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ELECTROANALYTICAL STUDIES OF DYES

by

KWANG-SIK YOO

A thesis submitted to the Loughborough University of Technology in partial fulfilment of the requirements for the degree of Ph.D.

March 1979

Supervisor: A. G. FOGG, Ph.D., A.R.T.C.S.
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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Dr. A. G. Fogg, for his friendly interest and constant guidance throughout the course of this work.

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I should also like to thank my parents and my wife whose love and concern have always been a source of encouragement during my stay in this country.
Acid and basic dyes and food colours have been determined by electroanalytical techniques, i.e., amperometric titration, potentiometry using ion selective electrodes developed here, and differential pulse polarography.

Several procedures for the determination of the food colours Sunset Yellow FCF and Tartrazine in sparkling orangeade, Green S and Tartrazine in sparkling limeade, Amaranth and Green S in blackcurrant health drink, and Chocolate Brown HT, Green S and Tartrazine in sparkling dandelion & burdock have been developed by differential pulse polarography in Britton-Robinson buffer. Tetraphenylphosphonium chloride removes the large polarographic maximum obtained with Tartrazine at pH values greater than 4 and causes the peak potential of the colour to be shifted towards more negative potentials. The addition of tetramethylammonium chloride improves the polarographic baseline for the peaks of colours by suppressing polarographic maxima. The food colours in each drink could be simultaneously determined by the control of pH in addition to the use of tetraphenylphosphonium chloride.

PVC and liquid state ion-selective electrodes have been developed for the determination of acid and basic dyes. The electrodes are based on basic dye 12-tungstosilicate, basic dye tetraphenylborate, tetraphenylphosphonium 12-tungstosilicate, quinoline phosphomolybdate and CI Basic Orange 30:1-Reineckate.
PVC electrodes based on Crystal Violet tetraphenylborate and tetraphenylphosphonium 12-tungstosilicate have produced satisfactory results for the potentiometric titration of several acid dyes with Crystal Violet and basic dyes with sodium tetraphenylborate. The response of the electrodes was sufficiently fast that the electrodes could be used in connection with an automatic titrator. In general, the slope factor of these PVC electrodes was Nernstian, but it gradually decreased with time.

Liquid state electrodes using a natural rubber membrane containing Crystal Violet 12-tungstosilicate or Crystal Violet tetraphenylborate as active material dissolved in o-dichlorobenzene have also been applied successfully to the potentiometric titration of dyes. The life of these electrodes is relatively long because of their easy regeneration.

Amperometric titration employing differential pulse polarography showed a promising applicability for the determination of dyes. The end points for the titration curves of acid dyes with a standard Crystal Violet solution and basic dyes with a sodium tetraphenylborate solution were easily determined by a simple graphical method. The high solubility of the precipitate formed causes the curves to be rounded and also the end point to be reached late.
GENERAL INTRODUCTION

Most analytical publications on synthetic dyes(11) and food dyes(11,136,187) are concerned with identification of dyes usually by the method of Thin Layer Chromatography(TLC). Spectrophotometric quantification can be applied after TLC separation.

Food dyes and their intermediates have been determined by liquid-solid, ion exchange and steric exclusion forms of High Performance Liquid Chromatography(HPLC)(11). But more recently better results have been obtained using the newly introduced paired-ion chromatography(18B,189).

For TLC/UV or HPLC determination dyes are first extracted from the material to be tested. In the case of beverages and water-soluble foods the dyes are adsorbed first onto wool or polyamide and are then re-extracted into an organic solvent. Dyes in more intractable foods undergo a more rigorous extraction with an organic solvent or liquid ion exchange resin, before being adsorbed onto wool or polyamide.

The present work was undertaken in order to assess the value of the differential pulse polarographic and potentiometric methods for the determination of synthetic dyes. Most dyes are reducible at the dropping mercury electrode and give distinct polarographic waves. Clearly dye samples obtained using the clean-up procedures described above could be determined using a differential pulse polarographic finish. The peak potential
observed could be used as partial confirmation of the identity of the dye. Dyes with the same reduction group, e.g. the azo group, do tend to reduce at similar potentials, however, so that identification cannot usually be made unequivocally and the analysis of dye mixture can be difficult without prior separation.

A wide range of ion pairing phenomena has been discussed at the Symposium held in Syracuse, New York from May 30 - June 2, 1978(347).

The study of reactivity and stereochemistry of ions and ion pairs in solution would supply useful information for separation techniques.

Potentiometric determinations of dyes have been reported using ion-selective electrodes responsive to dyes(316) and potassium ion-selective electrodes(305). The well defined titration curves indicated their applicability to the assay of dye samples. Triphenylmethane dyes(289,313,340) were used as the ion-exchange site in liquid membrane electrodes responsive to anions such as salicylate, benzoate, aromatic sulphonate and chlorate. So far no electrodes particularly selective to dye molecules are reported in the literature.

The development of dye electrodes is important from the practical point of view for the control of dye purity and therefore this was made an important aspect of this work.

Amperometric titrations of dyes have been studied by means of the conventional DC polarography(78,79). When the current measurements are made on unstirred
solutions, true polarographic diffusion currents are observed. The end point is obtained from the intersection of two lines or tangents. The reactions were mainly based on ion/ion combination or combination of charged complexes, giving water-insoluble products.

Classical DC polarography has been used widely for chemical analysis for both inorganic and organic compounds. It is not satisfactory at concentrations below about $10^{-5} \text{M}$, and resolution becomes very poor in the presence of a relatively large concentration of a more easily reduced depolarizer.

Osteryoung and Hasebe\(^{(191)}\) have reviewed pulse polarography which was first developed by Barker\(^{(190)}\). Differential pulse polarography is one of the best extensions of DC polarography for the purpose of improving sensitivity and resolution. The main idea behind the pulse technique is that the capacitive current which flows at an electrode in response to a potential pulse decays exponentially, while any faradaic current decays at a much slower rate. The current can be measured at some time after pulse application which is long enough so that the capacitance current is negligible while the faradaic current is still appreciable. The normal pulse polarographic mode employs a series of pulses, each of greater amplitude than the previous one by the same amount, so that the envelope of the potential-time curves is a linear potential scan.

In the differential pulse mode, the potential-
time curve consists of a linear potential scan on which is superimposed a series of constant amplitude pulses. The difference current measurement is essential to this mode, but in the case of the normal pulse mode the signal is just the average current measured near the end of the pulse life. In contrast the peak height in differential pulse polarography, while proportional to analyte concentration, also depends on the electrochemical reduction rate and therefore is sensitive to the exact matrix. Therefore we must be very careful to specify whether we are dealing with a fast(reversible) electro-chemical reaction.

Studies on the choice of pulse amplitude in differential pulse polarography have been reported by several workers(191,211). Generally, the sensitivity increases with increasing pulse amplitude, but the resolution degrades with increase in pulse amplitude. The lower capacitance current in the differential pulse mode permits detection limits in the 20nM range to be attained, that is, about ten times lower than normal pulse polarography. The effects of kinetic and adsorption complications on peak heights in differential pulse polarography(DPP) are not clearly understood.

Current-potential-time relationships in DPP have been rigorously treated by Birke(192) considering the effects of electrode expansion and drop sphericity and current averaging. The difference current expression at peak of the wave in DPP has been
derived and the peak potential where the peak current occurred is shown as

\[ E_p = E_{1/2} - \frac{\Delta E}{2} \]

where \( E_p \) is peak potential, \( E_{1/2} \) is half wave potential and \( \Delta E \) indicates the pulse height applied.

There is an increasing concern by the public as well as by the dyestuff and chemical industries over the misuse of potentially hazardous substances. Recently Simmons et al. reported in an article some environmental properties of dye carriers. In the paper, he states that the environmental impact of a compound should always be judged on the basis of the combination of all four key parameters - movement, degradation, bioconcentration and toxicity. The potential hazard from each compound to men should be considered not only in terms of overall biological activity, but also in terms of the type and length of exposure most likely to be encountered.

Direct differential pulse polarographic procedures for the determination of four food colour mixtures have been developed. Several electroneutalitical methods such as DPP, potentiometry using ion-selective electrodes have been developed, and amperometric titration has been assessed for the determination of acid and basic dyes and some food colours.
CHEMISTRY OF SYNTHETIC DYES

INTRODUCTION

Although synthetic dyes dominate the field nowadays, before their development natural dyes and mineral pigments were used for the colouring of textiles.

The synthetic dyes industry was founded in 1857 by W. H. Perkin (193) when he set up a factory, at Greenford Green near London, for the manufacture of Mauveine from coal tar benzene. Mauveine became accepted by dyers, and a relatively simple process for dyeing cotton after first treating it with tannic acid was soon developed by Perkin and others.

The most important natural dye is logwood (CI natural Black No 1, CI 75290). Other natural colouring products are fustic, quercitron, cutch, turmeric, cochineal, lac dye, and madder (now completely replaced) (194).

Almost all dyes are now derived synthetically, i.e. by known chemical steps, from raw materials - principally the hydrocarbons, benzene, toluene, naphthalene and anthracene - obtained from coal tar produced by distillation of coal out of contact with air, and also, to an increasing extent, from certain operations in the petroleum industry. These hydrocarbons, known as aromatic hydrocarbons, provide the molecular framework for the final dye molecule, such aromatic nuclei or other conjugated systems being essential to the structures of substances having the property of
colour.

It has been estimated that there are in excess of 2,000 individual dyes available today to dyers and other consumers.

Molecular rotation is responsible for the absorption of radiation in the far infrared. A combination of molecular rotation and vibration causes absorption in the near infrared. Absorption in the visible and ultraviolet regions, with which dyes and related substances are concerned, is electronic in origin. For any substance to be coloured its molecule must contain mobile electrons which can be raised from ground to excited states at values of $\Delta E$ which lie between 297 (400nm) and 148.5(800nm)kJ/mole. Structural factors determine whether or not a molecule will absorb in the visible region and they also decide where such absorption will occur. According to the theory of O. N. Witt (1876) the dye molecule is regarded as a combination of an unsaturated kernel with certain groups called chromophores, such a combination being called a 'chromogen', and one or more characteristic substituent groups called auxochromes, the function of which is to intensify colour and to improve the substantivity of the dye for the substrate (fibre, yarn, cloth, plastic or in fact any material which is to be coloured).

Examples of chromophores are: $-N\equiv N-$; azo group, $-NO$; nitroso group, $-NO_2$; nitro group, $>C=O$; characteristic of the triarylmethane system, $>C=O$; anthraquinone dyes
Auxochromes are: \(-\text{NH}_2\), \(-\text{NHMe}\), \(-\text{NMe}_2\), (as such or as cations, e.g. \(+\text{NMe}_2\text{Cl}\)), \(-\text{SO}_3\text{H}\), \(-\text{OH}\), \(-\text{COOH}\) (often as anions, e.g. \(\text{O}^-\), \(-\text{SO}_3^-\)).

In azobenzene \((\bigcirc\text{-N=N-\bigcirc})\) "benzenoid unsaturation" is present as is the azo chromophore. No auxochrome is present, however, and although coloured the substance is useless as a dye, having no aptitude for imparting colour to a substrate. On the other hand the substance \((\bigcirc\text{-N=N-\bigcirc\text{-NMe}_2})\) which contains the dimethylamino group, \(-\text{NMe}_2\) as auxochrome, is strongly coloured and is used as a dye.

In the progression benzene - naphthalene - anthracene, the excitation energy \((\Delta E)\) of a molecule becomes less and hence absorption shifts towards longer wavelengths as the molecule becomes longer, i.e. as the space in which the \(\pi\) electron is free to move increases. A monoazo dye prepared by Brode \((193)\) in 1952 is noteworthy in that the cis - form changes to a coloured trans - form on standing. Certain cases of transient fading in azo dyes brought about by light (phototropy) are known to involve trans - cis conversion, reversal and restoration of colour occurring on storing in the dark. The number of axes of polarizability (direction of oscillation) decides the number of absorption peaks.

The major function of a dye is to give rise to a particular hue in association with a substrate. Natural and synthetic fibres form the most important
substrates in dyeing and printing, though fur, leather, plastics and polymers must be included as substrates also.

The use of certified, or natural colour is permitted in food products to enhance their appearance and make them more pleasing to the eye.

Dyes can be classified in different ways; as by chemical structure, by method of application (as mordant, vat), or by utilization. Azo, triarylmethane, xanthene, thiazine, anthraquinone dyes etc. belong to the classification according to constitution.

The classification by application includes the terms of acid, basic, direct, mordant, disperse, vat, sulphur, leuco, esters of vat dyes, insoluble azo, acetate, pigments, oxidation, natural and inorganic dyes.

Neither system of classification is satisfactory by itself; the same chromophoric system may be present in dyes differing widely in usage and application, the presence or absence of solubilizing groups, proton-accepting groups, long-chain alkyl groups, etc., being among the factors determining dyeing characteristics and suitability for a particular technical purpose.

In general pigments are inert, stable, coloured substances, insoluble in water, which are used for imparting colour by incorporation within an article during manufacture, e.g. moulding from an evenly coloured mass of plastic material or by application in the form of paint.
The CI distinguishes among 'pigments', 'lakes' and 'dyes for lakes' for the purposes of classification. Lakes of acid dyes are precipitated from aqueous solution by organic acids such as tannic acid, or inorganic complex acids such as phosphotungstomolybdic acid. The metal salts of mordant dyes are also used as lakes.

Anionic acid dyes contain as active groups in their chemical structure both a chromophoric group and a water-solubilizing group. Usually the solubilizing group is the sulphonic radical, -SO₃H. The commercial dye appears in the form of the sodium salt normally standardized, that is, diluted to a standard effective concentration with anhydrous sodium sulphate. In the few cases where solubility is not due to a sulphonic group the dye contains either a carboxyl groups or a hydroxyl group, the latter being associated with a nitro, nitroso, or another hydroxy group.

Acid dyes are characterized as follows(194):
(1) they are quite soluble in dilute solution and are much more soluble than the direct dyes, which are also aromatic compounds with sulphonic radicals. (2) a dilute solution contains a relatively larger proportion of dye in the form of ions, simple molecules, and simple micelles than a solution of a direct dye, which contains a greater proportion of more complex micelles. (3) an acid dye has little or no affinity for pure cotton, even in the presence of 1/2% sodium chloride or sodium sulphate based on the weight of the solution.
The acid dyes can be divided into three types:

(i) The simple acid dyes are those that do not contain polyvalent metals in their composition.

(ii) Mordant acid dyes are acid dyes that can combine simultaneously with a mordanting substance, most generally a metal ion or hydrated chromic hydroxide, and with the fibre. Such dyes always contain in their composition a hydroxyl group in a position ortho to an azo group or to another hydroxyl group. The mordant acid dyes are characterized on the whole by superior wet fastness and superior light fastness on both wool and silk. In general, however, there is a lack of bright colours among the mordant acid dyes and the application is more involved than the application of the simple acid dyes.

(iii) Premetallized acid dyes are applied like the leveling simple acid dyes, except that on wool the premetallized dyes require even more strong acid and longer boiling for application than the simple acid dyes. They are characterized by excellent fastness to light and good fastness to washing, perspiration, and medium fulling.

Basic dyes contain unsubstituted or substituted amino or imino groups, which cause the dye to function as a positive ion in dilute acid solution. This class probably comprises the smallest number of dyes and is of least importance for textiles. The first synthetic dye, mauve, prepared by Perkin in 1856, was a basic dye.
Most basic dyes are offered in the form of water-soluble chlorides, but some appear as acetates, oxalates, sulphates, or zinc double salts.

In general, basic dyes are characterized by high tinctorial value and often by exceptional brightness. Only simple acid dyes with analogous structures equal basic dyes in brightness.

Generally dyeing is carried out in aqueous solution. The process of attachment of the dye molecule to the fibre is one of adsorption. There are four kinds of forces by which dye molecules are bound to the fibre:

1. Ionic forces.
3. Van der Waals' forces.
4. Covalent linkages.

Ionic forces are the mutual interactions between positive centres in a fibre and negative centres in a dye molecule or vice versa.

Hydrogen bonds result from the acceptance by a covalently bound hydrogen atom of a 'lone pair' of electrons from an electron donor atom. In the dyeing of wool, silk and the man-made fibres hydrogen bonding is involved in the attachment between dye and fibre. The substantivity of a dye for cellulose must be explained on other grounds.

Van der Waals' forces are those existing between atoms or molecules of all substances and are small compared with the other interatomic forces present.
In the dyeing process they are the result of second-order wave mechanical interaction of the \( \pi \) orbitals of dye and fibre molecules. These forces are especially effective when the dye molecule is linear, i.e., long and flat, and can thus approach close enough to the fibre molecule or molecular unit (cotton substantivity), and when dye and fibre both contain alkyl or aryl groups as is the case with certain wool dyes and with the majority of polyester dyes.

Covalent linkages are actual chemical bonds between dye and substrate molecule. They are brought about by chemical reaction between a 'reactive' dye molecule and, for example, a hydroxyl group of a cotton fibre.

The fastness property of a dye is defined as the ability or otherwise of a dye, in association with a given substrate, to withstand the various agencies: sunlight, washing with soap or detergent, dry cleaning, water, perspiration, and, so on, in processing or in use. For example, by colour fastness is meant the resistance of the hue of textiles to the different agencies to which they may be exposed during manufacture and subsequent use.

The manufacture of a dye from benzene, toluene, xylene, naphthalene, anthracene and other primary raw materials involves a number of prior synthetic stages and transformations such as nitration, reduction, halogenation, amination, sulphonation, diazotization, oxidation and others. The products, precursors of the
dyes themselves, are collectively known as "intermediates".

Disperse and cyanine dyes have been reviewed by Leverenz (219) and anon (220) respectively. Mitsuishi (180) has provided a review on the association or aggregation of dye molecules in dye solutions. Nemoto and Funahash (231) explained the interactions between dyes and surfactants in terms of thermodynamic parameters, complex formation by means of several binding forces, solubility and dispersion.

Many authors have observed that the light fastness of certain dyes applied alone to textile material is much better than when applied as mixtures. This phenomenon has been termed "catalytic fading". The catalytic fading of some yellow azo dyes in the presence of blue, violet or red anthraquinonoid dyes has been investigated by Rembold and Kramer (232). They have shown that the phenomenon occurs only in the presence of oxygen and is suppressed by adding typical singlet oxygen quenchers, such as nickel-dibutyldithiocarbamate (NBC). Bromophenol blue, Crystal Violet, Malachite Green, and phenolphthalein all undergo a slow decolorization (235) upon combining with hydroxide ion. When phenolphthalein (Ph) is added to an alkaline solution it first undergoes a rapid irreversible conversion to the quinoid form (Ph=) which has a pink colour (absorbance peak of 550nm). The quinoid form then slowly and reversibly reacts with hydroxide ion to form the nonresonant (hence colourless) carbinol form (PhOH²). However, the other dyes mentioned above fade irreversibly.
ANALYTICAL CHEMISTRY OF DYES

The determination of environmental quality and the disturbance of the ecological balance have aroused international concern in recent years. Some trace metals in small doses are essential to sustain life, but can be toxic in higher concentrations. The availability of sensitive and rapid analytical methods owing to the remarkable growth in sophisticated analytical instrumentation has generated considerable interest to probe into ecological systems more deeply.

The sources for metal contaminants in dyes are due to residual catalyst, corrosion of equipment, raw water, raw materials, and intermediates.

At present there are neither comprehensive data nor suitable tests available to define precisely the effect on aquatic life of residual dyes in plant effluents from dye production or application.

Chemical carcinogens are of increasing concern to the dye industry. A few aromatic amines used as dye intermediates have been classified by governmental agencies as cancer-suspect agents. The first compounds recognized as chemical carcinogens in 1930-32 belong to this class: benz(a)pyrene (3,4'-benzpyrene) was isolated from coal tar pitch and dibenz(a,h)anthracene (1,2,5,6'-dibenzanthracene) prepared synthetically. Carcinogenic activity is very sensitive to small changes in structure; for example, methyl groups greatly enhance the activity of benz(a)anthracene in some positions but not in others.
Activity is generally destroyed by hydroxyl groups but can be strongly enhanced by methoxyl.

In 1932 prolonged feeding of o-aminoazotoluene (\(\text{CH}_3\text{N} = \text{N} - \text{CH}_3\text{H}_2\)) to rats was found to cause liver tumours. Since then, much experimental work has been carried out with related azo dyes in an attempt to elucidate the mechanisms of carcinogenesis. The most widely studied dye has been 4-dimethylaminoazobenzene (\(\text{CH}_3\text{N} = \text{N} - \text{CH}_3\)) , formerly used extensively in some countries as a food colourant.

Weisburger\(^{(209)}\) reported an article about cancer induction by aromatic amines, azo dyes, nitrosoamines, and mycotoxines.

The first commercial dye establishments were founded between 1860 and 1880 in the Rhine valley of Germany and Switzerland. In 1895, the German physician Rehn noted three cases of bladder cancer, not in a random population, but in employees of a factory making dye intermediates. Rehn attributed these cancers to his patients' occupation, from which evolved the label aniline cancer which is now considered a misnomer.

The dyes which are used in food, clothing, and cosmetics must be harmless as we touch them in our daily lives. Most of the commercial dye molecules have polar substituents that make them innocuous.

As a result of the responsibilities of the Food and Drug Administration or its counterparts in other countries, dyes have been examined with respect to their...
safety, particularly from the viewpoint of carcinogenity.

Liver tumors have been induced in rats by compounds such as Ponceau 3R—the azo dye derived from 2',4',5'-trimethylaniline (H₃C-N=N-N=CH₃ SO₃H) and R acid, 2-naphthol-3,6-disulphonic acid— and by Trypan blue.

\[
\text{H}_2\text{N OH} \quad \text{N=N} \quad \text{N=NH}_2 \\
\text{SO}_3\text{Na} \quad \text{CH}_3 \quad \text{SO}_3\text{Na}
\]

Benzidine and 2-naphthylamine are now recognized as two of the most potent human carcinogens. Major dye manufacturers have recognized the need for additional information concerning toxicological, ecological, and analytical problems common to synthetic dye industries.

A work has been reported on the chronic toxicity of 4 certified food colourings, amaranth, tartrazine, new coccine and Sunset Yellow, in rats (201). The results indicated that female rats may be more sensitive to some synthetic coal-tar dyes than males as far as effect on growth is concerned.

Biological studies for ecological impact have been done for an assessment of the effects of dyes on the environment (11). The fathead minnow (pimephales promelas) was used to study detrimental effects on fish. The data indicate that many of the dyes are not likely to present a practical fish toxicity problem because the dye threshold values are far above concentrations.
that are likely to be acceptable in streams from the standpoint of colour. This may not be true for cationic dyes, which appeared to be the most toxic class, at least with respect to the fathead minnow.

A dye to be identified may be available either in substance or on a textile fibre or other substrate. According to Venkataraman (11) the examination of an unknown dye involves (a) the determination of its application class, (b) chromatography to check its homogeneity, (c) separation of the constituent dyes, and (d) determination of the chemical class by colour reaction or "spot tests". Finally, the dye matches with a dye having a CI generic name using a suitable method. He mentioned chromatography and spectrophotometry as the most rapid and dependable procedure for proving identity.

The separation and analysis of synthetic dyes have been reviewed in a few books (11,186,187,214).

Gasparic and Pikhart (1) have developed a method for the determination of the inorganic salts content (Na$_2$SO$_4$, NaCl) in acid and direct dyes. Many dyestuffs contain up to 60% of these salts which would be introduced in the final product as the result of the sulphonation and neutralization process or of the salting out operation involved in the dye preparation technology. 0.25% aqueous solution of the dyestuff to be tested was introduced to the top of the strong acid exchanger column. Then elution was carried out with water and the eluate was allowed to pass through the column filled with polyamide.
In the eluate sulphate or chloride were determined as barium sulphate or silver chloride respectively, using common procedures of inorganic analysis. In the case of sulphonphthalein dyes the inorganic salts were separated using Sephadex G25 by Jirsa and Hykes (2).

