^{-1}$, making a much higher type of extraction.
However, Methylene Blue methods are far from perfect: the blanks are high, there are many interferences, reagent concentrations and solvent purities are often critical and the procedures tend to be long and involved.

The method was first proposed by Ducret (62) in 1957. The boron-containing sample was digested in a polythene bottle with a mixture of ammonium bifluoride and hydrofluoric acid for 18 hours, the Methylene Blue added and the resultant complex extracted into dichloroethane. To prevent interference by Methylene Blue fluoride, the organic phase was washed with water. The absorbance of the organic phase was measured at 645 nm. and the method calibrated by the method of standard additions. The temperature had to be carefully controlled to give consistent results but in spite of all precautions, perchlorate, thiocyanate, permanganate and nitrate were found to cause considerable interference. Ducret recommended that the dichloroethane be distilled and only the fraction at 83 ± 1°C be used. Strizovic and Caldwell (63) however, found that it was sufficient to wash the solvent with sodium hydroxide solution, followed by sulphuric acid and finally water. By this procedure, molar absorptivities of 80,000 l.mol⁻¹.cm⁻¹ were obtained with aqueous samples and 75,000 l.mol⁻¹.cm⁻¹ with silica samples; the difference probably being either due to the extraction procedure or interfering elements present in the samples. However, absorbance blanks of about 0.33 were obtained in spite of the back-washing procedure.

Pasztor et al. (64) used the method outlined above to determine boron in steel samples. They concluded that the optimum pH for the aqueous phase was pH 1 which was a compromise between the optimum pH for dissolution of the steel (pH < 1.5) and the pH of maximum extraction (pH > 2.0). A calibration graph was used instead of the method of standard additions and the absorbance was measured at 660 nm. They concluded that the Methylene Blue boron tetrafluoride was a 1:1 complex.

Rosotte (65) pointed out that as iron catalyses the formation of
the BF$_4^-$ ion, the digestion time could be reduced to 30 minutes for steel samples. She described a procedure applicable to stainless steels and a pneumatic separation device for the separation of the two layers.

Vernon and Williams (66) dissolved steel in sulphuric acid and then added sodium fluoride which is safer than the dissolution in hydrofluoric acid which is very highly corrosive. They noted that the high blanks were not due to the extraction of Methylene Blue fluoride, as the size of the blank was not dependent on the fluoride concentration in the aqueous phase, but rather that the high blanks were due to the high sulphate concentration. They stated that the blank could be reduced to 0.14 by having ortho phosphoric acid in the aqueous phase instead of sulphuric acid, but stated that this was not possible when steel samples were analysed. Both Kammori et al. (67) and Bhargava et al. (68) employed hydrogen peroxide to speed up dissolution of the sample and oxidise the iron.

Klitina (69) used Methylene Blue to determine boron in titanium alloys but found that molybdenum and vanadium caused considerable interference. Methylene Blue has also been found to be a better reagent than triphenylmethane dyes for the determination of boron in uranium alloys, due to its stability at the low pH values necessary to suppress the hydrolysis of the UO$_2^{2+}$ ion (70).

Skaar (71) investigated the use of Methylene Blue for the determination of boron in plant materials and found that many metal ions interfered. In a later study of a series of oxazine and thiazine dyes for the determination of boron, he found that the dye Capri Blue (C.I.51000) gave better results (29). Stanton and McDonald (72) applied Methylene Blue to the determination of boron in soils and rock samples and in a similar study Weir and Jones (73) noted that the resultant complex was extremely stable, giving no change in absorbance over a period of 6 hours.
Methylene Blue is far from an ideal reagent for boron. The blanks tend to be high; the lowest value reported being 0.14 absorbance units. This is due to either extraction of the dye itself or to possible interference from foreign ions in the sample matrix. Indeed, many common ions extract under similar conditions to boron. The purity of the solvent is critical and the use in many cases of hydrofluoric acid to form the complex anion is not ideal because of the additional experimental care needed because of its dangerous properties. When applied to such matrices as glass, fertilisers and rocks etc., an iron catalyst must be added to enable the $\text{BF}_4^-$ ion to be formed quickly.

However, the method has also certain advantages. Compared with previous standard methods, based on curcumin, quinalizarin and carminic acid, it is more sensitive and less subject to interferences, which means that less pretreatment of the sample is needed. The complexes are stable and compared with other methods the procedure is fairly quick: about 6 samples can be analysed in two hours.

2.5.2 Methylene Blue as a reagent for Gallium, Indium and Thallium

Kish and Bukovich (74) studied the extraction of gallium as a Methylene Blue tetrachlorogallate from a sulphuric acid medium into organic phases which consisted of benzene and another solvent (ketone, ether or a nitro-compound). They obtained 80 - 89% extraction in a single operation and found Beer's law to hold from 0.1 - 4.0 ppm gallium. They found that metals in Groups I and II and zinc, indium and silver did not interfere. Tarayan et al. (75) studied the extraction of the tetrahalogallate complexes with a series of thiazine dyes and decided that the extraction of Methylene Blue tetrachlorogallate from a hydrochloric acid medium into a mixture of dichloroethane and trichloroethane was the best system. They obtained complete extraction in a single operation. Bagbanly et al. (76), in contrast, used a
mixture of chloroform - acetone (3 : 1) to extract the same complex, but found that a triple extraction was needed to obtain total recovery of the complex.

Kish et al. (77) also proposed a method for the determination of indium by the extraction of Methylene Blue tetra bromoindate from an acid medium into a mixture of benzene and nitrobenzene. The most serious interferences were caused by zinc, tin, aluminium and thallium.

A similar procedure was proposed by Tarayan et al. (78) for the determination of thallium. After oxidation of thallium(I) to thallium(III) with chlorine water, a Methylene Blue tetrachlorothallium(III) complex was extracted into a similar organic phase. The interferences were the same as for the indium determination.

On the face of the literary evidence, the methods for the determination of gallium, indium and thallium all appear to be good, giving high molar absorptivities and good recoveries. However, the size of the blanks is not mentioned except that Tarayan states that they are lowest for the extraction of gallium from a hydrochloric acid medium. This suggests that possibly the values may be high.

2.5.3 The determination of other ions as Methylene Blue - halogen complexes

Kish and Onishchenko (79) noted that Methylene Blue had never been tried as a reagent for antimony. They proposed a method whereby the Methylene Blue hexachloroantimonate complex was extracted from an aqueous phase containing sodium chloride in 9 M sulphuric acid into a benzene - nitrobenzene (5 : 1) mixture. They obtained molar extinction coefficients of 69,000 l.mol.⁻¹.cm⁻¹ and blanks of about 0.05 absorbance units. Interfering ions were Ga³⁺, Tl³⁺, Au³⁺, I⁻, SCN⁻, NO₃⁻.

Tarayan and Mikaelian (80) determined gold by extracting a tetrachloroaurate of Methylene Blue from a hydrochloric acid medium into a mixture of dichloroethane and trichloroethane. They claimed
an apparent molar extinction coefficient of 120,000 l.mol$^{-1}$cm$^{-1}$ but no mention is made of the size of the blank, or of possible interferences such as gallium, antimony, thallium etc. More recently, Ganchev and Dimitrova (81) have extracted gold into chloroform as a tetrachloroaurate – nitron complex, which was then given a colorimetric finish by shaking with Methylene Blue solution. They claimed that their procedure was sensitive and gave good recoveries. A procedure was suggested to remove the interference by thallium. A method has also been proposed for the determination of mercury as an iodo complex with the dye (82).

Onishi and Nagai (83) found that tantalum could be extracted from approximately 1 M sulphuric acid containing hydrofluoric acid, into dichloroethane. Beer's law was obeyed for 0.3 to 2.0 ppm. tantalum. Borate, perchlorate, and niobium were found to interfere, although the latter could be prevented by a pre-extraction procedure. The apparent molar extinction coefficient was found to be in the region of 100,000 l.mol$^{-1}$cm$^{-1}$, although the method suffers from high blanks (0.15 absorbance units). A mention was made of Methylene Blue as a reagent for tantalum by Tarayan et al. (84). She noted that the molar extinction coefficients could be increased from 60,000 to 90,000 l.mol$^{-1}$cm$^{-1}$ by the addition of oxalate to the aqueous phase. Even so, Methylene Blue as a reagent for tantalum compares unfavourably with Methyl Green (C.I.42585) which gives apparent molar extinction coefficients of 120,000 l.mol$^{-1}$cm$^{-1}$.

2.5.4 The Determination of Oxyanion complexes with Methylene Blue

Methylene Blue as a gravimetric reagent for perchlorate in the presence of chlorate was mentioned by Attack in 1915 (43), and was subsequently used for the determination of perchlorate in Chile Saltpetre (sodium nitrate)(85).
It was not until 1958, that a solvent extraction procedure was put forward by Boltz (86). The Methylene Blue perchlorate was extracted from a solution of pH 5 to 7 into chloroform. Five extractions were performed on each sample and after drying the combined extracts with anhydrous sodium sulphate, the absorbance was measured at 655 nm. Iwasaki et al. (87) investigated the method and found it very inadequate, having a low extraction yield, bad reproducibility and sensitive to even slight temperature variations. He and his colleagues put forward a similar method (88) using dichloroethane as the organic phase, which was more sensitive and gave more reproducible results. The aqueous phase was made slightly acidic with sulphuric acid and it was found that one extraction gave a blank of 0.054 absorbance units with 96% recovery. The organic solutions were stable over at least 24 hours. Many ions interfered, but they were removed by either back-washing with a Methylene Blue – sulphuric acid wash or masked with mercury(II). However, the following interferences proved unavoidable: chromate, tungstate, nitrite, nitrate, periodate, chlorate, selenate, cyanide, magnesium, strontium, silver, cadmium, lead and nickel.

Shortly after Boltz's work, Nabar and Ramachandran (89) published a spectrophotometric method whereby a known amount of Methylene Blue was used to precipitate the perchlorate in the sample, and the excess determined after filtration, by a direct absorbance measurement on the filtrate. A correction factor was applied to allow for the solubility of the dye perchlorate. This and the fact that each determination takes about four hours makes the method of little practical value.

Leitsin et al. (90) reported that a 1 : 1 complex of Methylene Blue perrhenate was formed in aqueous solution, but this has not apparently been adapted to analytical use.
2.5.5 Other Solvent Extraction Methods

Kochan and Protzenko (91) investigated the determination of cerium by extraction of a Methylene Blue - cerium(IV) complex from an alkaline aqueous phase into various organic solvents. Optimum conditions were given as pH 12.6, using benzene as the organic phase, and the absorbance was measured at 510 nm. They stated that Beer's law was obeyed for $5 \times 10^{-5}$ to $10^{-3}$ M cerium(IV) and that two moles of cerium(IV) reacted with each mole of dye. Goto and Kakita (92) came to a similar conclusion. However, Vernon (93) using radio-active cerium-141 discovered that the colour in the organic phase was not due to a cerium(IV) complex, but rather due to an oxidation product of the dye. Interferences noted were persulphate, permanganate, and chromate, which also oxidised the Methylene Blue and iron(II) which reduced the cerium(IV).

Recently, Kuroda et al. (94) have published a quantitative method for the determination of palladium based on the extraction of a palladium(II) - azide - Methylene Blue ternary complex into chloroform from an acetate buffered aqueous phase. The blank values were low, but molar extinction coefficients of only 58,000 l.mol$^{-1}$.cm$^{-1}$ were obtained. However, this value is said to compare favourably with other reagents for the metal. Predictably, gold, chromium, molybdenum, rhenium and the platinum metals interfere seriously.

The literature is seriously lacking in a good method for the determination of sulphate by a solvent extraction method. Ducret and Ratouis (100) produced an indirect method whereby the sulphate solution to be determined was passed through an ion-exchange column, quantitatively releasing thiocyanate ions which complex easily with Methylene Blue, and which can be extracted easily in the presence of acid into dichloroethane. However, the blank was extremely high (0.50 absorbance units) and the method was temperature dependent and sensitive to light. For these reasons alone it is of little practical use.
2.6 Determinations, based on the Reduction of Methylene Blue to its Leuco Derivative

These methods are of two types: those based directly on the quantitative reduction of Methylene Blue by the unknown ion and those based on the catalytic effect of an unknown ion on some other reduction system.

Nemodruk and Bezrogova (95) observed that Methylene Blue was reduced during irradiation with ultra-violet light in the presence of uranium and ethanol.

\[ \text{U (VI)} + \text{EtOH} \xrightarrow{h\nu} \text{U (IV)} \]

\[ \text{U (IV)} + \text{dye} \rightarrow \text{U (VI)} + \text{leuco-dye} \]

The solutions were deoxygenated by blowing carbon dioxide through them. There were many significant variables: pH, solution concentration, irradiation times and oxygen content. The overall error was stated to be 15%.

Both titanium and tin have been determined by reducing the metals to the titanium(III) and tin(II) states respectively with zinc in hydrochloric acid. The reduced metal solutions are then titrated in the absence of oxygen with standard Methylene Blue solutions, which they decolourise quantitatively (9). A similar means is used to determine molybdenum (96).

Goto and Hirayama (97) determined selenium by the addition of a solution of sodium sulphide, followed by Methylene Blue. The concentration of selenium was said to be proportional to the time taken for the solution to be decolourised at a fixed temperature.

Rao and Dutt (98) have used the catalytic effect of palladium on the reduction of Methylene Blue by sodium hypophosphite as a spot test for palladium. They claim that few other metals produce a similar effect.
2.7 Determinations, based on the Oxidation of Leuco Methylene Blue to the Blue form of the Dye

Iron, chromium and vanadium can be determined by procedures based on the oxidation of leuco Methylene Blue and the subsequent determination of the blue form of the dye by titration with titanium(III) chloride, as outlined above (9).

Leuco Methylene Blue, prepared by the action of sodium thiosulphate on Methylene Blue in an aqueous hydrochloric acid solution, may be used for the detection of peroxides which quantitatively oxidise the dye to its blue form again (99).

2.8 The Methylene Blue method for the Determination of Sulphide (fig. 2)

The formation of Methylene Blue was first used to estimate gaseous sulphide by Mecklenburg and Rosenkränzer in 1914 (101). This is an unusual method amongst Methylene Blue analyses in that it depends on the quantitative production of the dye by reaction of sulphide with p-amino-N,N-dimethylaniline or a similar reagent in the presence of iron(III) chloride rather than on the addition of Methylene Blue as a reagent. Much work has been done on these methods and they have been well-summarised by Boltz (86).

Among the more recent papers is one by Zutshi and Mahadevan (102) who absorbed the hydrogen sulphide in a calcium hydroxide suspension, and added the reagent in 1.5 M sulphuric acid, along with the iron(III) chloride. They measured the absorbance of the Methylene Blue produced at 749 nm., a peak which has an intensity about 20% greater than the peak at 675 nm., which is usually used. The method is also applicable to the determination of sulphide in water. Sinclair et al. (103) have recently used this method for the determination of sulphur in beer samples.
Determination of Sulphide as
Methylene Blue

(Figure 2)
2.9 Some Examples of Organic and Pharmaceutical Uses of Methylene Blue as an Analytical Reagent

(1) Determination of Picrates and Picrolonates

It has already been noted that Methylene Blue forms a complex with picric acid which is relatively insoluble in water. This property has been used by Bolliger (104) to devise a volumetric determination. The picrate is titrated with 0.001 N dye and the Methylene Blue picrate is extracted into chloroform, giving a green colour. Since Methylene Blue is not appreciably extracted into chloroform, the method is self-indicating.

(2) Determination of Saccharin and Salicylic Acid by salt formation with Methylene Blue

Shih and Teare performed an investigation into the salts formed between various organic acids and Methylene Blue (105). The complexes formed were extracted into dichloroethane. It was found that saccharin, salicylic acid and phthalic acid all extracted well. They went on, in a later paper (106) to specify a method of analysis for the former two. In neither case was a linear calibration curve obtained. Each determination took about 1.5 hours. Values for the molar extinction coefficients were 11,000 and 15,000 l.mol$^{-1}$.cm$^{-1}$ for salicylic acid and saccharin respectively, and the blanks were about 0.06 absorbance units. Neither method is very satisfactory when considered in terms of other basic dye analyses, but they compare very favourably with other methods, such as the iron(II) complex method for these ions, and they are at least partially selective.

2.10 Conclusions on the Published Methods of Analysis using Methylene Blue

Of all the basic dyes, probably Methylene Blue has the longest history as an analytical reagent in spite of the fact that in many
cases, it is far from ideal. It has a large tendency to give high blanks, especially with chlorinated solvents: a fact which many authors neglect to mention. It extracts a multitude of ions under similar conditions, and thus, involved procedures of pre-extraction, masking and back-washing must be used in order to eliminate the extraction of unwanted ions. It has a tendency to adsorb on glassware: this effect will be discussed more thoroughly in a later chapter. Like most other basic dyes, it can only be used in neutral or acidic conditions, as a pink form of the dye appears at pH values greater than seven. However, it is much more tolerant to strong acids than many basic dyes such as Brilliant Green. In volumetric methods, which are probably of less importance these days, it must always be pre-standardised as its exact composition with respect to water is not known.

However, unlike many basic dye solutions, Methylene Blue solutions are usually stable, both in aqueous solution and as dye complexes in organic solvents. It is also readily available in a sufficiently pure form.

2.11 Taylor's Blue (1,9 Dimethylmethylene Blue) (fig.1)

In 1969, Taylor and Jeffree developed a new thiazine dye, 1,9 Dimethylmethylene Blue (107) which they used as a histochemical stain. No instance of its use in chemical analysis has been found in the literature. It is for this reason that study of it has been made herein.

Experimental Work

2.12 Spectra of Methylene Blue and Dimethylmethylene Blue

The visible spectrum of Methylene Blue is well known, and well documented (54). It consists of a major peak at about 668 nm. with a shoulder at about 620 nm., which is less prominent in ethanol solutions than in aqueous solutions, due to a lesser proportion of the
Visible Spectrum of Taylor's Blue

Visible Spectrum of Methylene Blue
dimeric form of the dye (fig. 3).

Dimethylmethylene Blue (Taylor's Blue) in water consists of two peaks of almost equal size, one at 606 nm and one at 660 nm. However, in ethanol, the spectrum is similar to that of the parent dye, Methylene Blue, having a main peak at about 666 nm and a shoulder peak at 606 nm. It seems reasonable to assume that the peak at 606 nm is again due to the formation of a dimer (fig. 3).

However, unlike Brilliant Green where the change from the dimeric form to the monomeric form can cause experimental complications, as discussed in chapter six, no difficulties were experienced with the thiazine dyes, even though aqueous solutions were used.

2.13 The Formulae of the Dyes

The uncertainty in the hydration of Methylene Blue has already been referred to. Because a knowledge of the exact molecular weight of the dyes was not a critical factor in these experiments, it was assumed that the B.P.C. (1973) molecular weight of the dye, which allowed for two molecules of water was correct. It was also assumed, as the dyes had such a closely related structure, that the Dimethylmethylene Blue had a similar degree of hydration.

In fact, when samples of Methylene Blue were dried for 24 hours in an oven, the mean weight loss was found to be 8.2% at 110°C and 14.0% at 120°C, which roughly corresponds to 2 and 3 molecules of water respectively, assuming that no decomposition of the dye had occurred. No disagreeable smells from decomposition products which have been reported by some workers were observed.

2.14 Dye Solutions

It was found that when aqueous solutions of dyes, rather than the usual ethanolic solutions were used, the blanks tended to be slightly lower, and the Dimethylmethylene Blue had less tendency to precipitate in acid solutions.
Although the effect of dimerisation is more noticeable in aqueous solutions, this appeared to cause no difficulty in the cases studied, and the standard dye concentrations of 0.05% in water were used throughout. However, in the development of any new method, optimum dye concentrations should ideally be determined with both new and aged dye solutions, so that any anomalies of concentration, due to dimerisation of the dye reagent may be detected.

2.15 Buffer Systems for Methylene Blue and Dimethylmethylene Blue Extractions

Like many other basic dyes, it is difficult to find a suitable buffering system for Methylene Blue and Taylor's Blue. It is desirable to have a range of buffers from about pH 1 to pH 9, which cause neither enhancement nor suppression of the absorbance value of the complex to be determined, so that a true idea of the dependence of the extraction on pH can be obtained. Unfortunately, this appears rarely possible.

A large range of buffering systems has been examined for these dyes: two universal buffers and a large number of individual buffer systems, applicable over a limited pH range.

B.D.H. Universal buffer consists of the anions phthalate, acetate, carbonate, borate and phosphate. The results obtained for blank extractions are shown in figure 4. Clearly, for the pH range 2 to 5 some component of the buffer is forming an extractable complex with both dyes. It is believed from tests with the appropriate ions, that this is due to the phthalate and the acetate ions.

Similar results were obtained when individual buffer systems were used. Their effect on the extraction of Dimethylmethylene Blue perchlorate into chlorobenzene is shown in figure 5. Phthalate buffers had already been shown to cause blanks of the order of 0.4 absorbance units over the pH range 3 to 5, so they were not re-examined in this
Extraction of Thiazine Dyes into Chlorobenzene from B.D.H. Universal Buffer

(Figure 4)
Dimethylmethylene Blue – Perchlorate

Extraction into Chlorobenzene from Various Buffer Solutions

(Figure 5)
Extraction of Thiazine Dyes into Chlorobenzene from Barbituric Acid Buffers

(Figure 6)

Taylor's Blue

Methylene Blue
test. It can be seen from the shape of the curves that any apparent pH dependence is at least partially caused by buffer interference. It is also notable that all the buffers cause the results for the extraction of dye perchlorate complexes to be lower than when the extractions are performed from an unbuffered aqueous phase.

However, more useful results were obtained using the barbiturate universal buffer of Johnson and Lindsey (108) as shown in figure 6. It is believed that these low figures for the blank values represent little extraction of a buffer - dye complex. However, it will be shown that even these buffers cause some suppression when compared with solutions extracted from unbuffered aqueous solutions. It is these buffers which have been used in the further work undertaken.

An investigation was also carried out into the extraction of the dyes from strong acid solutions into chlorobenzene. Sulphuric acid was used for this purpose, as it was found in preliminary tests that the sulphate ion did not form an extractable complex with either dye. Neither dye was found to extract into the organic phase over the range 1 to 16 N sulphuric acid. However, Dimethylmethylene Blue formed precipitates over the complete range of acidities with sulphuric acid, and was subsequently noted, to do so, under other strongly acidic conditions. For this reason, it is not considered a useful reagent in such circumstances.

2.16 Extraction Tests on Various Anions with Taylor's Blue and Methylene Blue

Initially, qualitative tests were performed, using boiling tubes rather than separating funnels. Chlorobenzene (2 ml), buffer (2 ml), dye (0.2 ml, 0.05%) and the anion to be tested (about 5 mg solid of either the sodium or potassium salt) were shaken together in a test tube, allowed to settle and then the organic layer examined for any sign of
a blue colour indicating that the anion had formed an extractable complex. The following ions were tested with both dyes at pH 2.6 and at pH 7.0, and with Methylene Blue in 0.2 M and 6.0 M hydrochloric acid:

\[
\begin{align*}
F^-, Cl^-, Br^-, I^-, ClO_3^-, BrO_3^-, IO_3^-, ClO_4^-, IO_4^-, S^{2-}, S_2O_3^{2-}, \\
SO_4^{2-}, HSO_4^-, S_2O_8^{2-}, S_2O_3^{2-}, S_2O_4^{2-}, SO_3^{2-}, CO_3^{2-}, MnO_4^-, \\
WO_4^{2-}, MoO_4^{2-}, CrO_4^{2-}, Cr_2O_7^{2-}, SCN^-, Fe(CN)_6^{3-}, Fe(CN)_6^{4-}, HPO_4^{2-}, \\
H_2PO_4^-, B_4O_7^{2-}, BF_4^-, NO_3^-, NO_2^-, H_2AsO_4^-, P_2O_7^{4-}, ReO_4^-, \\
Acet^-, Phth^{2-}, Oxal^{2-}, Tart^{2-}, N_3^-.
\end{align*}
\]

For those anions which showed any sign of blue colour in the organic phase further tests, on a quantitative scale were performed and the results are summarised in table 2.

It can be seen from these results that few of the common anions extract in any appreciable quantity with these dyes under the conditions shown. A notable exception however, is the perchlorate ion with Taylor's Blue. This extraction was subsequently developed into a method for the determination of perchlorate.

Other Solvents as Extractants for These Dye Complexes

Two other solvents, chloroform and dichloroethane were found to give blanks which were too high for analytical methods. Benzene on the other hand, a solvent of comparatively low dielectric constant, gave zero blanks but did not extract any of the likely ions, such as perchlorate, perrhenate and periodate in any appreciable amount.

2.17 Development of a Method for the Determination of Perchlorate.

Based on the Extraction of Dimethylmethylene Blue Perchlorate into Chlorobenzene

Sodium perchlorate was used throughout as the standard for test solutions.
Table 2
The Absorbance Values Obtained when Various Anions were Extracted into Chlorobenzene as Methylene Blue and Dimethylmethylene Blue Complexes

Formulation: 8 ml barbituric acid buffer \( \rightarrow \) Extracted into 10 ml 
2 ml anion solution \( \rightarrow \) chlorobenzene 
1 ml dye (0.05% in water) \( \lambda = 650 \text{ nm} \).

<table>
<thead>
<tr>
<th>Anion</th>
<th>Conc. ppm</th>
<th>Taylor's Blue</th>
<th>Methylene Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 2.6</td>
<td>pH 7.0</td>
<td>pH 2.6</td>
</tr>
<tr>
<td>Cl\text{O}_4^-</td>
<td>100</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.368</td>
<td>0.340</td>
</tr>
<tr>
<td>I\text{O}_4^-</td>
<td>100</td>
<td>1.40</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.083</td>
<td>0.062</td>
</tr>
<tr>
<td>Mn\text{O}_4^-</td>
<td>100</td>
<td>0.028</td>
<td>0.042</td>
</tr>
<tr>
<td>I^-</td>
<td>100</td>
<td>0.084</td>
<td>0.058</td>
</tr>
<tr>
<td>CN^-</td>
<td>100</td>
<td>0.202</td>
<td>0.182</td>
</tr>
<tr>
<td>Re\text{O}_4^-</td>
<td>64</td>
<td>1.11</td>
<td>1.05</td>
</tr>
<tr>
<td>Phth\text{H}_2^-</td>
<td>100</td>
<td>0.074</td>
<td>0.032</td>
</tr>
<tr>
<td>Cr\text{O}_4^-</td>
<td>100</td>
<td>0.116</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cr\text{O}_7\text{O}_2^-</td>
<td>100</td>
<td>0.037</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NO_2^-</td>
<td>100</td>
<td>0.008</td>
<td>0.024</td>
</tr>
<tr>
<td>HPO_4\text{O}_2^-</td>
<td>100</td>
<td>0.048</td>
<td>0.032</td>
</tr>
<tr>
<td>Fe(CN)_3\text{O}_6^-</td>
<td>100</td>
<td>0.004 p</td>
<td>0.007 p</td>
</tr>
<tr>
<td>Fe(CN)_4\text{O}_6^-</td>
<td>100</td>
<td>0.013</td>
<td>0.015</td>
</tr>
<tr>
<td>BF_4^-</td>
<td>100</td>
<td>0.568</td>
<td>0.450</td>
</tr>
<tr>
<td>IO^-</td>
<td>100</td>
<td>0.028</td>
<td>0.058</td>
</tr>
</tbody>
</table>

p indicates that precipitation occurred
Optimisation of pH

The difficulties in finding an appropriate buffer system for these determinations has already been described. Therefore extractions at a range of pH values obtained with barbituric acid buffers were compared with a non-buffered system.

Method

Into a 100 ml separating funnel were placed perchlorate solution (2 ml, 10 ppm.), buffer or water (8 ml), and Dimethylmethylene Blue (1 ml, 0.05%). Chlorobenzene (10 ml) was added and the funnel shaken for 1 minute. The organic layer was removed and filtered through a Whatman number 41 filter paper into a 25 ml standard flask. The aqueous layer was extracted with a further 10 ml chlorobenzene which was filtered into the same flask. The combined extracts were made up to 25 ml with solvent and the absorbance measured at 650 nm, against a solvent blank, using silica cells.

Results

The results for the buffered systems are shown in figure 7. It will be seen that the optimum pH is about pH 3, when the absorbance of the complex solution is greatest and the blank smallest.

<table>
<thead>
<tr>
<th>System</th>
<th>$A_{\text{complex}}$</th>
<th>$A_{\text{blank}}$</th>
<th>$A_{\text{net}}$</th>
<th>$\epsilon \text{ (M}^{-1}\text{cm}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffered pH 3</td>
<td>0.273</td>
<td>0.018</td>
<td>0.255</td>
<td>32,000</td>
</tr>
<tr>
<td>Buffered pH 7</td>
<td>0.208</td>
<td>0.020</td>
<td>0.188</td>
<td>23,000</td>
</tr>
<tr>
<td>Water diluted</td>
<td>0.315</td>
<td>0.002</td>
<td>0.313</td>
<td>39,000</td>
</tr>
</tbody>
</table>

Conclusion

It can be seen from the results that the attempt to control pH by the addition of buffer solutions, appears to cause considerable suppression of the extraction. For this reason, further work was performed on aqueous solutions alone. If a buffering system is considered essential for any reason, then the optimum pH with this system is pH 3.0.
Extraction of Dimethylmethylene Blue-

Perchlorate into Chlorobenzene at Various pH Values

(Figure 7)
(2) **Shaking Time**

Experiments were performed using shaking times varying from 15 seconds to 4 minutes at each stage of the experiment. It was found that providing the shaking was vigorous, the time was not critical within these limits. A shaking time of 30 seconds for each stage of the extraction was used, therefore, as a standard for further work.

(3) **Stability of the Coloured Chlorobenzene Solutions to Light**

Solutions of Dimethylmethylene Blue perchlorate in chlorobenzene were exposed to light in the laboratory. They were found to be quite stable, undergoing a deterioration of only about 1% over a period of 2½ hours. Compared with many basic dye complexes, this is unusually stable. Nevertheless, it must be recommended that if the solutions need to be left for any length of time before their absorbance values are measured, it would be a wise precaution to store them in the dark, for greatest accuracy.

(4) **Job's Plot to find the Structure of the Complex**

**Method**

Appropriate amounts of sodium perchlorate and Dimethylmethylene Blue (both $1.0 \times 10^{-4}$ M) were placed in a separating funnel and extracted with 10 ml chlorobenzene. The absorbance of the extract was measured directly in 1 cm silica cells at 650 nm, against a solvent blank. As a check, the experiment was repeated using $0.4 \times 10^{-4}$ M solutions.

**Results**

The results obtained are shown graphically in figure 8.

**Conclusions**

The maximum absorbance was obtained using 5 ml perchlorate solution and 5 ml dye solution, which indicates a 1 : 1 complex. The slight non-symmetry of the curves is probably due to the uncertainty in the degree of hydration of the Dimethylmethylene Blue, resulting in the solutions being of a slightly incorrect concentration. Thus the complex
JOBS PLOTS

Equimolar Solutions of Taylor's Blue and Sodium Perchlorate

(Figure 8)
most probably consists of 1 molecule of Dimethylmethylene Blue and 1 molecule of perchlorate.

(5) Optimisation of Dimethylmethylene Blue Concentration

Assuming that the complex is 1 : 1 as shown above,

10 ppm perchlorate is \( 1.005 \times 10^{-4} \) M

5 ml of this solution will need:

\[ 1 \text{ ml of } (1.005 \times 10^{-4} \times 5) \text{ M dye solution} \]

\[ = 1 \text{ ml of } 0.0182\% \text{ dye solution} \]

Method

Perchlorate solution (5 ml, 10 ppm), water (5 ml) and Dimethylmethylene Blue (1 ml, various concentrations) were placed in a separating funnel and extracted with two 10 ml portions of chlorobenzene. After filtration, the combined organic extracts were made up to 25 ml and the absorbance was measured at 650 nm. as before.

Results

The results are summarised in figure 9

Conclusions

The standard solution of 0.05% dye is well within the semi-plateau region of the graph, and seems satisfactory for these extractions.

(6) Standard Deviation of the Method

Method

A series of extractions were performed as in method 6, except that standard 0.05% Dimethylmethylene Blue was used throughout, and the concentration of the perchlorate sample was 5 ppm.

Results

Absorbance values at 650 nm. for a series of independent extractions:

\[
\begin{align*}
0.403 & \quad 0.404 & \quad 0.403 & \quad 0.403 \\
0.405 & \quad 0.396 & \quad 0.398 \\
0.404 & \quad 0.412 & \quad 0.412 \\
\end{align*}
\]

\[
\bar{x} = 0.404 \quad \sigma = 0.0051 \quad \%\sigma = 1.26\%
\]
The Effect of Varying the Concentration of Dimethylmethylene Blue on the Absorbance of the Chlorobenzene Extract

(Figure 9)

![Graph showing the absorbance of the Chlorobenzene Extract against varying concentrations of Dimethylmethylene Blue. The graph demonstrates a 1:1 complex formation.]
Conclusion
The precision of this experiment compares well with other standard methods for perchlorate. This figure is about what would be expected for a good solvent extraction method, using a basic dye reagent.

(7) The Effect of the Source of the Chlorobenzene

Method
Into a clean 100 ml separating funnel was placed the perchlorate sample (5 ml), water (5 ml) and Dimethylmethylene Blue (1 ml, 0.05%). The mixture was extracted with two 10 ml portions of chlorobenzene which, after filtration, were combined and made up to 25 ml. The absorbance of the chlorobenzene solutions was measured at 650 nm. as before.

A separate Beer's law plot was obtained for each brand of chlorobenzene.

Results
The graphs obtained are shown in figure 10

Conclusion
Surprisingly, the less pure brand of chlorobenzene (Fison's 95%) gives higher absorbance values than the purer product (Koch-Light 99%). This must be due to an enhancement of the extraction properties by the impurities in the solvent. Because the type and quantity of impurities is not controlled by the manufacturer, it was decided to recommend the Koch-Light 99% chlorobenzene, in spite of the fact that it gave less extraction, as the results were more likely to be consistent from batch to batch.

(8) The Effect of Chloride and Chlorate on the Extraction

As it is often necessary to determine perchlorate in the presence of chloride and chlorate, it was necessary to see what effect these ions would have on the determination.

Method
Perchlorate solution (2 ml, 10 ppm), chlorate or chloride (2 ml of the required concentration added as the sodium salt), water (6 ml) and
Beer's Law Plot for DMMB-ClO₄⁺ from Aqueous Solutions into Two Different Grades of Chlorobenzene

(Figure 10)

- Fisons 95%
- Koch-Light 99%
dye (1 ml, 0.05%) were placed in a separating funnel. The solution was extracted with two 10 ml portions of chlorobenzene which were made up to 25 ml with fresh solvent, and the absorbance determined as before.

Results

<table>
<thead>
<tr>
<th>Ion added</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perchlorate</td>
<td>0.313</td>
</tr>
<tr>
<td>Perchlorate + chlorate (4000 ppm)</td>
<td>0.318</td>
</tr>
<tr>
<td>Perchlorate + chloride (4000 ppm)</td>
<td>0.238</td>
</tr>
<tr>
<td>Perchlorate + chloride (100 ppm)</td>
<td>0.307</td>
</tr>
</tbody>
</table>

Conclusion

Even a four hundred times excess of chlorate ion only causes a difference in absorbance of about 1% which is within the limits of experimental error and can thus be ignored. A similar four hundred times excess of chloride however, causes about a 25% decrease in the absorbance value and thus constitutes a serious problem. However, with only a ten times excess of chloride, the suppression is only about 2%.

(9) The Effect of Other Foreign Ions on the Extraction Method

Perchlorate solution (3 ml, 10 ppm), water (7 ml), dye solution (1 ml 0.05%) and the ion under test (about 10 mg solid, added as either the sodium or potassium salt) were placed in a separating funnel. The solution was extracted with two 10 ml portions of chlorobenzene which were made up to 25 ml and the absorbance determined as before.

Results

<table>
<thead>
<tr>
<th>Ion added</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.434 ± 0.006</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>0.436</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>0.178 (precipitation)</td>
</tr>
<tr>
<td>I$^-$</td>
<td>0.177 (precipitation)</td>
</tr>
<tr>
<td>F$^-$</td>
<td>0.408</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>0.348</td>
</tr>
</tbody>
</table>
## Conclusion

As was indicated by the difficulty in finding a suitable buffer system, many common anions interfere with the determination by suppressing the result when present in macro quantities, so any application of the method will be limited.

(10) **Final Comment**

The obvious course to prevent halide interference is the precipitation of the interfering ion with a silver or mercury salt. However, neither silver nitrate nor silver acetate which are the two most soluble common silver salts can be used because of interference by the anion. An attempt was made to use silver sulphate but it was not found to be sufficiently soluble to deal with a four hundred times excess of chloride, even when added as a solid. Due to the difficulty which it caused in the separation of the two phases, its addition was not considered worthwhile even to deal with small quantities of halide. Additionally, silver perchlorate is soluble in chlorobenzene to some extent and this provides another possible source of error. Various other solvents were tried, along with back-washing techniques, to try and eliminate the halide interference, but without success.

### 2.18 Recommended Procedure

The sample (5 ml), water (5 ml) and Dimethylmethylene Blue (1 ml, 0.05% in water) are placed in a 100 ml separating funnel. Chlorobenzene (10 ml, 99%) is added and the funnel shaken for 30 seconds. After separation of the two layers, the chlorobenzene layer is removed.
and filtered through a Whatman 41 filter paper into a 25 ml standard flask. A second extraction is performed with a further 10 ml chlorobenzene, which is filtered and combined with the first extract. The volume is made up to 25 ml with fresh solvent and the absorbance measured at 650 nm in 1 cm silica cells using a solvent blank. About 50% extraction and a molar extinction coefficient of 40,000 l.mol.⁻¹.cm.⁻¹ is obtained.

If it is thought necessary to use a buffer system, the initial 5 ml water, should be replaced by a similar quantity of barbiturate buffer at pH 3.0, but it must be remembered that due to suppression of the extraction, a new calibration plot will be required.

Similarly, if no buffer is required a 10 ml sample may be used, and no water added if this is allowed for during the calibration of the method.

2.19 Final Comments

As a potential reagent, Dimethylmethylene Blue has certain advantages over the parent dye and certain disadvantages. In many cases it gives a greater degree of extraction for common ions, but its chief disadvantage is its tendency to precipitate in acid solution and with certain other anions. However, both dyes produce complexes which are extremely stable even in the presence of sunlight which is an unusual feature for basic dyes.

The method which has been developed for the determination of perchlorate, compared with many Methylene Blue determinations has low blanks and a comparatively high recovery, but like the parent dye, its tolerance to foreign ions, as displayed by the difficulty in finding a suitable buffer system, is very low. Because of this, it must not be regarded as an absolute method but rather as a method which may be useful under certain limited circumstances. One possible application is in the determination of
the perchlorate content of commercial chlorate samples which are to be used for the production of pyrotechnics.
Chapter Three

The Preparation and Analytical Use of Butyl Rhodamine B, compared with Rhodamine B

3.1 Introduction

The Rhodamine group of basic dyes, with the exception of Rhodamine S and Rhodamine 5G, are all alkyl amino derivatives of fluorane (fig. 11).

![Fluorane](image)

Figure 11

Fluorane

The most prominent member of the group, both from the commercial and the analytical viewpoint is Rhodamine B (Basic Violet 10, C.I.45170), first prepared by Cérésole in 1887 and initially marketed by B.A.S.F. (109). Its major role as a fabric dye was found in the dyeing of silks, wools and tannin-mordented cottons, to which it imparted a fluorescent bluish-pink shade (110). It has been used for the colouring of paper, and has found several applications as a biological stain (5). In the past, it was also used to impart the characteristic pink colour to seaside rock and other foods, but this was discontinued some years ago, due to a suspicion that it may be carcinogenic (111).

The ethyl ester of Rhodamine B, known as Ethyl Rhodamine B or Rhodamine 3B (Basic Violet 11, C.I.45175) was first prepared by Monnet in 1891 (112). It is a bluer and more basic dye than Rhodamine B. Although it is of some slight interest to the analyst, it has not been produced commercially for some years.
Butyl Rhodamine B has no commercial history, and has only appeared in the literature with reference to its synthesis and its properties as an analytical reagent.

3.2 The Analytical Uses of Rhodamine B and Butyl Rhodamine B found in the literature

3.2.1 Rhodamine B

The analytical uses of Rhodamine B are numerous and have been summarised in several textbooks and reviews. A summary of spot tests and earlier methods has been given by both Welcher (9) and Feigl and Anger (10). The more recent work has been reviewed by Blyum and Oparina (11) and by Fogg, Burgess and Thorburn Burns (11). For this reason, only examples of the various determinations for which Rhodamine B has been used will be given here, along with details of the more recent papers which have been published on the subject.

In general, Rhodamine B and other xanthene dyes tend to give higher blanks than the other most commonly used group of dyes, the triphenylmethanes, when used in solvent extraction procedures. In spite of this apparent disadvantage, they are still widely used with varying degrees of success. Because of the comparative rigidity of the Rhodamine B molecule, discouraging the loss of absorbed energy by vibration and rotation, both absorption and fluorescent finishes to extraction procedures are possible.

By far the greatest number of papers on the analytical use of Rhodamine B has been concerned with the determination of antimony. The first paper appeared as long ago as 1921, when Eegriwe described how, after oxidation of antimony(III) to antimony(V) with nitrite, the addition of Rhodamine B produced a characteristic violet colour (114). This has been the basis of most subsequent determinations. The most recent papers have been based on the oxidation of antimony with cerium(IV)
followed by the extraction of a hexahaloantimonate—Rhodamine B complex from hydrochloric acid into an organic phase, followed by the measurement of the absorbance or fluorescence. Interfering elements have been removed by a variety of pre-treatments such as the co-precipitation of antimony with manganese dioxide and determinations have been published for such matrices as blood, urine, solder, semi-conductors, soils and rocks. One of the most recent papers liberates antimony from rock samples as stibine, which is absorbed into a mercury(II) chloride solution, oxidised with a cerium(IV) solution and finally extracted into benzene as a Rhodamine B—chloroantimonate complex (115).

Thallium and gallium are determined by a similar means to antimony, usually by extraction of the tetrachlorometal complex of Rhodamine B from an acid solution into an organic solvent. In the case of thallium the oxidation step is usually achieved with bromine—water or hydrogen peroxide, reflecting the greater stability of the thallium(I) state. In the case of gallium determinations, the interference of antimony and thallium is usually prevented by reducing them to lower oxidation states which do not extract with Rhodamine B, by the addition of titanium(III) chloride. The gallium is stable in the higher oxidation state and is unaffected by the treatment. Other halocomplexes which have been extracted with Rhodamine B include those of indium, tantalum and tin (11). Zine has been determined as a thiocyanate complex.

Gold has been determined as the oxynion complex with Rhodamine B. The procedures have all been based on that of MacNulty and Woollard (116), who after oxidation of the gold to the stable tervalent state, extracted the Rhodamine B complex into iso-propyl ether. Interferences, such as antimony, thallium, platinum, and vanadium were prevented by initial precipitation with manganese dioxide, with which they co-precipitated, leaving the gold still in solution. The procedure has been used for
the determination of gold in copper concentrates and ores.

Other metal ions which have been determined with Rhodamine B include mercury and iron for which procedures have been developed by Imai et al. (117,118). A procedure for phosphate has been developed by Kirkbright et al., where the Rhodamine B molybdophosphate complex is extracted into a chloroform - butanol mixture (119). Similarly, Golkowska has used Rhodamine B to determine traced amounts of silicon by the extraction of a Rhodamine B molybdisilicate into chloroform (120). After optimisation of the conditions, she obtained a procedure giving about 50% extraction (121).

The most recent work with Rhodamine B, has involved the formation and extraction of larger ternary complexes, unlike the simple sample or complex anions which have been used in the past. The greater selectivity thereby achieved has meant that the reagent has found application in the determination of the rare earth metals and others which are normally less easily determined. The first method to appear was the determination of uranium as a uranyl - benzoic acid - Rhodamine B complex, extracted into benzene, by Anderson and Hercules (122). Poluektov and Bel'ntyukova subsequently showed that, using a similar technique, improved selectivity could be obtained using nicotinic acid (123).

Pilipenko et al. have proposed a procedure for gadolinium, whereby the metal solution is extracted with salicylic acid and Rhodamine B from a medium at pH 6.2 into benzene, using a fluorimetric finish (124). Poluektov et al. have developed a procedure for the determination of lanthanum using 2,phenylquinoline-4-carboxylic acid and Rhodamine B (125). However, they admit that such elements as beryllium, zinc, aluminium, scandium, zirconium, hafnium, lead, bismuth, and copper interfere considerably, and several other metals are tolerable only in small amounts. In view of this, other similar methods which do not detail interferences should be regarded with suspicion. Neodymium has been
determined as a ternary complex by Toei and Nakato using 8-hydroxy-5,7-dinitro quinoline with Rhodamine B (126).

Of the transition elements, ternary complex methods have been published for zirconium, using benzoic acid and Rhodamine B and for scandium using 2-phenyloinchoninic acid and Rhodamine B, both by Bel'tyukova et al (127,128). In the former case, uranium, scandium, molybdenum, tungsten and iron are said to interfere but no details were given for the latter.

Other recent publications include a determination of ozone (129) with Rhodamine B, and by way of an example of the many organic determinations for which the dye is used, with which we are not principally concerned here, a method for the determination of cannabis (130).

3.2.2 The Esters of Rhodamine B

The ethyl ester of Rhodamine B has been used very little for analytical purposes, probably because it has only slight advantage over the parent compound, and it is difficult to obtain commercially in a sufficiently pure form. Methods have been published for the determination of indium (131), rhenium (132), and tellurium (133) using procedures similar to those using Rhodamine B itself.

However, in 1959 Kuznetsov and Bol'shakova prepared a series of Rhodamine B esters to determine which, if any, had better analytical properties than the parent acid, Rhodamine B itself. They reasoned that the esterification of the carboxylic acid group would produce, without too much difficulty, a dye with more affinity for organic solvents, thus giving greater extraction, whilst not greatly affecting the basicity of the molecule. Using the thallium tetrachloride ion for test purposes, they compared the n-butyl, isopropyl, n-octyl, and benzyl esters with Rhodamine B, and recommended the Butyl Rhodamine B, as it gave the highest selectivity and sensitivity (134). During the last ten years
there has been a succession of papers giving determinations for various
anions with this dye, all of which have come from the workers in
Eastern Europe.

Blyum and Shebalkova used the dye for the determination of
tantalum by the extraction of the fluorotantalate - dye complex from
6 M sulphuric acid into benzene and claimed that the method was very
selective (135). Pavlova and Blyum used a similar method, precipitating
interfering elements which formed fluoro complexes with ammonium
oxalate (136). They found that the presence of titanium, aluminium,
zirconium, and tungsten tended to give low results and that under certain
conditions, nitrate, iodide, bromide and chloride caused high blanks.
Noticing that niobium formed fluoroniobiate complexes with Butyl Rhodamine I
in a similar manner to tantalum, they also developed a method for the
determination of niobium in ores (137). Dorosh determined tantalum in
tartrate solutions stabilising his dye - complex solutions with acetone (138).