Pyridinol azo-dyes (252) have been suggested for use as visual indicators for the titration of lead(II) with EDTA, and molybdate and phosphate with lead nitrate solution. Neutral Red (CI Basic Red 5) (256) has been used as a titrant for the spectrophotometric determination of iron(III). The use of Alcian Blue (CI Ingrain Blue 1) has been reported for assaying polyanions of algal acid and sulphated polysaccharides such as agar, carrageenan, alginic acid and pectin spectrophotometrically (259).

Many basic dyes form coloured complexes with metal cations and may be determined spectrophotometrically. Most of them form either coloured complexes with unidentate or chelating ligands or ion-association complexes. Fogg, Burgess and Burns (185) reviewed the use of basic dyes in the determination of anions. They emphasized that the advantage of using basic dyes as extractants is that many of them have high molar absorptivities (0.6 - 1.2 x 10^5 l mole^-1 cm^-1).

Five triphenylmethane dyes (3), Xylenecyanol FF (CI 42135), Setoglaucine O (CI 42025), Setocyanine Supra (CI 42140), Fast Green FCF (CI 42053), and Night Blue (CI 44085) have been successfully used as redox indicators in the titration of iron(II), uranium(IV), molybdenum(V),
hydroquinone and thallium(I) with sodium vanadate in sulphuric, hydrochloric, phosphoric and perchloric acid media. Rao and Viswanath(4) have reported some triphenylmethane dyes used as redox indicators in the titration of antimony(III) with cerium(IV) sulphate. Erioglaucine A, Erio Green B, Patent Blue, and Xylene Carbinol FF have been recommended by them for use in the titration in 1 - 2M $\text{H}_2\text{SO}_4$ using iodine as a catalyst, and in 1-2M HCl with no catalyst but using manganese sulphate solution to prevent dye decomposition. Metal complex pigments and dyes has been reviewed by Yamamoto(151).

Azine dyes(47) such as phenosafranine, Methylene Violet(MV), Amethyst Violet(AV), Safranine T, Wool Fast Blue BL, and Aposafranine(AS) were used as redox indicators in the cerimetric titrations in acid media of Fe(II), As(III), V(IV), hydroquinone, and ascorbic acid with ammonium hexanitratocerate(IV). Of the six indicators, MV, AV, and AS are the best. The results obtained are in good agreement with those obtained by using standard indicators.

Electrosensitive and heat-sensitive image recording materials(48) contain a leuco base of a triphenylmethane or fluoran dye as a colour former and ZnO as a colour developer. The use of ZnO as a colour developer eliminates the use of an additional electric conductor. Thus, ZnO 6 parts was dispersed in a mixture of Crystal Violet lactone 3, a 10% butyral resin solution in methanol 6 and ethanol 10 parts and coated on an Al.
support to give a heat-sensitive image recording sheet.

Hosono(123) has reviewed the use of aminonitrophenylmethane dyes, thiazine dyes, and Xanthene dyes for the determination of metals and nonmetals.

An extraction spectrophotometric method(140) has been described for the determination of acidic drugs with 12 different dyes. The method was most sensitive to drugs containing an -SO3H group, such as analgin, Vikasol and Sergosin. Chromopyrazole dyes gave the most satisfactory results with the sulphonlic acid containing drugs.

Z. Gregorowicz et al(146,158) have reviewed the use of synthetic organic dyes as analytical reagents and instrumental techniques in dye analysis. Anabasine azo dyes, their absorption characteristics, acidity constants, and application in spectrophotometric determination of metals have been reviewed by Talipov et al(150).

The extraction of anionic bromo complexes of silver by the cationic forms of the triphenylmethane dyes(152), Malachite Green(I), Brilliant Green(II), Methyl Violet(III), Crystal Violet(IV) and ethyl Violet(V) was studied photometrically. The extracting power of the dyes followed the order V > IV > II > III > I. The Ag-Br-dye ratio in the complexes was 1:2:1. Triphenylmethane dyes(44): Crystal Violet, Malachite Green, and Fuchsine have been recommended for use in the spectrophotometric determination of vanadate, chromate and tungstate in 1M H2SO4.
The investigation on Crystal Violet by Turgeon and Lamer showed that this dye gave perceptible carbinol precipitation when the original dye concentration was of the order of $5 \times 10^{-6}$ mole/l in aqueous media. The absorption peak (at 590 nm) of the Crystal Violet ion has been observed to shift towards a longer wavelength on the addition of acetone or dioxane to an aqueous solution of the dye.

Crystal Violet was formed by the reaction between chloronil, tetrahalogenated quinones and N,N-dimethyl-aniline. The two intermediates, diamagnetic donor-acceptor complexes and semiquinone radicals have been observed spectrophotometrically in the reaction.

The use of Titan Yellow (CI Direct Yellow 9) as an analytical reagent has been reviewed by Werner. Tananaiko and Bilenko reported the complexes of zinc and cadmium with 1,10-phenanthroline and Rose Bengal B (CI Acid Red 94). It was shown that Zn or Cd forms a 1:2:1 (metal:1, 10-phenanthroline:Rose Bengal B) ion-association complex that was extractable from aqueous phosphate buffered medium of pH 7 to 8 into CHCl₃.

The method based on these facts was used for determining 0.1% of Zn in an aluminium alloy.

The physical and chemical applications of dyestuffs have been reviewed in detail.

Absorption spectrometry is regarded as an important method in the dye industry for purposes of identification and assessment. Pallotti et al. have reported the spectrophotometric determination of synthetic water-
soluble dyes in foods and beverages. The method with relative errors of 3 to 8% was applied to liqueurs, aperitifs, carbonated beverages, syrups, caramels and water ices.

The electronic absorption spectra of disperse dyes in liquid ammonia were given by Kalinnikov et al (222). Disperse dyes are highly solvated in liquid ammonia which is related to a universal and specific interaction between the dye molecule and ammonia. The dyes in ammonia obeyed the Lambert-Beer law which suggested complete dissociation in solution.

A vector matrix (223) was obtained for comparing visual and spectrophotometric methods of colour evaluation. The applicability of the matrix tested using the visual and spectrophotometric data obtained for a textile product dyed with Saturn Scarlet LGC.

Spectrophotometric analysis of multicomponent dye solutions which do not obey Beer's law has been carried out by Werthemann (15). He described a method for computing the dye concentrations of a multidye solution from measured absorbances. It was based on an iterative procedure, using calibrated excitation functions to vary the extinction coefficients which are used for a least squares calculation. Examples were given for the mixtures of CI Basic Yellow 45, CI Basic Red 46 and CI Basic Blue 41 and of CI Acid Orange 67, CI Acid Red 293, and CI Acid Blue 260 respectively.

Erythrosine and other permitted red dyes are added
to many canned fruits in order to mask the disappearance of the naturally occurring red pigments (anthocyanins) during storage. Adams and Butler (55) have developed a method for the qualitative identification of Erythrosine in cans of raspberries, loganberries, strawberries, plums and rhubarb spectrophotometrically.

The changes that occur in the visible absorption spectrum of aqueous Rhodamine B on changing its concentration have been described previously by Suzuki and Tsuchiya (84). The absorption spectrum of the dimeric species of acridine orange hydrochloride in aqueous solution was interpreted in terms of exciton coupling (85) between the vibronic levels of the monomeric dye molecules.

The spectral changes with concentration of Rhodamine B (36) in near-neutral aqueous solutions, studied in the visible region, have been interpreted in terms of a monomer-dimer equilibrium and the corresponding species spectra have been derived. The monomer spectrum has been fitted to a progression involving a single vibrational mode. The dimer spectrum has been analysed using a vibronic exciton coupling theory suitable for degenerate interactions.

A simple procedure for the determination of the amount of covalently bound Cibacron Blue F3GA dye present in preparations of blue sephadex and blue dextran-sepharose has been described by Chambers (91). The method involves hydrolysis of the sephadex and sepharose dye derivatives in 6M hydrochloric acid followed by spectrophotometric determination at 515nm of the dye released.
The use of triphenylmethane dyes for extraction and photometric determination of traces of phosphate has been studied by Babko et al.(121). The molar extinction coefficients of the molybdophosphate complex with Crystal Violet was 270,000(at 582nm); that of the complex with Malachite Green was 170,000(at 610nm).

Goshima et al.(142) reported a rapid determination of Rhodamine B, Malachite Green and Auramine in water-soluble dye mixtures. Acid Red and Rose Bengal interfered in the determination of Rhodamine B.

Absorption curves for 25 water soluble food colours permitted in Spain were reported by Carballido and Villanua(157). Honkawa(160) determined the food colour mixtures with the function generator by two-wavelength spectrophotometer. The spectral properties of heterogeneous aggregates of dye molecules have been studied by Levshin et al.(161). The occurrence of association between methylene blue(I) and Acridine Orange (II) and I and Rhodamine 6G extra(III) in aqueous solution was shown by UV spectroscopy and by the quenching of the luminescence of II and III by I.

Spectrophotometric studies of dye aggregation have been carried out by Tull(162), Ghosal and Mukherjee(163).

The amount of Crystal Violet and iodine(164) taken up by bacteria, wool, nerve- and muscle-fibres and certain proteins was determined quantitatively. The reactions of triarylmethane, azine, oxazine, and thiazine dyes with antimony were studied in aqueous solutions by the method of spectrophotometry.
A method for evaluating the diffusion capacity of reactive dyes has been described by Rosenberg\(^{(181)}\). The diffusion of fibre reactive dyes was measured by using a multi-layered cellophane(I) band wrapped around the end of a glass rod and tied by a polyester thread. The dye absorption of each layer was measured at the wavelength of maximum absorption. A mathematical approach for the determination of dye concentration in mixtures has been proposed by Saguy et al\(^{(226)}\). The procedure is based on a nonlinear curve fitting of the visible spectrum of the pigments with a predicted function of the individual dyes. The logarithmic normal distribution function showed a remarkable fitting with the pigments tested (Amaranth, Tartrazine and Yellow 2G) and was used as a mathematical model for the curve fitting process.

Association complexes\(^{(251)}\) of azo compounds with long-chain quaternary ammonium salts exhibit bathochromic shifts of 100 to 200nm, as well as enhanced intensity, relative to the free azo-compounds. Some spectrophotometric titration results for azo compounds\(^{(10mM)}\) were obtained with 0.1 to 0.2mMethylpyridinium chloride.

Resonance Raman spectrometry\(^{(229)}\) has been applied for the detection and identification of industrial fabric dyes. A 200ppb level could be detected and identified on doped samples of river and seawater.

Perdih\(^{(14)}\) has reported his work on the liberation of acid dyes from their quaternary ammonium salts on chromatograms. Quaternary ammonium compounds that are sufficiently bulky can be used for the isolation of acid
dyes from food and some cosmetic preparations. The extracted compounds cannot be directly chromatographed on paper until the starting line is impregnated with arylsulphonates, which liberate the dyes from their quaternary ammonium salts. The three compounds; hexachlorophene (HCP), tetrapropylenebenzenesulphonic acid (TPBS), and sodium tetraphenylboron (Bph₄) liberated three dyes; CI 15630, CI 16290, CI 42051. The most suitable one was sodium tetraphenylborate, which was used as a solution of 2g in a mixture of 100ml methanol or ethanol, 2ml of water and 0.2ml of glycerol for the 1-2cm wide impregnation of the starting line of the chromatogram. He has recommended the following mixtures for food dyes: nitromethane-acetonitrile-water (1:8:3), nitromethane-acetonitrile-formic acid (or acetic acid)-water (10:80:1:3:) and triethylamine-acetonitrile-water (5:1:2). Determination of dyes used for artificial colouring in beverages was made by ascending paper chromatography after isolation of the dyes by the official Brazilian method using Whatman No.1 paper.

Gilhooley et al. described a method for the extraction of synthetic water-soluble food colours using a polyamide column for the purification of the dye extracts. An attempt has been made to produce a method for the quantitative determination of the colour content of foodstuffs. The method has been applied to jellies, jams, sweets, cakes, canned meats and sausages.

Application of column chromatography to the analysis
of intermediates and dyes has been described by Lawniczak and Tomczyk (154). Isatin was isolated from the crude product by passing it through a SiO$_2$ column and eluting with acetone; the absorbance of the eluate was measured at 413 nm.

Soap chromatography (210) using the cationic detergent, cetyltrimethylammonium bromide, C$_{15}$H$_{33}$(CH$_3$)$_3$NBr, has been applied to study the chromatographic behaviour of a number (33) of food colourings. The chromatograms show that most common food colourings can be separated on the SASHypersil column. However, brown and some of red colours require other means of identification, e.g. TLC or paper chromatography.

12 fat soluble tar dyes for cosmetic products were separated by the isocratic elution technique of HPLC (213). Eight tar dyes, CI No. 12120, 26100, 12085, 26105, 11680, 11380, 11390 and 47000, were separated with chloroform and n-hexane mixtures as eluent. Diethylether /n-hexane = 4/96 mixture for CI No. 12140 and 12100 and acetone/n-hexane = 9/91 mixture for CI No. 12075 and 12315 have been tried for their separations, respectively. High pressure, reversed-phase liquid chromatography has been applied for the simultaneous analysis (188) of tartrazine and its uncombined intermediates adding tetrabutylammonium hydroxide, tetraethylammonium hydroxide, and tridecylamine to increase affinity of the dye and its synthetic intermediates for a lipophilic stationary phase.

Martin et al (215) analyzed nine synthetic acid
Fast dyes in alcoholic products by HPLC. The authors stated that by using their wool extraction procedure, all the dyes examined could be recovered and detected by HPLC at the concentration ranges of 1 ppm to 5 ppm which was more sensitive than the visual examination of TLC plates.

High speed liquid chromatography or high performance liquid chromatography (HPLC) which has been developed by Kirkland (5) in 1969 has been highly recommended by Ishida (6) especially for the analysis of sulphonic acids and their dye intermediates. The analytical conditions of some intermediates of dyes and pigments by ion-exchange, adsorption, gel and partition chromatography are presented in a table.

Masa and Mihashi (7) have applied HPLC for the separation of some synthetic organic food colours. Red No. 2, 3, 102, 104, 105 and 106, Yellow No. 4 and 5, and Blue No. 1 and 2 which are allowed for use in Japan have been separated by HPLC with octadecylsilane as stationary phase and 0.2% (NH₄)₂CO₃·CH₃OH as a mobile phase. Values of retention time and column capacity are given for some of these colours.

Louis J. Papa (8) has reviewed some chromatographic separations of anthraquinone dyes, azo dyes, ionic dyes (cationic, sulphonic acid dyes) and FD & C. He has emphasized that HPLC is far superior in precision to GC, TLC, and PC for dye analysis.

Many dyes used in food and pharmaceutical preparations were separated and quantitatively determined by
high pressure liquid chromatography\(^{(155)}\) using an anion-exchange column. The Merck dyes El02, El04, El22, El10, El23, El26, and El31 were analyzed as pure compounds, and in mixtures, Vitamin tablets, and syrups.

The principles of thin layer chromatography(TLC) were first described in the late 1930's; In 1938, Izmailow and Shraiber\(^{(9)}\) separated mixtures of organic compounds on loose layers of adsorbent powders spread on microscopic slides. Kirchner\(^{(10)}\) was one of the first to use adsorption chromatography on impregnated filter paper and later used glass fibre paper coated with silicic acid or alumina.

Zweig and Sherma\(^{(183)}\) reviewed the application of TLC in the cosmetic industry for the analysis of dyes and perfumes.

Today one can readily obtain plates precoated with silica gel, alumina, cellulose, polyamide, reversed phases, charcoal, graphite, magnesium oxide, and magnesium hydroxide\((0.10-2\text{mm thickness})\). The coating may be hard or soft, with or without a binder or fluorescent indicator, polar or nonpolar, neutral, acidic or basic, impregnated with silver nitrate or any other complexing agent. The use of binders such as starch, gypsum, or carboxymethyl cellulose causes the layers to adhere to the plate surface.

The TLC process is divided into four parts: application of sample using a micropipet, microsyringe, or capillary tube, development with solvent or reagent, immobilization of adsorbate, and visualization. The
technique became popular since Stahl(184) built a convenient spreader for the preparation of the plates in 1956. Since the early 1960's, many textile materials have been identified and separated by TLC, but the largest number of publications have involved dyes.

Venkataraman(11) has edited a book including the application of TLC for the separation of dyes, FD & C colours and pigments with solvent systems and \( hR_f \) values which are \( R_f \) values multiplied by 100.

Perry and Landers(12) have published their work on the use of thin layer chromatography as a means of separating and identifying common dye carriers. The ten possible dye carrier chemicals; (perchloroethylene, 1,2,4-trichlorobenzene, 1-methylnaphthalene, biphenyl, diphenyl oxide, methyl salicylate, butylbenzoate, methylbenzoate, o-phenylphenol and methyl p-toluene/benzoate) has been separated, identified, and discussed. The optimum TLC system was with hexane; diethyl ether(98:2) as the developing solvent, silica gel as the adsorbent and an S-chamber for development. The dye carrier chemicals were finally identified by a comparison of \( R_f \) values and using the visualizing agents.

The control of the synthesis and commercial production of the basic dyes for polyacrylonitrile fibres requires more rapid and effective analytical methods to be used, such as TLC.

Arsov et al(53) have investigated more suitable solvent systems for the TLC separation of 23 basic dyes.
on silica gel. They suggested that the systems described could also be used for the preparative isolation of dye compounds in order to study their chemical structures.

The Sudan dyes are widely used for the histological demonstration of fats(58). Highly effective TLC system for the quality control of Sudan dyes has been described by Marshall(57). The developing solvent consisted of benzene-chloroform(10:1 v/v). Several reports, e.g. those of Schweppe(59), and Jordan(60,61), indicate that good separation of Sudan dyes may be obtained on thin layers of silica using benzene as the developing solvent. Sudan Black B is an extremely heterogeneous product. Coloured impurities in this dye have been detected by many authors using gel-filtration chromatography(62), paper chromatography(63,64), and TLC(59,60,61).

Identification of 29 food and cosmetic dyes by means of thin layer chromatography has been effected by Schmidiger(65). He has proposed a two step thin-layer method using standardized plates coated with cellulose powder for the separation and identification of these dyes.

Malkus(91) extracted benzidine, 2-naphthylamine, aniline, and 4-aminozobenzene with chloroform from 9 azo dyes and separated them by TLC on silica gel.

The content of the carcinogenic or toxic amines was found below the permissible limit of 0.001 % as required by Czechoslovak standards.

Organic dyes, e.g. Riboflavine, Erythrosine, and
Acid Violet were identified by TLC using a methylcellulose stationary phase on a plastic support and a mobile phase of 2.5% sodium acetate or 2% sodium citrate, both in 5% ammonia.

Tewari et al. (93) applied a thin layer electrophoretic technique for the separation and identification of synthetic dyestuffs present in liquors and beverages. 32 dyes were identified by comparing colour shade sequences and migration distances with standards at the different pHs. Pleskonics Szabo et al. (99) reported the rapid semiquantitative determination of synthetic food dyes present in foods by TLC method. One of the TLC systems was cellulose MN 300 and the solvent system of phenol: acetic acid: water = 150:2:48.

One particular class of dyes known as cyanines shares a high degree of interest in dye laser work (67) because of their wide distribution of fluorescent wavelengths over the visible spectrum. Kues and Teague (66) have reported the results obtained for each TLC system tried, along with $R_f$ values for 23 cyanine dyes and the impurities in these dyes. Two solvent systems that seemed to give the best results and were most suited to cyanine group in general have been proposed as 100% methanol and a propanol-formic acid (80:20) mixture. Several cyanine dyes are noted for rapid decomposition in some solvents and it was thought this might be a problem (68).

Synthetic dyes (95) in dragee coatings, tablets,
and syrups were extracted with water, adsorbed on alumina and eluted with 0.5% ammonium hydroxide, and identified by comparison with standards after thin-layer chromatography on CM cellulose.

Separation and identification of certain xanthene and other red basic dyes (105), food dyes (106, 228) and organic dyes (130) in foodstuffs have been reported.

Paper chromatography of acid triphenylmethane dyes (5 to 8μg) has been done using the system butanol-pyridine-water as solvent (109).

Andrzejewska (114) developed a method for the identification of organic dyes in food products in the presence of natural pigments, fats, and other components. The detectability of tartrazine, Sunset Yellow FCF, and amaranth was 0.06μg, Brilliant Black BN, 0.14μg, and indigotine, 0.23μg. The recovery rate of indigotine and Ponceau 4R ranged from 50.5-67%, and that of the remaining 4 dyes from 77-90%. All dyes tested were added to fruit products and honey in the form of a 6-dye mixture.

Azo dyes prevalent in various cosmetic formulations are identified by TLC (116). 4 pigments were separated on SiO₂ plates by using benzene and n-hexane:benzene:pyridine (70:20:5) as solvents.

Churi et al. (119) used barium sulphate as an adsorbent in the TLC of 1:2 metal complex dyes, e.g. Lanacyn Gray 2BL, and found it suitable for separation and semiquantitative estimation. Pearson (120) reported Rf values for 16 oil-soluble colours, after employing reverse-phase chromatography.
Cuzzoni et al (128) used TLC with programmed multiple development (with heat-control unit) to test the purity of food dyes. They found that the technique afforded better separation of impurities (homologues or isomers) from the major dye components than does conventional chromatography under similar conditions.

Tewari et al (129) applied thin layer electrophoresis for the separation and identification of synthetic dyestuffs in the forensic analysis of liquors.

Separation and detection of water soluble acid dyes on polyamide thin layer (133) (pyridine-methanol-28% aqueous ammonia-water = 5:6:1:16) with magnesium and aluminium hydroxide zone papers (135) and azo dyes on precoated adsorbents fixed with fused glass (136, 137) have been studied by TLC method.

Basic dyes, including Rhodamine B, adulterating food (141) were identified by extraction with ether from alkaline aqueous solution, acidifying with acetic acid, evaporating, dissolving in water, and separating by electrophoresis on paper. Tanner and Brunner (153) described 3 solvent systems (a solution of 4% tri sodium citrate in 5% NH₄OH, acetic acid-formic acid-ethanol-water (1:1:1:7, v/v) and butanol-pyridine-water (6:4:3) for the detection of artificial dyes in purple hibiscus extract. The identification and analysis of fibre reactive dyes on natural and synthetic textile fibres has been described by Desai (156).

Different methods were tested for the analysis of natural red dyestuffs (159) used for the preparation of
lacquers in ancient times.

IR, visible, and UV spectrophotometry were successfully used to identify dyestuffs on wool. However, TLC was the most sensitive method for small samples such as scrapings from illuminated manuscripts. Sweeny (240) discussed the separation of dye mixtures for analysis. Solvent systems for TLC and solvents useful for dye-extraction from fibres are included in the paper. He also mentioned that TLC is a simple, fast, low cost and versatile technique.

Dyestuffs added to alcoholic liquors have been separated and identified by Thin-Layer-Electrophoresis (241). The reporters said that the technique was found to be particularly suitable for distinguishing fake samples from genuine ones.

Substantive dyes, sulpho group containing hydrophilic dyes (177), were distinguished from basic and disperse dyes by their pH dependent chromatographic behaviour on cellulose acetate; they were distinguished from hydrophilic acid and metal complex dyes by their lack of mobility on cellulose. The individual substantive dyes were separated by chromatography on silica gel. The purity of o-hydroxy-substituted thiazolylazo dyes (242) has been tested by TLC method. The azo dyes migrate as undisassociated species and the formation of colour complexes with metal impurities from commercial silica gels was suppressed with their solvent systems.

The bromination reaction of azo compounds with
N-bromosuccinimide has been studied in acetic acid medium by Pande and Gopal (56). The reaction study has been applied to the determination of 2-10 mg of azo compounds; Chrysoidine G, Butter Yellow, Congo Red, Chrysamine G, Methyl Orange, Mehtyl Red. The reaction ratio of the azo compounds and N-bromosuccinimide has also been presented in the paper. The deviation of the results were within ±1%.