A method for the determination of tellurium in the presence of
selenium was proposed by Ivanova and Blyum in 1961, based on the
extraction of the bromo-complex of tellurium with Butyl Rhodamine B,
into benzene, with a photometric finish (139). However, a molar
extinction coefficient of only 35,000 l.mol⁻¹ cm⁻¹ was obtained and the
mean experimental error was said to be 10-15%. Vladimirova et al. used
a similar method with a fluorimetric finish, and found that extraction
with a mixture of benzene and butyl acetate gave higher sensitivity (140).
Alekseeva et al. used the chloro-complex of tellurium for the determination
of the metal in the dust from copper smelting works (141).

Butyl Rhodamine B methods have also been published for the
determination of antimony as a hexachloroantimony complex (142), gallium
as a tetrachlorogallium complex (143), indium as a tetrabromoindium
complex (144) and tin as a chlorostannate (145). In each case, an
extraction procedure is given for the determination of the metal in
ores. Amongst the oxyanion complexes, a method has been proposed for the determination of perchrenate with Butyl Rhodamine B (146) and its use for the determination of perchlorate has been investigated (147) but in this case, triphenylmethane dyes were found to be better. For the determination of arsenic an arsenomolybdate – Butyl Rhodamine B complex is precipitated by addition of ether to the aqueous phase. After separation of the phases, the addition of acetone to the organic phase dissolves the precipitate to form pink solutions. This is the basis of a method developed by Babko et al. in 1966 (148).

The most recent papers, have used a triphenylmethane dye, which is more selective, to extract the anion to be determined and then replaced it with Butyl Rhodamine B in order to give a low background level for the subsequent fluorimetric finish. Methods of this type have been developed for the determination of tantalum (149), mercury (150) and gold (151).

In reading through the various determinations with Butyl Rhodamine B it will be noted that the only samples which have been examined are of a geological nature. As Rhodamine B has been used successfully with numerous different matrices, there is no reason to suppose that the same will not be true of the butyl ester.

3.3 Experimental Work

The object of this work was to compare the extraction properties of Rhodamine B with Butyl Rhodamine B, for a series of simple ions and solvents. Much of this work was of a qualitative nature. Additionally, a more detailed investigation has been undertaken on the extraction and determination of a few selected anions.
3.4 Preparation of Butyl Rhodamine B

(According to the method of Kuznetsov and Bol'shakova (134))

Reagents

Rhodamine B (B.D.H. Spot-test reagent)
1-Butanol
1-Bromobenzene
Sodium hydroxide (pellets)
Hydrochloric acid (S.G. 1.18)

Apparatus  (figure 12)

Method

(a) Preparation of the Sodium Salt of Rhodamine B

Rhodamine B hydrochloride (50 gm, 0.105 moles) was dissolved in distilled water (200 ml) by heating on a water bath. The dark, rose-coloured solution was filtered through a hot Buchner funnel, containing a Whatman number 1 filter paper to remove any undissolved residue.
Preparation of Butyl Rhodamine B.

(Figure 12)

According to the method of Kuznetsov and Bol'shakova (134)
Sodium hydroxide (25 gm, 0.625 moles) was dissolved in distilled water (100 ml) and added to the hot solution. The deep pink sodium salt precipitated out immediately. It was filtered, washed with distilled water and dried in air.

(b) **Preparation of Butyl Rhodamine B**

Dry Sodium Rhodamine B (30 gm, 0.645 moles), n-butyl bromide (54 ml) and butanol (200 ml) were put into a flanged-topped reaction vessel, and heated under reflux on an oil-bath at 120°C for 12 hours. At the end of this period, the mixture was poured into an evaporating basin and the excess n-butyl bromide and butanol was removed by gentle heating, in a fume cupboard.

(c) **Preparation of Butyl Rhodamine B hydrochloride**

The thick oil, left after evaporation of the excess solvent was dissolved in hot water and acidified with concentrated hydrochloric acid (about 2 drops) until an acid reaction to Congo Red (red to blue) was obtained at pH 3. The syrupy product separated out above the water layer and was removed by decantation. The product was dried at room temperature for several weeks.

(d) **The Product**

The Butyl Rhodamine B prepared, consisted of deep raspberry-red crystals with a greenish-yellow fluorescence. The product was soluble in ethanol and acetone but not extremely so in water. The absorbance maximum was at 560 nm, compared with Rhodamine B at 555 nm. Analysis of the product by paper chromatography, showed that it was free from Rhodamine B and the sodium salt of Rhodamine B. The chromatographic method used is described in the next chapter.

(e) **Elemental Analysis of the Product**

When the product was sent away for analysis to Manchester University,
Department of Chemistry, no trace of chloride was found, and it appeared that the product existed as a bromide rather than a chloride.

### Theoretical Results for Chloride vs. Bromide

<table>
<thead>
<tr>
<th>Atom</th>
<th>M.Wt.</th>
<th>%</th>
<th>M.Wt.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>384.35</td>
<td>72.0</td>
<td>384.35</td>
<td>66.3</td>
</tr>
<tr>
<td>H</td>
<td>39.0</td>
<td>6.7</td>
<td>66.3</td>
<td>99.9</td>
</tr>
<tr>
<td>O</td>
<td>48.00</td>
<td>9.0</td>
<td>48.00</td>
<td>8.3</td>
</tr>
<tr>
<td>N</td>
<td>28.02</td>
<td>4.8</td>
<td>28.02</td>
<td>4.8</td>
</tr>
<tr>
<td>Cl</td>
<td>35.46</td>
<td>6.7</td>
<td>79.22</td>
<td>13.8</td>
</tr>
</tbody>
</table>

**Total**

| Chloride   | 535.14 | 100.2|
| Bromide    | 579.60 | 99.9 |

**Analysis Results**

<table>
<thead>
<tr>
<th>Atom</th>
<th>%</th>
<th>Molar ratio</th>
<th>Number of Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>63.6</td>
<td>63.6 / 12 = 5.30</td>
<td>32</td>
</tr>
<tr>
<td>H</td>
<td>7.0</td>
<td>7.0 / 1 = 7.00</td>
<td>41</td>
</tr>
<tr>
<td>N</td>
<td>4.7</td>
<td>4.7 / 14 = 0.335</td>
<td>2</td>
</tr>
<tr>
<td>Br</td>
<td>12.8</td>
<td>12.8 / 80 = 0.160</td>
<td>1</td>
</tr>
</tbody>
</table>

It will be seen that for the Butyl Rhodamine B Bromide, the carbon, nitrogen and bromide content is correct, and only slight variation in the hydrogen content is present. The remaining unaccounted for percentage, is made up of the oxygen known to be present (3 atoms, 7.5%), some sodium as detected by a flame test and possibly some water. Thus the sample is seen to be at least 95.6% pure, assuming that it is Butyl Rhodamine B bromide.

### 3.5 Reagent Solutions of Rhodamine Dyes

#### 3.5.1 Stability of the Dye Solutions

When concentrated aqueous dye solutions (0.05%) were diluted to the point where their absorbance values could be measured (0.0005%) they attained a maximum absorbance value immediately and did not display the
strange dilution effects of Brilliant Green, which are described in chapter six. In the case of aqueous solutions of Rhodamine B this was 95% of the theoretical value, and in the case of aqueous solutions of Butyl Rhodamine B, 93% of the theoretical value. When diluted with ethanol, the values were 70% and 63% respectively. The lesser values obtained with Butyl Rhodamine B may have been due to the fact that the dye solutions had not completely dried out at the time the absorbance values were measured. If kept in the dark, both aqueous and ethanolic solutions were stable over a period of 2 hours but in the light, the ethanolic solutions deteriorated 11% and the aqueous solutions, 5%.

In practice, dilute solutions are never allowed to stand this long, and over the length of time of an average experiment (10 minutes) the effect is slight and not likely to affect the results.

When old aqueous solutions of Butyl Rhodamine B were diluted with buffer solutions from 0.05% to 0.0005%, solutions which were stable in the dark over a period of four days were obtained over the pH range 1 to 9, but above this value (at pH 11) deterioration over the four day period amounted to 15%. However, over the short term the deterioration was slight and in any case, extractions are rarely done at such a high pH value.

3.5.2 Spectra of Rhodamine B and Butyl Rhodamine B (shown in fig. 13)

Apart from a slight shift in the absorbance maximum from 555 nm. in the case of Rhodamine B, to 560 nm. in the case of Butyl Rhodamine B, the spectra for the two dyes in both water and ethanol are remarkably similar. The main peak is thought to be that of the R+ form of each dye. When the solutions are acidified, a new series of peaks occur which are characteristic of the orange RH2+ form of the dyes. These phenomena have been discussed fully by Ramette and Sandell in a paper on the equilibria of Rhodamine B (152).

However, it was found that when the dyes were dissolved in acetone
Spectra of Rhodamine Dyes in Various Solvents (Figure 13)

1. Rhodamine B

2. Butyl Rhodamine B
the spectra were very different. In the case of Rhodamine B, the spectrum closely follows that in hydrochloric acid, whilst in the case of Butyl Rhodamine B it is similar to that of the aqueous and ethanolic solutions. This is thought to be a consequence of the difference in basicity of the two dyes.

Because of the unprotected carboxylic acid group, Rhodamine B is the less basic of the two dyes and therefore, has a greater affinity for protons and other positive species. When dissolved in acetone, it is believed that the polar carbonyl group in some way reacts with the dye cation either to form a new compound or loose ion-associate, which blocks the resonance in one benzene ring in a similar manner to the addition of a proton in the $\text{RH}^2+$ form found in acid solution, thus producing a similar species. It is, in fact, more probable that the $(\text{R}^+ - \text{acetone})$ species is an ion-associate, as the addition of water to the solution quickly regenerates the characteristic pink colour of the $\text{R}^+$ form of the dye.

Butyl Rhodamine B is more basic than its parent acid, and has therefore less affinity for positive species. Thus acetone, is not sufficiently polar to react with it, and the spectrum is characteristic of the pink $\text{R}^+$ form rather than the $\text{RH}^2+$ form of the dye.

3.6 Extraction of Rhodamine B and Butyl Rhodamine B dyes, themselves into Various Solvents.

A series of aqueous phases were used as being typical of those used for basic dye extractions and for Butyl Rhodamine B extractions in particular, the conditions for which are shown in table 3. The observations were of a qualitative nature, as this was sufficient to determine whether any significant amount of dye was extracting into the organic phase. The proportions of buffer, dye solution and solvent were chosen as typical of an analytical extraction.

Method

Barbiturate buffer or hydrochloric acid (2 ml), the solvent under test (2 ml...
and the dye solution (0.2 ml, 0.05%) were placed into a test tube and shaken for 30 seconds. After being allowed to settle, the organic phase was inspected for pink colouration.

**Results**

These are summarised in table 4.

**Conclusions**

Butyl Rhodamine B is much more restricted in the solvents which are ideal for anion extraction than is Rhodamine B. This is due to the tendency for most solvents, to extract the dye in significant amounts, thus leading to high blanks.

The only solvents which were found to be suitable over the whole pH range were carbon tetrachloride, cyclohexane and n-heptane, which are all non-polar with dielectric constants in the region of 2.0. Ether (dielectric constant = 4.34) extracted Butyl Rhodamine B only with the buffered systems.

The use of toluene and benzene for the extraction of thallium and gallium by the Russian authors, would thus appear to be by no means ideal but the use of carbon tetrachloride for the antimony extraction seems valid.

Xylene, which only extracts Butyl Rhodamine B slightly, and does not extract Rhodamine B at all, is not a good solvent to use, because a constant isomeric composition is not guaranteed. For further study it was decided to use cyclohexane and ether.

3.7 Extraction of Various Simple Anions into Organic Solvents with Rhodamine B and Butyl Rhodamine B

**Method**

For the initial qualitative work, the same method was used as in the previous experiment, except that solid anion samples were added in about 10 mg amounts of the sodium or potassium salt. For those
### Table 3

**Extraction Conditions for Butyl Rhodamine B**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Acidity</th>
<th>Solvent(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>arsenic</td>
<td>$0.3 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene, toluene</td>
<td>148</td>
</tr>
<tr>
<td>tin</td>
<td>$8.5 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene</td>
<td>145</td>
</tr>
<tr>
<td>thallium</td>
<td>$2.0 \text{ N } \text{HCl}$</td>
<td>toluene</td>
<td>134</td>
</tr>
<tr>
<td>gallium</td>
<td>$6.0 \text{ N } \text{HCl}$</td>
<td>toluene, benzene</td>
<td>143</td>
</tr>
<tr>
<td>mercury</td>
<td>$12.0 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene</td>
<td>150</td>
</tr>
<tr>
<td>tellurium</td>
<td>$10.0 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene - butyl acetate</td>
<td>140</td>
</tr>
<tr>
<td>gold</td>
<td>$0.5 \text{ N } \text{HCl}$</td>
<td>benzene</td>
<td>151</td>
</tr>
<tr>
<td>antimony</td>
<td>$8.0 \text{ N } \text{HCl}$</td>
<td>carbon tetrachloride</td>
<td>142</td>
</tr>
<tr>
<td>niobium</td>
<td>$10.0 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene</td>
<td>137</td>
</tr>
<tr>
<td>tantalum</td>
<td>$12.0 \text{ N } \text{H}_2\text{SO}_4$</td>
<td>benzene</td>
<td>135</td>
</tr>
</tbody>
</table>
### Table 4

Extraction of Rhodamine B and Butyl Rhodamine B into Various Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rhodamine B</th>
<th></th>
<th>Butyl Rhodamine B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8M HCl</td>
<td>3M HCl</td>
<td>pH 2.6</td>
<td>pH 7.0</td>
</tr>
<tr>
<td>Ether</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Isopropyl Ether</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Benzene</td>
<td>0</td>
<td>0</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Toluene</td>
<td>0</td>
<td>0</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Xylene</td>
<td>0</td>
<td>0</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>m</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>s</td>
<td>2</td>
<td>2</td>
<td>d</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>d</td>
</tr>
<tr>
<td>Dibromomethane</td>
<td>0</td>
<td>3</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Dichloroethane</td>
<td>3</td>
<td>4</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>2</td>
<td>4</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>o-Dichlorobenzene</td>
<td>1</td>
<td>3</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>1</td>
<td>1</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>0</td>
<td>0</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0</td>
<td>4</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>Isobutyl methylketone</td>
<td>0</td>
<td>4</td>
<td>d</td>
<td>d</td>
</tr>
</tbody>
</table>

**Key:**
- 0 - No visible extraction
- 1 - Faintest indication of colour
- 2 - Small amount of colour
- 3 - Large amount of colour
- 4 - Complete extraction of dye from aqueous phase
- d - Decolourisation of aqueous phase, no extraction
- m - Phases miscible
- s - Very slow phase separation
Table 5
Dye – Anion Complex Extraction with Rhodamine Dyes

(1) Complexes which extract into Chlorobenzene

<table>
<thead>
<tr>
<th>Anion</th>
<th>Rhodamine B</th>
<th></th>
<th>Butyl Rhodamine B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8M HCl</td>
<td>3M HCl</td>
<td>pH 2.6</td>
<td>pH 7.0</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IO₄⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S₂O₅²⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MnO₣⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CrO₄²⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cr₂O₇²⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SCN⁻</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

(2) Complexes which extract into Ether

<table>
<thead>
<tr>
<th>Anion</th>
<th>Rhodamine B</th>
<th></th>
<th>Butyl Rhodamine B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8M HCl</td>
<td>3M HCl</td>
<td>8M HCl</td>
<td>3M HCl</td>
</tr>
<tr>
<td>I⁻</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>BrO₃⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>S₂O₅³⁻</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>CrO₄²⁻</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cr₂O₇²⁻</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>ReO₄⁻</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: see table 4
Table 6
Extraction of Various Anions at the 100ppm level into Ether

<table>
<thead>
<tr>
<th>Dye (0.05%)</th>
<th>Anion</th>
<th>Acidity of the Aqueous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(100 ppm)</td>
<td>1M HCl</td>
</tr>
<tr>
<td>Butyl</td>
<td>Cr(<em>{2}O</em>{7}^{2-})</td>
<td>1.165</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>Cr(<em>{4}O</em>{4}^{2-})</td>
<td>0.915</td>
</tr>
<tr>
<td></td>
<td>Cl(_{4}^{2-})</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>SCN(^{-})</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>I(^{-})</td>
<td>0.098</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>Cr(<em>{2}O</em>{7}^{2-})</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>Cr(<em>{4}O</em>{4}^{2-})</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Cl(_{4}^{2-})</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>SCN(^{-})</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>I(^{-})</td>
<td>0.158</td>
</tr>
</tbody>
</table>
### Table 7

**Extraction of Chromate and Dichromate at the 10 ppm. level into Ether**

<table>
<thead>
<tr>
<th>Dye (0.05%)</th>
<th>Anion</th>
<th>Acidity</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl</td>
<td>Cr₂O₇²⁻</td>
<td>1M HCl</td>
<td>0.152</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>CrO₄²⁻</td>
<td>1M HCl</td>
<td>0.126</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>Cr₂O₇²⁻</td>
<td>1M HCl</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>CrO₄²⁻</td>
<td>1M HCl</td>
<td>0.018</td>
</tr>
</tbody>
</table>

### Table 8

**Extraction of Various Anions into Cyclohexane**

<table>
<thead>
<tr>
<th>Dye (0.05%)</th>
<th>Anion</th>
<th>Absorbance Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(100 ppm)</td>
<td>1M HCl</td>
</tr>
<tr>
<td>Butyl</td>
<td>NO⁻</td>
<td>-</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>SCN⁻</td>
<td>0.004</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>NO₂⁻</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SCN⁻</td>
<td>0.006</td>
</tr>
</tbody>
</table>
anions which showed any sign of extracting under these conditions of excess, further quantitative tests were performed by using 5 ml test anion solution (100 ppm), 5 ml buffer or acid, 1 ml dye and performing a double 5 ml extraction with the appropriate organic solvent. This combined extract was diluted to 25 ml with fresh solvent and the absorbance measured as before.

Results

The following anions were tested and the results for those giving any positive extraction are shown in tables 5 to 8:

**Anions Tested**

- $F^-$, $Cl^-$, $Br^-$, $ClO_3^-$, $BrO_3^-$, $IO_3^-$, $ClO_4^-$, $IO_4^-$, $AsO_2^-$
- $S_2^-$, $S_2O_3^-$, $HSO_4^-$, $SO_4^{2-}$, $S_2O_6^{2-}$, $S_2O_7^{2-}$, $SO_3^{2-}$, $CO_3^{2-}$
- $HCO_3^-$, $MnO_4^-$, $WO_4^-$, $CrO_4^{2-}$, $Cr_2O_7^{2-}$, $Tart^-$, $Oxal^-$, $Phth^-$
- $SCN^-$, $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, $MO_4^-$, $H_2PO_4^-$, $HPO_4^{2-}$, $B_4O_7^{2-}$
- $BF_4^-$, $Cit^-$, $NO_2^-$, $NO_3^-$, $HAsO_4^-$, $Ac^-$, $ReO_4^-$, $SeO_4^-$

Conclusions

Of all the anions tested in the ether series, including some which could be expected to extract and others which were unlikely to extract, only chromate and dichromate seemed sufficiently promising to test at the 10 ppm level and at an acid concentration of 1 M (Hydrochloric acid) which seemed from the results reported in table 4 to be near the optimum. When the molar extinction coefficients were calculated, they were found to be $16,400$ $1.\text{mol}^{-1}.\text{cm}^{-1}$ for the dichromate ion and $7,000$ $1.\text{mol}^{-1}.\text{cm}^{-1}$ for the chromate ion. As chromate is converted to dichromate in acid solution, it is reasonable that the solution added as chromate will have a molar extinction coefficient about half that of the dichromate solution. Neither of these values is sufficiently high for an analytical method. Although the blank is negligible,
the recovery is too low. However, further optimisation of the conditions, later yielded more promising results. These will be discussed shortly.

When cyclohexane was used as the organic phase, neither of the ions tested quantitatively, extracted sufficiently to be of any use (table 8).

3.8 An Experimental Assessment of the Determination of Perrhenate with Butyl Rhodamine B, according to the method of Blyum and Dushina (146)

This method was published in 1962, and it was claimed that the method was very sensitive and suitable for the determination of rhenium in ores. As the present work is not concerned with the extraction of the anion from the raw material, the sample solutions were made up in pure water. For ease of dissolution, the Butyl Rhodamine B solutions were made up in ethanol rather than in water, as used by the previous authors.

Reagents

Sulphuric acid, 5.0 M
Phosphoric acid, 6.0 M
Butyl Rhodamine B, 0.1% in ethanol
Potassium perrhenate, 6.214 μg/ml (ie 4 ppm. ReO₄⁻) in water
Benzene, A.R. grade

Procedure

Into a separating funnel were placed 5 ml acid, 5 ml perrhenate solution, and 1 ml Butyl Rhodamine B solution. The mixture was extracted with one portion of 10 ml benzene, which after removal from the aqueous phase was filtered through Whatman 41 filter paper and made up to 25 ml in a standard flask with fresh benzene. The absorbance was measured at 565 nm in 1 cm silica cells against
Extraction of Butyl Rhodamine B - Perrhenate into Benzene

(Figure 14)

Phase Ratio 5:2

Phase Ratio 1:1
a solvent blank. For the experiments where the phase ratios were varied, the aqueous phase was diluted to an appropriate volume with a 50:50 mixture of the acid and distilled water, before extraction.

Results

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Acid</th>
<th>Absorbance</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample (4 ppm)</td>
<td>blank</td>
<td>net</td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>sulphuric</td>
<td>0.658</td>
<td>0.180</td>
<td>0.478</td>
</tr>
<tr>
<td>2nd</td>
<td>sulphuric</td>
<td>0.331</td>
<td>0.163</td>
<td>0.168</td>
</tr>
<tr>
<td>1st</td>
<td>ortho-phosphoric</td>
<td>0.912</td>
<td>0.042</td>
<td>0.870</td>
</tr>
<tr>
<td>2nd</td>
<td>ortho-phosphoric</td>
<td>0.107</td>
<td>0.037</td>
<td>0.070</td>
</tr>
</tbody>
</table>

from these figures, the following data can be deduced, for a single extraction:

<table>
<thead>
<tr>
<th>Acid</th>
<th>Experimental</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>ortho-phosphoric</td>
<td>40,500</td>
<td>40,000</td>
</tr>
<tr>
<td>% extraction</td>
<td>92%</td>
<td>96%</td>
</tr>
<tr>
<td>sulphuric</td>
<td>22,250</td>
<td>30,000</td>
</tr>
<tr>
<td>% extraction</td>
<td>65%</td>
<td>70%</td>
</tr>
</tbody>
</table>

The Beer's Law plots obtained, for the Butyl Rhodamine B perrhenate system for two different phase ratios are given in figure 14.

Observations and Conclusions

The original authors recommended that the organic phase be centrifuged and allowed to stand for two hours before measurements of absorbance were made. Using pure aqueous samples, this was not found to be necessary. After filtration of the organic phase through Whatman number 41 filter paper, it was found that 10 minutes was ample time to allow for full colour development. There seems no reason why this simplified technique should not be applicable when ore samples are used.
It can be seen from the results, that although reasonable agreement with the quoted data is obtained with the ortho-phosphoric acid system, some variation occurs when the sulphuric acid medium is used. This may be either due to optimism on the part of the previous authors or variation in the quality of the reagents or laboratory conditions.

From the Beer's Law plots in figure 14, it can be seen that the best phase ratio is 1:1, which gives the best extraction and the more linear plots for both acid systems.

However, of the two media recommended by the authors, it is clear that only an aqueous phase which is 3.0 M in ortho-phosphoric acid gives useful results. For the sulphuric acid medium, the blanks are high and the molar extinction coefficients are comparatively low. In contrast, the ortho-phosphoric acid system gives linear plots over the range 0 to 20 µg perrhenate in the 5 ml sample, the blank is low (0.065 absorbance units) and the recovery good, even for a single extraction. It is therefore only this system which can be recommended.

3.9 An experimental Assessment of the Determination of Gallium with Butyl Rhodamine B according to the method of Skrebkova (143)

This method, published in 1961, was one of the first applications of Butyl Rhodamine B in analytical chemistry. Like the parent acid, Rhodamine B, the butyl ester is stable in a coloured form in strong acid solution, and thus can be used successfully in the determination of gallium as the tetrachlorogallate. The ester has the advantage of extracting the complex into toluene to a high degree, unlike Rhodamine B itself, for which an ideal solvent system for the extraction of the tetrachlorogallate has yet to be discovered. Selectivity, with respect to other metals in the same group is achieved by the addition of titanium(III) chloride solution, which reduces these metals to an unreactive lower oxidation state without affecting the oxidation state of the gallium. As before, only pure aqueous solutions were studied.
Reagents
Gallium solution, 0.77 ppm in 6.0 M hydrochloric acid, made by dissolving gallium metal in hydrochloric acid (S.G. 1.18)

Hydrochloric acid, 6.0 M
Titanium(III) chloride, 20% in 6M hydrochloric acid
Butyl Rhodamine B, 0.1% in 6M hydrochloric acid, (BRB)
Ethyl Rhodamine B, 0.1% in 6M hydrochloric acid, (ERB)
Toluene, A.R. grade

Procedure
Into a separating funnel was placed 5 ml gallium solution, 2 ml hydrochloric acid and 2 ml titanium(III) chloride solution. The mixture was shaken for 30 seconds. Then, 1 ml dye and 10 ml toluene were added and the funnel shaken for two minutes. The organic phase was filtered through a Whatman number 41 filter paper to remove any traces of water, and its absorbance was measured at 565 nm. in silica cells against a solvent blank.

Results

<table>
<thead>
<tr>
<th>Dye</th>
<th>Extraction</th>
<th>Absorbance</th>
<th>% Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Complex</td>
<td>Blank</td>
</tr>
<tr>
<td>Butyl Rhodamine B</td>
<td>1st</td>
<td>0.575</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.060</td>
<td>0.052</td>
</tr>
<tr>
<td>Ethyl Rhodamine B</td>
<td>1st</td>
<td>0.467</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.087</td>
<td>0.000</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>1st</td>
<td>0.042</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.014</td>
<td>0.022</td>
</tr>
</tbody>
</table>

from these results the following data may be deduced:

For BRB = 93,500 1.mol.\(^{-1}\).cm.\(^{-1}\) (single extraction)

For ERB = 84,500 1.mol.\(^{-1}\).cm.\(^{-1}\) (single extraction)

= 99,800 1.mol.\(^{-1}\).cm.\(^{-1}\) (double extraction)

Quoted literary value for BRB = 90,000 1.mol.\(^{-1}\).cm.\(^{-1}\)
Job's Plot for the Determination of Gallium with Butyl Rhodamine B

(Figure 15)
In both cases, it was found that full colour development occurred in 3 minutes. The organic solutions were stable for a period of at least 6 hours, even when exposed to sunlight.

The comparatively long shaking time of two minutes was found to be necessary to achieve equilibrium between the two phase.

For Job's Plot shown in figure 15, both the Butyl Rhodamine B and the gallium solutions were $1.44 \times 10^{-4}$ molar. The maximum absorbance occurred when 3.3 ml Butyl Rhodamine B was extracted with 6.6 ml gallium solution, indicating a 1 : 2 complex. In acid solution, the dye exists in the Rh$^{2+}$ form which will combine with 2 molecules of gallium solution to give a complex of the form $(H\text{-BRB})^{2+}(GaCl_4^-)_2$.

Comments and Conclusions

From the results it can be seen that both the esters of Rhodamine B give good recoveries and high molar extinction coefficients. It is known that the Ethyl Rhodamine B sample contained a certain amount of Rhodamine B as shown in the next chapter, but this does not seem to interfere with the extraction.

Of the two esters, Butyl Rhodamine B gives the greatest degree of extraction (98%) along with a high molar extinction coefficient (93,500 1.mol.$^{-1}$.cm.$^{-1}$) and a tolerably low blank (0.057 absorbance units). From the literature, the Beer's law plot is also satisfactory, being linear from 0 to 1.0 ppm gallium, and the organic solutions are light stable. The method can, therefore be highly recommended.

3.10 The Determination of Dichromate as a Butyl Rhodamine B complex

As was indicated in the earlier anion extraction tests, Butyl Rhodamine B showed some promise as an extractant for dichromate into ether. This was therefore investigated further.
1. Optimisation of the Acidity of the Aqueous Phase

Method

Into a separating funnel were placed 5 ml dichromate solution (25 ppm), 5 ml hydrochloric acid of the required concentration, 1 ml Butyl Rhodamine B solution (0.05%). This was extracted with two 10 ml portions of ether, and the combined extracts, after filtration through a Whatman number 41 filter paper, were made up to 25 ml with fresh ether. The absorbance of this solution was measured at 565 nm in silica cells against a solvent blank.

Results

<table>
<thead>
<tr>
<th>Acidity of the Aqueous Phase</th>
<th>Absorbance Dichromate</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M HCl</td>
<td>0.324</td>
<td>0.014</td>
</tr>
<tr>
<td>0.5 M HCl</td>
<td>0.406</td>
<td>0.020</td>
</tr>
<tr>
<td>0.3 M HCl</td>
<td>0.532</td>
<td>0.018</td>
</tr>
<tr>
<td>0.2 M HCl</td>
<td>0.553</td>
<td>0.018</td>
</tr>
<tr>
<td>0.1 M HCl</td>
<td>0.495</td>
<td>0.016</td>
</tr>
</tbody>
</table>

These results are shown graphically in figure 16

Conclusion

The maximum extraction occurs when the aqueous phase is 0.2 M in hydrochloric acid. The blank is largely unaffected by variations in acidity within this range. Therefore in further experiments, an aqueous phase which was 0.2 M in hydrochloric acid was used.

2. Determination of the Extraction Characteristics of Butyl Rhodamine B Dichromate into Ether

Method

This method was used for all subsequent experiments in this section. Into a separating funnel were placed 5 ml dichromate solution (<10 ppm.), 1 ml hydrochloric acid (2.0 M), 4 ml water and 1 ml Butyl Rhodamine B
Optimisation of the Acidity of the Aqueous Phase for the Determination of Dichromate.

(Figure 16)
(0.05% in ethanol). This was extracted with two 10 ml portions of ether. The combined extracts were dried by filtration through a Whatman number 41 filter paper, and made up to 25 ml in a standard flask with fresh ether. The absorbance of the solution was measured at 565 nm in silica cells against a solvent blank.

Results
Concentration of dichromate solutions = 8 ppm.

1st 10 ml extraction without dilution  Absorbance = 1.215
2nd 10 ml extraction without dilution  Absorbance = 0.404
3rd 10 ml extraction without dilution  Absorbance = 0.134

Hence with a single extraction  \[ \varepsilon = 65,700 \text{ l.mol}^{-1}\text{cm}^{-1} \]
\[ \% \text{ extraction} = 66\% \]

With a double extraction  \[ \varepsilon = 87,400 \]
\[ \% \text{ extraction} = 88\% \]

Conclusion
If a double extraction procedure, such as outlined above, is used this method is potentially useful. Therefore its development was considered.

3. Job's Plot for the Dichromate Determination

It was expected that the complex would consist of two molecules of dye per molecule of dichromate. As a symmetrical peak is the easiest to examine and identify, it was therefore decided to make the dye solution twice as concentrated as the dichromate solution.

Method
A solution of potassium dichromate (10 ppm., 4.62 \times 10^{-5} M) and a solution of Butyl Rhodamine B (9.24 \times 10^{-5} M, 0.00456\%) were prepared using 0.2 M hydrochloric acid as solvent in both cases. Portions of the two reagents were mixed so that their combined volumes added up to 10 ml. This mixture, in a separating funnel, was extracted with 10 ml
Job's Plot for the Extraction of

Butyl Rhodamine B - Dichromate

into Ether

(Figure 17)

The concentration of BRB is double that of the $\text{Cr}_2\text{O}_7^{2-}$, therefore the complex is $(\text{BRB})_2\text{Cr}_2\text{O}_7^-$. 
ether, and the absorbance of the organic phase was measured directly at 565 nm, using 1 cm silica cells and a solvent blank.

**Results**

The data obtained is shown graphically in figure 17.

The graph is symmetrical, having a maximum absorbance when equal volumes of the two reagents were present.

**Conclusion**

As the Butyl Rhodamine B solution is twice the molarity of the potassium dichromate solution, the formula of the complex is \((\text{BRB}^+)\_2\text{Cr}_2\text{O}_7^{2-}\).

4. **The Precision of the Determination**

**Method**

A series of determinations were performed, using the method described in section 2. The standard deviation and coefficient of variation for the method were calculated.

**Results**

Absorbance values for solutions containing 8 ppm dichromate.

<table>
<thead>
<tr>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.552</td>
<td>0.548</td>
<td>0.568</td>
<td>0.566</td>
</tr>
<tr>
<td>0.566</td>
<td>0.564</td>
<td>0.570</td>
<td>0.564</td>
</tr>
<tr>
<td>0.560</td>
<td>0.562</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \bar{x} = 0.562 \]

\[ \sigma = 0.00663 \]

\[ \% \sigma = 1.2\% \]

**Conclusion**

The coefficient of variation is about 1%, which is what would be expected for a spectrophotometric dye solvent extraction procedure, using pure aqueous solutions. It is likely that the figure would be higher, if the method were used on practical matrix samples.

5. **Interferences in the Determination by Foreign Ions**

**Method**

In the initial qualitative tests, standard dichromate solutions (4 ml. 10pp
water (5 ml), hydrochloric acid (1 ml, 2M), Butyl Rhodamine B (1 ml, 0.05%), and about 50 mg of the anion under test added as either the sodium or the potassium salt, were placed in a separating funnel and extracted with two 10 ml portions of ether. The combined extracts were filtered through a Whatman number 41 filter paper into a 25 ml standard flask and after being made up to volume with pure solvent the absorbance was measured at 565 nm. in the usual way.

For the quantitative tests, 4 ml of the interfering ion of the appropriate concentration and 1 ml water were used instead of the original 5 ml water for the extraction.

Results
The results for the qualitative tests are given in table 9 and those for the quantitative tests are summarised in table 10.

Conclusions
The qualitative tests show that in excess quantities many ions interfere slightly and a number interfere very significantly. Chloride, bromide, iron(III), fluoride and chlorate can be considered to be non-interfering. If it is taken into account that in the qualitative tests, the foreign ions are present at concentrations of about 7,000 ppm. in the sample, the ions, nitrate, phosphate and tungstate can be considered as virtually non-interfering, but obviously neither nitric nor phosphoric acids should be used to dissolve any sample as they would be then present in a very large excess. The interference of iron(II) is not significant, because if present, it would be oxidised to the non-interfering iron(III) during the oxidation of the chromium to dichromate.

The quantitative tests show that all the ions which interfere seriously on a macro scale, have a significant effect even at the 100 ppm. level. However, at the 10 ppm. level, perchlorate may be considered as tolerable but molybdate, permanganate, nitrite, and sulphate still

......page 76
Table 9

Interferences in the Determination of Dichromate with Butyl Rhodamine B

Qualitative Results

<table>
<thead>
<tr>
<th>Ion</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(added as 50 mg Na or K salt)</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>0.562 ± 0.006</td>
</tr>
<tr>
<td>Cl(^{-})</td>
<td>0.566</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>0.648</td>
</tr>
<tr>
<td>NO(_3^{-})</td>
<td>0.582</td>
</tr>
<tr>
<td>Br(^{-})</td>
<td>0.554</td>
</tr>
<tr>
<td>I(^{-})</td>
<td>1.55</td>
</tr>
<tr>
<td>WO(_4^{2-})</td>
<td>0.584 (precipitation)</td>
</tr>
<tr>
<td>MoO(_4^{2-})</td>
<td>0.716</td>
</tr>
<tr>
<td>CrO(_4^{2-})</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>MnO(_4^{-})</td>
<td>0.004</td>
</tr>
<tr>
<td>ReO(_4^{-})</td>
<td>0.986</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>0.049</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>0.572</td>
</tr>
<tr>
<td>F(^{-})</td>
<td>0.578</td>
</tr>
<tr>
<td>NO(_2^{-})</td>
<td>0.636</td>
</tr>
<tr>
<td>PO(_4^{3-})</td>
<td>0.624</td>
</tr>
<tr>
<td>ClO(_4^{-})</td>
<td>1.42</td>
</tr>
<tr>
<td>ClO(_3^{-})</td>
<td>0.560</td>
</tr>
</tbody>
</table>
### Table 10

**Interferences in the Determination of Dichromate with Butyl Rhodamine B**

**Quantitative Results**

<table>
<thead>
<tr>
<th>Anion</th>
<th>Absorbance</th>
<th>100 ppm.</th>
<th>10 ppm.</th>
<th>2 ppm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td></td>
<td></td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td></td>
<td>0.621</td>
<td>0.667</td>
<td>-</td>
</tr>
<tr>
<td>MnO$_4^-$</td>
<td></td>
<td>0.017</td>
<td>0.470</td>
<td>-</td>
</tr>
<tr>
<td>ReO$_4^-$</td>
<td></td>
<td>1.075</td>
<td>0.680</td>
<td>-</td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td></td>
<td>0.840</td>
<td>0.538</td>
<td>-</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td></td>
<td>0.630</td>
<td>0.566</td>
<td>-</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td></td>
<td>0.604</td>
<td>0.558</td>
<td>-</td>
</tr>
<tr>
<td>CrO$_4^{2-}$</td>
<td></td>
<td>&gt; 2</td>
<td>1.145</td>
<td>0.796</td>
</tr>
</tbody>
</table>
cause problems. It should be noted that any chromium present in the sample as chromate rather than dichromate will seriously affect the result, as it will be converted to dichromate in the aqueous phase.

6. **Beer's Law Plot for the Determination of Dichromate**

**Method**

The sample (5 ml), water (4 ml), hydrochloric acid (1 ml, 2.0 M), and Butyl Rhodamine B (1 ml, 0.05%) were placed in a separating funnel and extracted with two 10 ml portions of ether. The combined extracts were filtered through a Whatman number 41 filter paper and made up to 25 ml with fresh solvent. The absorbance was measured at 565 nm, using silica cells and a solvent blank.

**Results and Conclusion**

The linear plot obtained is shown in figure 18, showing that the system obeys Beer's law from 0 to 12 ppm dichromate in the sample solution. This corresponds to approximately 0 to 6 ppm chromium.

3.11 **Recommended Procedure**

**Reagents**

- **Hydrochloric Acid**: 2.0 M
- **Potassium Dichromate Solution**: 0.1361 gm of potassium dichromate are diluted to 1 litre with water. 10 ml of this solution are diluted to 100 ml to make a 10 ppm dichromate solution. This solution deteriorates quickly and should be prepared freshly each day.
- **Butyl Rhodamine B**: 0.05% in ethanol
- **Ether**

**Method**

Into a 100 ml separating funnel is placed 5 ml sample, 4 ml water, 1 ml hydrochloric acid and 1 ml Butyl Rhodamine B solution. Ether (10 ml)
Beer's Law Plot for the Extraction of Butyl Rhodamine B - Dichromate into Ether

(Figure 18)
is added and the funnel shaken for 1 minute. The aqueous layer is
drained into a clean beaker and the ether layer run into a 25 ml
standard flask through a Whatman number 41 filter paper. The aqueous
solution is returned to the separating funnel and extracted with a
further 10 ml ether in a similar manner. This second extract is
combined with the first and the solution is made up to 25 ml with
fresh ether. The absorbance of this organic solution is measured
at 565 nm using silica cells and a solvent blank.

It should be noted that if the calibration graph is prepared
accordingly, it may be convenient to use a 9 ml sample instead of
adding a volume of water, as described above.

3.12 Conclusions

Butyl Rhodamine B seems to be a very useful reagent. It has
probably been largely ignored in the West because it is not commercially
available. However, the preparation is quite simple and one batch would
provide sufficient dye for several months work.

In general, it gives high molar extinction coefficients and
good recoveries with low blanks, under suitable conditions. In spite
of the misgivings of several authors, the interferences do not seem
dramatically higher than those for the parent acid, Rhodamine B.

The method which has been developed for the determination of
dichromate is an illustration of the potential use of the reagent.
As neither the chloride nor the fluoride anions, nor iron(III) interfere,
the method may be suitable for the determination of chromium in certain
steel samples, alloys and ores. As it stands, it would unfortunately
not be universally applicable because of the significant interference
by molybdenum, rhenium and manganese. Particular examples where the
method may be used are: in the determination of chromium in stainless
steels where the proportion of chromium to other metals which may
interfere is of the order of 20:1; in the determination of chromium
in ferro-chromium alloys where the proportion is about 100 : 1; and in the determination of chromium in ores such as Grecian chrome ore.
Chapter Four

Chromatography of Basic Dyes, Particularly Xanthenes

4.1 Background

Compared with other dye users, the analyst has little importance from the commercial viewpoint, as his usage generally amounts to less than a few hundred grams of dye each year. The commercial dyes, which are used to dye fabrics, are the most impure grades available to the analyst, because the manufacturer is concerned to achieve a constant, reliable and fast colour, rather than a pure product. Thus their products often contain related dyes added for colour adjustment, organic impurities from the manufacturing process, metal ions and other additives. Sometimes the dyes obtained from such sources have been prepared many years previously and may have been stored for a considerable period of time. This is made worse, due to the fact that many dyes of interest to the analyst, such as the Rhodamines, have long-since been superceded as fabric dyes.

All commercial dyes which have been obtained for use as reagents, should be regarded with suspicion by the analyst until their identity and purity are established. For example, commercial Methylene Blue is generally a double salt of zinc chloride and Methylene Blue, whilst the medicinal grade is a zinc-free product. Both are sold as Methylene Blue, and it is often left to the individual to determine which he has; in this case, combustion of a small sample is all that is required to ascertain the presence or absence of zinc.

Another useful source of dyes for analytical purpose is those dyes manufactured as biological stains. The relative purity of these dyes is normally much higher than the commercial dye grades, but again other dyes are sometimes added in small quantities, or the dye left in a semi-pure state in order to retain a particular staining property.
For example, Polychrome Methylene Blue contains small quantities of the Azure dyes, which enhance its properties as a blood stain (5).

Medicinal grade of dye often meet analytical requirements, but occasionally do not. These dyes must conform to given standards, set out in an appropriate Pharmacopoeia or Codex. For example, Methylene Blue and Brilliant Green are classified in the British Pharmaceutical Codex (153). Such standards are more concerned with toxicity and medicinal value than with absolute chemical purity. Fogg, Burgess and Thorburn Burns (154) examined several samples of Brilliant Green, and found that the results obtained in the determination of gold, varied considerably according to the sample of Brilliant Green used. The B.P. grade was not sufficiently pure for this purpose, although it was suitable for the determination of antimony, perrhenate and perchlorate (155,156).

It is therefore necessary for the analyst to have adequate and rapid methods to check the purity of dye samples. Failure to ensure the necessary purity of a dye may result, for example, in unexpected shifts in the absorbance maximum, low recoveries, low molar extinction coefficients, and in the extreme a total failure of the method. It has already been noted that a certain ambiguity exists in the nomenclature of dyes, and any errors thereby caused should also be made apparent if all new samples are checked.

The immediate reason for this work being undertaken, was two-fold; firstly to check the purity of a sample of Rhodamine 3B, which had been obtained from a commercial source, and secondly to check the purity of a sample of Butyl Rhodamine B, which had been prepared in this laboratory.

4.2 Chromatographic Methods found in the Literature

In spite of a detailed search of the available literature and abstracts, it was found, with some surprise, that chromatographic
methods for basic dyes, and in particular xanthene dyes, were few in number. A specific search was made for paper chromatographic methods, as it was hoped to avoid the purchase or preparation of thin layer plates, and also it was felt desirable to be able to keep the chromatograms easily, for future reference.

Initially, a standard text-book was consulted for general methods of separating dyes, (157). The solvents recommended are listed as numbers 3 to 6 in the experimental section. None of them were found to be satisfactory. Four further solvent systems were also investigated. Ciglar, Kolsek and Perpar (158) proposed a method whereby basic dyes could be separated on paper which had been pretreated by impregnation with cetyl alcohol and a mixture of ethanol – aqueous ammonia (S.G. 0.88) - water (2 : 1 : 1) was used to develop the chromatogram.

Kuypers and Kiel proposed, in a series of papers, a complete analysis of a wide range of dyes by chromatography. They recommended a mixture of ethanol – methanol (4 : 1) as being a suitable medium for separating basic dyes (159). Holaster suggested a mixture consisting of water – ethanol – ethyl acetate – aqueous ammonia (S.G. 0.88) in the ratio (60 : 25 : 12 : 30) for similar use (160).

The only solvent system which proved at all satisfactory, was that of Taylor (161). He proposed a method to separate xanthene dyes for the purpose of studying the elimination of derivatives of Rose Bengal (C.I. Acid Red 94) and other fluorescent dyes, by the liver. He used a solvent mixture consisting of ammonia solution (S.G. 0.33) – ethanol – water (1 : 2 : 17) on Whatman number 1 chromatography paper.

4.3 Preliminary Work

Materials and Method

1. Chromatographic Solvents

Mixtures of solvents were freshly prepared according to the following compositions:-
1. ethanol - ammonia solution (S.G. 0.88) - water, (2 : 2 : 1)
2. ethanol - methanol, (4 : 1)
3. isopropanol - ammonia solution (S.G. 0.88) - water, (10 : 1 : 1)
4. n-butanol - ammonia solution (S.G. 0.88), (1 : 1)
5. glacial acetic acid - water, (1 : 9)
6. glacial acetic acid - water, (15 : 85)
7. glacial acetic acid - water, (1 : 4)
8. water - ethanol - ethyl acetate - ammonia solution (S.G. 0.88),
   (60 : 25 : 12 : 30)
9. hydrochloric acid, (1.0 M)
10. ammonia solution (S.G. 0.88) - ethanol - water, (1 : 2 : 17)

2. Paper
Whatman number 1 (25 x 25 cm) was used throughout the work as supplied
by the makers, except that with solvent system 1, it was first
impregnated with cetyl alcohol by soaking in a 5% solution made with
ethanol and drying. Solvent 9 was subsequently also tried with
Whatman number 20 chromatography paper.

3. Method
The dyes were made up into approximately saturated solutions using a
mixture of ethanol - water (1 : 1). The samples, about 0.5 μl in
volume, were applied to the paper with a drawn-out capillary tube,
approximately 3 cm from the base of the paper. The chromatogram was
run in an enclosed tank, saturated with solvent vapour by lining
the walls of the tank with solvent-impregnated filter paper, using
the required solvent. After the solvent front had reached a height
of about 15 cm (about 45 minutes) the paper was removed and dried in
a current of warm air.

General Results
Solvent systems 2, 3, 4, 5, 6 and 8 were total failures, giving no
separation at all. System 7 gave a very slight separation. System 1 also gave slight separation after a period of two days, but because of the great length of time involved and because of the unevenness of the solvent front, this system was also rejected. System 10 differentiated between Rhodamine B and its esters, but did not resolve the individual esters, namely Ethyl Rhodamine B and Butyl Rhodamine B. In spite of difficulties due to tailing and in seeing the spots, it was decided to investigate solvent system 9 further.