An amperometric titration between the surfactant (Manoxol OT), and the dye (Methylene Blue) has been proposed by Buchanan and Griffith (79). However it was found that sufficient time for complete reaction and settling of the precipitate should be allowed before polarographic analysis (78). Saikina et al (97) have studied amperometric titration of some monoazo dyes with cobalt and nickel salts.

Procedures have been developed for determining dyes (13, 122, 126) containing azo and nitro groups based on reducing them with titanium(III) sulphate and then titrating the excess titanium(III) sulphate potentiometrically with iron ammonium alum (6 active dyes), or on a biamperometric indication (2 disperse dyes) during titration with titanium(III) sulphate in a glycerol-sulphuric acid medium without using an inert atmosphere. The authors have found that reduction of the dye by Ti(III) to the amine was speeded up in a citrate buffer (pH 4.85) and quantitative at room temperature.

Nikolcev et al (98) have reported the potentiometric
determination of azo dyes with Mohr's salt in a glycerol medium. Lyande et al (115) described a method for the determination of active chlorothiazine dyes. The total content of dye in the sample could be determined by direct titration of the water-soluble anthraquinone derivatives with 0.1M \( \text{H}_2\text{SO}_4 \) potentiometrically. Heliogen Blue SBL (74) and 5 triphenylmethane dyes (73,110) have been determined potentiometrically with cerium(IV) sulphate.

A 0.2M bromate-bromide solution was used for the direct potentiometric determination of triphenylmethane dyes by Matrka (108). Reductimetric titration of triphenylmethane dyes (111) has been done with vanadium(II) sulphate in the presence of sodium xylenesulphonate. A method was developed for automatic potentiometric titration of cationic dyes (113) in acetic anhydride with 0.1M perchloric acid in acetic acid.

Direct, azo and sulphon dyes could be analysed by the introduction of potentiometric titration (132) with sodium nitrite of the excess of benzidine. The sample of dye (0.05-0.1g) was dissolved in 15-30ml of water and the solution at 50°C was treated with twice the theoretical amount of 0.1M benzidine hydrochloride. The precipitated salt was filtered off and a suitable aliquot of the filtrate was titrated with 0.01M sodium nitrite. However the method could not be recommended for use now because of the carcinogenicity of benzidine hydrochloride.

Tandon and Mehrotra (145) reported the reduction of dyestuffs which yield leuco compounds with chromous
The addition of ethanol was advantageous because it prevents some dyestuffs adhering to the walls of the flask.

A quantitative determination method of Brilliant Green was described by Vaisman and Filenko (166). The method was based on the reaction of Brilliant Green with iodine to form the periodide, C_{29}H_{34}O_{4}N_{2}·HI·4I_{2}. 1ml of 0.1N iodine solution = 0.00593g B.G.

The method developed by Puente and Valdeperas (113) can be used for determination of dyes of known molecular weight in compound preparations for determining the molecular weight of unknown pure dyes. The potential reached on adding half the reagent required for the first inflexion also gives an indication of the reactivity of the dye toward acrylic fibers in nonaqueous media, which is centrally dependent on the anion.

Coulometric titration with electrolytically generated titanous ion has been applied for the determination of the dyes, Orange II, Tartrazine, p-aminocarbazolene, Amaranth, and Methyl Violet (54). The average current efficiency of 31 titrations was 99.8%, with a standard deviation for a single value of ±0.15%. The rate of reaction of titanous ion with the dye may be somewhat slower by the coulometric titration, as the titanic ion is about 20 times the titanous ion added. The function of the buffer is not only to regulate the hydrogen ion concentration but also to promote the reduction of the dye.
Makoto described the coulometric titration of dyestuffs with electrolytically generated dithionite. The optimum pH range for Methylene Blue and Indigo Carmine was 3 to 5, and milligram amounts of dye were titrated with an average error of 0.5%.

Unterhalt and Kruetzig examined the dye content in a series of cough syrups in Germany.

Ivanova and Peneva studied the electric conductivity of some organic dyes. Usually the organic dyes are amorphous. Their electrical properties depend on the presence of gases such as hydrogen, oxygen, etc. The resistance of the organic dyes considered are of the order of $10^9 - 10^{11}$Ω. The following nine dyes were investigated: type B; Bismark Brown, Water Blue, Basic Fuchsin, Eosin Bluish, Eosin Water-soluble, type A; Methyl Red, Alizarin Yellow GG, Crystal Violet, Ruby S. The specimens were prepared as follows: four electrodes, two gold and two aluminium ones, 1mm apart were evaporated in vacuo on a glass substrate. The organic dye layer was deposited on the electrodes by means of evaporation from an aqueous solution. The current-voltage(I-V) characteristics were taken in darkness by transmitting constant voltage to the specimen and the current was measured electrometrically by the voltage drop on standard resistances. A number of authors have studied the phenomena connected with the injection of current carriers in organic semiconductors.
Morozevich et al. (31) have studied theoretically the possibility of a photoinduced reversible change in pH in aqueous solutions of dyes, whose acid-base properties change appreciably on excitation. The region of the optimum conditions in which fairly large changes in pH can be obtained ($\Delta pH \approx 0.02$) is indicated. The time characteristics of these processes have been calculated.

Chambers et al. (43) have carried out the effect of dimer formation on the electronic absorption and emission spectra of ionic dyes. The visible absorption, excitation, fluorescence, and phosphorescence spectra have been measured for the monomeric and dimeric forms of rhodamine B and sulphorhodamine B. The phosphorescence emission spectra of Acridine Orange, Acriflavine, Proflavine and Eosin Y were obtained using $10^{-5}M$ solutions in 1:1 ethanol-water containing $10M$ LiBr for the monomers and $10^{-5}M$ solutions in water containing $10M$ LiBr for dimer spectra at 77$^0$K respectively.

Yagi (96) has reviewed the properties of photosynthetic bacteria and treatment of dye waste water by photosynthetic non-sulphur bacteria.

Yasui et al. (100) studied the degradation of azo dyes in food by sodium ascorbate. The content of azo dyes in processed foods decreased markedly to approximately 10% of the amount permitted by the law with the addition of sodium ascorbate as an antioxidant. Other ascorbate related compounds were effective also.

The effect (103) was studied of adding water-miscible organic solvents to an aqueous solution of Brilliant
Orange and Crystal Violet in which the 550 - 660nm absorption of Crystal Violet was reduced, and a new absorption at 494nm was produced.

The IR spectra of several dyes have been discussed by Etaiw et al(147) with special reference to characteristic bands of OH, N:N, C-S, and sulphone groups. An OH group in the para position of the phenyl substituent causes the dye to dimerize by formation of an intermolecular H bond, whereas an OH group in the ortho position forms an intramolecular H bond with the-azo group.

Samples of wool dyed with a range of 9 dyes were exposed in a microscale fading lamp. The colour changes accompanying light fading(172) were followed visually and instrumentally and characterized in terms of light-fastness grades, grey-scale differences, colour differences in CIELAB colour space, and in Munsell colour coordinates. The light-fastness grades were similar to those previously reported for day light exposure. The various methods of expressing colour change gave detailed and quantitative measures of the effects of light on the colour of natural dyes; this type of information could be of value in predicting the original colour of ancient textiles.

The dependence of the fluorescence efficiency of the dyes Crystal Violet and Auramine O on solvent viscosity at room temperature has been investigated using pressure to alter the viscosity(216). Many non-rigid aromatic molecules in which the aromatic groups are linked by bonds of partial multiple order or polyene chains exhibit
a marked enhancement of fluorescence efficiency in fluid solution as the viscosity of the solvent is increased. This effect has been observed in the di- and tri-phenyl-methane dyes, the polymethine cyanine dyes, substituted benzophenones, and sterically hindered stilbenes and tetraphenylbutadienes. Their result did not contain any detailed information on the mechanism but one could gain some insight from peak shifts and half-width changes in terms of a single configuration coordinate diagram. The coordinate in question would presumably involve the interaction of the dye with neighboring solvent molecules.

The behaviour of several basic azo dyes (225) in solutions was studied from pH 14 to 17.5 M H₂SO₄, to find conditions for the existence of their ionic, protonated, and hydrolysis constants. Depending on dye structure, the absorption spectra showed with increasing dye concentration in aqueous solutions either a shift of the absorption maximum to shorter wavelength, or a decrease of absorption intensity. Those dyes which often form various polymeric structures depending on concentration, can be regarded as monomers in ionic associations with metal halo complexes.

Militky and Rais (230) derived an equation for determining the diffusion coefficient of a dye from its
time of half-dyeing. Although diffusion coefficients obtained are only estimates of integral diffusion coeff., they characterize the whole dyeing process and are suitable for comparing various dyeing systems.

Azo compounds have been the subject of a number of studies utilising the dropping mercury electrode. As a result, reduction process in aqueous media that lead to formation of the corresponding hydrazo compound(1) and formation of amines(2) have been recognized.

\[
\text{Ph-N}=\text{N}-\text{Ph'} + 2\text{e}^- + 2\text{H}^+ = \text{Ph-NH-NH-Ph'} \quad (1)
\]

\[
\text{Ph-N}=\text{N}-\text{Ph'} + 4\text{e}^- + 4\text{H}^+ = \text{PhNH}_2 + \text{Ph'}\text{NH}_2 \quad (2)
\]

Later studies, however, included the reduction of some well characterized dyes such as Orange II, Metanil Yellow(40), Methyl Red(41), Methyl Orange(42), the dyestuff complexed with Cu(II)(131,139), some monoazo dyes with cobalt and nickel ions(148), and iron(III)(246).

Allen and Powell(39) have investigated the oxidation of the leuco base Crystal Violet at a rotating platinum electrode polarographically. It was found that in an acidic medium of pH 1.1 that the compound underwent a one electron change to form a rather stable, triphenylmethane-type free radical. Hindawey(16) has described the polarographic behaviour of several azo dyes containing the benzo(b)thiophene nucleus. In general, increasing the dye concentration in 0.1M sodium hydroxide as supporting electrolyte, the half-wave potential shifts to more negative values. This behaviour reveals that the
reduction is accompanied by an overvoltage, indicating that it proceeds irreversibly\(^{(17)}\). He has also explained the relation between the reactivity of the azo center towards reduction and the group bonded to the azo center. The reactivity increases in the order of phenyl < benzo-thiophene < naphthol. It has been observed that the reduction of a compound containing the \(-\text{OH}\) group in a position ortho to the \(-\text{N}=\text{N}-\) centre is much easier than that of the compound which has the \(-\text{OH}\) group in a position para to the reducible centre by Hussey and Diefenderfer\(^{(18)}\).

Duff et al\(^{(25)}\) have used polarography to investigate the effects of structural differences on the aggregation of groups of closely related monoazo acid dyes. Polarography has been used during the last ten years in dye-aggregation studies\(^{(26,27,28,149)}\) giving results which agree well with those using other techniques both for dyes such as the cationic Methylene Blue\((\text{CI Basic Blue }9)\), which forms mainly dimers\(^{(29,30)}\), and for the anionic Congo Red\((\text{CI Direct Red }28)\), which forms aggregates containing many thousands of molecules\(^{(26)}\).

Powell and Snowden\(^{(34)}\) have studied some polarographic behaviour on the dye Fast Red E, disodium 2-hydroxy-1,1'-azonaphthalene-4',6-disulphonate which no longer appears on the list of permitted food colours. The dye is reduced at the dropping mercury electrode in a single four electron wave over the pH range 2.0 - 11.3, with the half wave potential\((E_{1/2})\) values ranging from -0.03 to -0.61V vs. SCE. The \(E_{1/2}\) values tend to
become slightly more negative with increasing depolariser concentration, e.g., by 40mV for a ten-fold concentration change at pH 4.3. Similar trends have been reported in the reduction of both Amaranth and azobenzene.

The two xanthene-type food colours, Eosine (Food Red 103) and Phloxine (Food Red 104) have been studied polarographically by Mizunoya and Omori. The electrolytic solution was prepared by adding 4ml of buffer solution, 1ml of 1M tetramethylammonium chloride as a supporting electrolyte and 4 drops of 1% gelatin solution as a maximum suppressor to 5ml of the solution of Eosine or Phloxine dissolved in dioxane-water(3:1) mixture. Eosine and phloxine in this electrolytic solution at pH 3.8 showed the well defined polarographic reduction waves without any anomalous waves, and their half-wave potentials were -0.61 and -0.65V vs. SCE, respectively. The polarographic waves of these two food colours could be considered as the reduction of their quinoid form, but it could not be concluded that their reductions were reversible electrode reactions because their relationships of log(i/(i_d-i)) to E did not show the exchanges of two electrons as expected. The diffusion currents of these two colours were proportional to the concentrations from about 10^{-3}M to 10^{-4}M of their two compounds, and their diffusion current constants were 1.46 and 1.60, respectively.

Amongst the azo dyes two types of dyes which are the azosalicylic acid and o,o'-dihydroxy azo dyes were studied in order to understand their redox behaviour by
Kalik and Gupta. From their data the following empirical relationship between half wave potential ($E_{1/2}$) - pH holds good for the four dyes tested; Solochrome Yellow 2GS, Solochrome Black PVS, Solochrome Red FRS, and Solochrome Black WDFA.

$$E_{1/2} = 0.068 - 0.31pH \pm 0.003$$

The waves of the four dyes were found to be irreversible. In the case of o,o'-dihydroxyazobenzene which gives a totally reversible wave, the $E_{1/2}$ and the standard potential ($E^0$) values were identical. A detailed mechanism indicating the effect of substituents on the $E_{1/2}$ of these dyes has also been suggested. The authors have determined the aggregation number of these four dyes by the method of Hillson and McKay. At low concentrations (1.0 x 10^{-5} M to 9.0 x 10^{-5} M) of Solochrome Yellow 2GS, $\Delta \log_{10} d$ was constant, indicating thereby the existence of the dye in the monomeric form in this concentration range. In general the tendency of rapid polymerization at higher concentration is probably due to existence of colloidal aggregates similar to those observed in the case of Congo Red.

Carcinogenic azo dyes have been used for many years for the production of liver tumors in rats, particularly p-dimethylaminoazobenzene (DAB) and various substituted derivatives such a 3'-methyl-p-dimethylaminoazobenzene (3'-Me-DAB). DAB, when administered to rats is metabolized by the liver resulting in such DAB derivatives as N-methyl-p-aminoazobenzene (NAB), p-aminoazobenzene
(p-AB), N-hydroxy-MAB and 3-methylmercapto-N-methyl-p-aminoazobenzene (3-methylmercapto-MAB). Carruthers(45) have examined polarographically azocarcinogens and related azo compounds which are p-aminoazobenzene, p-dimethylaminoazobenzene, 3'-methyl-p-dimethylaminoazobenzene, p'-amino-p-dimethylaminoazobenzene, p'-amino-p-aminoazobenzene, and p'-amino-N-methyl-p-aminoazobenzene. Finkelshtein et al.(46) reported that the \( E_1^{\frac{1}{2}} \) of six derivatives of DAB in an alcohol-buffer solution of pH 7.76 was shifted to more negative values in the order of the substituents: m-Me, -H, p-Cl, -Br, m-Br and o-Br.

The anodic AC polarographic properties of Methylthymol Blue (MTB)(52) were investigated in the supporting electrolyte of potassium nitrate containing buffer. The difference in the peak potentials of MTB and EDTA is a good measure of the difference in the stabilities of both chelates. The stability constant of mercury(II)-MTB complex was successfully determined.

The antibacterial triphenylmethane dyes Crystal Violet, Brilliant Green, and Malachite Green, and the bacteriostatic diphenylmethane dye Auramine have been extensively studied for their polarographic reduction by Kaye and Stonehill(49). All four dyes were reducible in aqueous solution giving polarograms distorted by dye adsorption on the mercury. The inclusion of 50% by volume of ethanol in the base(supporting electrolyte) solution and the addition of 0.01 - 0.02% of methyl
cellulose have been applied to eliminate the adsorption effects and the maximum respectively. They have reported the polarograms exhibiting two well-separated one-electron steps which indicate the formation at the first step of a semiquinone free radical of apparently very great stability for Crystal Violet, Brilliant Green and Auramine.

Bengtsson(69) has continued the polarographic studies of basic triarylmethane dyes especially for 2-Thiophene Green(2-TG), p-Methoxy Malachite Green(p-MeOMG), and Crystal Violet(CV). The similarity between the polarographic behaviour of these dyes was not very pronounced. There was a tendency towards a splitting of the over-all reduction process into two one electron steps with increasing depolarizer concentrations and pH-values. With p-MeOMG and CV the reduction gives rise to one polarographic wave at low depolarizer concentration and low pH-values. The basic triarylmethane dyes, previously studied by classical polarography(71), have been studied at a stationary mercury electrode(mercury pool electrode)(70). The current-voltage curves recorded indicated that the formation of a semiquinone in the first step was a reversible process whereas the further reduction seemed to be irreversible. The appearance of the anodic branch of the current-voltage curves of these dyes has been interpreted as due to a dimerization of the semiquinone.

Azo compounds are commonly used as dyestuffs and food additives and hence can be encountered in both the aqueous environment and in body fluids. Voltammetric
methods for the determination of azo compounds have been reviewed by Smyth and Smyth. The article states that the acidic supporting electrolyte for the polarographic determination of these compounds is superior than alkaline media where the height of the differential pulse polarographic peak can be decreased owing to repulsion of deprotonated hydroxyl groups by the negatively charged mercury surface and also the negatively charged sulphonate groups.

The light fading mechanism of dyes in a solution containing EDTA-Fe(III) has been studied by the polarographic method. The light fading scheme recognized that EDTA-Fe(III) was reduced to EDTA-Fe(II) by light in the first step, then the latter reduced the dyes to form leuco dyes. The reaction mechanisms suggested were as follows:

1. Dye-1, 3-methyl-1-phenyl-4-(p-diethylaminophenyl) imino-2-pyrazoline-5-one at pH 4.5 - 7.0.

\[
\text{EDTA - Fe(III)} \xrightarrow{hV} \text{EDTA - Fe(II)}
\]

\[
\begin{array}{c}
\text{N} \\
\text{CO - C - N - N(C}_2\text{H}_5)_2 \\
\text{N} = \text{C - CH}_3
\end{array} + 2\text{EDTA-Fe(II)} + 2\text{H}^+
\]

\[
\rightarrow \begin{array}{c}
\text{N} \\
\text{CO - C - N - N(C}_2\text{H}_5)_2 \\
\text{N} = \text{C - CH}_3
\end{array} + 2\text{EDTA-Fe(III)}
\]

Leuco dye

2. Dye-2, 2-acetamido-N-(p-diethylaminophenyl) benzoquinone monooimine at pH 5.6 - 7.0.

\[
\begin{array}{c}
\text{N} \text{HCOCH}_3 \\
\text{N} \\
\text{N(C}_2\text{H}_5)_2
\end{array} + 2\text{EDTA-Fe(II)} + 2\text{H}^+
\]
Leuco dye

The individual aminophenoxazones could be detected oscillographically[72] even in their mutual mixtures and in the presence of starting substances used for the synthesis of these dyes. The determination was best carried out in alkaline Britton-Robinson buffers, 1M-KSCN, 1M-NaHCO₃, and 0.5M-NaF. Phenoxazine dyes among which are many basic components of certain natural substances (omochrome, actinomycines, orceins, litmus) have been hitherto studied with respect to their use as acid-base and redox indicators. In strongly alkaline media the phenoxazine dyes undergo hydrolysis under opening of the ring system at the place of the oxygen bridge, or, even a total cleavage under formation of the corresponding o-aminophenol and dihydroxyquinone can occur. The oscillographic depolarization of the dropping mercury electrode by a series of galloxyanine dyes has been studied by Kotoucek[75]. 5 x 10⁻⁴M dye solutions with Britton-Robinson buffers were used containing methanol and inorganic salts as electrolytes. The solutions were purged with an inert gas in order to prevent the air-oxidation of the reduced form of the dye.

The basic triarylmethane dye, Methyl Green, has been studied by conventional polarography[76]. Spectrophotometric and polargraphic values of the equilibrium constants and rate constants of Methyl Green
were presented in the paper. The work function of 19 photocative dyes has been estimated from their polarograms (77).

The polarographic method has been used to determine Alizarin Red S (134), Cyanine dyes (168), and the binding of the acidic dyes, Congo Red and Alizarin Red S, to cationic surfactants (cetylpyridinium bromide-CPB, and cetyltrimethylammonium bromide-CTMAB) and that of the basic dye, Methylene Blue to the anionic surfactant, dioctyl sodium sulphosuccinate (Monoxol OT). The calibration curve of surfactant concentration vs. decrease in wave height of a dye was used to determine the concentration of anionic and cationic surfactants. They reported the binding ratio between dye and surfactants as follows:

- Congo Red: CTMAB or CPB = 1:2
- Alizarin Red S : CTMAB = 2:1
- Alizarin Red S : CPB = 1:10
- Methylene Blue : Monoxol OT = 5:1

Hishiki and Ueda (80) measured the polarographic half-wave potentials of the unsymmetrical carbocyanine dyes (Quinoxaline and Pyrazine dyes) containing one nucleus having sensitizing ability and the other one having a desensitizing effect. They reported that these dyes exist among the range of sensitizing dye and desensitizing dye. A few dyes having no ability to sensitize or to desensitize silver halide have been suggested for use as anti-irradiation dyes.
The electrochemical oxidation-reduction behaviour at the platinum electrode in liquid sulphur dioxide of four triphenylmethane dyes (Crystal Violet, Ethyl Violet, Malachite Green and Brilliant Green) \(^{(81)}\), the leuco form of Crystal Violet, a possible oxidation product; \(N,N,N',N'\)-tetramethylbenzidine has been investigated using voltammographic (polarographic) and cyclic voltammetric techniques. The oxidation of the dyes in liquid sulphur dioxide was quite different from that observed in acidic aqueous solution \(^{(82,83)}\). Galus and Adams \(^{(82)}\) reported that the oxidation of Crystal Violet and Malachite Green leads to the formation of the oxidized form of \(N,N,N',N'\)-tetramethylbenzidine. The polarography of Chrome Azurol S \(^{(143)}\), hydroxytriphenylmethane dyes \(^{(144)}\) has also been reported.

The polarographic behaviour of eight azo dyes \(^{(90)}\) used in foods has been examined in a basal solution of tetramethylammonium chloride containing various buffer mixtures. The \(E_{1/2}\) values decrease with increase in pH, and reversible two-electron waves appear at a certain pH at which the limiting current is diffusion controlled. The calibration graphs were rectilinear for \(10^{-4}\) to \(10^{-3}\) M solution in 0.1M tetramethylammonium chloride (10ml) containing 1ml of McIlvaine buffer solution of approximate pH and 0.2ml of 1% gelatin solution.

Polarographic half-wave potentials of 26 disperse azo dyes \(^{(102)}\), as well as monosubstituted nitrobenzene and azobenzene, were measured in dioxane-Britton-Robinson
buffer solution at pH 5.0. The relation between the chemical structure and the values of half-wave potential were also studied. Nucleophilic groups increase the values of \(-E_{1/2}\), while electrophilic groups decrease them.

Nazario and Zenebon\(^{(104)}\) studied the polarographic behaviour of 10 permitted dyes in food and found only azo dyes to give well defined polarographic waves.

Matrka and Navratil\(^{(107)}\) reported some polarographic results for 4 acid anthraquinone dyes in 0.2M acetate buffer containing 0.001% of gelatin. Three synthetic basic dyes\(^{(112)}\), forbidden for use in food in France, Auramine O, methyl Violet and Malachite Green, could be detected in suitable mixtures of all three by polarography of a solution in methanol of 50 to 100 µg of dye mixed with potassium hydrogen phthalate buffer (pH 5).

The possibility of using second-harmonic alternating current polarography in the analysis of organic compounds (Methylene Blue)\(^{(117)}\) was considered, and with the use of both stationary and dropping mercury electrodes. A method of obtaining a differential polarogram by subtracting the interfering signal, previously recorded on a magnetic tape was suggested.