4.4 Detailed Study of the Hydrochloric acid system

Optimum results were obtained by the following method:-

Small spots of Rhodamine B, Ethyl Rhodamine B, Butyl Rhodamine B and a mixture of the three dyes, were spotted onto the Whatman number 1 chromatographic paper about 3 cm from the base. A Shandon chromatography tank was lined with paper, damped with 1.0 M hydrochloric acid and about 200 ml solvent poured into the bottom.

The chromatography paper was then thoroughly dampened with distilled water using a Shandon spray held at a distance of about 1 metre away from the paper, until the paper was distinctly damp to the touch. This pre-damping procedure was found to reduce considerably the tailing of the spots. The paper was inserted into the tank and left until the solvent front had climbed to a given height. Various distances were tried and about 16 cm found to be the optimum, giving the greatest difference in Rf values and taking between 45 and 60 minutes to run. These results are summarised in table 10.

The paper was removed, dried in a current of warm air until almost dry, when it was exposed to ammonia fumes to develop the spots. The leading edges of the spots were marked and the Rf values calculated from there.

Whatman number 20 chromatography paper is a grade recommended by the manufacturers to reduce the tailing of spots, and it was
Table 10

Variation of Rf value with Distance travelled by the solvent front

(1) Whatman number 1 paper, with 1.0 M Hydrochloric Acid

<table>
<thead>
<tr>
<th>Dye</th>
<th>Rf values for each solvent front height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 cm</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>0.60</td>
</tr>
<tr>
<td>Ethyl Rhodamine B</td>
<td>0.52</td>
</tr>
<tr>
<td>Butyl Rhodamine B</td>
<td>0.43</td>
</tr>
</tbody>
</table>

(2) Whatman number 20 paper, with 2.0 M Hydrochloric Acid

<table>
<thead>
<tr>
<th>Dye</th>
<th>Rf values for each solvent front height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11 cm</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>0.48</td>
</tr>
<tr>
<td>Ethyl Rhodamine B</td>
<td>0.45</td>
</tr>
<tr>
<td>Butyl Rhodamine B</td>
<td>0.38</td>
</tr>
</tbody>
</table>
### Table 11

**Approximate Rf Values**

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Colour Index Name</th>
<th>Rf Values</th>
<th>1.0 M HCl</th>
<th>Taylor's solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxilon Red BL</td>
<td>Basic Red 22</td>
<td>0.68</td>
<td>(0.79)</td>
<td>0.41</td>
</tr>
<tr>
<td>Maxilon Red GRL</td>
<td>Basic Red 46</td>
<td>0.58</td>
<td>(0.64)</td>
<td>0.14 (0.33)</td>
</tr>
<tr>
<td>Sevron Bordeaux G</td>
<td>Basic Red 16</td>
<td>0.63</td>
<td>(0.42)</td>
<td>0.84</td>
</tr>
<tr>
<td>Sevron Brilliant Red D</td>
<td>Basic Red 19</td>
<td>0.48</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Sevnon Brilliant Red B</td>
<td>Basic Red 15</td>
<td>0.46</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Sevnon Red GL</td>
<td>Basic Red 18</td>
<td>0.32</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Sevnon Brilliant Red 4G</td>
<td>Basic Red 14</td>
<td>0.31</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Deorlene Brilliant Red R</td>
<td>Basic Red 27</td>
<td>0.25</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td>Deorlene Brilliant Red 3B</td>
<td>Basic Red 26</td>
<td>0.81</td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>Deorlene Brilliant Red 4G</td>
<td>Basic Red 14</td>
<td>0.31</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Deorlene Fast Red 2G</td>
<td>Basic Red 18</td>
<td>0.33</td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Rhodamine S</td>
<td>Basic Red 11</td>
<td>0.32</td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Rhodamine 6G</td>
<td>Basic Red 1</td>
<td>0.45</td>
<td></td>
<td>0.29 (0.49)</td>
</tr>
<tr>
<td>Rhodamine 6GD</td>
<td>Basic Red 1</td>
<td>0.44</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Rhodamine 3B</td>
<td>Basic Violet 11</td>
<td>0.50</td>
<td>(0.59)</td>
<td>0.28 (0.65)</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>Basic Violet 10</td>
<td>0.59</td>
<td></td>
<td>0.66</td>
</tr>
<tr>
<td>Rhodamine 3G</td>
<td>Basic Red 3</td>
<td>0.49</td>
<td></td>
<td>0.25 (0.54)</td>
</tr>
<tr>
<td>Butyl Rhodamine B</td>
<td>-</td>
<td>0.34</td>
<td></td>
<td>0.25</td>
</tr>
</tbody>
</table>

The Rf values were calculated from the leading edge, this being the easiest to measure in cases where tailing of the spots had occurred. Minor components are given in parentheses.
therefore tried with the hydrochloric acid system. It was found that equally good results could be obtained with this paper by increasing the concentration of hydrochloric acid to 2.0 M, but no improvement in the tailing effect was noted. It was also observed that the calculated Rf values were more dependent upon the height to which the solvent front rose, resulting in less dependable results, (table 10). As Whatman number 1 paper is the grade which is normally stocked, there seems little point in using number 20 paper to achieve similar results.

Since successful results had been obtained with the required dyes, it was decided to see if the method was applicable to other of the Rhodamines, and some of the newer cationic red dyes, which have been developed for the synthetic fabrics. The results are summarised in table 11.

4.5 Discussion of the Results

The identity of samples of Basic Red 14 (Sevron Brilliant Red 4G and Deorlene Brilliant Red 4G) and samples of Basic Red 18 (Sevron Red GL and Deorlene Fast Red 2GL) from two different sources was confirmed; also the difference between Rhodamine 6G and Rhodamine 6GD was illustrated. A commercial sample of Rhodamine 3B was found to contain substantial amounts of Rhodamine B, and therefore of little use in analytical chemistry without purification. The prepared sample of Butyl Rhodamine B was shown to be free from coloured impurity.

Compared with the results obtained by Logar, Perkavec and Perpar (162), improved resolution has been obtained for the Deorlene range of dyes. It should however, be noted that in the hydrochloric acid method proposed here, the spot for Deorlene Brilliant Red 3B appears only after the paper has been drying for about 30 minutes, and can thus be easily missed if care is not exercised.

In an ideal system, the Rf value obtained should be independent of the distance travelled by the solvent front. Indeed this is the
value of quoting such a figure. The variation in this case is probably due to the tailing effect, but as this is, like all chromatographic methods, a comparative technique the effect is of no great importance, as long as care is taken to run standards and also to eliminate edge effect.

4.6 *Adaption of the Method to the Column Chromatographic Purification of Rhodamine 3B*

Because of the difficulty in the purification of a dye, which is contaminated by a closely related dye by normal chemical methods, it was decided to attempt to produce a small amount of pure Rhodamine 3B using a column adaption of the paper chromatographic method. A 3 cm diameter glass column was prepared with a tap at the bottom. It was packed with an aqueous slurry of Whatman chromatography-grade cellulose, which was allowed to compact, but not dry out. A piece of filter paper was placed on the top of the slurry to protect it and to give a flat surface on which to apply the sample.

The sample of impure dye was dissolved in a small quantity of water and applied to the top of the column. Molar hydrochloric acid was added in small quantities until the sample had passed sufficiently far into the column, so as not to be diluted by the large quantities of solvent subsequently added. The solvent was then added in bulk until the first fraction, the Rhodamine B, had been eluted. The second fraction, the Rhodamine 3B was then eluted with ethanol. The column was washed with distilled water before reuse for a second separation.

Although after running twice through the column, a satisfactory pure sample of the Rhodamine 3B was obtained, which was checked by the normal paper chromatographic method, it was felt that the method was unsuitable for bulk production, even at the scale required for colorimetric use. The weight of sample with which the column could
Table 12

Paper Chromatography of Thiazine Dyes

<table>
<thead>
<tr>
<th>Dye</th>
<th>Colour Index Name</th>
<th>Rf Values</th>
<th>Taylor's solvent</th>
<th>1.0 M HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thionin</td>
<td>-</td>
<td>0.065</td>
<td>0.058 (0.117)</td>
<td></td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>Basic Blue 9</td>
<td>0.099</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Methylene Green</td>
<td>Basic Green 5</td>
<td>0.15</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Dimethylmethylene Blue</td>
<td>-</td>
<td>0.024</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>Azure A</td>
<td>-</td>
<td>0.080</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>Azure B</td>
<td>-</td>
<td>0.088</td>
<td>0.099 (0.40)</td>
<td></td>
</tr>
<tr>
<td>Azure C</td>
<td>-</td>
<td>0.064</td>
<td>0.010</td>
<td></td>
</tr>
</tbody>
</table>

The Rf values were calculated from the leading edge, this being the easiest to measure in cases where tailing of the spots had occurred. Minor components are given in parentheses.
cope was very small, even in spite of its large diameter. After about 30 hours continuous use, it was estimated that only about 0.01 gm Rhodamine 3B had been produced.

4.7 Chromatography of Thiazine Dyes, using Taylor's solvent and the Hydrochloric Acid method

As thiazine dyes have been another principal source of study during this work, it was decided to investigate the possibility of using this method to separate them also. The paper chromatographic methods used were similar to those used for the xanthene dyes, and the results are summarised in table 12. Unfortunately, tailing was a severe problem in spite of all the precautions described earlier in this chapter. Taylor's solvent gave negligible separation, and the hydrochloric acid method was satisfactory, only in the absence of the Azure series of dyes. As such, neither method can be recommended for use with thiazine dyes.

4.8 Conclusions

The chromatographic method developed is simple and easy to use, giving improved resolution for a number of xanthenes and other cationic red dyes. It is suggested that in conjunction with the method described by Taylor (161), a satisfactory means of examining the purity of these types of dye has been developed. It has achieved all the aims for which it was evolved and has produced separations in the Deorlene range of dyes, which compare favourably with those in the literature.
Chapter Five

An Investigation into the use of Onium Compounds as Masking Agents in Basic Dye Methods of Analysis

5.1 Introduction

Basic dye methods of analysis are potentially very useful because of the high sensitivities and good recoveries which can be obtained. However, in general, basic dye methods of analysis are not very selective and this can be a serious disadvantage, when applied to complex matrices. Therefore an attempt was made to add various onium compounds, which in previous work had been shown to display some degree of selectivity (166,167) to well-established systems, to see if any masking effect could be obtained without affecting the desirable qualities of the original method.

The systems chosen for study were the analyses of antimony, thallium, perchlorate and perrhenate with Brilliant Green, which were developed by Fogg, Burgess, Thorburn Burns et al. These methods had been used in the Department and were believed to be straightforward and reliable (154,155,156,163).

The onium compounds used, were all of a commercial manufacture with the exception of diphenyliodonium hydrogen sulphate, which was prepared by Miss S. Soleymanloo in this Department.

According to the table of extraction properties of onium compounds given by Narcus and Kertes (164), the extraction of onium complexes is largely independent of pH. Thus with few exceptions, where the structure of the anion is pH dependent, a complex which extracts from an alkaline medium will do so from an acidic medium, although the extent to which it extracts may vary slightly. On the basis of this information, it was felt justifiable to add the onium compound at the same pH as the Brilliant Green.
Initially, the onium compounds were added, where their solubility permitted, as 0.01 M solutions, as this was roughly the concentration of the Brilliant Green reagent (0.5%, 0.0124 M) and therefore a direct comparison of effects could be obtained. In the cases where the Brilliant Green reagent was only 0.05%, the onium reagent was similarly diluted tenfold. The concentrations and abbreviations used to designate the onium compounds are given in Table 13.

5.2 The Addition of Onium Compounds to the Brilliant Green Method of Analysis for Antimony (155,163)

The details of reagent preparation and the development of the original method are given in the literature, so only brief details will be given here. The method was that used successfully by Al-Sibbai and Fogg in a study of the stabilities of standard solutions (165).

Procedure

Into a separating funnel, were placed antimony solution (5 ml, 4 ppm), hydrochloric acid (5 ml, S.G. 1.18), and cerium(IV) sulphate (15 drops, 0.1 N in 10% sulphuric acid). The mixture was swirled for one minute and then diluted with water (25 ml). The excess cerium(IV) sulphate was reduced by dropwise addition of hydroxyammonium chloride (1%) until the original yellow colour had been discharged. Toluene (10 ml), Brilliant Green (0.5 ml, 0.5%) and the onium compound (0.5 ml, 0.01 M) were added and the mixture shaken for 1 minute; the toluene layer was filtered into a 25 ml standard flask through a Whatman number 41 filter paper. This was repeated with a further similar portion of toluene with further additions of dye and onium compound solution. The combined toluene extracts were made up to 25 ml with fresh solvent and the absorbance of a portion of this solution was measured at 640 nm using 1 cm silica cells and a solvent blank.
<table>
<thead>
<tr>
<th>Onium compound</th>
<th>Abbreviation</th>
<th>0.01 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraphenylarsonium chloride</td>
<td>TPAC</td>
<td>0.418%</td>
</tr>
<tr>
<td>Methyltriphenyl phosphonium bromide</td>
<td>MTPB</td>
<td>0.357%</td>
</tr>
<tr>
<td>Tris(3-chlorophenyl)ethyl phosphonium iodide</td>
<td>TCEPI</td>
<td>*0.521%</td>
</tr>
<tr>
<td>Tetraphenylphosphonium chloride</td>
<td>TPPC</td>
<td>0.374%</td>
</tr>
<tr>
<td>Methyltriphenyl arsonium iodide</td>
<td>MTAI</td>
<td>0.448%</td>
</tr>
<tr>
<td>Tris(3-chlorophenyl)methyl phosphonium iodide</td>
<td>TCMPI</td>
<td>*0.507%</td>
</tr>
<tr>
<td>Triphenyl-n-butylphosphonium bromide</td>
<td>TBPB</td>
<td>0.363%</td>
</tr>
<tr>
<td>Tetrabutylphosphonium chloride</td>
<td>TBPC</td>
<td>0.294%</td>
</tr>
<tr>
<td>3,5 di-t-butylhydroxy benzyl triphenyl-</td>
<td>BHTPB</td>
<td>*0.547%</td>
</tr>
<tr>
<td>phosphonium bromide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tri-n-butylmethylphosphonium iodide</td>
<td>TBMPI</td>
<td>0.344%</td>
</tr>
<tr>
<td>Triphenylmethallylphosphonium chloride</td>
<td>MPC</td>
<td>0.353%</td>
</tr>
<tr>
<td>Diphenyliodonium hydrogen sulphate</td>
<td>DIB</td>
<td>*0.376%</td>
</tr>
</tbody>
</table>

The compounds marked * were insufficiently soluble to produce 0.01 M solutions and were therefore used as saturated solutions.
Table 14

Effect of the Addition of Onium Compounds on the Brilliant Green Method for Antimony

<table>
<thead>
<tr>
<th>Onium Salt</th>
<th>Concentration</th>
<th>Absorbance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>0.576</td>
<td></td>
</tr>
<tr>
<td>TPAC</td>
<td>0.01 M</td>
<td>0.038 0.082</td>
<td></td>
</tr>
<tr>
<td>MTPB</td>
<td>0.01 M</td>
<td>0.260 0.3250.458 0.188</td>
<td></td>
</tr>
<tr>
<td>TCEPI</td>
<td>saturated</td>
<td>0.143 0.6870.482 0.618</td>
<td></td>
</tr>
<tr>
<td>TPPC</td>
<td>0.01 M</td>
<td>0.063 0.050</td>
<td></td>
</tr>
<tr>
<td>NTAI</td>
<td>0.01 M</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>TCMPI</td>
<td>saturated</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>TBPB</td>
<td>0.01 M</td>
<td>0.263 0.2440.172 0.188</td>
<td></td>
</tr>
<tr>
<td>TBPC</td>
<td>0.01 M</td>
<td>0.434 0.3380.144 0.143</td>
<td></td>
</tr>
<tr>
<td>BHTPB</td>
<td>saturated</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>TBMPI</td>
<td>0.01 M</td>
<td>&gt;2.0</td>
<td></td>
</tr>
</tbody>
</table>
Results

These are summarised in table 14.

Discussion and Conclusions

Initially, some difficulty was experienced with the fundamental method, which gave very erratic results, usually approximately 50% lower than the expected values. The reason for this was eventually found to be impurities in the toluene. It is therefore recommended that only B.D.H. analytical grade should be used. All other analytical grades should be purified, as described below, before use. It is also suggested that as the Brilliant Green is quickly converted to the yellow protonated form in acid solution, the temperature should not be allowed to rise above 25°C during the course of the extraction. In practice, as the principal heat-generating step is the initial addition of hydrochloric acid to the sample, the required temperature control can be achieved by cooling the sample and the hydrochloric acid to below 5°C before commencement of the procedure.

From the results it can be seen that the absorbance sometimes increased and sometimes decreased but in all cases the results were very erratic, which means that the possibility of using the onium compounds as masking agents in this system is negligible. However, as tetraphenylarsenium chloride, tetraphenylphosphonium chloride and 3,5-di-t-butyl-4-hydroxybenzyltriphenyl bromide prevented any extraction of the Brilliant Green – antimony(V) complex, there was the possibility of using them to mask antimony in other procedures.

In the case of the onium iodides, all gave high absorbance values. This was thought to be due to the extraction of Brilliant Green iodide. In fact, it was shown experimentally that under the conditions of this experiment and under the milder conditions used for the determination of perrhenate and perchlorate, Brilliant Green would extract the iodide ion into toluene. Therefore the use of onium
iodides was discontinued, and the results obtained with onium bromides were treated with caution.

**Method of Purification of Toluene** (based on the procedure of Stanton and McDonald (72))

Into a suitable separating funnel (2 litre) was placed 800 ml toluene, and 20 ml saturated potassium dichromate solution. The funnel was shaken for about 30 minutes, although not continuously. The dichromate was drained off, and a fresh portion added. Both layers were drained into a large covered beaker and left overnight in a covered solvent can. The liquids were replaced in the separating funnel and the dichromate layer removed. The toluene was washed with four successive portions of distilled water (200 ml each) and then drained into a suitable bottle through a thick layer of Whatman number 41 filter paper, in order to remove the remaining small amount of water.

5.3 **The Addition of Onium Compounds to the Brilliant Green Method of Analysis for Thallium**

**Procedure**

Thallium(I) solution (2 ml, 10 ppm) and hydrochloric acid (8 ml, HCl : H₂O = 2 : 1) were cooled and placed into a separating funnel. Cerium(IV) sulphate reagent (10 drops) was added and the flask swirled for 1 minute. The excess cerium(IV) sulphate was reduced with hydroxy-ammonium chloride and the procedure completed in a similar manner to the antimony determination.

**Results**

The results are summarised in table 15

**Comments and Conclusions**

With the exception of triphenyl-n-butylphosphonium bromide, the results were very similar and just as erratic as those obtained for
### Table 15

**Effect of the Addition of Onium Compounds on the Brilliant Green Method for Thallium**

<table>
<thead>
<tr>
<th>Onium Salt</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>-</td>
<td>0.416 0.412 0.418</td>
</tr>
<tr>
<td>TPAC</td>
<td>0.01 M</td>
<td>0.082 0.021 0.040</td>
</tr>
<tr>
<td>TPPC</td>
<td>0.01 M</td>
<td>0.053 0.062</td>
</tr>
<tr>
<td>TBPC</td>
<td>0.01 M</td>
<td>0.240 0.124</td>
</tr>
<tr>
<td>MTPB</td>
<td>0.01 M</td>
<td>0.336 0.266</td>
</tr>
<tr>
<td>TBPB</td>
<td>0.01 M</td>
<td>0.068 0.064</td>
</tr>
<tr>
<td>BHTPB</td>
<td>saturated</td>
<td>0.011 0.008</td>
</tr>
<tr>
<td>DIB</td>
<td>saturated</td>
<td>0.366 0.366</td>
</tr>
</tbody>
</table>

### Table 16

**Effect of the Addition of Onium Compounds on the Brilliant Green Method for Perchlorate**

<table>
<thead>
<tr>
<th>Onium Salt</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>-</td>
<td>0.682 0.680</td>
</tr>
<tr>
<td>TPAC</td>
<td>0.001 M</td>
<td>0.704 0.702</td>
</tr>
<tr>
<td>MTPB</td>
<td>0.001 M</td>
<td>0.700 0.718</td>
</tr>
<tr>
<td>TPPC</td>
<td>0.001 M</td>
<td>0.672 0.660</td>
</tr>
<tr>
<td>TBPB</td>
<td>0.001 M</td>
<td>0.635 0.670</td>
</tr>
<tr>
<td>TBPC</td>
<td>0.001 M</td>
<td>0.600 0.622</td>
</tr>
<tr>
<td>BHTPB</td>
<td>20% sat.</td>
<td>0.476 0.468</td>
</tr>
</tbody>
</table>
antimony. In the case of triphenyl-n-butylphosphonium bromide, complete suppression of the thallium(III) - Brilliant Green complex occurred, whereas in the antimony method, partial extraction of the antimony(V) - Brilliant Green complex occurred. In a colorimetric method, the triphenyl-n-butylphosphonium bromide would not be a practical masking agent for thallium as the sensitivity of the Brilliant Green - antimony determination would be reduced by too much, even assuming that consistent results could be obtained.

5.4 The Addition of Onium Compounds to the Brilliant Green method of Analysis for Perchlorate (156)

As there has been some errors in the published list of reagents for this method, namely in the concentration of the potassium perchlorate solution and in the formulation of the buffer, they will be given here in detail.

Reagents

**Brilliant Green**: 0.05% in ethanol

**Benzene**: B.D.H., A.R. grade

**Standard Potassium Perchlorate, 5 ppm (equivalent to 3.58 ppm ClO₄⁻)**

Dissolve 0.500 grams of potassium perchlorate in water, and dilute the resulting solution to 1 litre in a calibrated flask. Dilute 10.00 ml of this solution to 1 litre in a calibrated flask as required.

**Buffer Solution, pH 6.5**: Dissolve 6.80 grams of potassium dihydrogen orthophosphate in water and add 13.9 ml of 0.2 M sodium hydroxide; dilute to 1 litre with water. If an impure sample of Brilliant Green is used, then 5 grams ascorbic acid may be added to the buffer, in which case 39.4 ml 1.0 M sodium hydroxide must be added before dilution to return the pH to 6.5. In view of the findings in chapter 7, it is recommended that the use of ascorbic acid is to be avoided if
Onium Compounds: These were diluted to 0.001 M to approximately correspond to the concentration of the Brilliant Green.

Procedure
Potassium perchlorate solution (5 ml, 5 ppm) was pipetted into a separating funnel and buffer solution added to make the volume up to 10 ml. Brilliant Green (1 ml) and Onium solution (1 ml) were added and the complex extracted with two successive 10 ml portions of benzene. The combined extracts were filtered into a 25 ml standard flask through a Whatman number 41 filter paper and the solutions made up to the mark with benzene. The absorbance of the extract was measured at 640 nm in 1 cm silica cells against a benzene blank.

Results
The results are summarised in table 16 on page 96.

Comments and Conclusions
Whilst the general results were not sufficiently precise to be very useful, it should be noted that they are considerably more precise than for the antimony and thallium methods. This may be a reflection of the greater reliability of this fundamental method due to the greater stability of the green species of Brilliant Green under the nearly neutral conditions required by this determination.

However, whilst there is the possibility of the salts being used to mask other ions in this method, none of these onium salts are of any use as masking agents for perchlorate interference under these or similar conditions, such as those required by the Brilliant Green determination of perrhenate.