The polarographic behaviour of the dyes Bright Red 5SRh, Golden Yellow K3R, Orange 6-436 and Violet 4K (each of which contains an azo group and a chlorinated triazine moiety, and the last named also contains Cu) was studied by Shkorbatova and Pegusova\(^{(119)}\). They suggested a method for the determination of the dyes in
Quantitative analysis of 8 azo dyes mixed with their Cr, Co, or Cu complexes were determined by AC, DC, and oscillography by Koch, Wolf, and Keil (124). Eriochrome Violet B (CI 15670) was studied polarographically in water-ethanol mixture and acetate buffer at pH 4.85 by Ioan (125). A polarographic method (127) was developed for determining 4 synthetic azo dyes used for colouring pharmaceutical preparations in Poland. Acetate buffer at 4.5 and Britton-Robinson buffer at pH 3.3 were used for the study of $10^{-5} - 2 \times 10^{-4}$ M dye concentration range. Smyth and Hassanzadeh (169) have described the application of differential pulse polarography to the analysis of diazo dyes and aromatic nitro compounds directly in certain aquarium situations. Differential pulse polarographic data on Acid Red 73, Direct Blue 84, Direct Red 24 and Direct Orange 34 together with a series of nitrogen-containing organic compounds has been given in the Table.

Florence et al (170) have investigated the electro-reduction of a range of pyridyl and thiazolyl azo dyes and compared their polarographic behaviour with the benzene analogues. They have concluded that the major differences between the polarographic behaviour of heterocyclic and aromatic azo compounds are as follows: Naphthaleneazo compounds having a hydroxy or an amino group ortho to the azo linkage invariably produce single waves at DME which have a height corresponding to four electrons, i.e. during the lifetime of the drop reduction
proceeds essentially completely to the amines at all pH values. Para-hydroxybenzeneazo dyes also yield 4-electron waves, but in acid solutions only. Pyridyl and thiazolyl hydroxyazo compounds, on the other hand, usually yield 2-electron waves in acid media and 4-electron waves only in strongly alkaline solution. Only in the case of 3-pyridyl compounds are pH values greater than 2 obtained in acid solution. The hydrazo derivatives of the heterocyclic dyes are more stable with respect to disproportionation, which is attributed to the strongly electron-attracting properties of the pyridyl and thiazolyl groups. In addition, their disproportionation is base-catalyzed, whereas earlier studies showed that aromatic azo dyes undergo an acid-catalyzed reaction.

The appearance of additional polarographic peaks in the polarograms of 3,3'-diethyl-5,5'-diphenyl-9-ethyloxatrimethinecyanine(I)(173) were examined by recording the DC polarogram and the oscillogalapolarograms of I in CH$_3$CN, CH$_3$OH, and 0.1M sodium perchlorate in the presence of varying amounts of water. The peaks were due to the formation of molecule I aggregates, especially in the presence of H bond-favouring H$_2$O. A column chromatographic purification method was given.

The anodic and cathodic half-wave potentials of 9 groups of vinylogous polymethylene dyes(174) were determined and related with their spectral properties and stabilities.

Analysis of monoazo dyes(176), the determination of dye diffusion coefficients(179) in solutions have
been studied polarographically. Aggregation of the Cr complex dye Acid Pink M (CI Acid Red 186) was accelerated in pH 10 - 11 solutions.

Azo dyes which have two donor groups ortho to the azo linkage are powerful chelating agents, and when complexed with metals may exhibit two discrete reduction waves, one due to free dye, and the other to metal-dye complex(182). Ethanol depressed the limiting currents of hydroxy substituted dyes in alkaline media, and this effect is believed to result from a decrease in adsorption of the dye on the electrode surface in the presence of high ethanol concentrations, and from electric repulsion by the electrode of the negatively charged dye species. Nitro-substituted azo dyes, like pyridylazo compounds, produced hydrazo derivatives which were relatively stable in acid solution.

A polarographic investigation of carcinogenic and several closely related non-carcinogenic aminoazobenzenes was carried out by Carruthers(217).
POLAROGRAPHIC STUDIES OF SYNTHETIC DYES

PREFACE

Reducible compounds, showing a cathodic wave and oxidizable compounds, giving an anodic wave are the two kinds of electroactive compounds.

Organic compounds which are reduced at the dropping mercury electrode must contain a highly polar or unsaturated bond. Zuman\(^{(237)}\) indicated that some organic substances exert a catalytic effect upon hydrogen evolution. All substances which cause catalytic waves in unbuffered acid or buffered solutions should be Brönsted acids with a pKa value in the medium range of the pH-scale. Some dyestuffs\(^{(236)}\) have been found to give a catalytic wave. Catalytic currents possess the form of a rounded or sharp maximum and can be observed either in simple buffer solutions or buffers containing heavy metals. It is well known that the heights of catalytic waves are very sensitive to pH, buffer composition, buffer concentration, ionic strength etc.

In the absence of a sample of pure compound, some indication of the amount of the compound present can be obtained by comparison with polarographic waves of a compound of similar structure. An extension of the conjugated system, or a substantial steric effect can change considerably the polarographic behaviour. The introduction of an additional active grouping in the immediate neighbourhood of the polarographically active group, can have a substantial effect on the behaviour of the latter. This effect can result not only in a shift of the half-wave potential, but in
a change in reduction mechanism as well.

It is surprising how little polarography has been applied to the control of dye production and in the supervision of applications of dyestuffs in the textile industry. Most dyes and intermediates used are polarographically active. Numerous papers have been devoted to the polarography of dyestuffs, but only the mechanism of the electrode process and its relation to structure have been reported.

Most studies of substituent effects are based on shifts of half-wave potential. The half-wave potential ($E_{1/2}$) for a reversible process depends chiefly on the difference between the standard free energies of the oxidized and reduced forms. The equation (238)

$$E_{1/2} = E^\circ - \frac{RT}{nF} \ln \left( \frac{D_{ox}}{D_{red}} \right)^{1/2} - \frac{RT}{nF} \ln \frac{f_{red}}{f_{ox}}$$

shows the relation between them. In the equation R is the gas constant, T is the absolute temperature, n is the number of electrons transferred, F is the Faraday constant, $D_{ox}$ and $D_{red}$ are the diffusion coefficients of the oxidized and reduced forms, respectively, and $f_{ox}$ and $f_{red}$ are their activity coefficients.

On the other hand, the half-wave potential for an irreversible process depends on the free energy of activation for the potential determining step of the sequence. The equation

$$E_{1/2} = E^\circ - (2.3RT/daF) \log 0.336 \times e^{(t_1/D)^{1/2}}$$

is given for the half-wave potential of slow electrode
processes, where $K^0$ is the heterogeneous rate constant of the electrode process, $\alpha$ is the transfer coefficient and $t_1$ is the drop time.

It was shown by Malik et al (239) that the reduction of some azo compounds, i.e. 2-amino-4-methyl-5-arylanthiazoles or 3,5-diphenyl-4-arylazopyrazoles at the dropping mercury electrode takes place at $-N=N-$ group in preference to $C=C$ or $C=N$ groups. They proposed that the reductions of these compounds take place with two electron transfer.

In the present work an attempt was made to develop analytical methods for dyes of practical importance using differential pulse polarography.

The dye samples obtained were proved to be chemically pure by TLC identification method and were used without further purification. The results are shown on Table 1 and 2.

The dyestuffs used in this work are listed on Table 3.
Table 1. hRf values of acid and basic dyes in different solvent systems on silica gel.

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<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Colour of spots</th>
<th>S5</th>
<th>S6</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Acid Yellow 199</td>
<td>yellow</td>
<td>62</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Acid Blue 62</td>
<td>blue</td>
<td>75</td>
<td>26</td>
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<td>brown</td>
<td>57</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Acid Red 114</td>
<td>reddish</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown</td>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Basic Yellow 59</td>
<td>yellow</td>
<td>61</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>Basic Yellow 28</td>
<td>yellow</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>Basic Orange 30:1</td>
<td>orange</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Basic Red 14</td>
<td>red</td>
<td>44</td>
<td>35d</td>
</tr>
<tr>
<td>9</td>
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<td>blue</td>
<td>43</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
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<td>blue</td>
<td>53</td>
<td>42</td>
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<td>11</td>
<td>H • 87034</td>
<td>yellow</td>
<td>81</td>
<td>45</td>
</tr>
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<td>12</td>
<td>Catechol Violet</td>
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<td>2</td>
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<tr>
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<td>Crystal Violet</td>
<td>violet</td>
<td>76</td>
<td>50d</td>
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<td>14</td>
<td>Brilliant Green</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>blue</td>
<td>83</td>
<td>50</td>
</tr>
</tbody>
</table>

Solvent systems: 

S5 = Chloroform:isopropanol:pyridine:glacial acetic acid:water(6:8:3:1:2)  

* hRf values are Rf values multiplied by 100.  
d = diffuse spot; t = tailing; u = unsatisfactory.
Table 2. hRf Values of dyes on several carriers

in different solvent systems.

Solvent systems: $S1 = \text{Isopropanol-water-carbontetrachloride}(60:25:15)$

$S2 = \text{Isopropanol-water-ethanol 98-100\%}(30:60:10)$

$S3 = \text{Isopropanol-water-ethanol-carbontetrachloride}(45:25:10:10)$

$S4 = \text{Isopropanol-water-carbontetrachloride-ammonia}(65:20:20:5)$.

Carriers:

A = High performance TLC on silica gel

B = TLC on cellulose

C = TLC on silica gel

D = TLC on alumina.

<table>
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<th>No</th>
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<th>Colour of spots</th>
<th>$S1$</th>
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<th>$S3$</th>
<th>$S4$</th>
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<td>pink</td>
<td>67</td>
<td>98</td>
<td>90</td>
<td>86</td>
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<td>2</td>
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<td>44090</td>
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<td>42</td>
<td>89</td>
<td>47</td>
<td>70</td>
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<td>orange</td>
<td>54</td>
<td>78</td>
<td>64</td>
<td>87</td>
</tr>
<tr>
<td>No</td>
<td>Commercial Name</td>
<td>Colour Index No</td>
<td>Colour of spots</td>
<td>S1</td>
<td>S2</td>
<td>S3</td>
<td>S4</td>
</tr>
<tr>
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<td>orange</td>
<td>55</td>
<td>70</td>
<td>64</td>
<td>85</td>
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<td>19140</td>
<td>yellow</td>
<td>42</td>
<td>15</td>
<td>54</td>
<td>87</td>
</tr>
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<td>6</td>
<td>Lissamine fast Yellow</td>
<td>18965</td>
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Note: The values in the S1, S2, S3, and S4 columns represent the intensity or concentration levels of the respective commercial names.
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<th>S4</th>
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<td>B</td>
<td>C</td>
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* hR_f values are R_f values multiplied by 100.

u = unsatisfactory; dash(-) = not tested with that system;
t = tailing; d = diffuse spot.
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- Amperometry,
- Polarography,
- Potentiometry using ion-selective electrode, and
- Thin Layer Chromatography.

\[
\begin{align*}
\text{C} & \quad \text{N}(\text{C}_2\text{H}_5)_2 \\
\text{N}^+ & \quad \text{N}^+\text{(C}_2\text{H}_5)_2^- \text{HSO}_4
\end{align*}
\]
INTRODUCTION

The use of certified, or natural colour is permitted in food products to enhance their appearance and make them more pleasing to the eye. If the colouring of a particular food product is prohibited, no colour, whether certified or of natural origin is permitted. The term "colour additive" (195) is defined as a material which is a dye, pigment, or other substance made by a synthetic process or extracted from natural products.

The colour additives that are used for colouring foods, drugs, and cosmetics are divided into three groups: (1) synthetic organic colouring matters; (2) natural colouring matters, which may be of either vegetable or animal origin; (3) inorganic colours, which may be synthetic or of mineral origin. Synthetic organic colours form by far the largest proportion of colouring matters used. Almost all these synthetic organic chemical compounds which are known as "certified colours" are derivatives of the azo, nitroso, nitro, pyrazolone, indigoid, xanthene, anthraquinone, pyrene, fluoran, quinoline, and triphenylmethane classes of dyes.

Light, acids, and alkalies may act on certified colours causing various reactions, such as fading or changing of shade. The elements such as excessive sunlight, bleaching agents (sulphur dioxide being the most common); tin and iron should be avoided as much as possible.
Dyes are coloured chemical compounds which exhibit their colouring power or tinctorial strength when dissolved in a solvent. Pigments are insoluble materials which colour by dispersion. The FD & C lakes consist of a substratum of alumina hydrate on which the dye is adsorbed or precipitated. Use of dyes involves addition of a solvent, generally water. However, the lakes can be incorporated dry and the material directly compressed.

Various studies in teratology\(^{196,197}\), mutagenic activity\(^{198}\), toxicities and carcinogenicities\(^{199,201}\) etc. on food colouring matters tend to indicate that the use of dyes in foodstuffs should be controlled.

The number of colouring agents previously permitted has been considerably reduced now. The decision\(^{200}\) of the United States Food and Drug Administration to withdraw approval for the use of Amaranth (FD and C Red 2) is just one example of the trend towards limitation.

The use\(^{227}\) of Orange G (CI 16230) and Chocolate Brown FD (Food Brown 2) in the U.K. has been withdrawn from 1 January 1978. Yellow 2G (CI 18965) is probably to be deleted by the end of 1979. Red 2G (CI 18050) is under review.

In view of the desire to control and restrict the usage of synthetic food colourings, there exists a need to develop methods of analysis for these compounds, which will indicate not only the nature of the added colouring but also the amount present.

The analytical chemistry of synthetic dyes, including
food applications, has been extensively reviewed in a recent monograph (11). Numerous schemes have been proposed for qualitative analysis mostly by paper and thin-layer chromatography. Quantitative analysis has been attempted by spectrophotometry (202), titration with titanous chloride solution (203), electrophoresis on polyacrylamide gel (204), HPLC (205-207) and paired-ion chromatography (188, 189, 210).

The work described here has been concentrated on developing the differential pulse polarographic method which is more attractive in its selectivity, sensitivity and rapidity than the other methods mentioned for the determination of food colourings.

Procedures have been developed for the simultaneous determination of colouring mixtures in four types of drinks: (1) Orange squash or Sparkling Orangeade; (2) Sparkling Limeade; (3) Blackcurrant health drink; and (4) Sparkling Dandelion & Burdock. The procedures have been tested on standard dye solutions prepared with uncoloured natural fruit syrups. A number of food colourings also have been studied polarographically and relevant data is presented.
EXPERIMENTAL

APPARATUS

A Princeton Applied Research (PAR) 174 polarographic analyser was used in connection with a Y-t recorder (Tarkan). A three electrode system was employed with the polarograph using a dropping mercury electrode (DME) as a working electrode, a thin platinum plate (1 x 1 cm$^2$) as counter electrode and a saturated calomel electrode (SCE) as reference electrode. The pH of a solution was measured using a Radiometer pHM 64 research pH meter.

The solution temperature in the polarographic cell (50 ml) with water jacket was adjusted by pumping water from a thermostated water bath (type SU6, Grant Instrument Ltd.).

The solution was deoxygenated using nitrogen gas passed through gas cleaning trains containing 10% vanadous sulphate in 4M sulphuric acid and zinc amalgam while a magnetic stirrer unit was operated in order to deaerate the solution effectively.

A two way tap was connected at the end of the deoxygenation train to keep the nitrogen gas over the solution throughout the polarographic work.

For the differential pulse operation, the forced drop time was 1 s, the pulse height 50 mV and the scan rate 2 mVs$^{-1}$ unless otherwise stated.

All potentials are reported vs the SCE.
REAGENTS AND SAMPLES

Britton-Robinson (B-R) buffer (pH 1.9; 0.04 M in each constituent) was prepared by dissolving 2.5 g of boric acid in 500 ml of distilled water containing 2.3 ml of glacial acetic acid, and adding 2.7 ml of orthophosphoric acid and then diluting to 1 litre with water. The pH of the buffer was adjusted as required by means of 0.2 M or 4 M sodium hydroxide solution.

Tetramethylammonium chloride (TMAC) (1 M) and tetraphenylphosphonium chloride (TPPC) (0.01 M) were prepared from the reagent grade of BDH chemicals.

Samples of Amaranth, Sunset Yellow FCF, Tartrazine, Green S and Chocolate Brown HT and of uncoloured blackcurrant health drink syrup and uncoloured lemonade syrup were kindly provided by Beecham Products Ltd.

Methyltriphenylphosphonium chloride (MTPPC), tetraethylammonium chloride (TEAC), Triton X-100 were reagent grade chemicals.
RESULTS AND DISCUSSION

I. DETERMINATION OF SUNSET YELLOW FCF AND TARTRAZINE IN SPARKLING ORANGEADE.

An uncoloured orangeade was prepared according to the Beecham's guidelines using the syrup provided: 15ml of uncoloured lemonade syrup (Beecham's No. DB8/7) was diluted to 100ml with distilled water previously carbonated using dry ice. A blank polarographic solution was prepared by mixing 5ml of uncoloured orangeade, 5ml of 0.01M tetraphenylphosphonium chloride (TPPC) and 20ml of B-R buffer, adjusting to pH 9 with 0.2M and 4M sodium hydroxide solution and diluting to 50ml. An aliquot (20ml) of this solution was pipetted into the polarographic cell, deoxygenated for 10 minutes and polarographed. In the method development work successive aliquots of concentrated solutions of Sunset Yellow FCF (420ppm) and Tartrazine (200ppm) were added to the blank solution by means of a 100μl syringe.

The solution was stirred using a magnetic stirrer while the solution was being deoxygenated for 1 minute after each addition.

The effect of adding the TPPC on the polarograms of a mixture of Sunset Yellow FCF and Tartrazine is shown in Fig. 1. On the addition of TPPC, the peak potential of Sunset Yellow FCF remains unchanged but the peak height is suppressed by 50%. The peak
Fig. 1. Effect of tetraphenylphosphonium chloride on the differential pulse polarogram of a mixture of Sunset Yellow FCF and Tartrazine using the recommended procedure (i) with the addition of TPPC and (ii) without it.
potential of Tartrazine is shifted to a more negative potential and the peak current is increased. The effect of the Tartrazine peak on the gradual addition of TPPC is shown in Fig. 2. In the absence of TPPC the main peak had a big polarographic maximum and a small peak presumably caused by adsorption of the colour on the mercury electrode or by an intermediate species appeared at -0.56V vs SCE, prior to the main peak. The shift of its peak potential to a more negative potential vs SCE could be explained by the formation of a less polar compound between the Tartrazine anion and the tetraphenylphosphonium cation. The effect on the peaks of the individual colours of the addition of TPPC is illustrated by the data in Table 4.

The peak potentials of both colours shift towards more negative values with increasing pH (see Fig. 3). The temperature coefficient was in the range of 1.24 - 1.70 % deg$^{-1}$ as shown on the Table 5. The peak heights were found to be linear with respect to $\sqrt{h}$ where $h$ is the height of mercury column (see Table 6). All these facts point to the diffusion controlled nature of the peaks for Sunset Yellow FCF and Tartrazine.

Calibration graphs for both colourings are linear and not affected by the presence of the other colouring in proportions normally found in fruit drinks. Typical polarograms to obtain a calibration graph for Tartrazine in the presence of Sunset Yellow FCF are shown.
Fig. 2. The effect of tetraphenylphosphonium cation on the differential pulse polarogram of 3.6 ppm Tartrazine Supra at pH 9.02. The concentration of tetraphenylphosphonium chloride: (I) to (VI); 0.0, 18, 37, 56, 187, and 887 ppm respectively.
peak current / $\mu$A

applied potential / V

-0.80 -0.60 -0.80 -0.60 -0.80 -0.60

0.2 $\mu$A

(IV) (V) (VI)
Table 4. Effect of tetraphenylphosphonium (TPPC) concentration on the d.p. polarographic peaks of Tartrazine and Sunset Yellow FCF. Tartrazine concentration = 4 ppm; Sunset Yellow FCF concentration = 8.4 ppm.

<table>
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<tr>
<th>TPPC conc./ppm</th>
<th>0</th>
<th>9</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>90</th>
<th>100</th>
<th>380*</th>
<th>900</th>
<th>1200</th>
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<tbody>
<tr>
<td><strong>i_p (Tartrazine)</strong> /µA</td>
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<td>0.67</td>
<td>1.49</td>
<td>1.65</td>
<td>1.65</td>
<td>1.68</td>
<td>1.67</td>
<td>1.68</td>
<td>1.67</td>
<td>1.68</td>
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<tr>
<td>***i_p (Sunset Yellow FCF) /µA</td>
<td>5.4</td>
<td>4.5</td>
<td>3.20</td>
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<td>2.10</td>
<td>2.50</td>
<td>2.60</td>
<td>2.56</td>
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</table>

* TPPC concentration used in recommended procedure.
** E_p = -0.73 V (in absence of TPPC), -0.80 V (at all concs. here).
*** E_p = -0.65 V (in presence or absence of TPPC).

Fig. 3. Relationship between peak potential and pH for the colours.
Table 5. Temperature effect on the peak currents of Sunset Yellow FCF and Tartrazine at pH 9 and $h_{Hg} = 50cm$.

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<th>30.0</th>
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<tr>
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<td>$(di/dT)%/°C$</td>
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<td>1.70</td>
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<td>Tartrazine</td>
<td>Current, $i/μA$</td>
<td>1.54</td>
<td>1.67</td>
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<tr>
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<td>-1.44</td>
<td>0</td>
<td>1.66</td>
<td>1.50</td>
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** Sunset Yellow FCF concentration = 8.4 ppm and Tartrazine concentration = 4.2 ppm.**

Table 6. The effect of the height of mercury column on the peak currents at 25°C and pH 9.

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<th>mercury height, $h/cm$</th>
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<td>$√h$</td>
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<td></td>
</tr>
<tr>
<td>Sunset Yellow FCF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current, $i_d/μA$</td>
<td>2.33</td>
<td>2.62</td>
<td>2.89</td>
</tr>
<tr>
<td>$i_d/√h$</td>
<td>6.32</td>
<td>7.07</td>
<td>7.74</td>
</tr>
<tr>
<td>$i_d/√h$</td>
<td>0.369</td>
<td>0.370</td>
<td>0.373</td>
</tr>
<tr>
<td>Tartrazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current, $i_d/μA$</td>
<td>1.65</td>
<td>1.82</td>
<td>2.00</td>
</tr>
<tr>
<td>$i_d/√h$</td>
<td>6.32</td>
<td>7.07</td>
<td>7.74</td>
</tr>
<tr>
<td>$i_d/√h$</td>
<td>0.261</td>
<td>0.257</td>
<td>0.258</td>
</tr>
</tbody>
</table>
in Fig. 4.

The recommended procedure for the determination of Sunset Yellow FCF and Tartrazine in sparkling orangeade is as follows:

Pipette 10ml of sparkling orangeade into a 50ml beaker. Add 5ml of 0.01M tetraphenylphosphonium chloride (TPPC) solution and 20ml of pH 1.9 B-R buffer. Adjust to pH 9 with 4M and 0.2M sodium hydroxide solution and dilute to 50ml in a volumetric flask. Deoxygenate a portion of this solution in a polarographic cell for 10 minutes and polarograph it between -0.3 and -1.0V vs SCE.

The procedure was tested using a standard sparkling orangeade (42ppm Sunset Yellow FCF, 20ppm Tartrazine) prepared from uncoloured lemonade syrup and the colouring samples. The result obtained for ten determinations was 41.4ppm Sunset Yellow FCF (coefficient of variation = 1.4%) and 20.5ppm Tartrazine (coefficient of variation = 1.0%).

The method can be directly applied to a orange drink (Quosh; Beecham's product) for the simultaneous determination of Tartrazine and Sunset Yellow FCF. An initial attempt was made to ion-pair extract these food colours into chloroform using TPPC. After evaporating the chloroform and dissolving the colours in pH 9 B-R buffer two distinct polarographic waves were obtained. Subsequently the extraction step was found to be unnecessary because neither the solid matter nor sweetening components interfered with the determination. Fig. 5 shows the typical polarograms of Sunset Yellow FCF. The presence
Fig. 4 Differential pulse polarograms produced in obtaining a calibration graph for Tartrazine in the presence of Sunset Yellow FCF (12 ppm). Tartrazine concentration in measured solution: (i) to (v) 0.0, 1.0, 2.9, 5.1 and 7.5 ppm respectively.