5.5 The Addition of Onium Compounds to the Brilliant Green Method for the Determination of Perrhenate (154)

Procedure
Potassium perrhenate solution (5 ml, 5 ppm), Buffer solution (Phosphate,
pH 6.0, 5 ml), Brilliant Green (1 ml, 0.05% in ethanol) and the
Onium compound (0.001 N, 1 ml) were placed into a separating funnel
and extracted with benzene (10 ml, 5 ml and 5 ml successively).
The combined extracts were filtered through a Whatman number 41 filter
paper into a 25 ml standard flask and made up to the mark with benzene.
The absorbance of the extracts were measured in 1 cm silica cells
against a solvent blank using the peak at 640 nm. The series of
experiments was repeated using Onium solutions which were ten times
more concentrated to see what effect this made.

Results
These are summarised in table 17.

Comments and Conclusions
The results were again not very predictable, and similar to
those obtained for the prechlorate determination, so there is no
possibility of using onium compounds to mask either ion in the
presence of the other.

With the exception of 3,5 di-t-butylhydroxybenzyltriphenylphosphonium bromide, an increase in the concentration of the onium
salt leads to an increase in the amount of coloured compound extracted.
The results are not sufficiently precise to show whether there is
any difference between the chloride and bromide compounds, but it seems
likely that again, a Brilliant Green halide is extracting into the
organic phase.

5.6 Review of the Work and General Conclusions
In nearly all cases, a preferential extraction of the Brilliant
Green complex occurs, except for a few cases, namely, tetraphenyl-
arsonium chloride, tetraphenylphosphonium chloride and 3,5 di-t-butyl-
hydroxybenzyltriphenylphosphonium bromide, all with the antimony system.
In these cases, the toluene was evaporated off, and the resultant solid
Table 17
Effect of the Addition of Onium Compounds to the Brilliant Green Method for the Analysis of Perrhenate

A. Molar Ratio of Brilliant Green : Onium Compound = 1 : 1

<table>
<thead>
<tr>
<th>Onium Salt</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>-</td>
<td>0.552</td>
</tr>
<tr>
<td>TPAC</td>
<td>0.001 M</td>
<td>0.570</td>
</tr>
<tr>
<td>MTPB</td>
<td>0.001 M</td>
<td>0.570</td>
</tr>
<tr>
<td>TPPC</td>
<td>0.001 M</td>
<td>0.572</td>
</tr>
<tr>
<td>TBPC</td>
<td>0.001 M</td>
<td>0.518</td>
</tr>
<tr>
<td>BHTPB</td>
<td>20% sat.</td>
<td>0.464</td>
</tr>
<tr>
<td>MPC</td>
<td>0.001 M</td>
<td>0.530</td>
</tr>
<tr>
<td>DIB</td>
<td>0.001 M</td>
<td>0.372</td>
</tr>
</tbody>
</table>

B. Molar Ratio of Brilliant Green : Onium Compound = 1 : 10

<table>
<thead>
<tr>
<th>Onium Salt</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPAC</td>
<td>0.01 M</td>
<td>0.552</td>
</tr>
<tr>
<td>MTPB</td>
<td>0.01 M</td>
<td>0.668</td>
</tr>
<tr>
<td>TPPC</td>
<td>0.01 M</td>
<td>0.648</td>
</tr>
<tr>
<td>TBPC</td>
<td>0.01 M</td>
<td>0.578</td>
</tr>
<tr>
<td>BHTPB</td>
<td>saturated</td>
<td>0.312</td>
</tr>
</tbody>
</table>

Triphenylmethallylphosphonium bromide and Diphenyliodonium bisulphate are too insoluble to even approach 0.01 M.
dissolved in chloroform. An ultra-violet spectrum showed the presence of benzene bands around the 270 nm region, which were thought to be due to the onium salt, rather than to traces of Brilliant Green or toluene.

In spite of this, it can be stated that for the Brilliant Green systems studied, there is no possibility of using Onium compounds as masking agents as, in no case, was sufficient selectivity and reproducibility found.
Chapter Six

The Stability of Various Basic Dye Solutions, with Particular Reference to Brilliant Green Solutions

6.1 Introduction

For many years, chemists have been aware that when aqueous dye solutions are diluted and the absorbance measured, Beer's law is not usually obeyed. That is, the absorbance at the maximum wavelength of the dye is not directly proportional to the concentration of dye in solution.

In a study of thiazine dyes, Rabinowitch and Epstein (169) showed that this was due to association between dye molecules, and showed that the main region of the visible spectrum comprised of two overlapping peaks, one for the monomeric form of the dye and one at a shorter wavelength due to the dimeric form of the dye; the difference between the two wavelengths being 30 to 50 nm.

Poluektov, Bel'tyukova and Meshkova (170) studied the effect for a whole series of basic dyes in common analytical use. They tabulated all the monomer and dimer wavelengths and showed that polymerisation beyond the dimeric form did not occur in any of the cases examined.

Coates, in a general study of the theoretical phenomena associated with dye aggregation effects suggested that the formation of the dimer took place by maximum overlap of the two ring systems, along with maximum separation of charge (171). Based on this, a possible type of structure for Brilliant Green is illustrated in figure 19.

Priestman (172) during the course of his work on the determination of tantalum, observed that Brilliant Green solutions were slow to reach a maximum absorbance value when diluted to the point where their
The Brilliant Green Dimer

--- upper

--- lower

(Figure 19)
absorbance values could be measured in a 1 cm cell. This was attributed to de-dimerisation of the Brilliant Green dimer.

The present short chapter is the record of an attempt to illustrate some of the practical implications of this effect to the analyst working with basic dyes.

6.2 Brilliant Green

Initial studies were made by comparing the absorbances of freshly diluted Brilliant Green solutions made directly from the solid dye, with the absorbances of solutions which had been prepared by dilution of a concentrated dye solution which had been prepared the previous day. Both aqueous and ethanolic solutions were used, and all solutions were stored in the dark.

The results of the preliminary studies are summarised in table 18. The theoretical absorbance maximum of 1.045. It can be seen that on immediate dilution from the solid, high absorbance values were obtained for both ethanolic and aqueous solutions (92.7% and 96.8% of the theoretical maximum respectively) which would give an adequate amount of Brilliant Green monomer in any practical extraction.

However, when a concentrated stock solution (0.05%, 1.03 x 10^{-3} M) such as is used in nearly all determinations with Brilliant Green, was diluted to the point at which an absorbance value can be measured (0.0005%, 1.03 x 10^{-5} M) the results for the two solvents varied. In the case of ethanolic solutions, a high absorbance value similar to that obtained from direct dilution, was obtained immediately upon dilution from the concentrated solution. However, in the case of the aqueous solutions the initial absorbance value, obtained immediately upon dilution was only about 27% of the theoretical maximum rising to about 80% after a period of about two hours. This effect is illustrated by the data plotted in figure 20. It is
Table 18
Comparison of Brilliant Green Solutions made by direct dilution, with those prepared from a concentrated dye solution

A. Aqueous Solutions

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Solution</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Directly from solid to 0.0005%</td>
<td>1.005</td>
</tr>
<tr>
<td>2</td>
<td>18 hrs at 0.05% then dilution to 0.0005%</td>
<td>0.286</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 18 hrs</td>
<td>0.960</td>
</tr>
<tr>
<td>3</td>
<td>42 hrs at 0.05% then dilution to 0.0005%</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 24 hrs</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 42 hrs</td>
<td>0.982</td>
</tr>
</tbody>
</table>

B. Ethanolic Solutions

<table>
<thead>
<tr>
<th>Day of Experiment</th>
<th>Solution</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Directly from solid to 0.0005%</td>
<td>0.962</td>
</tr>
<tr>
<td>2</td>
<td>18 hrs at 0.05% then dilution to 0.0005%</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 18 hrs</td>
<td>0.152</td>
</tr>
<tr>
<td>3</td>
<td>42 hrs at 0.05% then dilution to 0.0005%</td>
<td>1.002</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 24 hrs</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>Dilute solution (0.0005%) stored for 42 hrs</td>
<td>0.076</td>
</tr>
</tbody>
</table>
Aged Brilliant Green (0.05%), diluted to 0.0005% with water

\[ \lambda = 640 \text{ nm}. \]
Dedimerisation of Brilliant Green

\[ \log_{10}(A_{\text{mm}} - A) \]

Time (mins)
believed that during the period of storage in concentrated aqueous solution a Brilliant Green dimer is formed, which slowly dedimerises upon dilution, causing the gradual rise in absorbance at the wavelength of the monomer. It is interesting to note that Rabinowitch and Epstein (169) found that Methylene Blue formed dimers easily in aqueous solutions but not in ethanolic solutions.

When in dilute solutions (0.0005%), it can be seen that the ethanolic solutions deteriorate badly over 18 hours in spite of being stored in the darkness. Aqueous solutions, however, seem much more stable, and would be useful for extraction of anions even after two days. Exposure to light seems to accelerate the deterioration of aqueous solutions as illustrated by the data plotted in figure 22. Even so, after a period of one hour the solutions would be quite suitable for extraction purposes.

An attempt was made to compare the size of the monomer and dimer peaks in concentrated and dilute aqueous solutions, both before and after equilibrium had been reached. The results are given in table 19. It can be seen that in concentrated solutions and unequilibrated dilute solutions the ratio of dimer to monomer peak intensity is greater than in the equilibrated dilute solutions, confirming that this slow attainment of equilibrium on dilution is probably due to dimerisation. Due to peak overlap, these values are not representative of actual concentration ratios, but nevertheless serve as an indication of what is occurring.

It was found that when the dye was equilibrated in 1.0 molar potassium hydrogen sulphate, there was no difference in the ratio of the monomer and dimer absorbances. A slight weakening of the whole spectrum occurred, similar to that observed by Rabinowitch and Epstein for Thionin (169), which they ascribed to the formation of undissociated Thionin chloride molecules. This confirms again that
Stability of Directly-Diluted Brilliant Green Solutions

Freshly made solutions, diluted directly from solid, and exposed to light.

- 0.0005% in EtOH
- 0.0005% in H₂O
Table 19
Monomer - Dimer Ratios for Brilliant Green

A. Comparison of Freshly Diluted and Equilibrated Dilute Aqueous Solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>Absorbance</th>
<th>Ratio $A_{\text{monomer}}$ to $A_{\text{dimer}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibrated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0001%</td>
<td>0.77</td>
<td>0.33</td>
</tr>
<tr>
<td>Diluted 5 mins</td>
<td>0.27</td>
<td>0.13</td>
</tr>
</tbody>
</table>

B. Comparison of Concentrated and Dilute Solutions at Equilibrium

For constant (concentration x path length)

1. 4 cm cell, 0.0001% aqueous Brilliant Green

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Absorbance</th>
<th>Ratio $A_{\text{m}}$ to $A_{\text{d}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>625 nm. (monomer)</td>
<td>0.58</td>
<td>0.46</td>
</tr>
<tr>
<td>585 nm. (dimer)</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

2. 0.1 cm cell, 0.004% aqueous Brilliant Green

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Absorbance</th>
<th>Ratio $A_{\text{m}}$ to $A_{\text{d}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>625 nm. (monomer)</td>
<td>0.46</td>
<td>0.51</td>
</tr>
<tr>
<td>585 nm. (dimer)</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>
the dimer is of the form \((\text{Brilliant Green})^2_{2} \cdot 2\text{HSO}_4\) rather than an anion – cation association. It should be noted that the concentration of the hydrogen sulphate ion in this experiment was \(10^5\) times greater than in the aqueous 0.0005% solutions. As the spectral weakening was only 14%, under these extreme conditions, the effect can be discounted for all normal experiments.

An attempt was made to form a complex molecule, Brilliant Green – Ethyl Violet in a concentrated solution, in the hope of obtaining a new absorbance peak which was easily distinguishable from those of the individual monomers. This dye was chosen as its structure is very similar to that of Brilliant Green itself (figs. 19 and 35). However, it appeared that Ethyl Violet did not itself dimerise under the same conditions as Brilliant Green and effectively blanked out the spectrum, so that it was impossible to tell whether or not the inter-dye complex had been formed.

Burgess et al. (154) indicated that the stability of the \(R^+\) form of Brilliant Green (the green singly-charged monomer) was pH dependent. It was therefore decided to allow a concentrated solution of Brilliant Green (0.05%) to dedimerise in various barbituric acid buffers. From past experience, it was known that no Brilliant Green – buffer complexes were formed with this type of buffer. Figure 23 shows the results which were obtained when absorbance readings were taken over a period of time for various pH values and figure 24 shows the general maximum absorbance values obtained for a whole series of pH values.

It is possible, as indicated by the results in table 18 that the maximum absorbance value which is obtained at pH 6, occurs somewhere between 2 and 22 hours and is not indicated in figure 24. Above pH 8 a colourless precipitate appears after a few hours, without any initial increase of the green colour. This is due to the formation of a neutral carbinol form of Brilliant Green. Below pH 2, the solution dedimerises
Dilution of 0.05% Aqueous Brilliant Green to 0.0005% with Barbituric Acid Buffers.

- pH 3
- pH 5
- pH 7

Time (mins)
Dilution of Brilliant Green (0.05%) with Barbituric Acid Buffers to 0.0005%
to the yellow double-positively charged form of Brilliant Green, which also fades after a few hours, probably due to an attack on the molecular structure by the acid.

As it was obvious that when aqueous solutions of Brilliant Green were first diluted, only about 20% of the monomeric form was available to complex anions, the effect of the use of aqueous Brilliant Green solutions on a typical determination was examined. The Brilliant Green - perrhenate system was chosen as this was known to be very reliable. The results are shown in table 20.

Table 20

<table>
<thead>
<tr>
<th>Brilliant Green solution</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05% in ethanol (immediately after extraction)</td>
<td>1.115 1.117</td>
</tr>
<tr>
<td>0.05% in water, aged (immediately after extraction)</td>
<td>0.552 0.528</td>
</tr>
<tr>
<td>0.05% in water, aged (1/2 hr after extraction)</td>
<td>0.550 0.528</td>
</tr>
</tbody>
</table>

It can be seen that only the monomer form of Brilliant Green, almost totally present in the ethanolic solution, but only partially so in the aqueous solution, takes part in the extraction. In this case, there was an excess of anion in the aqueous phase due to the dimerisation effect of the aqueous Brilliant Green, so low results were obtained. As the absorbance of the benzene extract did not increase on standing, it must be presumed that the dimer plays no part in the extraction.

The Kinetics of the Dedimerisation Effect of Brilliant Green

The kinetics of the reaction were investigated by reference to the data in figure 20. By extrapolation in figure 20, the maximum absorbance

\[ A_{\text{max}} = 0.85 \text{ absorbance units} \]
For first order kinetics:

If \( x \) is the concentration of the dimer in solution, 

\[
\frac{dx}{dt} = -k x
\]

By integration of this equation

\[
\log x + c = -kt
\]

The amount of dimer present in solution at any time will be proportional to \((A_{\text{max}} - A)\), where \( A \) is the absorbance of the monomer peak.

\[
\log (A_{\text{max}} - A) + c = -kt
\]

Figure 21 shows that when \( \log (A_{\text{max}} - A) \) is plotted against time, a straight line is obtained, confirming that the equilibration of Brilliant Green in water obeys first order kinetics, as would be expected for a dedimerisation effect.

Conclusions and Recommendations

Due to the formation of a Brilliant Green dimer in aqueous solution it is recommended that whenever possible, ethanolic solutions of the dyes are used in any determination. However, it has already been shown that in the case of thiazine dyes this may not always be possible due to the enhancement of precipitation effects and adverse effects on the size of the blanks. Some dyes, in fact can only be used in ethanol solutions due to slight solubility in water.

In the absence of other constraints on pH imposed by the anion under determination, such as the necessity to extract the Brilliant Green - hexachloroantimonate at a high concentration of hydrochloric acid because of the type of anion employed, the optimum pH for Brilliant Green extractions will be pH 6, as this is the region of greatest stability of the important Brilliant Green singly-charged monomer.

In the case of most typical Brilliant Green determinations, the anion to be determined is at a concentration of about \( 5 \times 10^{-5} \) M in the aqueous phase, while the concentration of Brilliant Green is
about \( 1 \times 10^{-4} \) M in the aqueous phase. With only about a two-fold excess of Brilliant Green reagent, this does not leave any margin for inactive species, as shown by table 20. Therefore aqueous solutions of the dye reagent, where an uncertain amount of the active species is present, should not be used. Only ethanolic solutions, where dimerisation does not occur at the required concentration, should be used.

6.3 Other Dyes

The following dyes were also examined in a similar manner: Rhodamine B, Butyl Rhodamine B (as discussed briefly in chapter 3), Rhodamine 6G, Rhodamine 3G, Sevron Brilliant Red D, Sevron Brilliant Red 4G, Sevron Bordeaux G, Methylene Blue, Dimethylmethylene Blue and Crystal Violet. It is known that most of these dyes display aggregation effects at high concentrations. However, at the dye concentrations involved in most extraction-photometric procedures, the dimerisation effect did not prove troublesome for any dye other than Brilliant Green. Solutions prepared by dilution from concentrated solutions gave similar absorbance readings to those prepared directly from the solid, even immediately after dilution.

If however, concentrations greater than 0.05% are ever used as stock solutions, it would be wise to check for any dilution effects similar to those described for Brilliant Green, before use in any anion determination.
Chapter Seven

The Effects of Adsorption of Basic Dyes on Cuvette Walls and Experimental Glassware

7.1 Introduction

In the course of experimental work, with most analytical methods using basic dyes, it can be observed that the dye is adsorbed onto the glassware and spectrophotometric cells, to a greater or lesser extent. Most workers ignore this effect on the assumption that the error caused thereby, is insignificant.

In other branches of chemistry, similar adsorption phenomena in relation to dyes are well-known, although not necessarily in relation to glassware. In a study of the dyeing properties of Methylene Blue, Paneth and Radu (173) calculated that the amount of dye taken up by cellulose acetate rayon was 0.027%, which corresponded to the formation of an adsorbed unimolecular layer of dye on the surface of the fabric. It has also been suggested that dyes can be used as an approximate method for estimating the surface area of various adsorbing materials (174).

Reusmann (175) in a study of the Brilliant Green procedure for the determination of perchlorate evolved by Golosnitskaya and Petrashen (147) claimed that he was unable to obtain reproducible results due to the adsorption of perchlorate on the glassware and because of oxidation of the dye by dissolved molecular oxygen. Fogg, Burgess and Thorburn Burns (156) studied the adsorption effect. They performed the perchlorate determination in untreated glassware, silanised glassware and polypropylene ware, and concluded that these effects were not serious in the pH range 3 to 6. Davidson indicated that the adsorption of Methylene Blue can cause considerable errors by deposition on colorimetric cells (54). He noted that the effect
was more marked on ground glass surfaces and suggested that if acid
solutions of the dye were used, the error was considerably reduced.

According to Glasstone (174), very little theoretical data
appears to be available concerning adsorption effects onto solids
from solutions. However, an approximate indication of the tendency of
a dye to adsorb on a given surface, may be obtained from the surface
tension of the solvent. Thus dyestuffs are more readily adsorbed by
charcoal from aqueous solutions (surface tension of water = 72.7 dyne cm$^{-1}$)
than from alcohol solutions (surface tension of ethanol = 22.8 dyne cm$^{-1}$)
in fact alcohol may be used to desorb the dye and clean the surface
again.

7.2 Studies of the Deposition of Methylene Blue and Taylor's Blue
on Cell Walls

This series of experiments was an attempt to elucidate the
effect of deposition of Methylene Blue and Dimethylmethylene Blue
(Taylor’s Blue) on cell walls, which had been observed in previous
studies. Three types of cell were used namely glass, silica and
plastic. The latter were made from polystyrene and were supplied
by W. Sarstedt (U.K.) Ltd., Leicester.

Experimental Work

All experimental glassware was washed in Decon (2%), then
rinsed thoroughly with distilled water and air-dried. The silica
and glass cells were soaked in ethanol, rinsed with distilled water
and air-dried. The plastic cells could not be cleaned with organic
solvents, as they dissolved and hence new cells were used for each
experiment.

Solutions of Methylene Blue (0.05% aqueous) and Taylor's
Blue (0.05% aqueous) were allowed to stand in 1 cm cells of each type.
Absorbance values were measured on the cells after emptying, rinsing
thoroughly and refilling with distilled water. Afterwards, they were
refilled with fresh dye solution and left for a further period of time. It should be noted that these solutions were about ten times as strong as those found in the aqueous phases of most solvent extraction determinations, but they were used to obtain quicker results.

A second similar series of experiments was performed using dye solutions made up in 0.1 M acetic acid. This was the medium suggested by Davidson (54) from which he claimed that no adsorption took place.

Finally, the effect of variation in the concentration of the dye solutions was investigated, by leaving dye solutions of various concentrations in a series of glass cells for a period of 28 hours. As before, the cells were rinsed with distilled water before their absorbance values were measured.

In all cases the absorbance value of the empty cell was subtracted from the value obtained, in order to find the quantity of dye in the cell.

Results
These are summarised in tables 21 and 22 and are shown graphically in figures 25, 26, 27 and 28.

Comments and Conclusions
Before any discussion of these results, it seems reasonable to point out the assumptions which have been made in obtaining the data. Firstly, it is assumed that the absorbance increases linearly with the amount of dye deposited on the cell surface. There are no real justifications for this assumption; Beer's law applies to dilute solutions and therefore is not strictly applicable. However, in spite of this, reasonable results have been obtained at the very low concentration levels used in this experiment.

Secondly, as each glass or other surface is unique in its micro-surface area, duplicate results cannot be obtained satisfactorily.
VARIATION OF RESIDUAL ABSORBANCE WITH TIME (Methylene Blue)
VARIATION OF RESIDUAL ABSORBANCE WITH TIME (Taylor's Blue)

Hours

Ab.

0.06
0.05
0.04
0.03
0.02
0.01

glass (H₂O)
silica (H₂O)
glass (HAc)
plastic (H₂O)
plastic (HAc)
silica (HAc)
EFFECT OF CONCENTRATION ON ADSORPTION OF

TAYLOR'S BLUE (28hr equilibrium)

Ab.

0.05

0.04

0.03

0.02

0.01

0.005

% Aqueous Concentration
EFFECT OF CONCENTRATION ON ADSORPTION OF TAYLOR'S BLUE

(Log Plot)

\( \log_{10} A \) vs. \( \log_{10} \) (molarity)
<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Methylene Blue (0.05% aqueous solution)</th>
<th>Glass</th>
<th>Plastic</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>0.021</td>
<td>0.004</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.020</td>
<td>0.003</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.022</td>
<td>0.005</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.028</td>
<td>0.008</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.041</td>
<td>0.018</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.014</td>
<td>0.004</td>
<td>0.027</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Methylene Blue (0.05% in 0.1 M acetic acid)</th>
<th>Glass</th>
<th>Plastic</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>0.002</td>
<td>0.004</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.003</td>
<td>0.004</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.004</td>
<td>0.005</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.003</td>
<td>0.004</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.004</td>
<td>0.010</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Taylor's Blue (0.05% aqueous solution)</th>
<th>Glass</th>
<th>Plastic</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>0.028</td>
<td>0.007</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.030</td>
<td>0.009</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.033</td>
<td>0.011</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.047</td>
<td>0.022</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.058</td>
<td>0.036</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Taylor's Blue (0.05% in 0.1 M acetic acid)</th>
<th>Glass</th>
<th>Plastic</th>
<th>Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>0.018</td>
<td>0.011</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.019</td>
<td>0.011</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.018</td>
<td>0.010</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.032</td>
<td>0.015</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>0.028</td>
<td>0.027</td>
<td>0.021</td>
<td></td>
</tr>
</tbody>
</table>
Table 22
Effect of Concentration on the Adsorption of Taylor's Blue
(28 hr equilibrium)

<table>
<thead>
<tr>
<th>% concentration (aqueous solution)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.047</td>
</tr>
<tr>
<td>0.02</td>
<td>0.032</td>
</tr>
<tr>
<td>0.005</td>
<td>0.024</td>
</tr>
<tr>
<td>0.002</td>
<td>0.020</td>
</tr>
<tr>
<td>0.0005</td>
<td>0.013</td>
</tr>
<tr>
<td>0.0002</td>
<td>0.012</td>
</tr>
</tbody>
</table>

\[
\log_{10} \text{(molarity)} \quad \log_{10} \text{(Absorbance)}
\]

| \(-2.910\) | \(-1.328\) |
| \(-3.310\) | \(-1.495\) |
| \(-3.910\) | \(-1.620\) |
| \(-4.310\) | \(-1.699\) |
| \(-4.910\) | \(-1.886\) |
| \(-5.310\) | \(-1.921\) |
Even if this were not so, the comparatively large number of cells involved in the experiment, would make duplication virtually impossible for practical reasons.

It appears from the results, that the deposition of dyes on cell walls may be the cause of serious error, due to the build-up of adsorbed dye on the optical surfaces. For example, taking a figure of 0.03 absorbance units, which can be deposited after only one hour; this would amount to a 10% error on an absorbance reading of 0.30 which is a typical figure in the minimum error region as indicated by a Twyman-Lothian plot. The decrease in absorbance of an individual solution, caused by this adsorption is considered to be slight, as the average solution is probably only in the cell for a matter of about a minute in the normal way. If however, a reagent blank is used, which may be in the cell for a longer period, the decrease in solution absorbance caused by adsorption of the dye on the cell wall may be significant. The need to clean the cells very regularly is clearly illustrated. This has also been emphasised by Ashton, Fogg and Thorburn Burns (176) who noticed a gradual build-up of material on the spectrophotometric cells, during the determination of tin with Catechol Violet and cetyltrimethylammonium bromide.

An unrelated effect, which was observed during this present study was the tendency for the solvent to evaporate from the surface of the solution, even with the cell stoppers in place, causing the deposition of solid material around the top of the cell. This dissolved when a fresh solution was placed in the cell, causing yet a further spurious increase in absorbance.

Stearns (177) claims that it is possible to allow the cells to become plated with the dye and then to correct for it in all subsequent determinations, as once a monolayer has formed, it remains on the surface of the glass unless powerful cleansing agents are used to remove it.
However, from the results obtained here, it will be seen that after varying amounts of time, over about 24 hours, the dye tends to desorb, to a greater or lesser extent. It is therefore recommended that this practice is not followed, and that the cells are cleaned each time they are used.

With regard to the best type of cell to use, the plastic ones are undoubtedly the best as far as the prevention of adsorption is concerned. Their chief disadvantage is their lack of tolerance to organic solvents. However, many Methylene Blue experiments are performed on aqueous solutions, where the tendency to adsorb is large, and therefore in these circumstances such cells are highly recommended. Where a conventional cell must be used, silica should be the choice: its tendency to adsorb falling about midway between that of glass and plastic. However in all cases, it is better to clean the cells, or in the case of plastic one to obtain new ones, before each reading is taken.

As Davidson suggested, plating-out occurs less from 0.01 M acetic acid solutions. However, many basic dye analyses are very pH dependent and it may not always be possible to use such conditions. In the case of Dimethylmethylene Blue, the dye tends to precipitate in acid solution, so further increase in acidity could not be tolerated.

Finally, if the results obtained when the variation of absorbance with concentration are examined and compared with the Freundlich adsorption isotherm equation, as shown below, it will be seen that the original assumption, that the amount of dye adsorbed is proportional to the absorbance reading obtained, would appear valid.

Let the the mass of dye adsorbed be $x$
the concentration of dye in solution be $c$
the mass of adsorbant be $m$
n, $k$, $k'$, $k''$ be constants
1. The volume of solution is large and of relatively high concentration, and therefore the concentration of the solution after adsorption has occurred will be considered the same as the solution added.

2. Assume that $A$ is proportional to $x$

Hence $A = k' x$

Freundlich's equation states

$$\frac{x}{m} = k' c^{1/n}$$

$$\log x - \log m = \log k' + \left(\frac{1}{n}\right) \log c$$

But

$$x = \frac{A}{k}$$

Therefore

$$\log A = k'' + \frac{1}{n} \log c$$

If we examine the plot of $\log A$ against $\log c$, it will be seen to be rectilinear as predicted by this equation (fig. 28).

Therefore, the assumption that the Absorbance is proportional to the amount of dye adsorbed on the cell wall, seems to be valid.

7.3 Characteristics of Plastic Cells

If a plastic cell is examined, it will be seen that in most cases, the face of the cell is not optically perfect. At first sight, it seems that the error caused thereby could be serious. However, on examining about 40 such cells, by measuring the absorbance at the wavelength maxima of various solutions; namely acid dichromate (350 nm) copper sulphate (830 nm) and alcoholic Rhodamine B (555 nm), it was found that the coefficient of variation in all three cases was only about 1% which can be attributed to path length and alignment errors, and operator error on the instrument. Thus it can be seen that optically these cells compare reasonably with conventional cells.
7.4 An Investigation into the Effects of Adsorption in the Brilliant Green Determination of Perchlorate

This series of experiments was an attempt to elucidate the reason for the deterioration of solutions with time, in the determination of perchlorate with Brilliant Green. Three possible reasons for the effect have been considered: adsorption of the reagent onto the surface of the apparatus, oxidation of the dye by dissolved oxygen and photodecomposition. As this chapter is principally concerned with the effect of adsorption and its significance, this is considered first.

Experimental

A bulk extract of Brilliant Green perchlorate in benzene was prepared as follows. Perchlorate solution (5 ppm, 21 ml), phosphate buffer (pH 6.5, 49 ml) and Brilliant Green (0.05% in water, freshly made, 7 ml) were extracted into 70 ml benzene using a 500 ml volume separating funnel. The benzene extract was filtered into a conical flask through a Whatman number 41 filter paper. A second similar benzene extraction was performed and added to the first extract. To the combined extracts was added 35 ml fresh benzene and the absorbance of the resulting solution was measured at 640 nm in 1 cm silica cells against a solvent blank.

Weighed quantities of glass beads (60 - 80 mesh) were placed in new 30 ml glass sample tubes, 20 ml of the bulk benzene extract was added and the tubes stored in the dark for $3\frac{1}{2}$ hours. Stoppers were placed on the tubes to reduce evaporation, being loosely applied due to the high benzene vapour pressure. After this time, the absorbances of the solutions in the tubes were measured in the usual way. A further set of tubes was left for 15 hours under similar conditions.
Table 23

Fall in Absorbance after \(3\frac{1}{2}\) hours in the dark

<table>
<thead>
<tr>
<th>Wt beads (gm)</th>
<th>Absorbance after (3\frac{1}{2}) hours</th>
<th>Fall in Absorbance</th>
<th>% Fall in Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.380</td>
<td>0.074</td>
<td>16.3</td>
</tr>
<tr>
<td>0.489</td>
<td>0.374</td>
<td>0.080</td>
<td>17.6</td>
</tr>
<tr>
<td>1.039</td>
<td>0.366</td>
<td>0.083</td>
<td>19.4</td>
</tr>
<tr>
<td>1.720</td>
<td>0.360</td>
<td>0.094</td>
<td>20.7</td>
</tr>
<tr>
<td>2.209</td>
<td>0.355</td>
<td>0.099</td>
<td>21.8</td>
</tr>
<tr>
<td>2.861</td>
<td>0.350</td>
<td>0.104</td>
<td>22.9</td>
</tr>
</tbody>
</table>

These results are displayed graphically in figure 29 and 30.

The best straight line was calculated using a least squares procedure.

\[ y \text{ intercept} = 0.0752 \text{ absorbance units} = 16.6\% \]
\[ x \text{ intercept} = 7.139 \text{ grams} \]

Table 24

Fall in Absorbance after 15 hours in the dark

<table>
<thead>
<tr>
<th>Wt beads (gm)</th>
<th>Absorbance after 15 hours</th>
<th>Fall in Absorbance</th>
<th>% Fall in Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.084</td>
<td>0.297</td>
<td>78.1</td>
</tr>
<tr>
<td>0.731</td>
<td>0.062</td>
<td>0.319</td>
<td>83.9</td>
</tr>
<tr>
<td>1.957</td>
<td>0.002</td>
<td>0.379</td>
<td>99.8</td>
</tr>
</tbody>
</table>

These results are displayed graphically in figure 30.

Calculating the best straight line as before,

\[ y \text{ intercept} = 85.1\% \]
\[ x \text{ intercept} = 8.160 \text{ grams} \]
Plot of Weight of Beads Against
Drop in Absorbance
(over $3\frac{3}{4}$ hours)
Comparison of Adsorbance Decrease for Various Weights of Beads after 3\(\frac{3}{4}\) hrs and 15 hrs.
Results

The results are summarised in tables 23 and 24 and are shown graphically in figures 29 and 30.

Initial Absorbances of the bulk benzene extracts,

\[ A_{3 h} \text{ hr sample} = 0.454 \]
\[ A_{15 \text{ hr sample}} = 0.381 \]

Calculations

To find an equation for the increase in glass surface area, available for adsorption, on the addition of a given volume of glass beads.

The volume of the cylinder of liquid = 20 ml

Effective volume in contact with sample tube walls, when beads are added = \((20 + b)\) ml

volume of beads added, \(b = \frac{\text{mass of beads}}{\text{density of beads}} = \frac{w}{d}\)

therefore the effective volume \(= \left(20 + \frac{w}{d}\right) = \pi R^2 h'\) (1)

where \(h'\) is the liquid height with beads present

\(h\) is the liquid height with no beads

\(R\) is the radius of the sample tube

(1) Initially, when no beads are present,

\[ \pi R^2 h = 20 \quad \text{therefore} \quad h = \frac{20}{\pi R^2} \]

therefore the surface area of liquid in contact with the tube, denoted as \(S_0\)

\[ = 2\pi Rh + \pi R^2 \]
\[ = 2\pi R \cdot \frac{20}{\pi R^2} + \pi R^2 \]
\[ = \frac{40}{R} + \pi R^2 \] (2)
When beads have been added, from eqn. (1) \[ \left(20 + \frac{w}{d}\right) = \pi R^2 h' \]

\[ h' = \left(20 + \frac{w}{d}\right) \cdot \frac{1}{\pi R^2} \]

Hence the surface area of liquid in contact with the tube, denoted as \( S_b \)

\[ S_b = 2 \left(20 + \frac{w}{d}\right) + \pi R^2 \]

Therefore from eqns. (1) and (2)

Increase in surface area, \( \Delta S = S_b - S_o \)

\[ \Delta S = 2 \left(20 + \frac{w}{d}\right) + \pi R^2 - \frac{40}{R} - \pi R^2 \]

\[ \Delta S = \frac{40}{R} + \frac{2w}{Rd} - \frac{40}{R} \]

\[ \Delta S = \frac{2w}{Rd} \]

This is the increase in surface area available to the liquid on the sides of the sample tube due to the addition of beads.

Let the radius of the beads be \( r \)

Therefore the volume of each bead = \( \frac{4}{3} \pi r^3 \)

If the density of glass is \( d \),

The mass of each bead, \( w = \frac{4}{3} \pi r^3 d \)

\[ \therefore \text{the number of beads per gram} = \frac{3}{4 \pi r^3 d} \]
Surface area of each bead, \( a = 4 \pi r^2 \)

Therefore surface area per gram of beads, \( A = 4 \pi r^2 \cdot \frac{3}{4 \pi r^3} \frac{1}{d} \)

\[ A = \frac{3}{r d} \]

Hence, the total increase in surface area of glassware available for adsorption,

\[ = A + \Delta S \]

\[ = \frac{2 \pi r}{R d} + \frac{3}{r d} \]

\[ = \frac{1}{d} \left\{ \frac{2 \pi r}{R} + \frac{3}{r} \right\} \]

Consider the glass beads

density of glass = 2.4 to 2.8 gm cm\(^{-3}\)

mesh of beads used = 60 to 80

Therefore the possible minimum and maximum surface area available can be calculated. The results are shown in table 25

<table>
<thead>
<tr>
<th>Physical Properties of the Glass Beads</th>
<th>Minimum</th>
<th>Mean</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of beads (mesh) (microns)</td>
<td>60</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>Radius of beads (microns)</td>
<td>177</td>
<td>213</td>
<td>250</td>
</tr>
<tr>
<td>Density of glass (gm cm(^{-3}))</td>
<td>2.4</td>
<td>2.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Surface area of beads per gram (cm(^2)) (from eqn.(4))</td>
<td>14,070</td>
<td>10,770</td>
<td>8,590</td>
</tr>
</tbody>
</table>

The mean values were used for further calculation purposes,
Consider the sample tube

Radius of the sample tube, \( R = 1.15 \) cm

From eqn. (2)

\[
S_0 = \frac{40}{1.15} + \frac{\pi (1.15)^2}{1.15}
\]

\[
= 34.8 + 4.16
\]

\[
= 39.0 \text{ cm}^2
\]

When 3 grams of beads are added,

\[
\Delta S = \frac{2w}{Rd} = \frac{2 \times 3}{1.15 \times 2.6}
\]

\[
= 2.0 \text{ cm}^2
\]

Hence, the increase in surface area due to the greater contact with the walls of the tube by the liquid, can be ignored in comparison with the surface area of the beads themselves (see table 25).

Consider the Absorbance Values in Table 23

For the addition of 2 grams of beads,

Increase in glass surface area = \( 2 \times 10,770 \) cm\(^2\)

\[
= 21,540 \text{ cm}^2
\]

From the graph (figure 29), this corresponds to an absorbance decrease of 0.021 absorbance units.

Hence, over three hours, the absorbance decrease due to the tube walls,

\[
= 0.021 \times \frac{39}{21,540}
\]

\[
= 4 \times 10^{-6} \text{ absorbance units}
\]

Consider the Absorbance Values in Table 24

Let figure 30, the Comparison of Adsorbance Decrease for Various Weights of Beads after \( 3\frac{3}{4} \) hours and 15 hours, be represented in diagramatic form as shown in figure 31. Assuming that the adsorption on the walls of the container was negligible in comparison with the adsorption on the beads, as has been shown; if the only effect
Diagramatic Versions of Figures 29 and 30

(Figure 31)

(Figure 32)
causing the solution to deteriorate was adsorption by the glass beads, figure 31 could be represented in the form shown in figure 32.

Then for a given addition of beads, \( w \) (from fig. 30)

\[
\begin{align*}
    t_1 &= 3.75 \quad t_2 = 15.0 \\
    w &= 3.0 \quad w = 3.0 \\
    d_1 &= 7.0 \quad d_2 = 29.
\end{align*}
\]

\[
\begin{align*}
    \frac{t_2}{t_1} &= \frac{15}{3.75} = 4.0 \\
    \frac{d_2}{d_1} &= \frac{29}{7.0} = 4.14
\end{align*}
\]

Thus \( d \) is proportional to \( t \)

Therefore within the limits of experimental error, the decrease in absorption is proportional to time.

\[
i.e. \quad \frac{d}{dt} = -kt
\]

Thus the adsorption effect is first order, as would be expected. However, as has already been shown, the surface area available from the container, which is about twice that of a spectrophotometric cell, is negligible, so a large proportion of the absorbance decrease is due to some other effect. As these experiments have been conducted in the dark, this unknown effect could well be oxidation.

When no beads have been added, \( w = 0 \)

\[
\begin{align*}
    t_1 &= 3.75 \quad t_2 = 15.0 \\
    D_1 &= 16.6 \quad D_2 = 85.1
\end{align*}
\]

\[
\begin{align*}
    \frac{t_2}{t_1} &= \frac{15}{3.75} = 4.0 \\
    \frac{D_2}{D_1} &= \frac{85.1}{16.6} = 5.14
\end{align*}
\]
Thus $D$ is proportional to $t$

Therefore within the limits of experimental error, which is expected to be large for reasons discussed previously, the decrease in absorbance is proportional to time,

\[ \frac{d}{dt} (\text{oxidation}) = -k' \cdot t \]

Thus, the other effect, suspected of being oxidation, is first order also. It should be noted that if this secondary effect were second order, the value $\frac{D_2}{D_1}$ would need to be 16. It would thus be impossible for $w_1$ and $w_2$ to be anywhere near the same value, as they would be if experimental error did not occur here.

Conclusions

For this experiment, errors are expected to be large for two reasons. Firstly, although we can calculate the macro area of the surface of the glass beads, the adsorption takes place at a molecular level. It is therefore necessary to assume that the area which can be calculated is proportional to the actual surface area, when indentations and irregularities on the molecular scale are considered. Secondly, error is to be expected due to the localised variations in concentration of the Brilliant Green perchlorate solution, as adsorption takes place. However, as straight line plots have been obtained, it was thought that this effect was not of great significance. It should be pointed out that, as the results obtained were so clear cut in terms of several factors of ten, they were thought to be reliable.

Four conclusions can be drawn from these experiments:–

1) From the calculations on the results obtained in table 23, it is clear that the part played by adsorption on the decomposition of the Brilliant Green complex on experimental glassware from a benzene solution is negligible.

2) As the experiment was conducted in darkness, photodecomposition can
be rules out in this situation.

3) The deterioration of the solutions on standing must be ascribed to oxidation or other unspecified effect.

4) Both the unknown effect and the adsorption process follow first order kinetics.

7.5 Other Effects causing the Deterioration of Solutions of Brilliant Green Perchlorate

In order to examine effects other than adsorbance on the Brilliant Green perchlorate system, various other tests were performed.

7.5.1 Adsorption from the Aqueous Phase

As the surface tension of water (72.7 dyne cm\(^{-1}\)) is much greater than that of benzene (28.8 dyne cm\(^{-1}\)), it is more likely that adsorption would occur from the aqueous phase. However, after leaving the aqueous solutions in contact with the separating funnels for varying lengths of time, it was found to be impossible to detect any difference in the absorbance of the subsequent benzene extracts. If any difference exists, it must be of the same order as the experimental error; at least over a period of one hour, which was the time limit of the data obtained. As no extraction with Brilliant Green takes this long, the effect can be ignored, providing the separating funnels are thoroughly cleaned between each experimental extraction.

7.5.2 The Effect of Light

When the effect of light was investigated, it was found that over a period of 2\(\frac{1}{2}\) hours, solutions stored in the sunlight in glass cells deteriorated about 2\(\frac{1}{2}\) times faster than those kept in the darkness. This is illustrated by the results shown in table 26.

In practice, in the case of most Brilliant Green extractions, the solutions are not allowed to stand before measurement, as the colour
**Table 26**

Comparison of Benzene solutions kept in the Darkness and in Sunlight in Glass Cells over a period of 2½ hours

<table>
<thead>
<tr>
<th>Initial Absorbance</th>
<th>Final Absorbance</th>
<th>Difference in Absorbance</th>
<th>Mean Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darkness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.428</td>
<td>0.396</td>
<td>0.032</td>
<td>0.027</td>
</tr>
<tr>
<td>0.430</td>
<td>0.408</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>Sunlight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.428</td>
<td>0.368</td>
<td>0.060</td>
<td>0.064</td>
</tr>
<tr>
<td>0.422</td>
<td>0.354</td>
<td>0.068</td>
<td></td>
</tr>
</tbody>
</table>

**Table 27**

Comparison of Extracts stored under Oxidising and Non-oxidising Conditions

<table>
<thead>
<tr>
<th>Gas</th>
<th>Buffer</th>
<th>Initial Abs'nce</th>
<th>After 4 hrs Abs'nce</th>
<th>Difference</th>
<th>After 3 days Abs'nce</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>air</td>
<td>Ascorbic</td>
<td>0.413</td>
<td>0.368</td>
<td>0.045</td>
<td>0.176</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>no &quot;</td>
<td>0.424</td>
<td>0.406</td>
<td>0.018</td>
<td>0.208</td>
<td>0.216</td>
</tr>
<tr>
<td>$O_2$</td>
<td>Ascorbic</td>
<td>0.410</td>
<td>0.344</td>
<td>0.066</td>
<td>0.039</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td>no &quot;</td>
<td>0.402</td>
<td>0.330</td>
<td>0.072</td>
<td>0.277</td>
<td>0.135</td>
</tr>
<tr>
<td>$N_2$</td>
<td>Ascorbic</td>
<td>0.410</td>
<td>0.392</td>
<td>0.018</td>
<td>0.364</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>no &quot;</td>
<td>0.410</td>
<td>0.392</td>
<td>0.018</td>
<td>0.356</td>
<td>0.054</td>
</tr>
</tbody>
</table>
is present instantaneously. Therefore, apart from the fact that exposure to bright sunlight should be avoided during the course of the experiment, the effect of light can be ignored or at the worst be considered as part of the unavoidable experimental error.

7.5.3 The Effect of Dissolved Oxygen in Solutions

An attempt was also made to determine the consequences of the dissolved oxygen in the solution, and the effect of having ascorbic acid as 'anti-oxidant' in the solutions. Various benzene Brilliant Green - perchlorate extracts were made from ascorbic acid buffers and buffers at a similar pH, but containing no ascorbic acid. The extracts, after treatment with oxygen or nitrogen gas, were stored in the darkness in glass cells and the absorbance values were measured after varying periods of time. The results are summarised in table 27.

In the case of benzene solutions which were treated by passing nitrogen through them before storage in the dark, it was found that the extracts from ascorbic acid buffers and those from buffers without ascorbic acid produced similar results. In both cases about a 5% decrease in absorbance occurred over four hours and about a 13% decrease over 3 days.

However, when dissolved oxygen was present in the benzene extracts, far from preventing deterioration of the solutions, the ascorbic acid accelerated the deterioration, this being particularly noticeable after 3 days. In the case where the solutions were saturated with oxygen and ascorbic acid was present in the aqueous phase during extraction, the greatest deterioration occurred. It is therefore strongly recommended that ascorbic acid should not be used as an anti-oxidant in the Brilliant Green perchlorate determination. At best, it is useless and at the worst it has a definite adverse effect.

The difference between the results obtained for the nitrogen-saturated solutions and for the oxygen-saturated solutions, clearly
show the effect of oxygen on the deterioration of the solutions. The evidence supports the assumption made in the previous section, that oxidation is most likely the chief factor involved in solution deterioration.

7.6 Conclusions and Recommendations

In respect of the findings in this chapter, the following recommendations are made:

1) When any absorbance measurements are made with basic dyes, the cells should be cleaned before each measurement with acetone or ethanol, to avoid the error caused by a build-up of deposit on the cells.

2) Whenever possible, plastic cells should be used, but where the choice of solvent prevents this, silica ones are the next choice.

3) It is not recommended that the solutions are stored for any length of time, but if they must be left for a few minutes, they should be kept in the dark. If the organic extracts must be kept for more than about 15 minutes, bubbling nitrogen through the solutions will further prevent deterioration, but this should not be regarded as an absolute measure.

4) The use of ascorbic acid in buffers should be discontinued, as it seems to accelerate the deterioration of the Brilliant Green perchlorate extracts in benzene.
Chapter Eight

The Determination of Nitrate with Basic Dyes and the Investigation of a Nitrate-Sensitive Electrode, Based on Crystal Violet Nitrate

8.1 Introduction

Over the years, there has been a considerable search for an ideal means of determining the nitrate content of various samples, ranging from ores to sewage. Nitrate is one of the forms in which nitrogen appears in the natural nitrogen cycle; the others which occur in solution being ammonia and nitrite. As such, it is common for samples to contain substantial quantities of both the latter ions, so any analytical method should be suitably selective.