Modulation amplitude 50mV, scan rate 2 mVs⁻¹, and drop time 1s.
Fig. 5.
Differential pulse polarograms of Sunset Yellow FCF (CI 15985) obtained in pH 9.0 B-R buffer: (I) to (VIII): 0.0, 1.05, 2.09, 4.16, 6.41, 8.23, 10.24, and 12.23 ppm respectively.
Modulation amplitude 50 mV, scan rate 2 mVs⁻¹, and drop time 1 s.
of Tartrazine did not affect Sunset Yellow FCF peak. However, careful attention must be paid to the fact that sufficient amount of TPPC has to be added to the solution because of its effect on both colouring matters.

The spectra of those colouring extracts in pH 9 B-R buffer are given in Fig. 6. The calibration graphs for single component of each colouring showed a rectilinear relation. The spectrum of the Quosh extract, however, indicates that the analysis of colour mixtures can be difficult by spectrophotometry without further separation or a more complicated mathematical approach (223, 226).

The polarographic method for the determination of these colours is obviously more accurate and faster than spectrometry.

Tetraethylammonium chloride and Triton X-100 were studied in an attempt to improve the separation of the Sunset Yellow FCF and Tartrazine peaks. The peak heights were decreased by the addition of such surfactants. On the other hand methyltriphenylphosphonium chloride (MTPPC) produced reasonably good separation of the peaks at a distance of 0.11 V which is 0.04 V smaller than the case with TPPC. In the case of MTPPC the peak height of Sunset Yellow FCF was increased by 30 %, but that of Tartrazine was decreased by 15 %, compared with peak heights using TPPC.
Fig. 6. Absorption spectra of Sunset Yellow and Tartrazine:

a to g: 0.25, 0.50, 1.00, 1.20, 1.60, 2.00, 3.00 x 10^{-5} M respectively.

1 to 7: 0.25, 0.50, 1.00, 1.20, 1.60, 2.00, and 2.80 x 10^{-5} M respectively.
II. DETERMINATION OF TARTRAZINE AND GREEN S IN SPARKLING LIMEADE.

Tartrazine is best determined under the solution conditions above for sparkling orangeade, viz. at pH 9 in the presence of tetrapaenylphosphonium chloride. Under these conditions Green S is reduced at a similar potential to Tartrazine but fortunately because only a small portion of Green S relative to Tartrazine is normally present in sparkling limeade and because the Green S wave is depressed in the presence of the phosphonium salt, interference of Green S in the determination of Tartrazine is negligible.

Green S is best determined at pH 4 and the addition of tetramethylammonium chloride was found to improve the baseline considerably. This improvement seems to arise owing to a partial suppression of the polarographic maximum of the Tartrazine. Calibration graphs for Green S were obtained in the presence of Tartrazine and the absence of tetramethylammonium chloride. Fig. 7 shows the linear relation between the peak current and Green S concentration in spite of the effect of Tartrazine maximum.

A better result is brought about by the recommended method for the determination of Green S and Tartrazine in sparkling limeade as follows: Pipette 10ml of sparkling limeade into a 50ml beaker. Add 5ml of 1M tetramethylammonium chloride and 10ml of pH 1.9 B-R buffer and adjust to pH 4 with 0.2M sodium hydroxide solution. Dilute to
Fig. 7.
Differential pulse polarograms of Green S(CI 44090) obtained in pH 4.02 B-R buffer including Sparkling Limeade: (I) to (X); 0.0, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 2.4, 3.3, and 4.3 ppm respectively. Modulation amplitude 50mV, scan rate 2 mVs⁻¹, and drop time 1s.
50ml in a calibrated flask. Deoxygenate a portion of this solution in a polarographic cell and polarograph it between -0.45 and -0.85V vs SCE.

Carefully readjust the solution in the cell to pH 9 with several drops of 4M sodium hydroxide solution and add 10mg of TPPC. Pass nitrogen to aid dissolution of the solid and to deoxygenate the solution. The use of a magnetic stirrer unit might be more effective for this purpose. Stop stirring and passing nitrogen into the solution and polarograph it between -0.6 and -1.0 V vs SCE while the interior of the cell is being purged with nitrogen gas.

The procedure was tested using a standard sparkling limeade (20ppm Tartrazine and 2ppm Green S) prepared from uncoloured lemonade syrup and the food colour samples. Typical polarograms are shown in Fig. 8. The shift of Tartrazine peak potential is more pronounced than the one of Green S with changing pH. At pH 4.1 two adjacent peaks of Tartrazine appear at more positive potentials than the Green S peak. An enhanced and well-defined single peak of Tartrazine appears at pH 9.1. The peak contains the suppressed Green S peak at the foot of its positive side. At the levels of Green S in the limeade this Green S peak at -0.75V is negligible; at very high concentrations of Green S a shoulder appears on the Tartrazine peak.

The result of ten determinations was Green S 2.1ppm (coefficient of variation = 3.4 %) and Tartrazine 19.6 ppm (coefficient of variation = 1.9 %).
Fig. 8. Typical differential pulse polarograms of Green S and Tartrazine in limeade: (i) pH 4.1 and (ii) pH 9.2.
III. DETERMINATION OF AMARANTH AND GREEN S IN BLACKCURRANT HEALTH DRINK

The first comprehensive legislation in the USA was the Food and Drug Act of 1906 which listed seven dyes(\textsuperscript{[2]}) which were permitted for use in foods. They were as follows; Amaranth, Erythrosine, Indigotine, Light Green, Naphthol Yellow, Orange I, Ponceau 3R.

Although Amaranth no longer appears on the list of permitted food colours in America, it is still permitted for use in blackcurrant health drink in UK.

Amaranth was the subject of an investigation in which a four-electron reduction process to yield the corresponding amines(\textsuperscript{[3]}\textsuperscript{2}) was established. The reaction of a four-electron reduction under the influence of chemical reducing reagents forms the basis of the quantitative determination of Amaranth and similar azo colours by titanium trichloride reduction.

The sweetening component in the uncoloured blackcurrant health drink syrup gave a small peak at pH 4 at the same potential as Green S. At higher pH than 6 this peak disappeared. Amaranth gives a broad polarographic maximum under these conditions but this can be suppressed by the addition of tetramethylammonium chloride.

The recommended procedure for the determination of Amaranth and Green S in blackcurrant health drink is as follows: Pipette 5ml of blackcurrant health drink into a 50ml beaker. Add 5ml of 1M tetramethylammonium chloride solution and 10ml of pH 1.9 B-R buffer. Adjust
the pH to 7.8 with 4M sodium hydroxide solution and dilute to 50 ml in a calibrated flask. Transfer a portion of this solution to a polarographic cell, deoxygenate it for 10 minutes and polarograph the solution between -0.30 and -0.80 V vs SCE.

The temperature and mercury pressure effects on the peak currents of both colours by the method described are shown on the Table 7 and 8. The concentration of Green S at -0.72 is 10.0 ppm and for Amaranth at -0.49 V is 2.5 ppm.

The dependence of the limiting current on the mercury head for both colours is shown to be directly proportional to $\sqrt{n}$. The temperature effects on the peak currents were found to be diffusion controlled for Green S but the temperature coefficient was slightly high for Amaranth. A single electron reduction process appears to be taking place for Green S from a comparison of its peak current with that of Amaranth.

The recommended procedure was tested using a standard blackcurrant health drink (Amaranth 250 ppm and Green S 4 ppm) prepared from uncoloured blackcurrant health drink syrup (Beechan's DB 8/6) and the food colour samples. A typical polarogram is shown in Fig. 9. Clearly the Green S is being determined near its detection limit for the amount of Amaranth present but the procedure can be used as a limit test for this colour. The result of ten determinations for Amaranth with the standard blackcurrant health drink was 245 ppm with a coefficient of variation of 1.3%.
Table 7. Peak currents dependence on temperature at $h_{Hg} = 50\text{cm}$.

<table>
<thead>
<tr>
<th>Colours</th>
<th>Temperature, $T/°C$</th>
<th>$10.0$</th>
<th>$15.0$</th>
<th>$20.0$</th>
<th>$25.0$</th>
<th>$30.0$</th>
<th>$35.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current, $i/µA$</td>
<td>0.72</td>
<td>0.93</td>
<td>1.13</td>
<td>1.39</td>
<td>1.62</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>% increased</td>
<td>-48.20</td>
<td>-33.09</td>
<td>-18.70</td>
<td>0</td>
<td>16.55</td>
<td>30.94</td>
</tr>
<tr>
<td></td>
<td>$(di/dT)/°C$</td>
<td>3.02</td>
<td>2.88</td>
<td>3.74</td>
<td>0</td>
<td>3.31</td>
<td>2.88</td>
</tr>
<tr>
<td>Amaranth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Current, $i/µA$</td>
<td>1.05</td>
<td>1.18</td>
<td>1.23</td>
<td>1.43</td>
<td>1.58</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>% increased</td>
<td>-26.57</td>
<td>-17.48</td>
<td>-10.49</td>
<td>0</td>
<td>10.49</td>
<td>21.68</td>
</tr>
<tr>
<td></td>
<td>$(di/dT)/°C$</td>
<td>1.82</td>
<td>1.40</td>
<td>2.10</td>
<td>0</td>
<td>2.10</td>
<td>2.24</td>
</tr>
<tr>
<td>Green S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Dependence on the height of mercury column, $h$ at $25.0\text{°C}$.

<table>
<thead>
<tr>
<th>Colours</th>
<th>Mercury height, $h/\text{cm}$</th>
<th>$30$</th>
<th>$43$</th>
<th>$51$</th>
<th>$64$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current, $i_d/µA$</td>
<td>1.05</td>
<td>1.23</td>
<td>1.40</td>
<td>1.59</td>
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<tr>
<td></td>
<td>$\sqrt{h}$</td>
<td>5.48</td>
<td>6.55</td>
<td>7.14</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>$i_d/\sqrt{h}$</td>
<td>0.192</td>
<td>0.195</td>
<td>0.196</td>
<td>0.198</td>
</tr>
<tr>
<td>Amaranth</td>
<td>Current, $i_d/µA$</td>
<td>1.00</td>
<td>1.28</td>
<td>1.45</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>$\sqrt{h}$</td>
<td>5.48</td>
<td>6.55</td>
<td>7.14</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>$i_d/\sqrt{h}$</td>
<td>0.183</td>
<td>0.195</td>
<td>0.203</td>
<td>0.209</td>
</tr>
</tbody>
</table>
Fig. 9.

(i) Typical differential pulse polarograms for Green S and Amaranth in blackcurrant health drink. (ii) Polaro- graphic peak of 0.6 ppm of Green S in the presence of 2.5 ppm of Amaranth.
IV. DETERMINATION OF GREEN S, TARTRAZINE AND CHOCOLATE BROWN HT IN SPARKLING DANDELION & BURDOCK.

An uncoloured dandelion & burdock was prepared by diluting 15ml of uncoloured lemonade syrup (Beecham's DB 8/7) to 100ml with distilled water previously carbonated using dry ice as the same procedure for the orangeade.

The blank solutions for the polarography of the colours were prepared as follows:

(i) Chocolate Brown HT; 10ml of uncoloured dandelion & burdock, 5ml of 1M tetramethylammonium chloride, 10ml of B-R buffer (pH 1.9), 1ml of 200ppm Tartrazine and 0.5ml of 40ppm Green S were mixed together, adjusted to pH 4 and diluted to 50ml with distilled water.

(ii) Green S; 10ml of uncoloured dandelion & burdock, 5ml of 1M tetramethylammonium chloride, 10ml of B-R buffer (pH 1.9), 1ml of 200ppm Tartrazine and 1ml of 628ppm Chocolate Brown HT were mixed together, adjusted to pH 4 and diluted to 50ml in a calibrated flask with distilled water.

(iii) Tartrazine; 10ml of uncoloured dandelion & burdock, 10ml of pH 1.9 B-R buffer, 5ml of 1M tetramethylammonium chloride, 5ml of 0.01M TPPC, 1ml of 628ppm Chocolate Brown HT and 0.5ml of 40ppm Green S were mixed in a 50ml beaker, adjusted to pH 9 and diluted to 50ml in a calibrated flask with distilled water.

An aliquot (20ml) of the solution was pipetted into the polarographic cell, deoxygenated for 10 minutes using nitrogen gas and polarographed. For the calibration
polarograms successive aliquots of concentrated standard solutions of Chocolate Brown HT, Green S and Tartrazine were added to the relevant blank solution using a 100μl syringe, the solution being deoxygenated for 1 minute after each addition. A magnetic stirrer unit was employed to deoxygenate a solution more effectively.

Tartrazine and Green S are best determined under the solution conditions for sparkling limeade. Tartrazine produces a very well defined single peak at pH 9 as mentioned at the section of orangeade. Nevertheless the determination of Tartrazine at pH 9 would be slightly affected by the presence of Green S and Chocolate Brown HT. They cause the Tartrazine baseline to be raised even though both peaks of Green S and Chocolate Brown HT are depressed in the presence of the phosphonium salt.

The determination of Green S at pH 4 is slightly affected by the presence of Chocolate Brown HT which produces a long tail in its differential pulse polarogram. The tailing effect could be caused by the slow reduction rate of the bis azo group in the colour, Chocolate Brown HT.

At pH 4 Chocolate Brown HT gives two peaks at -0.18V and -0.32V respectively. The first peak is independent of the peak of the other colour. The second peak of Chocolate Brown HT is completely overlapped by the Tartrazine peak.

The overall view of the individual polarograms of the three food colours at pH 4 and pH 9 is shown in Fig. 10. The polarograms were produced using the concentrations of each colour which is normally present in the drink.
Fig. 10. Overall view of differential pulse polarograms of three food colours in sparkling dandelion & burdock.
The proposed procedure for the determination of Green S, Chocolate Brown HT and Tartrazine is as follows:

Pipette 5ml of sparkling dandelion & burdock into a 30ml beaker. Add 3ml of 1M tetramethylammonium chloride, and 5ml of pH 1.9 B-R buffer. Adjust to pH 4 with 0.2M sodium hydroxide solution and dilute to 25ml in a volumetric flask. Deoxygenate a portion of this solution in a polarographic cell and polarograph it between 0.0 and -0.8V vs SCE.

Carefully readjust the solution in the cell to pH 9 with 4M sodium hydroxide solution and add 10mg of TPPC. Pass nitrogen gas to aid dissolution of the solid and to deoxygenate the solution, and polarograph the solution between -0.6 and -1.0V vs SCE.

The procedure was tested using a standard sparkling dandelion and burdock (62.8ppm Chocolate Brown HT, 2.0ppm Green S and 20 ppm Tartrazine) prepared from uncoloured lemonade syrup and the food colour samples.

The calibration polarograms of Chocolate Brown HT in the presence of 0.4ppm Green S and 4ppm Tartrazine is shown on Fig.11. The relation between the peak current at -0.13V and concentration produce a linear graph. It is worth mentioning that the first peak height is proportional to the concentration of Chocolate Brown HT while the second peak at -0.32V overlaps the Tartrazine peak.

The Green S peak appears on an increased baseline when the concentration of Chocolate Brown HT is increased.

The result of ten determinations was Chocolate
Fig. 11  Differential pulse polarograms of Chocolate Brown HT obtained in pH 4.0 B-R buffer: (i) to (vi); 3.1, 6.2, 12.3, 18.3, 24.1 and 29.9 ppm respectively. Modulation amplitude 50mV, scan rate 2mVs$^{-1}$ and drop time 1s.
Brown HT 61.7 ppm with the coefficient of variation of 3.3%.

Fig. 12 shows the linear relationship between the peak current and concentration of Green S in the presence of 12.6 ppm Chocolate Brown HT and 4 ppm Tartrazine. The Green S peak is raised mainly due to the tail of the high concentration of Chocolate Brown HT. The coefficient of variation for 1.96 ppm Green S was 3.9% and considered to be quite applicable as a routine check.

The calibration polarograms of Tartrazine were obtained at pH 9 where the peaks of Chocolate Brown HT and Green S were considerably suppressed (Fig. 13).

Although the presence of the last two food colours raises the Tartrazine baseline, good results could be obtained using the recommended procedure. The Tartrazine content was determined as 20.4 ppm (coefficient of variation = 2.0%; ten determinations).
Fig. 12. Differential pulse polarograms of Green S obtained in pH 4.0 B-R buffer: (i) to (vii); 0.20, 0.40, 0.78, 1.16, 1.54, 1.90 and 3.80 respectively. Modulation amplitude 50mV, scan rate 2mVs⁻¹ and drop time 1s.
Fig. 13. Differential pulse polarograms of Tetrazine obtained in pH 9.0 B-R buffer in the presence of 0.4 ppm Green S and 12.56 ppm Chocolate Brown HT: (i) to (v); 1.00, 2.96, 4.88, 6.76 and 8.61 ppm respectively. Modulation amplitude 50 mV, scan rate 5 mVs⁻¹, and drop time 1 s.
V. BRIEF POLAROGRAPHIC INVESTIGATION OF SEVERAL FOOD COLOURS.

Most organic electrode processes are pH dependent\(^{(244)}\). The half-wave potential\((E_{1/2})\) is pH-independent for reversible redox and irreversible systems which do not involve proton transfer. The half-wave potential-pH plot for a reversible system gives information on the number of electrons\((n_e)\) and the number of protons\((m_H)\) transferred during the electrode process. At 25°C the slope \(dE_{1/2}/dpH\) is given by 0.059 \(\times m_H / n_e\) for a system where dissociation occurs only for the reduced form. The intercepts of the linear parts correspond to dissociation constants of the reduced and oxidized form, respectively.

There are three common patterns for the \(E_{1/2} - \text{pH}\) plot of irreversible systems:

(i) A pH-independent portion at low pH values,
(ii) A pH region where the half-wave potential is shifted to more negative values with increasing pH, and
(iii) pH independent region at high pH values.

In the pH range where the half-wave potential is pH dependent, the substance is present in the bulk of the solution in the conjugate base form, but only reduction of the protonized acid form occurs. A protonated form which is reducible is present in acid solution at low pH values. At high pH values, the reduction of the unprotonized form takes place in a pH-independent step.

The change in the concentration of the depolarizer causes a shift of half-wave potential which is not
pronounced. Slight effects on the half-wave potentials are also noticed with a change in the drop time particularly for certain irreversible systems (244).

Some basic studies have been carried out for several food colours in Britton and Robinson buffer. In general the calibration graphs for the colours were found to be linear at the $10^{-6}$ M level, but to deviate slightly from rectilinearity in more concentrated solutions ($>10^{-5}$ M) owing to adsorption effects at the dropping mercury electrode.

Table 9 furnishes some useful data for polarographic work on these food colours. For almost all systems the half-wave potentials are shifted towards more negative potential with increasing pH values except in two cases. One of them was the second and the third peaks of Erythrosine BS at around -1.0V and -1.5V respectively. The other one was the third peak of Indigo Carmine at about -0.96V vs SCE. They seem to be pH-independent in the pH region studied. At those stages the reduction of the unprotonized form should take place in a pH-independent step because the proton transfer reaction is not sufficiently fast.

Orange G, Lissamine Red and Ponceau 2R in the Table have already been used in food in the UK. Thiazine Red is not a food colour.

The first peak of Erythrosine BS overlaps the second peak at pH 7.8. Therefore the height of the combined peaks is higher than each single peak. Thus the pH was selected to give the combined peak as the optimum condition.

The conditions which are given in the Table would be useful
Table 9. The pH dependence on the differential pulse polarographic peak of colours.

<table>
<thead>
<tr>
<th></th>
<th>Colour</th>
<th>pH</th>
<th>$E_p$/-V</th>
<th>$I_p$/$\mu A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Orange G CI 16:30</td>
<td>4.7 5.3 5.7 6.0* 6.8 7.3 8.6 10.2</td>
<td>0.64 0.68 0.71 0.72 0.78 0.82 0.92 0.96</td>
<td>4.8 7.1 8.4 8.9* 9.2 8.0 7.0 6.8</td>
</tr>
<tr>
<td>2</td>
<td>Chocolate Brown HT CI 20285</td>
<td>3.8* 4.9 5.6 6.0 6.5 7.5 8.5 9.5</td>
<td>0.18 0.27 0.34 0.37 0.40 0.46 0.54 0.65</td>
<td>1.4* 1.3 1.1 1.06 1.0 0.8 0.6 0.4</td>
</tr>
<tr>
<td>3</td>
<td>Green S CI 44090</td>
<td>1.7 2.8 4.0* 4.8 5.5 6.3 7.6 9.1</td>
<td>0.52 0.60 0.67 0.72 0.78 0.81 0.90 0.96</td>
<td>7.5 7.7 7.5* 7.3 7.1 6.7 5.1 4.0</td>
</tr>
<tr>
<td>4</td>
<td>Erythrosine BS CI 45430</td>
<td>2.0 3.0 4.0 4.6 5.6 6.3 7.8* 11.3</td>
<td>0.45 0.54 0.60 0.66 0.75 0.84 1.00 1.26</td>
<td>0.4 2.9 4.4 5.4 5.9 8.2* 7.7</td>
</tr>
<tr>
<td>5</td>
<td>Yellow 2G CI 18965</td>
<td>2.8 4.5 5.8 6.7* 7.5 8.7 9.3 10.7</td>
<td>0.36 0.54 0.66 0.70 0.77 0.86 0.88 1.05</td>
<td>5.7 4.7 5.1 5.7* 5.5 4.0 2.0 0.3</td>
</tr>
<tr>
<td>6</td>
<td>Indigo</td>
<td>pH</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Carmine</td>
<td>E(_{p1})/V</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I(_{p1})/(\mu)A</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E(_{p2})/V</td>
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<td>0.17</td>
</tr>
<tr>
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<td></td>
<td>I(_{p2})/(\mu)A</td>
<td>3.5</td>
<td>3.4</td>
</tr>
<tr>
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<td>E(_{p3})/V</td>
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<tr>
<td></td>
<td></td>
<td>I(_{p3})/(\mu)A</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

| 7 | Lissamine Red | pH       | 2.3 | 3.5 | 4.5 | 5.8 | 6.7 | 8.3* | 9.1 | 10.5 |
|   | CI 18055 | E\(_{p1}\)/V | 0.86 | 0.94 | 1.00 | 0.92 | 1.19 | 1.33 | 1.38 | 1.43 |
|   |         | I\(_{p1}\)/\(\mu\)A | 1.8 | 2.1 | 2.2 | 2.2 | 2.1 | 2.5* | 2.5 | 1.4 |

| 8 | Ponceau 4R | pH       | 2.4 | 3.0 | 3.8 | 6.1 | 7.8* | 8.7 | 10.3 | 11.1 |
|   | CI 16255 | E\(_{p1}\)/V | 0.09 | 0.12 | 0.18 | -   | -   | -   | -   | -   |
|   |         | I\(_{p1}\)/\(\mu\)A | 6.9 | 4.3 | 3.2 | -   | -   | -   | -   | -   |
|   |         | E\(_{p2}\)/V | -   | 0.30 | 0.37 | 0.77 | 0.90 | 0.93 | 0.97 | 0.97 |
|   |         | I\(_{p2}\)/\(\mu\)A | -   | -   | -   | 4.0 | 7.0* | 7.0 | 3.7 | 2.7 |

| 9 | Red 2G  | pH       | 2.2 | 2.7* | 3.8 | 5.0 | 6.4 | 8.1 | 9.2 | 10.4 |
|   | CI 18050 | E\(_{p1}\)/V | 0.28 | 0.32 | 0.37 | 0.54 | 0.69 | 0.82 | 0.90 | 0.96 |
|   |         | I\(_{p1}\)/\(\mu\)A | 2.8 | 2.8* | 3.1 | 3.0 | 3.2 | 4.1 | 4.2 | 2.8 |

<p>| 10 | Brown FK | pH       | 4.0 | 4.8 | 5.6 | 6.5* | 7.6* | 8.7 | 10.1 | 11.2 |
|    |         | E(<em>{p1})/V | 0.16 | 0.24 | 0.32 | 0.39 | 0.49 | 0.57 | 0.63 | -   |
|    |         | I(</em>{p1})/(\mu)A | 0.8 | 0.8 | 0.8 | 0.7 | 0.9 | 0.7 | 0.9 | -   |
|    |         | E(<em>{p2})/V | 0.30 | 0.41 | 0.54 | 0.60 | 0.69 | 0.72 | 0.72 | 0.82 |
|    |         | I(</em>{p2})/(\mu)A | 6.3 | 5.9 | 5.0 | 9.2* | 9.3* | 8.6 | 7.3 | -   |</p>
<table>
<thead>
<tr>
<th></th>
<th>Amaranth CI 16185</th>
<th>pH</th>
<th>2.4</th>
<th>3.7</th>
<th>4.5</th>
<th>6.3</th>
<th>7.1</th>
<th>8.1</th>
<th>9.1</th>
<th>10.1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>EP/-V</td>
<td>0.11</td>
<td>0.26</td>
<td>0.30</td>
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<td>0.54</td>
<td>0.67</td>
<td>0.70</td>
<td>0.75</td>
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<tr>
<td></td>
<td></td>
<td>IP/µA</td>
<td>10.0*</td>
<td>6.0</td>
<td>5.5</td>
<td>5.0</td>
<td>4.8</td>
<td>5.6</td>
<td>5.8</td>
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<td>------</td>
</tr>
<tr>
<td>12</td>
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<td>pH</td>
<td>2.4</td>
<td>3.1</td>
<td>4.0</td>
<td>5.1*</td>
<td>7.7</td>
<td>8.4</td>
<td>10.2</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP/-V</td>
<td>0.12</td>
<td>0.17</td>
<td>0.26</td>
<td>0.36</td>
<td>0.55</td>
<td>0.60</td>
<td>0.75</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP/µA</td>
<td>2.2</td>
<td>2.7</td>
<td>2.6</td>
<td>2.6*</td>
<td>2.2</td>
<td>2.2</td>
<td>1.8</td>
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<td>------</td>
</tr>
<tr>
<td>13</td>
<td>Ponceau 2R CI 16150</td>
<td>pH</td>
<td>2.9</td>
<td>3.7</td>
<td>5.2</td>
<td>6.4</td>
<td>7.7*</td>
<td>8.9</td>
<td>10.2</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP/-V</td>
<td>0.21</td>
<td>0.34</td>
<td>0.61</td>
<td>0.76</td>
<td>0.85</td>
<td>0.93</td>
<td>0.93</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IP/µA</td>
<td>1.7</td>
<td>1.4</td>
<td>1.6</td>
<td>2.3</td>
<td>3.9*</td>
<td>3.8</td>
<td>2.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*The best condition studied in Britton & Robinson buffer at 25 °C.
The concentration of the colours used was 5 x 10⁻⁵ M in the buffer.
for the determination of each colour in a matrix. However, it always seems to be necessary to make a compromise among the conditions in order to determine simultaneously several colours. Usually more than two colouring matters were found to be used in a drink to improve the tint.