The determination of nitrate in water samples is important for two reasons. Firstly, a high level of nitrate can cause cyanosis in young babies, sometimes with fatal results. The problem occurs mainly in areas where boreholes are used for the local water supply, such as in East Anglia. A notable case was that of the North Lindsey water board, where an abnormally high nitrate concentration in the local water caused concern in the national press (118,119).

Secondly, a sudden increase in the nitrate content of any given water supply, may indicate a new form of organic pollution, such as fertiliser, manure, effluent or dead animals.

Another common type of sample is sewage. Here, the determination of nitrate serves as a guide to the efficiency of the oxidation process in the secondary treatment facilities. As this oxidation occurs by a bacterial process, care must be taken to determine the nitrate content of the sample as soon as possible after collection. This also applies to soil and plant samples.

Only a brief summary of existing methods for nitrate determination
will be given here as the subject has been reviewed in detail in a previous dissertation (180). The methods fall broadly into five categories: volumetric, gravimetric, gasometric, spectrophotometric and electrochemical.

The volumetric methods are nearly all based on a reduction process, where either the titrant is directly oxidised such as in the decolourisation of indigo carmine by nitrate (181) or where the nitrate is first reduced and the resultant ammonia determined by titration with standard acid. Such is the Devarda’s alloy method, first proposed in 1892 (181,182).

Few gravimetric methods exist because of the extremely high aqueous solubility of most simple nitrate salts. The most common reagent is nitron (diphenylendoanilino dihydrotriazole) in acid solution (183). To obtain satisfactory results, the conditions must be carefully controlled, but nevertheless the reagent is basically non-selective and expensive. Salem and Stephen made a detailed study of nitrate precipitants and have developed several new reagents (184).

Gasometric methods are generally unsatisfactory because of the need for carefully controlled conditions to obtain reliable results. Ohashi and Makishma (185) proposed a method based on the liberation of nitrogen with a phosphoric acid – iodic acid mixture, and a further method exists whereby the nitrate is converted to nitric oxide by reduction with an iron(II) salt (6).

Spectrophotometric methods fall into two categories, measurements in the ultra-violet region and measurements in the visible region. The former are usually based on direct measurement of the absorbance of the nitrate ion itself, which has peaks at 210 nm. and 303 nm. (186) but in previous work by the author a method was developed whereby the nitrate ion was extracted into chloroform as an ion-association complex with the tetraphenylphosphonium ion, giving an absorbance
maximum at 269 nm. (167).

The colorimetric methods available are numerous and varied. The nitrate content of the sample is either measured by direct interaction with the reagent or by the determination of the ammonia or nitrite produced by a controlled reduction process. Among the reagents which have been employed are phenol disulphonic acid (187); brucine (188); and Nessler's reagent after reduction to ammonia (189). Only two basic dye methods exist. These are discussed in detail later.

The electrochemical methods are of two types, polarography and ion-selective electrode. The former include a method based on the reduction of 4-nitro 2,6 xyleneol (nitrate and 2,6 xyleneol reagent) at a mercury - mercury(I) sulphate electrode (190) and a method based on the catalytic effect of the nitrate ion on the diffusion-controlled waves of molybdenum(VI) (191). As ion-selective electrodes have only been mentioned briefly in the previous survey, and are of prime interest in this study they will be discussed in more detail presently.

Although the preceding methods are not discussed in great detail here, most of those mentioned are long and tedious, even the simpler ones needing time of the order of half an hour to complete, assuming that the equipment, reagents and standards have been prepared previously. When this is added to the time required to obtain, transport and prepare samples, a considerable amount of time has elapsed before results can be obtained. This could be critical, both because of sample deterioration and because for example, in a sewage works or treatment plant a considerable amount of sub-standard effluent can be discharged before laboratory results indicate the fact. Additionally, it is desirable in many circumstances to have a method available which can be used easily by relatively unskilled people. A simple, selective and efficient electrode would be ideal in such circumstances.
8.2 Ion-Selective Electrodes for Nitrate

In spite of the great potential use for a nitrate electrode, only one principal type has so far been developed commercially. These electrodes are based on the use of a liquid ion-exchanger, absorbed into a filter membrane, which separates the test solution from the internal reference solution (fig. 33). Three examples of this type of electrode are commercially available; the Orion 92-07 nitrate electrode, the Beckman 39618 nitrate electrode, and the Corning 476134 nitrate electrode. The Orion 92-07 electrode uses a tris(substituted orthophenanthroline) nickel(II) ion exchanger, but no published information is available for the other two. The Orion electrode seems to have been most widely used in work reported in the literature. Examples of its use are the determination of nitrate in soils, waters, plant extracts and explosives; for potentiometric titrations and for the study of microbiological reactions (199).

The Orion and Corning liquid ion-exchangers have been incorporated into a polyvinyl chloride membrane by Davies, Moody and Thomas (192). The membrane was mounted as shown in figure 34. It was stated that iodide, chlorate and perchlorate constitute the most serious interference for both these electrodes and the commercially available ones based on ion-exchangers.

A further membrane electrode has been developed by Godievskii et al. based on the use of nitron dissolved in benzyl alcohol (193). The electrode is said to be sensitive to $10^0$ to $10^{-3}$ M nitrate and can be used in the presence of many foreign ions.

With the introduction of a Methylene Blue uranyl tribenzoate electrode for the determination of uranium by Entwistle and Hayes (194) interest has been aroused in ion-selective electrodes based on basic dye complexes. After the development of a Brilliant Green electrode by Fogg, Duzinkewycz and Pathan (195) which was sensitive to zinc,
Types of Ion-Selective Electrode

Liquid ion-exchange Electrode
(Figure 33)

Heterogeneous solid-state Electrode
(Figure 34)
and a further one by Fogg and Pathan sensitive to perchlorate (196), it was decided to investigate the construction of a nitrate electrode on similar lines.

Only two basic dye methods for the determination of nitrate are reported in the literature, neither of which appears to be very satisfactory as a colorimetric method. The first is based on the extraction of Nile Blue nitrate from 0.1 M sulphuric acid into 1,2 dichlorobenzene. The blanks and the percentage extraction are very dependent on the acidity of the aqueous phase and the apparent molar absorptivity is only 3,100 \( \text{l.mol}^{-1} \text{cm}^{-1} \) (197).

It was decided that the method of Yamamoto, Uchikawa and Akabori (198) should be examined further. This method is based on the extraction of Crystal Violet nitrate from a phosphate buffer at pH 6, into chlorobenzene. Few details are given in the original paper, but it seems more likely to produce a successful electrode than the Nile Blue method as the extraction conditions were nearer to neutrality.

8.3 Spectrophotometric Determination of Nitrate by extraction with Crystal Violet (according to the method of Yamamoto et al. (198))

According to the procedure given for this determination, the method is not good, according to the standards of most basic dye determinations: the blanks are high and the recoveries are low. However, as the procedure was one of a single extraction only, it was decided to adapt this to the double extraction procedure developed in the Brilliant Green determinations. A more detailed study than in the original paper, of the precision and interferences involved in the method was undertaken.

8.3.1 Reagents

Phosphate Buffer pH 6: Dilute 500 ml 0.1 M Potassium Dihydrogen
CRYSTAL VIOLET

Basic Violet 3  CI 42555

ETHYL VIOLET

Basic Violet 4  CI 42600
Sodium Nitrate Solution

Dilute 3.425 gm sodium nitrate to 1 litre with distilled water. For a working solution, dilute 10 ml of this solution to 1 litre. The final solution is 25 ppm nitrate ion.

Crystal Violet

0.04% (w/v) in water

Chlorobenzene

Koch-Light 99%

Procedure

Into a separating funnel, was pipetted phosphate buffer (5 ml), nitrate standard solution or unknown (5 ml), and Crystal Violet solution (1 ml). The solution was extracted with chlorobenzene (10 ml). The extract was run into a 25 ml standard flask through a Whatman number 41 filter paper. A second 10 ml extraction was performed on the same aqueous phase, and the combined extracts were made up to 25 ml with chlorobenzene. The absorbance was measured at 595 nm. against a solvent blank using 1 cm silica cells.

8.3.2 Conformity to Beer's Law

From the standard calibration plot given in figure 36, it will be seen that the solutions conform to Beer's law only from 0 to 15 ppm.

8.3.3 Interferences

(1) Nitrite

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ppm NO₃⁻</td>
<td>0.345 0.348</td>
</tr>
<tr>
<td>10 ppm NO₃⁻</td>
<td>0.370 0.374</td>
</tr>
<tr>
<td>10 ppm NO₂⁻</td>
<td></td>
</tr>
</tbody>
</table>

10 ppm nitrite causes an absorbance error of 0.025 a.u. This is equivalent to 1.25 ppm Nitrate.

At first sight, this may appear serious, but it should be remembered...
DETERMINATION OF NITRATE WITH CRYSTAL VIOLET (Beer's Law Plot) (Figure 36)
that nitrite is a transient oxidation state in the nitrogen cycle, and at least in water samples, is unlikely to be present at a concentration much more than 5 ppm. The error caused in a nitrate determination under a practical situation, is therefore likely to be quite small.

(2) Other Ions

Qualitative tests were performed by adding about 10 mg solid sodium or potassium salt of the appropriate ion to the extraction solution in the separating funnel.

<table>
<thead>
<tr>
<th>Ion Added</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>0.352</td>
</tr>
<tr>
<td>Cl</td>
<td>0.416</td>
</tr>
<tr>
<td>ClO₃⁻</td>
<td>&gt;2</td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Br⁻</td>
<td>1.415</td>
</tr>
<tr>
<td>I⁻</td>
<td>&gt;2</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>0.364</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.378</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>0.372</td>
</tr>
<tr>
<td>Ac⁻</td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

Even bearing in mind that the amounts of foreign ion added are large, it will be seen that the potential interferences in this method are numerous. Particularly serious is that of chloride, which is a common ion in many water samples. It is unfortunate that acetate causes such great interference, as this eliminates the possibility of precipitating the unwanted chloride with a soluble silver salt.

8.3.4 Precision of the Method and Recovery of the Sample

A series of determinations was performed under the same conditions, and a coefficient of variation of 2.25% was obtained for a series of ten
determinations at a nitrate concentration of 10 ppm. This is comparable to most basic dye determinations, in the absence of foreign ions, and on a pure aqueous sample.

The molar absorptivity calculated for the method was only \(7,000 \text{ l.mol}^{-1}\text{cm}^{-1}\), which is equivalent to only about a 7\% extraction. At first sight, this value does not agree with that in the published method, if it is assumed that the usual 1 cm cells were employed. However, if it is born in mind that this is a double extraction procedure and not a single one, it can be calculated that the original method used 5 cm cells for the measurement of the absorbance value.

8.3.5 A Possible Modification of the Reagents

As chloride interfered in this method but sulphate did not, an attempt was made to produce a hydrogen sulphate form of Crystal Violet to see if by reducing the chloride content of the reagent, a lower blank could be obtained. The method used was similar to that for obtaining Crystal Violet nitrate, which will be described later in this chapter. Unfortunately, the results obtained were similar to those for the original reagent.

An attempt was also made to use Ethyl Violet for the extraction but this produced even larger blanks and was less sensitive than the original reagent.

The use of barbiturate buffers, which are often found to be more suitable for basic dye determinations, met with a similar lack of success, in improving the original method.

8.3.6 Conclusions

In spite of the fact that this determination does not compare well with the general standards of basic dye determinations because of its high blanks and low recoveries, it is competitive with many of the general colorimetric methods available for the determination of
nitrate. For example, the phenol disulphonic acid method, in common use, although applicable over a wider range (10 to 400 ppm) is only precise to 5% and is plagued by interference from many common ions (107).

Therefore particularly in view of the improvements made by the introduction of a double extraction technique, and the general elucidation of the method, the determination may have much more to recommend it, than would appear at first inspection.

8.4 A Possible Nitrate Electrode Based on Crystal Violet Nitrate

8.4.1 Preparation of Crystal Violet Nitrate

Because many basic dye chloride salts do not precipitate with silver nitrate, an indirect method had to be used to prepare the Crystal Violet nitrate. The Crystal Violet chloride was allowed to react with sodium hydroxide solution in order to precipitate the carbinol base. This base was then dissolved in nitric acid and the pH adjusted in order to obtain the singly-charged purple form of the dye.

(a) Preparation of the Carbinol Base

A concentrated aqueous solution of Crystal Violet (100 ml, 0.5%) was filtered to remove undissolved solid and then mixed with sodium hydroxide solution (100 ml, 4 M). The precipitate, a greyish-purple solid, was filtered through a Buchner funnel, and washed with dilute sodium hydroxide (0.1 M) until no further chloride ion appeared in the filtrate. The test for this was by neutralising the washings with sulphuric acid and then adding silver nitrate. The Crystal Violet carbinol precipitate was sucked dry and then allowed to dry further, by being left in a warm atmosphere overnight.

(b) Preparation of the Crystal Violet Nitrate

The solid carbinol base was dissolved in nitric acid (100 ml, 1M)
and then the pH adjusted by the addition of more sodium hydroxide, until the required purple form of the dye was obtained about pH 4. The solution was then evaporated slowly, the pH being continually adjusted by the addition of more sodium hydroxide, until the total volume was approximately 30 to 40 ml. This was cooled, poured into a separating funnel and the dye product extracted by successive 20 ml portions of dichloromethane, until most of the product had been removed from the aqueous phase. The organic solvent was removed by evaporation over a waterbath in the fume cupboard.

8.4.2 Preparation of the Electrode Membrane

Solutions of Crystal Violet nitrate (1 x 10^{-3} M) in chlorobenzene and orthodichlorobenzene were prepared, and circular pieces of natural rubber sheeting, about 1 cm in diameter, were allowed to soak in them for two days, or until they had become completely impregnated with the dye compound. Some pieces were dried on a tissue and inserted directly into the electrode body and some were pre-conditioned by soaking for 24 hours in a 1.0 M sodium nitrate solution.

8.4.3 The Electrode Body and Other Apparatus

The electrode body used, was that developed by Entwistle and Hayes (194) and used successfully by Pathan et al. in this laboratory (195,196). It consists of a hollow P.T.F.E. tube, with a section cut off at the end, which was equipped with a screw thread in such a way that the membrane could be inserted into the bottom of the tube. Electrical contact with the back of the membrane was made with a carbon rod, which passed through the main body of the electrode. This was connected to the pH meter by means of a coaxial cable (fig. 37). A silver-silver chloride electrode was used as a reference electrode. The reaction vessel was maintained at 25°C by means of a water jacket connected to a thermostatted water bath.
8.4.4 Results

The results are summarised in table 28 and are displayed graphically in figures 38 and 39.

8.4.5 Comments and Conclusions

An ideal electrode, should be quick to respond, should give a linear Nernstian response, and give reproducible results. Sadly, this electrode falls short of nearly all these requirements.

The membrane which was soaked in chlorobenzene gave an almost linear response when it was first removed from the Crystal Violet nitrate soaking solution, but this response per decade change in molar concentration was only about 22 mV, compared with a theoretical value for a monovalent ion of 59 mV as predicted by the Nernst equation. When not in use, the electrode membrane was stored under
Table 28

Response of the Crystal Violet Nitrate Electrode

1. Chlorobenzene Organic Phase

<table>
<thead>
<tr>
<th>Molarity of Nitrate Solution</th>
<th>Response in mV of the electrode after ageing for various lengths of Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>262</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>248</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>227</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>206</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>182</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>148</td>
</tr>
<tr>
<td>$10^{0}$</td>
<td>119</td>
</tr>
</tbody>
</table>

2. Orthodichlorobenzene Organic Phase

<table>
<thead>
<tr>
<th>Molarity of Nitrate Solution</th>
<th>Response in mV of the electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh from Solvent</td>
</tr>
<tr>
<td></td>
<td>After 24 hrs conditioning in 1.0 M NaNO₃</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>292</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>273</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>240</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>229</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>225</td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>198</td>
</tr>
<tr>
<td>$10^{0}$</td>
<td>166</td>
</tr>
</tbody>
</table>

All these values are displayed graphically in figures 38 and 39.
Response of the Nitrate Electrode after Storage

Solvent: chlorobenzene
Effect of Conditioning the Membrane by Soaking in 1M Sodium Nitrate solution
water, but protected by an air bubble trapped at the base of the electrode body. However, when further calibration plots were attempted, completely different results were obtained, as shown in figure 38. The response time was very slow, varying from about 5 minutes for the more concentrated solutions to about 1 hour for the more dilute solutions.

It was noted that after use, the surface area of the membrane in contact with the aqueous solution, was noticeably decolourised. It was thought that much of the lack of success with this system was due to the lacking ability of the chlorobenzene to hold the dye-nitrate compound, which was gradually leached out into the test solutions. It was therefore decided to try a solvent of higher dielectric constant, which would hold more of the dye in the organic phase. Ideally, nitrobenzene (dielectric constant = 35.7) would have been the solvent of choice but it was found not to swell the rubber. Therefore, it was decided to use orthodichlorobenzene (dielectric constant = 9.9) as being the next available solvent immiscible with the aqueous phase.

The best results obtained, were those using a membrane which had been swollen in orthodichlorobenzene and then conditioned by soaking for 24 hours in a 1.0 M sodium nitrate solution, but even so the response was, at best, only about 35 mV per decade change in concentration, and the response was very slow (fig. 39).

Therefore, it was decided that this system was unsuitable for the development of a practical nitrate electrode.
Chapter Nine

Conclusions and Recommendations for Future Work

The aim of this study was threefold: firstly to investigate the use of Rhodamine and Thiazine dyes in solvent extraction procedures; secondly to investigate the possibility of using onium compounds as masking agents in existing basic dye determinations; and lastly to investigate some of the general practical difficulties involved in basic dye determinations which have been noted during the course of this study and by previous authors. This work is a continuation of that undertaken by Burgess (40) on the use of basic dyes in analytical chemistry.

A detailed survey of the uses of Methylene Blue in analytical chemistry has been undertaken, tracing the history of the subject from the development of the dye until the present day. A practical survey of the use of this dye and the recently developed Taylor's Blue (1,9 Dimethylmethylene Blue), revealed the possibility of using the latter for the determination of perchlorate. This has been developed into an analytical procedure, which is of use in the determination of perchlorates in the presence of chlorate. The method is applicable in the concentration range 0 to 10 ppm perchlorate ion and will tolerate at least a four hundred-fold excess of chlorate ion. Although the molar extinction coefficient is only 39,000 l.mol$^{-1}$cm$^{-1}$ the size of the blank and the precision of the method is very good. This appears to be the first solvent extraction procedure to be developed using Taylor's Blue.

Until the present work was undertaken, Butyl Rhodamine B has received little attention in the West, and most of the work in the literature originates from Eastern Europe. A survey of the anions for which it can be used as a reagent has been undertaken, and the methods
compared with those of the parent dye, Rhodamine B. The published procedures for the determination of gallium and perrhenate with Butyl Rhodamine B have been investigated and elucidated. A general survey of the extraction of simple ions with both dyes revealed a possible method for the determination of dichromate. This has been investigated further, and a procedure has been described for the determination of dichromate with Butyl Rhodamine B of possible application in the analysis of certain ores and alloys. The method is applicable in the concentration range 0 to 12 ppm. dichromate ion. The molar extinction coefficient is high (87,000 l. mol.⁻¹cm.⁻¹), the blank is small and precision is excellent. It is considered that Butyl Rhodamine B has more potential as an analytical reagent than the relatively small volume of literature suggests.

During the course of this work, it was noted that the dye samples obtained from commercial sources, in particular Ethyl Rhodamine B, did not always meet the required standards of purity. A paper chromatographic method for the examination of Rhodamine dyes was developed and compared with the method of Taylor (161). Additionally, both methods were used to examine a number of cationic dyes designed for use on synthetic fabrics.

The study of onium compounds, with respect to their possible use as masking agents for basic dye systems, in particular the Brilliant Green determinations, unfortunately met with little success. However, the study was useful in that the Brilliant Green determinations developed by Burgess et al. (154,156,163) were thoroughly tested and certain experimental difficulties resolved. Although determinations using other dyes were not investigated, it is not believed that further study in this field would be useful at the present time.

With regard to the studies undertaken into the stabilities of Brilliant Green solutions, certain conclusions have been drawn.
Firstly, that due to the formation of the Brilliant Green dimer in aqueous stock solutions, ethanolic stock solutions should always be used and never aqueous solutions. Secondly, that it is not altogether desirable to use the dye at very high or very low pH values if this can be avoided, due to the formation of the carbinol or the yellow acid form of the dye respectively. It is felt that further study would be valuable in this field, both with regard to Brilliant Green solutions and to other dye solutions.

The studies on the adsorption of dyes on cuvettes were interesting and illustrated certain desirable qualities of the disposable polystyrene cuvettes. Although the application of such cuvettes is limited by their solubility in organic solvents, their relative non-adsorbing properties, coupled with their cheapness make them highly desirable for use with aqueous solutions. Although simple visual inspection, shows that the optical quality seems poor, it has been shown that experimentally this is of little consequence in most determinations. For organic solutions of basic dye complexes, silica cuvettes are recommended on the grounds that they adsorb the dyes less than the glass cuvettes, which at first sight could be used for measurements in the visible region of the spectrum.

The reasons for the deterioration of Brilliant Green perchlorate solutions have been investigated. This deterioration has been shown to be largely due to oxidation, rather than adsorption effects, as had been previously postulated. The use of ascorbic acid, as an anti-oxidant in these determinations has been challenged.

Finally, the determination of nitrate with Crystal Violet was studied. The spectrophotometric method of Yamamoto et al. (198) was investigated and information as to the experimental conditions, not given in the original paper was obtained. It was shown that whilst by the standards of general basic dye determinations, the method leaves
much to be desired, in fact it compares quite favourably with many
other nitrate determinations. An attempt to produce a nitrate-selective
electrode based on this system was unsuccessful, due to the high
solubility of Crystal Violet nitrate in the aqueous phase. Compared
with their use in spectrophotometric methods, basic dyes have received
comparatively little attention in the field of ion-selective electrodes.
Amongst traditional basic dyes, the potential for research in the
solvent extraction spectrophotometric field is almost exhausted,
but many of the methods already published, particularly those involving
bulky anions and ternary complexes, may well prove suitable for
adaptation to ion-selective electrodes. Some work, in addition to that
described here, has already been undertaken in this field by Pathan
and Fogg (195,196) but many systems remain to be investigated.

In the field of spectrophotometry, the future research must
lie with either the Antipyrine dyes, which have been used extensively
in Eastern Europe or with the newer cationic dyes developed for
synthetic fabrics. Certain difficulties exist with the latter, namely
the uncertainty of the structure which exists in many cases and also
the fact that due to their commercial origin, many may be of questionable
purity. In the case of the Antipyrine dyes, an extensive programme of
synthesis may be needed, as none of them are apparently available
commercially in this country. Some of the anions, such as nitrate
and sulphate, which are difficult to determine with conventional
basic dyes, may be more amenable to determination with these dyes.
In fact, little work seems to have been undertaken, in the determination
of simple anions. The whole programme of work in the Eastern
European research schools, seems to be geared to the determination
of metals and metal complexes, with basic dyes.

Although, there is an increasing trend in analytical chemistry
towards mechanisation and automation, it is believed that for many
years to come, there will be a place and a need for such comparatively simple analytical techniques as the basic dye determinations described in this work.
Suppliers of Basic Dyes

   (stains and reagent grades)
   (commercial dyes)
   (commercial dyes)
   (commercial dyes)
5. B.D.H. Chemicals Ltd., Poole, Dorset.
   (stains and reagent grades)
6. Difco Laboratories, West Molesey, Surrey.
   (stains)
   (medicinal grades)
8. Evans Medical Ltd., Speke, Liverpool.
   (medicinal grades)
   (stains)
    (commercial dyes)

The author would like to express her thanks to those companies, who have willingly supplied information and in some cases numerous samples, during the course of this work.
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