Polarography in combination with TLC was carried out for several food colours. A silica gel TLC plate (no fluorescence indicator) with spots of 5nl each of \(1 \times 10^{-3}\)M Green S, Orange G, Sunset Yellow FCF, and Erythrosine BS was developed in the solvent system(65), iso-propanol: water:carbon tetrachloride = 60:25:15. Each spot of the different colours was scraped off from the plate, dissolved in 2.5ml of B-R buffer at the pH 4.6 for Green S, pH 6.2 for Orange G, and pH 10.2 for Sunset Yellow FCF respectively and polarographed in a micropolarographic cell (10ml) with a SCE system and Pt wire for the counter electrode connection. Quite reasonable recoveries were obtained for the colours at \(2 \times 10^{-6}\)M concentration.

The differential pulse polarographic peak current of the Orange G after TLC was \(3\%\) less than that obtained with a standard solution without the TLC process. \(35\%\) enhancement of the peak current resulted for Sunset Yellow FCF, but a suppressed peak current was produced for Green S solution. When a cellulose TLC plate with the spots of different colours was placed in the TLC tank( containing the solvent system described) for 3.5 hours, the colour of Tartrazine spot disappeared, that of Green S changed (from green to bluish), and that of Erythrosine BS faded.
However, the colours spotted on silica gel TLC plate seemed to be stable except for the fading of the Erythrosine BS colour. In any case Erythrosine BS was successfully separated from the others and provided no polarographic peak after scraping it off the TLC plate. It is possible that the structure of the colour, Erythrosine BS, is altered to a polarographically inactive form during the developing process in the solvent system.

The stationary phase material for TLC, e.g., silica gel, alumina, etc., may act as a "surface active agent" for certain compounds to increase their polarographic peak currents. The solvent on the TLC plate should be completely evaporated off before the colour spot is scraped off from the plate. Otherwise it will cause a change in the pH of the polarographic solution.
CONCLUSION

Several food colours have been determined by the differential pulse polarographic method without prior separation of the colours from the fruit drinks. The differential pulse polarographic peaks are sharp and afford some measure of identification. Precise results can be obtained in a shorter period of time by this simple polarographic method than by other methods described in analytical publications (11,187).

Although relatively costly equipment is required, HPLC is now the nearest approach to an ideal method for the identification and determination of food colours combining as it does efficient separation with precise but usually nonspecific quantification. HPLC methods, however, generally require separation of the food colours from even simple food matrices before application to a device.

Onium compounds, i.e., tetraphenylphosphonium chloride and methyltriphenylphosphonium chloride, form ion-pair compounds with acidic food colours. Whenever separation of the colours is necessary to avoid interference from subsidiaries in a foodstuff, it is advisable to ion-pair extract the compound into chloroform, remove the organic solvent in a fume cupboard and dissolve the colour in Britton-Robinson buffer before polarography. The suppression effect of onium compounds on the peaks of most food colours is extensive in basic solutions.
but only a slight effect was noticed in low pH solutions.

Some basic studies have also been carried out for several food colours in Britton-Robinson buffer using the polarographic method. For almost all systems the polarographic peak potentials are shifted towards more negative potentials with increasing pH values as would be expected.

The combination of polarography with TLC gave a quite promising result for some food colours. Each spot of the different colours was scraped off from the plate, dissolved in 2.5ml of B-R buffer at the appropriate pH mentioned for each colour, and polarographed.

The peak current of Orange G was reproducible at $2 \times 10^{-6}$ M but the result was 3% lower than the one obtained with a standard solution without chromatography. An enhancement (35%) of the peak current resulted for Sunset Yellow FCF, but a suppressed peak current resulted from the Green S solution after scraping off the silica gel TLC plate. Erythrosine BS was successfully separated from the others by TLC but was found to be easily faded in the TLC system. No polarographic peak was found for the colour, Erythrosine BS. The reason is considered to be due to the alteration of its structure during the TLC process. The approach could be worth trying in particular cases although satisfactory results were not obtained for all food colours tried.

The silica gel in the solution may act as a "surfactant" for certain compounds and increase or decrease their polarographic peak currents.
ACID AND BASIC DYES

INTRODUCTION

Studies on the polarographic reduction of azo dyes date back to 1926 when Conant and Pratt reported the reduction of about twenty seven dyes of rather doubtful purity.

Most commercial dyestuffs contain besides a reducible azo (in the case of anthraquinone dyestuffs C = O) group a further group which can be reduced under given conditions, e.g. -NO₂. Whereas the -N=N- group can be reduced in a two- or four-electron way, the nitro group can be reduced in a six-electron step. Gemzova and Jehlicka explained the reduction process taking an example of nitroazo dyestuff.

The diffusion coefficient must be the same irrespective of its being calculated from the diffusion current of either reduction process of the two reducible groups (-NO₂, -N=N-). Thus, if the ratio of two diffusion currents in a polarogram of a dyestuff equals 1:3, the only possible reduction processes taking place must involve two and six electrons respectively.

The effects of increasing hydrophobic character and the number and position of the hydrophilic sulphonate groups on the aggregation have been investigated for some monoazo acid dyes polarographically. The aggregation is promoted by increasing length of the chain and affected by increasing degree of sulphonation. The state of
aggregation could not be completely explained in terms of a simple monomer-dimer equilibrium.

Four commercially used diazo dyes\(^{(169)}\) discharged into effluents from textile works were studied to follow the decay of these substances using polarography. Acid Red 73 proved to be the most toxic by killing all the daphnæ and by decaying the plant life such that it sank to the bottom. It has also been shown that the most toxic dye in the series is the easiest to reduce polarographically\((-0.18\text{V in formate buffer})\) and this could be connected with the metabolic reduction of the azo group to yield toxic aromatic amines.

Present work was undertaken to determine some acid and basic dyes by the differential pulse polarographic method including an ion-pair extraction step.
EXPERIMENTAL

REAGENTS AND SAMPLES

The CI Acid and Basic dyes have been kindly provided by ICI with their assay values.

Acetate buffer (pH 5.5) contained 6ml of glacial acetic acid and 55g of potassium acetate in 1 litre of distilled water.

Carbonate buffer (pH 10) was prepared by dissolving 26.5g of sodium carbonate and 21g of sodium bicarbonate in 1 litre of distilled water.

Kane buffer (2M in hydrochloric acid, 1M in orthophosphoric acid, glycine (\(\text{NH}_2\text{CH}_2\text{COOH}\)) and citric acid). The pH of the buffer was adjusted using 5M or 10M sodium hydroxide solution.

Trifluoroacetic acid (0.1M)

Britton-Robinson buffer (pH 1.9)

Tetraphenylphosphonium chloride (0.01M)
RESULTS AND DISCUSSION

ACID DYES

The dyes of this class dye wool, silk, nylon, modified acrylic fibers, and leather from acidic or neutral baths but will not dye cotton. The demand for these dyes must be enormous judging from their uses. Simple methods for the determination of acid dyes are therefore urgently required to control the dyeing process as well as our environmental situation. Most dyes give polarographic waves mainly due to their chromophoric group. In most of the cases a complicated polarographic picture would result from a dye mixture so that each dye cannot be distinguished from another.

In this section a brief procedure is described to determine and separate acid dyes by a similar method to that mentioned for food colours; 1ml of $10^{-3}$ M acid dye solution, 1ml of 6M hydrochloric acid and 2ml of 0.01M tetraphenylphosphonium chloride were diluted to 30ml with distilled water in a 100ml separatory funnel. The onium-acid dye association compound was extracted twice with 10ml and 5ml of chloroform successively. The extracts, combined in a 100ml beaker, were evaporated on a water bath. Polarography was carried out after dissolving the dye residue in 20ml of carbonate buffer (pH 10), diluting to 100ml with water, and deoxygenating for 10 minutes using purified nitrogen gas.

The polarographic results for several acid dyes by
the procedure described above are tabulated on the following table.

Table 10. Differential pulse polarographic peak potential(V) of acid dyes.

<table>
<thead>
<tr>
<th>Acid dyes</th>
<th>$E_p$/V</th>
<th>$E_p$(I)</th>
<th>$E_p$(II)</th>
<th>$E_p$(III)</th>
</tr>
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<tbody>
<tr>
<td>Acid Red 151</td>
<td></td>
<td>-0.19</td>
<td>-0.77</td>
<td>-</td>
</tr>
<tr>
<td>Acid Red 114</td>
<td>*</td>
<td>-0.54</td>
<td>-0.93*</td>
<td>-1.44*</td>
</tr>
<tr>
<td>Acid Yellow 199</td>
<td></td>
<td>-0.14</td>
<td>-0.76*</td>
<td>-</td>
</tr>
<tr>
<td>Acid Blue 62</td>
<td></td>
<td>-0.17</td>
<td>-0.77</td>
<td>-</td>
</tr>
<tr>
<td>H 87034</td>
<td></td>
<td>-0.18</td>
<td>-0.98*</td>
<td>-</td>
</tr>
</tbody>
</table>

* indicates useful peak for the determination of the dye.

Acid Red 114, Acid Yellow 199, and H 87034 gave well-defined narrow differential pulse peaks under these conditions. Acid Red 151 and Acid Blue 62 could be better determined in acidic buffer.

The extraction ofonium-acid dye compound into chloroform using TPPC seemed to be complete judging from the lack of dye colour in aqueous solution. In the absence of TPPC most of the acid dye itself was not extracted from the aqueous layer into the chloroform. Acid Blue 62, however, completely extracted from acid solution at pH 2 into organic layer and H 87034 was extracted partially under the same conditions.
The paired-ion extraction of a large anion such as an acid dye into an organic solvent, e.g. chloroform, using TPPC, supplies a possible way of separating them from various matrices. The technique could help to improve the accuracy as well as the precision of their analytical determination.

**BASIC DYES**

One of the important applications of CI basic dyes, which are ammonium, sulphonium, or oxonium salts, is for conversion into pigments.

The analysis of the basic dye in a fibre needs special care because of their instability in an extraction solvent. The fading of cationic dyes\(^{(233)}\) in organic solvents has been reported to be caused by (a) conversion of the dye to a leuco base or loss of a proton due to a pH shift, (b) nonphotolytic degradation of the dye, or (c) photochemical degradation of the dye. In the absence of acid the fading is accelerated by traces of water in the solvent. The solvent acidity has been shown to be a critical variable determining the absorbance and stability of cationic dyes by Kissa\(^{(247)}\).

The present work was undertaken to give a brief indication towards the polarographic determination of basic dyes connected with the paired ion extraction into organic solvent using trifluoroacetic acid. The procedure developed was as follows; 1ml of \(10^{-3}\)M a CI basic dye
solution and 3ml of 0.1M trifluoroacetic acid solution were mixed and diluted to 30ml with water in a 100ml separatory funnel. The ion-association compound found in the solution was extracted twice with 10ml and 5ml of chloroform successively. The extracts were combined in a 100ml beaker and placed in a boiling water bath to evaporate the chloroform. The residue of the basic dye compound was dissolved in 20ml of acetate buffer (pH 5.5) and diluted to 100ml with water in a calibrated flask. An aliquot of the solution was transferred into the polarographic cell with a water jacket, deoxygenated for 5 minutes, and polarographed using the differential pulse mode.

No colour tinge was left in the final aqueous layer after the extraction processes.

Brilliant Green at \(10^{-3}\)M prepared in 0.1M citrate buffer (pH = 6.0) showed the most prominent differential pulse peak (which seemed to consist of the overlapped peaks) at \(-0.51\) V. Other ill-defined and half-overlapped peaks come at \(-0.83\) V and \(-0.95\) V at the same pH. Diluting the solution to \(10^{-4}\)M or less concentrated level of Brilliant Green resulted in a single and sharp peak at \(-0.80\) V in the citrate buffer. The formation of a semiquinone free radical of Brilliant Green will give rise to the appearance of the peak. The peak current decreased as time passed. The phenomenon seems to indicate the dimerization of the dye even at low concentration such as \(10^{-4}\)M. The effect of pH on the peak potential of
Brilliant Green is shown on Fig. 14. Similar results were obtained using the paired ion extraction procedure described. The maximum values of peak current and absorbance at 632nm were brought about at the pH between 4 and 6.

Crystal Violet among the basic dyes has been repeatedly studied voltammetrically by many workers as mentioned previously. At low concentration such as $1 \times 10^{-5} M$ the dye gave one polarographic wave in 10\% Kane buffer.

The emergence of electroreducibility for Crystal Violet has been suggested by Kaye and Stonehill(49) as due to the resonance between the carbonium ion structure and three other equivalent structures.

$$\text{Resonance}$$

The differential pulse polarographic peak current of Crystal Violet was almost constant at pH 2 to 7. However it decreased very rapidly at a lower pH than 2 by decreasing pH and gradually at pH range higher than 7 by increasing pH. The pH effect on its absorbance at 595nm and half-wave potential in the Kane buffer is shown in Fig. 15. The peak potential was displaced towards more negative values with increasing pH up to 7 and then was independent of pH. The same result was obtained
Fig. 14. Effect of pH on the peak potential of Brilliant Green.
Fig. 15. Effect of pH on the absorbance (A) and the peak potential of Crystal Violet.

using the recommended extraction procedure of Crystal Violet-trifluoroacetate compound. However the B-R buffer has been adopted for the acetate buffer because of the versatile change of its pH.

The peak currents at -0.82V are proportional to the concentration of Crystal Violet as shown in Fig. 16. The appearance of a prepeak to the main peak with adsorption characteristics could be excluded by decreasing the mercury drop time, i.e. from 2s to 0.5s.
Fig. 16. Differential pulse polarographic peaks obtained in pH 5.5 B-R buffer. Crystal Violet concentration: (i) to (ix); 0.0, 0.2, 0.5, 0.7, 1.0, 2.0, 5.0, 7.0 and $10.0 \times 10^{-6}$ M respectively. Drop time 0.5s.
It is a well known fact that the adsorption peak is caused by the formation of adsorbed layer of the semiquinone and its dimer on the surface of the mercury drop.

Several other basic dyes have been investigated for their determination using the procedure of ion-pair extraction. The calibration polarograms of Fig. 17, 18, and 19 indicate the possibility for the polarographic determination of CI Basic Orange 30:1, CI Basic Blue 141 and CI Basic Yellow 59 respectively. Trifluoroacetic acid did not interfere with the basic dye determination polarographically in acetate buffer or in B-R buffer.

The complicated behaviour of CI Basic Blue 3 is shown on Fig. 20. The peak potential of the first peak at around -0.2V is changing to more positive values with increasing depolarizer concentration. At higher concentration than $7 \times 10^{-5}$M the peak splits into two peaks which may indicate that the reduction proceeds in two steps with the formation of an intermediate. The second peak at about -1.0V is moving towards more negative potentials with increasing dye concentration and at $10^{-4}$M concentration merges with the background current.

A very complicated situation arises in the case of CI Basic Red 14 in pH 5.5 B-R buffer. At low concentration below $1 \times 10^{-5}$M the dye gives twin peaks in the potential range between -0.9 and -1.1V. The single peak(V) obtained for $2 \times 10^{-5}$M solutions at about -1.0V disappeared at the $1 \times 10^{-4}$M level as shown on Fig. 21. Two peaks, at -0.3V and -0.6V appear instead of the one
Fig. 17. Differential pulse polarograms obtained in pH 5.5 B-R buffer. CI Basic Orange 30:1: (i) to (vii); 0.0, 0.60, 1.7, 4.3, 6.0, 8.5, 17 x 10^{-6} M respectively. Modulation amplitude 50 mV, scan rate 2 mVs^{-1}, and drop time 1 s.
Fig. 18. Differential pulse polarograms and calibration graph obtained in pH 5.5 B-R buffer. CI Basic Blue 141: (i) to (vii); 0.0, 0.5, 1.0, 2.0, 5.0, 7.0, 10 x 10^{-5} M respectively.
Fig. 19. Differential pulse polarograms of CI Basic Yellow 59 obtained in pH 5.5 B-R buffer: (i) to (ix); 0.0, 0.2, 0.5, 0.7, 1.0, 2.0, 5.0, 7.0, $10 \times 10^{-5}$M respectively. Modulation amplitude 50mV, scan rate 2mVs$^{-1}$, drop time 1s.
Fig. 20. Differential pulse polarograms of CI Basic Blue 3 obtained in pH 5.5 B-R buffer: I to VII; 0.0, 0.7, 1.0, 2.0, 5.0, 7.0, 10.0 x 10^{-5}M respectively. Modulation amplitude 50mV, scan rate 2mVs^{-1}, drop time 1s.
Fig. 21. Differential pulse polarograms obtained in pH 5.5 B-R buffer. CI Basic Red 14; I to VIII; 0.0, 0.5, 0.7, 1.0, 2.0, 5.0, 7.0, 10.0 x 10^{-5} M respectively.
at -1.0V when the depolarizer concentration is increased. The phenomena could result from intermediate formation through intermolecular rearrangement. In fact the peak potential at about -1.0V was independent of the dye concentration until the peak disappeared.

10^{-4} M Catecholsulphonephthalein (Catechol Violet) solution gave two peaks in pH 10 B–R buffer at -0.13 and -0.85V respectively. The reaction mechanism for those polarographic peaks have not been proved yet but the second peak at -0.85V was very well defined and could be applicable for determining the dye. When the dye solution is left in air for several hours a rather broad second peak is produced at -1.08 V.
CONCLUSION

Some acid and basic dyes have been studied with a view to determining them by the differential pulse polarographic method including an ion-pair extraction step.

The acid dyes form an ion-pair compound with tetraphenylphosphonium chloride and this is quantitatively extracted into an organic solvent, e.g. chloroform. The polarographic solution was prepared by evaporating chloroform, and dissolving the residue, acid dye-tetraphenylphosphonium ion-pair compound, in carbonate buffer at pH 10.

Three of the dyes, Acid Red 114, Acid Yellow 199 and H87034 showed well defined and sharp differential pulse polarographic peaks under these conditions.

Basic dyes associated with trifluoroacetate anion were extracted into chloroform. After evaporating the chloroform and dissolving the basic dye in pH 5.5 acetate or B-R buffer the solution was ready for polarography. The excess of trifluoroacetic acid in the polarographic solution did not interfere with the basic dye determination by this method.

Most basic dyes have shown very well defined polarographic peaks. The calibration graphs using the peaks for the dyes were linear at the concentration range up to $10^{-5}$M. However, CI Basic Blue 3 and CI Basic Red 14 which gave different polarograms at different concentrations require further study.
The faster the mercury drop time the less resolution and the lower the sensitivity that was produced using differential pulse polarography. Relatively high temperature (30°C) of the solution and fast mercury drop time (0.5 s) was sometimes useful to decrease the adsorption of the depolariser on the mercury drop. An accurate adjustment of the mercury column height, solution temperature and pH of the solution gives rise to a reproducible result.

Analytical techniques such as spectrophotometry, titrimetry, and chromatography are currently used to determine total colour, intermediates, subsidiary colours etc.

The differential pulse polarographic method using the paired ion extraction approach for acid dyes with TPPC and basic dyes with trifluoroacetic acid into chloroform provided a convenient means of determining and separating or concentrating these dyes from a mixture.

The reduction mechanism may be slightly different from one dye to another even in the same buffer. Therefore, a suitable pH has to be chosen to get a satisfactory result for each dye and this often means readjusting the pH of the solution. The number of electrons involved in each reduction process has not been determined. To obtain the best shape of differential pulse polarographic peak for each dye has always been the aim in spite of possible decreases of the peak current owing to a smaller number of electrons being involved for the reaction.

Keeping in mind the decolourization of some tri-
phenylmethane type basic dyes\textsuperscript{(235)} and the catalytic fading of some azo dyes\textsuperscript{(232)} the polarographic method was reaffirmed as one of the most powerful tools to be studied for the determination as well as the elucidation of the reaction of dyes in aqueous solution.
Ion-selective electrodes and gas-sensing probes could still be considered to be new analytical tools.

Some features which make them very attractive are speed of measurement, utility for on line or in line analysis, non-destructive nature, and independence from sample volumes, colour, suspended solids etc.

The literature on the topic is still growing exponentially for fundamental, industrial, environmental and physiological applications. The most comprehensive reviews are those of Buck (261, 262, 266), Covington (263) and Koryta (264, 265).

The book written by Moody and Thomas (267) covers both theoretical and practical aspects of ion-selective electrodes. More recent books are by Bailey (268), Băiuțescu and Cosofret (269), Koryta (270), Lakshminarayanaiah (271), and Berman and Herbert (272).

A bibliography of ion-selective electrodes has been prepared by Pick (273). The proceedings of the September 1977 Budapest conference on Ion-Selective Electrodes (274) has recently been published in English.
The nomenclature established by the IUPAC committee(275) is helpful to sort out of the terms and symbols. Incorrect terms such as "specific ion electrode" or "ion-specific electrode" should not be used in papers on Ion-Selective Electrodes, the electrochemical sensors which respond to ionic activities according to the Nernst equation, sometimes with subtheoretical slopes. The symbol \( K_{AB}^{\text{pot}} \) is sanctioned to designate the potentiometric selectivity coefficient. Some useful suggestions have been made by Buck(261) on the selectivity coefficient.

Other reviews on enzyme electrodes(276), ion-selective electrodes(260,277,310), bioelectrodes(278), and coated-wire ion-selective electrodes(287) are also available.

The development of ion-selective electrodes started with the observations of Cremer who showed that a glass electrode responded to hydrogen ions in 1906(268). The so called sodium error for a pH glass electrode in alkaline solution was minimized by the alteration of glass compositions and led to the development of glass electrodes sensitive to metal ions over hydrogen ions at high pHs. It is worth knowing that Nicol'sky(279) first presented the form of the Nernst equation which describes the response of a hydrogen ion-selective electrode to both hydrogen and sodium ions:

\[
E = E^0 + \frac{2.303RT}{F} \log_{10}(a_{\text{H}^+} + K_{\text{HNa}\text{Na}^+})
\]

where \( E \) is the electrode potential in the solution containing both ions, \( E^0 \) is the standard potential of the electrode and \( R, T \) and \( F \) have their conventional meanings. This
equation is often referred to as the Nicolsky equation.

All ion-selective electrodes have the same basic construction composed of a thin membrane of the ion sensing material and electrical contact to the membrane directly or through a reference solution and internal reference electrode. This electrochemical half cell must be connected to another external reference electrode; i.e. SCE or Ag/AgCl electrode, to complete the circuit of the cell. As a whole the potential of the reference electrode assembly will consist of the reference electrode potential plus the potentials across the liquid junctions that should remain constant for the purpose.

Ion-selective electrodes can be classified by (1) the nature of the ion-sensing membrane(267,277), (2) the type of active material(268) used for the membrane, (3) the principles of the electrode function(280) or (4) their dynamic behaviour(284).

The first classification comprises five types of electrodes:

1. Glass electrodes,
2. Solid state electrodes
3. Liquid state electrodes,
4. Heterogeneous electrodes,
5. Sensitized-electrodes(gas-sensing and enzyme electrodes).

The second category has classified the electrodes by considering them from both practical and theoretical viewpoints. The five classes of sensor suggested are as follows:
1. Glass electrodes,

2. Electrodes based on inorganic salts: membranes of homogeneous form, i.e., single crystal, and heterogeneous forms of silicone rubber, PVC or polythene containing an active material fall into this class,

3. Electrodes based on organic ion exchangers and neutral carriers: electrodes with liquid or solid membranes belong to this group,

4. Gas-sensing probes,

5. Miscellaneous electrodes: sensors in this class include the enzyme electrodes, surfactant electrodes and ion-sensitive field effect transistors (ISFETs).

The third classification has been suggested by Pungor et al (280). Fundamental ion-selective electrodes and sensitized ion-selective electrodes are its two main groups:

I. Fundamental ion selective electrodes.

1. Electrodes operating on electron exchange reaction.
   a. The first kind (metal/metal ion) electrodes,
   b. Electrodes of the second kind,
   c. Electrodes of the third kind,
   d. Electrodes involving organic or inorganic redox couples.

2. Electrodes operating on ion-exchange reaction.
   a. Glass electrodes,
   b. Precipitate based ion-selective electrodes,
   c. Dissolved ion-exchange type electrodes.

II. Sensitized ion-selective electrodes.
2-1. Enzyme electrodes.
   a. Enzyme activity measuring electrodes,
   b. Substrate activity measuring electrodes,

2-2. Gas electrodes.
   a. Gas permeable membrane electrodes,
   b. Air gap electrodes.

The first group of this classification includes all the electrodes that function by an ordinary electrode process. In the second group, however, a selective chemical reaction or mass transport result in separation taking place ahead of the ordinary electrode process. The second subgroup of the fundamental ion-selective electrodes, electrodes operating on ion-exchange reaction are normally called membrane electrodes.

Morf, Lindner, and Simon classified ion-selective electrodes into two groups:

(1). Ion-exchange electrodes (Ion-selective electrodes with constant membrane composition: glass, solid and liquid ion-exchange based electrodes),

(2). Neutral carrier electrodes.

Although different authors tend to classify electrodes in different ways, the development of ion-selective electrodes is substantiated in the theory of glass electrodes.

The glass electrodes generally respond to monovalent cations and therefore they have low selectivity. The electrodes should be stored in water and reactivated in a 0.1M solution of the determinand (279). When the electrode
is immersed in an aqueous solution, the glass membrane absorbs water and a swollen hydrated layer in the range of \(10^{-2} \mu\text{M}\) is formed on the surface where the ion exchange reaction of cations takes place with the ion to be measured. The sodium glass electrode is one of the most successful examples of cation electrodes and has been widely studied for its applications. The cation selective electrodes have been used as sensors in enzyme electrodes and also for certain type of gas sensing probes.

Ion exchanger electrodes with electrically charged liquids have poor selectivity coefficients which change with time. Electrodes produced in the form of liquid membrane electrodes encounter some difficulties in maintenance and handling in their use.

The neutral carrier type electrodes with electrically uncharged ligands, neutral antibiotics such as valinomycin or nonactin, which were developed by Simon and Morf\(^ {281}\) offer a remarkable possibility of preparing highly selective electrodes for cations. The surrounding solvent molecules of the cation are replaced by the neutral carrier ligand to form a stable structure. Only the cation of adequate size can form the most stable complex with the ligand. The selectivity coefficient of this type of electrode is dependent on the ratio of the equilibrium constant of the ion exchange reactions of two ions.

The enzyme electrode is basically a modified cation selective glass electrode. In this electrode a thin hydrophobic enzyme layer (0.1 - 0.5 mm) immobilized in a gel covers the surface of the glass electrode. The layer
called the reaction layer or reaction film is generally separated by a dialysis membrane from the bulk of the solution.

Certain kinds of gas sensors are made of the pH-sensitive glass electrodes coated by a thin film of the appropriate electrolyte solution in high concentration (e.g. NaHCO₃, NaHSO₃ and NH₄Cl). A reference electrode is in contact with the electrolyte layer. The sensing element, reference electrode and the internal electrolyte are separated from the sample solution by means of hydrophobic membrane which is permeable to gases but impermeable to aqueous solutions. The sample solution should be adjusted to the pH that the equilibrium of the reaction is completely shifted to form free gas.

Another kind of gas sensor called air gap electrodes employ an air layer of constant thickness instead of hydrophobic plastic membranes such as teflon, fluorinated ethylenepropylene, polyvinylidene fluoride, rubber film etc.

Gas sensors are designed to measure dissolved gas concentration in solution. They can also be used to measure the gas concentration in moist gaseous streams. Furthermore the gas concentration above a solution can be determined by manipulating the sensitizing layer as long as it does not dry out and the gas phase is in equilibrium with the solution. The sensor can be highly specific by choosing a chemically specific internal electrolyte and a suitable condition for the sample.
A membrane may be defined as a phase that acts as a barrier to the flow of matter. In most cases, with the exception of pure liquids and solids, it is heterogeneous in structure. Conceptual heterogeneity can allow selective transport of some chemical species. The driving forces which cause to net transport to occur between two phases can come from differences in concentration, electrical potential, or pressure.

Kunin and Winger have defined a liquid ion-exchange membranes as a system consisting of a poorly water miscible liquid membrane interposed between two aqueous solutions and containing a dissociable acid or base. An ion can move across a liquid membrane either by dissolving in the liquid phase and diffusing across the membrane or it can combine with another ion or molecule to form a membrane-soluble complex which will diffuse across the membrane and dissociate at the other phase boundary. These different modes of transport can be used to classify liquid membranes, although to some extent both types will always be present simultaneously in every system.

The membrane potential of ion-selective membranes is generally formed by two fundamental contributions namely a membrane-internal diffusion potential and a boundary potential. The boundary potential is related to the ion-exchange processes at the phase boundaries between the membrane and the outside solutions.

The liquid junction potential is produced by the diffusion of ions across the aqueous contacting layer between sample solution and salt bridge solution.
Morf (312) has presented a new and less circuitous derivation of the Plank relation for the calculation of liquid-junction potentials and membrane potentials. He also mentioned that the inconsistancy of the liquid junction potential may lead to severe deviation as a change in the measured activity. The selectivity of membranes towards cations can be enhanced by the incorporation of different types of ion-exchange sites as shown by the result.

The passive membranes which are the most important part in ion-selective electrodes are classified as follows (282):

I. Site-free membranes.
   A. nonporous homogeneous solid-organic crystals, insulators, polymer films, uncharged lipid bilayers containing neutral carriers.
   B. nonporous heterogeneous solid-PVC and other films containing organic solvents and solvents with neutral carriers.
   C. nonporous liquids-low dielectric organic liquids, organic liquids with ion solubilizing neutral carriers such as valinomycin etc.
   D. porous homogeneous solids-cellophane, dialysis membranes, teflon and PVC film.
   E. porous homogeneous liquids-constrained liquid junctions.

II. Fixed-site membranes.
   A. nonporous homogeneous solids-glass membranes, doped
inorganic crystal membranes, such as AgX, LaF$_3$, such that only interstitials or vacancies carry current, highly cross linked ion-exchange resins, hydrophobic or oil-impregnated ion exchangers.

B. nonporous heterogeneous solids—binders (PVC, silicone rubber, etc.), supported particles of glass, AgX crystallites, ion-exchange resin beads, etc.

C. porous homogeneous solids—loosely cross-linked ion-exchange resins.

D. porous heterogeneous solids—glass and mineral particles compacted, ion-exchange resin beads

III. Mobile-site membranes.

A. homogeneous nonporous liquids—liquid ion exchangers, hydrophobic cation or anion-containing salts dissolved in organic liquids, including solvents that permit dissociation as well as ion pairing in the membrane.

B. homogeneous nonporous solids, such as AgX, in which both vacancies and interstitial ions may carry current.

The definitive interpretation of membrane potential in terms of ion activities is the basis for an analytical technique for a measurement. Membrane transport is also useful in analytical chemistry because the membrane-modified transport can lead to the development of electrostatic potential differences across membranes.

The potential generating processes explained by Buck are divided into three parts:

(1) At unblocked interfaces by electron and ion exchange.

(2) At blocked interfaces by dipole orientation, specific adsorption, and fixation or generation of charged
species.

(3) At real interfaces which are neither ideally reversible nor ideally blocked by combined processes.

The interfacial potential differences are dominated by faradaic processes. Nonfaradaic processes of adsorption and dipole alignment adjust their contributions to overall potential difference to be consistent with equality of electrochemical potential.

The dielectric constant of the solvent is an important factor influencing the selectivity coefficient of a membrane electrode. In particular, the preference of a membrane for monovalent ions over divalent ions\(^{283}\) is enhanced as the dielectric constant drops and vice versa. The dielectric constant of the solvent also affects the electrode response time. Morf, Lindner and Simon\(^{284}\) studied valinomycin based potassium electrodes and found that a change of dielectric constant from about 5 to 24 increased the response time at least tenfold.

A study of solvent mediator on the PVC membrane barium ion-selective electrodes has been carried out by Jaber, Moody and Thomas\(^{285}\). A more viscous solvent mediator than 4-nitroethylbenzene with dynamic viscosity of \(1.99 \text{mNscm}^{-2}\) at 25 °C has been required for long life of their PVC electrodes. The solvent mediators of low viscosity shrank the PVC membrane during storage. They also concluded that the low solubility of the sensor in the solvent mediator led to poor response.

Concentration hysteresis\(^{283}\) results when the electrodes are used in very strong solutions of determinand
for long periods, as the loading of determinand in the membrane is increased. Drift and deviation from the Nernst equation owing to this effect is more important than drift owing to the internal reference solution.

The response time of the electrodes must be one of the most important characteristics for their use in streaming solutions. The valinomycin-based potassium electrodes have been employed for the response time study\(^{(286)}\) of the ion selective electrodes. The initial slope of the response time curve of silicone rubber valinomycin based electrodes (at \(t = 0\)) was only one fifth of that obtained for PVC electrodes. The response rate of silicone rubber based carrier electrodes was uninfluenced by the flow rate of the solution, i.e., by the thickness of the stagnant diffusion layer in the solution. They modified their so-called "classical" carrier electrodes to contain a lipophilic anions as ion-exchange sites as well as the sensor component in order to reduce the permeability of the membrane towards sample ions. The neutral carrier membranes with tetraphenylborate anion showed more favourable electrochemical and dynamic characteristics than the classical membranes.

An article concerned with design of calcium ion-selective electrodes\(^{(288)}\) has described the mechanical design and the effect of selectivity principles and solvent mediators on electrode performance. The roles of the solvent mediator are: (1) dissolving the sensor; (2) adjusting the ultimate relative permittivity of the final organic phase; (3) adjusting the mobility of the ion-exchange/sensor sites according to the viscosity of
the solvent mediator; and (4) the adjustment of the site
density by variation of the concentration of the sensor
and the tendency towards dimerisation.

Tetrafluoroborate-selective liquid membrane electrodes\(^{(307)}\) have been prepared by soaking the pores of a
glass frit G4 attached to the end of glass tube in 0.2 %
tetraphenylphosphonium bromide solution in tetrachloroethane.
The electrode showed Nernstian response in the concentration
range of \(10^{-1}\) and \(10^{-4}\) M tetrafluoroborate ion when constant
ionic strength was maintained by sodium sulphate or dihydrogen
phosphate. Acetate and carbonate (0.1M) did not disturb
the electrode response to BF\(_4^-\), but bromide and chloride
interfered.

Another paper\(^{(308)}\) studied the response of the membrane-
electrode consisting of a Pt wire coated by a PVC membrane
containing dibutylphthalate and potassium tetraphenylborate
towards organic cations; alkaloids, vitamins, aminoacids.
The solubility product of the tetraphenylborate of the
respective organic cation has been established by means
of the electrode functions obtained in standard solutions.

Midgley\(^{(288)}\) interpreted the non-Nernstian responses
of ion-selective electrodes using several types of solid
state electrodes. The causes of nonideality suggested
are the presence of determinand in reagents added to the
solution, the presence of interfering species, and the
solubility of the material of the electrode itself. The
action of complexing agents or the establishment of steadys-
tate rather than equilibrium conditions are also considered
as secondary effects. The theory developed is also
applied to liquid membrane electrodes.

Materova, Ovchinnikova and Smekalova\(^{(289)}\) have studied membrane electrodes containing aromatic anions functioning to the anions, salicylate and benzoate. The salicylate and benzoate of tetradecylammonium and Crystal Violet were used as the exchangers in fabricating the membranes. The influence of the foreign ions; acetate, chloride, bicarbonate and sulphate anions, increased gradually as the activity of the principal ion was reduced. The salicylate electrode was found to be more selective than the benzoate one in the systems examined. They gave as the reason for the difference in electrode properties that the salicylate anion, due to the presence of conjugate bonds, is known to be less hydrated than the benzoate ion, and is more readily solvated in the organic phase. The importance of solvent has also been mentioned for the selectivity of liquid-ion-exchanger membrane.

Ion-selective electrodes in biological systems\(^{(290)}\) cannot be expected to behave at the same high level of quantitative response performance, established by calibration using ideal, interference-free electrolyte standards.

Hirst\(^{(291)}\) has mentioned that ion selective electrodes are not commonly used in most routine clinical chemistry laboratories, except in determinations of blood pH, \(pO_2\) and \(pCO_2\).

The virtues of conventional ion selective electrodes in biological applications\(^{(290)}\) follow from (1) the frequent need for only qualitative potential measurements, and (2) the possibility that nearly ideal quantitative results can
often be achieved after thorough characterisation and treatment of real systems. Limitations of ion-selective electrodes in biological systems are a result of limitations on the potential-determining processes. The electrode potential is not firmly established, but tends to drift or to become sensitive to all species that undergo ion exchange at comparable or faster rates.

The applications of ion-selective electrodes have been continuously reported for the measurement of calcium ion\(^\text{253}\) concentration in "heavy-metal" flotation pulps, sulphide ion\(^\text{254}\) by indirect potentiometric method, the potentiometric determination of iodine values of oil\(^\text{292}\), iodine determination in milk\(^\text{293}\).

Liquid PVC membrane electrodes selective to Cl\(^-\), Br\(^-\), I\(^-\), NO\(_3^-\), SCN\(^-\), BF\(_4^-\), and ClO\(_4^-\)\(^\text{294}\) were made by using phosphonium and tetraalkylammonium salts and Fe-o-phenanthroline and Ni-o-phenanthroline complexes as ion exchangers and chlorobenzene, nitrobenzene, di-butylphthalate and other esters as solvents. The selectivity coefficient was mainly determined by the ion-exchange constant.

Picrate-selective membrane electrode\(^\text{295}\) based on the water insoluble tetrapentylammonium picrate dissolved in 2-nitrotoluene has been reported by Hadjiioannou and Diamandis. The electrode was used for direct potentiometry and potentiometric titration of Ag in the presence of thiourea with picrate.

Silver/Silver sulphide ion-selective electrodes have been used for the determination of sulphate\(^\text{296}\) in phosphoric acid. The method is based on reduction of
sulphate by a mixture of hydriodic acid and hypophosphorous acid in HCl and determining the resulting $H_2S$ by the electrode. Methadone was determined in acidified (pH 2 to 3) urine samples using a miniaturized hydrophobic-cation-selective plastic membrane electrode.

Cheng and Chao\(^{(297)}\) have developed an arsenite-selective electrode. The porous membrane consists of 0.01 – 50 % $Ag_3AsO_3$ in $Ag_2S$. The membrane is substantially free of Ag.

Andreu and Morales\(^{(298)}\) have reported the complexometric titrations of magnesium(II), calcium(II), barium(II), and strontium(II) with amalgamated gold electrodes using EDTA as a titrant. The electrode was prepared by plating gold on a Pt wire and amalgamating by electrolysis in the presence of $Hg(ClO_4)$.

Best results were obtained at pH 10 by using an electrode containing 20 – 25 wt. % Hg.

Rhenium was determined in alloys containing Mo and W by using a $ReO_4^{-}$ selective liquid membrane electrode\(^{(299)}\). $Ph_4AsReO_4$ in nitrobenzene was used as the liquid exchanger solution; the inner reference solution was $1 \times 10^{-2}M NH_4ReO_4$ in 0.5M $H_2SO_4$ (or 0.1M $NH_4OH$); and a standard Ag-AgCl electrode was the reference electrode. The electrode was constructed from a teflon tube and also had a porous teflon membrane. The electrode response was linear for $10^{-5} - 10^{-1}M ReO_4^{-}$ with a slope coefficient of 57 mV. The potential was constant for a wide acidity range (from 3M $H_2SO_4$ to 5M $NH_4OH$) and in the presence of large amounts of $MoO_4^{2-}$, $WO_4^{2-}$, $SO_4^{2-}$, $V_2O_7^{3-}$, $Cl^{-}$, and $NO_3^{-}$. Constant potential readings were obtained after 3 – 5 mins. in dilute solutions and after 2 mins. in concentrated solutions with
A reproducibility of ±2mV.

A perchlorate ion-selective electrode\(^{(331)}\) was used to monitor titrations of organic compounds with 1,2,4,6-tetraphenylpyridinium acetate(TPPA). Some requirements for the successful potentiometric titration of those compounds have been suggested as follows: (1) the compound must be reasonably soluble in water or in dilute alkali solution, (2) it must form a sufficiently insoluble precipitate with TPPA, and (3) the perchlorate(nitrate or fluoroborate) ion-selective electrode must respond to the compound.

The application of halide ion-selective electrodes (Orion Model 94-17A, 35, 53) for the determination of quinones have been investigated by Hassan and Elsays\(^{(332)}\) in coloured solutions. The procedure is based on the formation of chlorohydroquinones resulting from the reaction between quinones with hydrochloric acid, and backtitration of the excess halide using the electrode as an indicator.

In order to correct the interference the two ion-selective electrodes for the same ionic species were applied for the determination of Na in the presence of large amounts of K in a computer-controlled flowing system\(^{(333)}\). Crystal-membrane electrodes responsive to calcium ion\(^{(334)}\) were prepared from 2mm-thick discs cut from undoped and Eu-doped calcium fluoride crystals.

Hulanicki et al\(^{(335)}\) used the solvents, 2-nitrophenoxyoctyl ether(I), 2-nitrophenoxyphenylether(II) and 2-nitrocymene(III) as internal solvent in wick- or polyvinylchloride type \(\text{NO}_3^-\) selective electrodes based on the tris(bathophenanthroline)nickel(II)\(\text{NO}_3^-\) ion-association complex. I and
II yielded electrodes with significantly longer lives as compared with that of III. The electrodes with these solvents were reported useful for more than 10 months.

A coated-platinum sulphate-selective electrode (336) was made by coating a commercial platinum electrode with a mixture of Aliquat-336 known as methyltricaprylylammonium chloride ($SO_4^{2-}$ form) and 4'-butyl-2,2,2-trifluoroacetophenone in a PVC matrix. The addition of 2-aminoperimidine sulphate or Ba$SO_4$ to the mixture was found to improve the selectivity for $SO_4^{2-}$ relative to $NO_3^-$. Liquid membrane electrodes for perchlorate, thiocyanate, tetrafluoroborate and nitrate (337), chlorate (340) based on triphenylmethane dyes, chloride sensitive homogeneous electrodes (338) in the presence of iron(III) with an on-line computer (339) and for bile salts (344) have been studied.

Alcohol, lactate and glutamate sensors (341) were prepared by sandwiching the oxidoreductases mixed with NAD$^+$ between a semi-permeable membrane and a platinum electrode. Measurements were made at 30°C and the sensors were stored at 4°C. Under these conditions, the sensitivities of the lactate and alcohol sensors deteriorated by 50 to 55% within 60 hours.

Amperometric enzyme electrodes (342) for glucose and L-amino acids have been constructed by the technique of glutaraldehyde coupling. The enzyme is co-crosslinked with bovine serum albumin using the bifunctional agent, glutaraldehyde.

A pyridinium ion-selective electrode (243) was prepared by mixing pyridiniummolybdoarsenate (13:7) with epoxy-resin
(Araldite), allowing the mixture to dry and cementing the hardened membrane equilibrated with 0.1M pyridinium nitrate for 3 to 4 days between the two limbs of a U-tube to form a two-compartment cell.

PVC membrane electrodes incorporating potassium tetrathylborate or a drug, e.g., ephedrine or propranolol have been evaluated with ephedrine hydrochloride (345).

Several pure methyltrialkylammonium chlorides were synthesized and evaluated as Cl⁻-selective components in liquid membrane electrodes (346). The electrode was applied for the measurement of chloride activity in blood serum.

Ion-selective membrane electrodes incorporating mercury(I) chloride has been used to determine chloride in the range of 0 - 20 µg/l in high-purity waters (248). The electrode was housed in a flow cell with a thermostatically controlled water jacket and was found to be about ten times more sensitive than the silver chloride electrode under these conditions.

Ion-selective, dry-operating electrodes (300), especially suitable for potentiometric determination of ion concentrations in body fluids, have been described. They consist of an internal metal/metal salt reference electrode, a wetproofed ion-selective membrane which contacts the reference electrode and consists of a binder with an ion carrier (dissolved in a solvent) distributed in it, and if necessary a support piece. The support piece consists of cellulose acetate, poly(ethylene terephthalate), a polycarbonate or a polystyrene. The binder of the membrane consists of
poly(vinyl chloride), polyurethane, carboxylated PVC, a copolymer of PVC and polyvinylacetate, a silicone elastomer, a polycarbonate, a cellulose ester, a copolymer of PVC and PVC, poly(vinyl butyral) or poly(vinyl formal). The ion carrier of the membrane comprises valinomycin, a cyclic polyether, tetrathenylborate, a tetralactone, a macrolidacetone, a cyclic polypeptide, a quaternary ammonium salt. The carrier solvent of the membrane is made up of aromatic ethers, aliphatic ethers, phthalates, adipates or sebacates.

A mercury ion(II)-selective liquid state electrode was described by Cosofret et al.(301). The membrane contained the Hg$^{2+}$ chelate of 1-(2-pyridinylazo)-2-naphthalenol dissolved in chloroform. The electrode has been recommended for the potentiometric titration of iodide, bromide, chloride and thiocyanate anions and used for the determination of pharmacological active halogenated organic compounds.

Mirkin et al.(302) reported on a nickel ion-selective electrode based on nickel dimethylglyoxime. Titration methods based on precipitate formation of the compound with tetrathenylborate ion were used for the determination of inorganic ions(303), i.e., K$^+$, Rb$^+$, Cs$^+$, Tl$^+$, Ag$^+$, NH$_4^+$ and alkaloid cations(304).

Univalent organic cations containing nitrogen atoms have been determined by potentiometric titration(305) using potassium ion selective electrode Crytur with valinomycin membrane. The author, Vitas explained the shape of the titration curves caused by the electrode selectivity for the given ion, solubility of the respective tetrathenyl-
borate, and the symmetry in the charge distribution. In case of Methylene Blue, \((\text{CH}_3)_2N^+\text{NO}_2^−\text{N}(\text{CH}_3)_2\) in which the positive charge distribution is symmetrical, the overall charge of the equilibrium voltage was bigger than the case for Methylene Green, \((\text{CH}_3)_2N^+\text{NO}_2^−\text{N}(\text{CH}_3)_2\) Where the symmetry of the cation is disturbed by the presence of nitro group.

Pucacco and Carter\(^{(306)}\) have manufactured a new \(\text{pCO}_2\) microelectrode with tip diameters ranging from 2 to 200\(\mu\)m by utilizing the recently developed glass-membrane \(\text{pH}\) microelectrode. The sensitivity(slope) was nearly theoretical, 56 to 60 mV/\(\text{pCO}_2\); the response time was 1-3 minutes, and the intercept stability(drift) was less than 3 mV/20min. time interval. The life time of this electrode was several days when it was stored correctly.

Gulens and Ikeda\(^{(308)}\) examined by a scanning electron microscope to reveal the factors which can alter the limit of Nernstian response for the \(\text{Ag}_2\text{S}\)-ion-selective electrodes. A dull film or tarnish on their surface\(^{(309)}\) has already been observed with use and accompanied by slow and non-Nernstian response of the electrode. The electrode response was again rapid and Nernstian at the \(3 \times 10^{-6}\) M level by polishing the electrode surface to remove the film and restoring the crystal to a shiny state. The super-Nernstian response observed at low sulfide concentration was attributed to the measurement of mixed potentials that arise as the result of various surface reactions: (i) porosity of electrode surface, (ii) kinetics of surface reactions, (iii) localized corrosion reactions, and (iv) influence
of redox reactions. The appearance of metallic silver at the membrane surface has been mentioned as the most important factor to the mixed potentials even though the influence of the factors on the potentials in a given solution will vary from one electrode to another manufactured by different process.

The determination of equivalence point in automated potentiometric titrations with ion selective electrodes\(^\text{322}\) has been critically discussed in terms of interactive graphic techniques, i.e. the inflection point, the full Gran plot, or an error function. The equivalence point and inflection point for acid-base titrations and potentiometric titrations using ion-selective electrodes do not necessarily coincide. The reasons are blamed on non-Nernstian response of the ion selective electrode which is caused by interference, slow electrode time-constants, and membrane characteristics at different stages of a potentiometric titration. The Gran plot of the chemical cell output at low concentration levels where the species has an activity coefficient of very nearly one yields a nearly straight line whose intersection of the titrant axis is a good estimate of the equivalence point. The influence of interfering ions becomes noticeable as the concentration of the measured species is reduced.

Frazer et al\(^\text{323}\) have developed the error function left(EFL) which is a technique to use the Gran plot to identify the region of a potentiometric curve where the electrochemical cell is most closely represented by the simplified Nernst equation.
\[ E = E_0 + \frac{RT}{ZF} \ln(a) \]

where \( E \), \( E_0 \) and \( Z \) are, respectively, the equilibrium potential, normal potential, and charge of the species with the activity, \( a \).

Six azo dyes\(^{(257)}\) have been determined potentiometrically by using a solution of Mohr's salt in glycerol. The results with less than 0.5 % coefficient of variation agreed with those of potentiometric titration with Ti(III).

Titration methods (including potentiometry) for dyes have been reviewed by Ashworth\(^{(325)}\).
DETERMINATION OF ACID AND BASIC DYES

INTRODUCTION

Many electrodes are now available for different ionic species which were previously difficult to determine, namely F⁻, NO₃⁻, S²⁻, K⁺, Ba²⁺ and so on.

An aromatic sulphonate ion sensitive membrane electrode has been developed by Ishibashi, Kohara and Horinouchi(313) using Crystal Violet as ion-exchange site. The liquid membrane electrode containing Crystal Violet-aromatic sulphonate, such as benzenesulphonate and α-naphthalenesulphonate, pair showed an approximately Nernstian response down to 10⁻⁴M sulphonate. An Orion liquid-membrane electrode barrel equipped with a Millipore filter sovinert membrane (pore size 0.25μm) was used for the study. Senkyr and Petr(314) have reported a liquid membrane electrode based on a triphenylmethane dye such as Crystal Violet for the determination of nitrate. The glass electrode body used contained a basic dye nitrate dissolved in nitrobenzene and the internal reference electrode system.

An attempt has been made to determine acid and basic dyes using liquid state ion-selective electrodes by Fogg et al(315,316). Several references for the determination of inorganic anions using the electrodes based on basic dye salts are listed in those papers. The electrode body used for those studies has been described previously(317).

The present work has also been concerned with the determination of dyes potentiometrically. At the initial stage the liquid state membrane electrodes mentioned
have been employed for the potentiometric determination of acid and basic dyes. Substantially the electrode is made up of a lightly cross linked natural rubber membrane containing the sensor dissolved in o-dichlorobenzene and the electrical connection made at the back of the membrane by means of a carbon rod. The liquid state electrodes based on Crystal Violet 12-tungstosilicate and Crystal Violet tetraphenylborate gave sharp end points for the potentiometric titration of Crystal Violet with sodium tetraphenylborate.

PVC membrane electrodes were prepared by dissolving sensors such as basic dye 12-tungstosilicate and tetraphenylphosphonium 12-tungstosilicate in the organic solvent, i.e. tetrahydrofuran and by sticking the PVC membrane formed at the end of PVC tube. Several plasticizers and solvent mediators have been tried to get better results. The electrodes were shown to be useful as indicator electrodes in the titration of acid and basic dyes with standard solutions of reactants.
A set of Radiometer pHM 64 Research pH meter, REA 160 Titrigraph Module and Autoburette ABU 11 used for the automatic potentiometric titrations of dyes using PVC membrane electrodes.

An electromagnetic stirrer and a glass cell with water jacket to control the temperature by means of a thermostatic water bath were utilized for the potentiometric measurements throughout the work. A sodium sulphate salt bridge or an agar bridge containing sodium nitrate provided stable and reproducible potential readings particularly for the work connected with the tetraphenylborate compounds.

Tetraphenylphosphonium chloride (TPPC), 12-tungstosilicic acid, and sodium tetraphenylborate were analytical grade. The Crystal Violet solution was prepared from the British Pharmacopoeia grade solid, and the other acid and basic dyes were obtained from ICI and used without further purification.

PREPARATION OF SENSORS

(1) Basic dye 12-tungstosilicate.

The procedure given by Burgess, Fogg and Burns (318) was followed for the preparation of these sensing materials: To a basic dye (0.3g) in 20ml of distilled water at 70°C was added 2 ml of 6M hydrochloric acid and then 20ml of 8% 12-tungstosilicic acid solution was added slowly with stirring. The mixture was digested for 2 hours at 70°C.
and filtered onto a glass filter (No. 4). After washing thoroughly with water the precipitate was dried in a vacuum oven at 50°C and 5mmHg for 3 hours.

(i) Basic dye tetraphenylborate.

Crystal Violet tetraphenylborate was prepared as follows: 300 ml of 10⁻² M sodium tetraphenylborate solution at 70°C was added slowly with continuous stirring to 250 ml of 10⁻² M Crystal Violet solution containing 10 ml of concentrated hydrochloric acid also at 70°C.

The precipitate was washed by decantation over a period of several hours and was filtered finally onto a No. 4 glass sinter and washed with water until colourless washings were obtained. The precipitate was dried under vacuum at 70°C and the melting point was found to be 185°C.

(iii) Tetraphenylphosphonium 12-tungstosilicate.

The sensor which is insoluble in water was prepared from analytical reagent grade 12-tungstosilicic acid and reagent grade tetraphenylphosphonium chloride. 50 ml of 3 % TPPC in water was slowly added with stirring to 100 ml of 30 % 12-tungstosilicic acid aqueous solution. The white and bulky mixture was left at room temperature for 30 minutes in order to form a complete precipitate. On adding 200 ml of acetone to the mixture a white crystalline precipitate was gradually deposited on the bottom of the beaker and was digested for 30 minutes at 50°C. The precipitate was separated by filtering through a glass
filter No. 4 from the excess of l2-tungstosilicic acid which was soluble in acetone. The precipitate was purified twice in acetone and dried in a vacuum oven at 10 mmHg and 50°C for 3 hours. The precipitate decomposed at 315°C.

(iv) Quinoline phosphomolybdate.

The compound was obtained by Lench's method (319) which is based on the precipitation of phosphorus as quinoline phosphomolybdate from a perchloric-hydrochloric acid solution after oxidation of phosphorus to orthophosphate. The standard deviation of 0.082 resulted from four gravimetric determinations of phosphate with a standard solution of sodium dihydrogen orthophosphate using the weighing form of \((\text{C}_9\text{H}_7\text{N})_3\text{H}_3(\text{PO}_4\cdot12\text{MoO}_3)\) in this work. The precipitate was dried at 120°C and 2 mmHg for 1 hour and used as a sensor for the quinoline phosphomolybdate PVC membrane.

(v) Basic Orange 30:1 - Reineckate.

50 ml of 2 % ammonium reineckate\((\text{NH}_4(\text{Cr(NH}_3)_2\cdot\text{SCN})_4\cdot\text{H}_2\text{O})\) (FISONs) was added to the solution containing 0.3g of Basic Orange 30:1 in 50 ml to prepare the compound. The mixture was digested for two hours at 70°C and filtered onto a No.4 glass sinter. The precipitate in the glass filter was washed thoroughly with distilled water and dried in a vacuum oven at 70°C and 5 mmHg for one hour. The product obtained was used for the PVC membrane electrode as a sensing material to the host ions.
PVC MEMBRANE ELECTRODES

The electrodes with PVC membrane incorporating sensors, i.e. Crystal Violet tetraphenylborate, basic dye 12-tungstosilicate, tetrapheylphosphonium 12-tungstosilicate and quinoline phosphomolybdate, were prepared according to the methods of Craggs, Moody and Thomas (320): 0.15g of sensing material was dissolved in 0.16 - 0.25 ml of solvent mediator such as 2-nitrotoluene or 2-nitrophenyl-n-butyrate, mixed with 0.02 - 0.12 ml of plasticizer (dinonylphthalate, di-iso-octylphthalate or di-n-butylphthalate), and finally mixed thoroughly with 3 ml or 6 ml of tetrahydrofuran solution containing 0.15 g PVC powder. The homogeneous mixture was poured onto a glass ring (3.6 cm i.d.) on a flat glass plate. A flexible PVC membrane is cured within a couple of days and has the thickness between 0.2 and 0.4 mm. A circular PVC membrane was cut out from the prepared master membrane using a cork borer No,8(1.3cm diameter) and stuck on one end of a PVC tube using a glue solution prepared by dissolving 0.5g of PVC powder in 5 ml of tetrahydrofuran.

The PVC membrane electrode was completed by dipping the silver (28 SWG)-silver chloride internal reference electrode in the PVC tube containing 0.1M potassium chloride.

LIQUID STATE ELECTRODES.

The fabrication of this electrode body has been described in detail in the previous work (321): The material of the membrane was natural rubber sheet which
was 2.2 mm in thickness and cut out with a cork borer of No.8 for fitting it to the electrode. The membranes were prepared by soaking the circular rubber discs overnight in a saturated solution of sensor in the mixture of nitrobenzene & o-dichlorobenzene(1:1). Whenever the membrane is required, it is simply wiped away from the excess of the organic solvent by means of medicinal tissue and placed in the electrode body. Polishing the surface of the carbon rod in contact with the membrane was needed to get a stable potential response. A saturated calomel reference electrode with a saturated sodium sulphate salt bridge was used during the potential measurements.
RESULTS AND DISCUSSION

The PVC matrix electrode based on the sensor tetraphenylphosphonium 12-tungatosilicate gave a fast and linear response to tetraphenylphosphonium cation from $10^{-2}$ M to $10^{-7}$ M. Four different membranes were prepared according to the compositions shown on Table II. Their behaviour towards to the onium cation is shown in Fig. 22. Near Nernstian responses were obtained within several seconds up to $10^{-6}$ M range of the cation. In very dilute solution of the onium cation such as $10^{-7}$ M the response of the electrodes was so slow that 5 minutes were required in order to get a relatively steady potential value. When the internal electrolyte of the electrode was filled with a mixture of 0.1M potassium chloride and $10^{-3}$ M tetraphenylphosphonium chloride solution instead of 0.1M potassium chloride solution, it was observed that the potential value was brought down about 170mV on the more negative side than the one for the single electrolyte.

Therefore great care must be taken in order not to change the composition as well as the concentration of the internal electrolyte of the electrode wherever the comparative potential value is important. The slope factor of the No.2 electrode to TPPC solution was constant at a near Nernstian value for 10 days but one of the others was considerably decreased for the same period of time.

In general the electrodes used gradually lost their function to the onium cation in about 5 weeks and eventually
Fig. 22. The responses of tetraphenylphosphonium 12-tungstosilicate PVC membrane electrodes to tetraphenylphosphonium cation.
Table 11. The compositions of the PVC membranes

<table>
<thead>
<tr>
<th>Components</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrapheny1phosphonium - 12-tungstosilicate (gr.)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>2-nitrophenyl-n-butyrate (ml)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>-</td>
</tr>
<tr>
<td>Polyvinyl chloride, PVC (gr)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Tetrahydrofuran (ml)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Diisooctylphthalate (ml)</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Dinonylphthalate (ml)</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Di-n-butylphthalate (ml)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>2-nitrotoluene (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
</tr>
</tbody>
</table>

did not respond to the cation.

The reasons for these phenomena are mainly considered to be due to the decomposition and loss of solvent mediator into the aqueous solution from the membrane.

The electrodes also responded to Crystal Violet cation as well as tetrapheny1borate anion. The response graphs for those species are not so good as the one for the onium cation as shown on Fig. 23. More reproducible and stable potential values could be obtained with a sodium nitrate agar bridge connected to the SCE than dipping the
Fig. 23. The responses of the tetraphenylphosphonium 12-tungstosilicate PVC membrane electrodes to (I) Crystal Violet (No. 1 electrode), (II) tetraphenylborate (No. 2 electrode).
reference electrode directly into the solution to be measured. A bigger potential fluctuation normally resulted in the latter case.

The response of the electrode to the same concentration of the species was not perfectly constant all the time due to changes in the junction potential, the position of the electrode in the solution and the stirrer speed. Its application to the potentiometric titration of dyes, however, was shown to give quite successful results.

Fig. 24 and 25 show some of the potentiometric titration results for the tetraphenylphosphonium cation and CI Basic Blue 141 with a standard sodium tetraphenylborate solution using the onium 12-tungstosilicate PVC electrode respectively. A big potential jump which was taken to be the end point was obtained with new membrane electrodes. Using the same electrode over again in the same system as well as a different system (old electrode) the potential jump was remarkably decreased for both cases. Either the consumption of the sensing material or the decomposition of the solvent mediator could affect the response of the electrode. It is noteworthy to mention that the steepest portions of the titration curves are the same for both electrodes, i.e. new and aged electrodes. More significant result would be expected from the titration work than the direct measurement of the potential.

In fact, several potentiometric titration curves were remarkably well established for some acid and basic dyes as shown in Figs. 26 and 27.
Fig. 24. Potentiometric titration curves of 5 ml of $10^{-2}$M tetraphenylphosphonium chloride using tetraphenylphosphonium 12-tungstosilicate PVC membrane electrode.
Fig. 25. Potentiometric titration curve of 5ml of $1 \times 10^{-2}$M CI Basic Blue 141 using tetraphenylphosphonium 12-tungstosilicate PVC membrane electrode.
Fig. 26. Potentiometric titration curves of 25ml of $1 \times 10^{-3}$ M sodium tetraphenylborate / ml

(I) Crystal Violet, (II) Basic Yellow 28 using tetraphenylphosphonium 12-tungstosilicate PVC membrane electrode.
Fig. 27. Potentiometric titration curves of $10^{-3}$M of (I) Acid Yellow 199 (25 ml) and (II) Acid Red 114 (15 ml) using tetraphenylphosphonium 12-tungstosilicate PVC membrane electrode.
The purities of those species were calculated estimating the equivalence points at the inflection point of their potentiometric titration curves as follows:

Basic Yellow 28 (24 %), Basic Orange 30:1 (89 %), Basic Blue 141 (32 %), Basic Yellow 59 (22 %)*, TPPC (92 %)*, Crystal Violet (97 %)*, Acid Yellow 199 (103 %)*, Acid Red 114 (80 %), H·87034 (99 %)*, Acid Blue 62 (91 %), Acid Red 151 (74 %).

A few of their assay values are in good agreement (*) with the manufacturer's result. However, the error could be diminished by a careful calibration process using the onium 12-tungstosilicate PVC electrode. The main sources of the deviations are considered to come from the slightly different mode of reaction between the determinands and the titrant, and also from dimerization as well as complex formation of dyes.

Taking as an example the amount of bromide found from the potentiometric titration of potassium bromide with silver nitrate using an ion selective electrode (322) increases monotonically as the titration time (from 48 minutes to 3 mins.) is decreased. The high results were explained principally on the basis of the relatively slow electrode and system time-response.

Frazer et al regarded the inflection point as a poor representation of the equivalence point when the valence of the titrant is different from that of the species being titrated.

Therefore, when the inflection point is used for routine work, complete working curves must be developed.
if quantitative results are required.

The electrode was not so greatly influenced by pH changes between 3 and 10 in the TPPC aqueous solution even though the solution colour turned to yellow at higher pH than 6.3. The response of the electrode to sodium and potassium cations was negligibly small in the concentration range of 1M and $10^{-4}$M. The deteriorating phenomenon of this PVC electrode appeared as a smaller Nernstian factor for the species to be measured as time went by. The response time, however, remained fast enough to trace the changing concentration of the species to be determined. One of the potentiometric titration results obtained from an automatic titration unit using the TPP 12-tungstosilicate PVC electrode is also presented in Fig. 28 as an example. The titration curve (i) was obtained from the electrode No.1 conditioned overnight in $10^{-3}$M TPPC. Reconditioning the electrode described in the titrant, $10^{-3}$M sodium tetraphenylborate solution for 30 minutes before the titration is performed, resulted in titration curve (ii) with a bigger potential jump. The electrode is reactivated to the tetraphenylborate anion as well as to the onium cation.

Several other PVC membrane electrodes based on basic dye (CI Basic Blue 3, CI Basic Blue 141, CI Basic Red 14 and CI Basic Yellow 28) 12-tungstosilicate have been prepared and tested for the response of the electrodes to their own dye cation at the concentration range between $10^{-2}$M and $10^{-4}$M. All the electrodes showed a near Nernstian response to their own host cation. Big potential
Fig. 28. Automatic titration of 0.001M Pph₄Cl (15 ml) using tetraphenylphosphonium 12-tungstosilicate PVC electrode with pH M 64 Research pH meter, REA 160 Titrigraph Module and Autoburette ABU 11.
fluctuations, as big as 20 mV, were observed for the Basic Yellow 28 and Basic Blue 141 12-tungstosilicate PVC electrodes.

A number of electrodes were prepared using different sensors, solvent mediators and plasticizers as shown in Table 12.

The PVC electrode based on Crystal Violet tetraphenylborate(C) showed a good looking response curve to the tetraphenylborate anion (see Fig. 29) with a super-Nernstian slope factor in the range of 10^-2 M and 10^-6 M. Using a saturated sodium sulphate salt bridge and a mixture of 0.1 M sodium chloride and 10^-4 M sodium tetraphenylborate solution as the internal electrolyte of the indicator electrode, more steady values of the electrode potential and faster response were obtained for tetraphenylborate solutions. Crystal Violet also causes a response in the electrode with the same compositions. The response curves, however, have a smaller slope factor than the one obtained from the electrode filled with the mixture (0.1 M potassium chloride and 10^-3 M Crystal Violet) solution as the internal electrolyte.

The application of the electrodes(C) to the potentiometric determination of dyes turned out to be promising as shown on Fig. 31 and 32. One of the most important requirements for the titration was the solubility of the compound formed between the reactants. The soluble couple between the determinand and titrant therefore did not produce a titration curve which is useful for the determination of the species.

The deposition of the products on the surface of the
Table 12. The compositions of the PVC membrane electrodes

<table>
<thead>
<tr>
<th>Components</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C5</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>Q6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Violet 12-tungstosilicate(g)</td>
<td>-</td>
<td>-</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Crystal Violet tetraphenylborate(g)</td>
<td>0.10</td>
<td>0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinoline phosphomolybdate(g)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>2-nitrophenyl-n-butyrate(ml)</td>
<td>0.15</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PVC(g)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Tetrahydrofuran (ml)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2.5</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Di-isooctylphthalate (ml)</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Di-nonylphthalate (ml)</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Di-n-butylphthalate (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>2-nitrotoluene (ml)</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
<td>0.15</td>
<td>0.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Fig. 29. The responses of Crystal Violet tetraphenylborate PVC membrane electrodes to tetraphenylborate ion.
Fig. 30. The responses of Crystal Violet tetraphenylborate PVC membrane electrodes to Crystal Violet (1) to (3); electrode Cl, C3 and C2 respectively.
Fig. 31. Potentiometric titration curve of 25 ml of $10^{-3}$ M Crystal Violet using Cl electrode.
Fig. 32. Potentiometric titration curves of 25 ml of $10^{-3}$ M (I) sodium tetraphenylborate, (II) Acid Red 151 and (III) H87034 using C3 PVC electrode.
PVC membrane was always observed for the successful couples at the end of the titrations. This heterogeneous layer normally causes irregular response and also decreases the slope factor and therefore the deposit has to be removed by means of a tissue before the next titration. The use of any organic solvent for the purpose is not recommended because of sensor dissolving out from the membrane. The potential value at every point for the potentiometric titration was recorded within 30 seconds after adding the titrant to the solution except in the end point area. The diluted concentration of Crystal Violet and tetraphenylborate, such as occurs in the end point area of the titration curve, showed such a slow response that three to five mins. were required to get a steady potential value.

The membranes for electrodes C were mechanically tough and the slope factor of the electrode to Crystal Violet and tetraphenylborate became smaller depending on the time passing. The electrode was always stored in 10^{-3}\text{M} Crystal Violet solution when not in use and conditioned in 10^{-3}\text{M} tetraphenylborate solution for one hour before use.

The electrode response was as fast as the TPP 12-tungstosilicate PVC membrane electrode so that a good looking titration curve with the potential jump about 300 mV was easily produced for the potentiometric titration of 10^{-3}\text{M} Crystal Violet with the titrant, 10^{-3}\text{M} tetraphenylborate using the automatic titration unit described previously. The C3 membrane electrode was used continuously for the determination of acid and basic dyes for two months and found to be useful as an indicator electrode for the
potentiometric titration of dyes. The life time of the membrane seems to be limited because of the decomposition of the solvent mediator, e.g., 2-nitrophenyl-n-butyrate. In fact the PVC electrode prepared without using solvent mediator as well as plasticizer did not give any significant response to its host ions.

A quinoline phosphomolybdate PVC membrane electrode was prepared according to the composition shown in Table 12. The sensing material was very well mixed with the solvent mediator but was immiscible with tetrahydrofuran containing PVC. Therefore small volume of tetrahydrofuran had to be added to the mixture of the sensor, solvent mediator and plasticizer and continuously stirred using a tiny glass rod until a uniform paste was formed. The pale greenish yellow PVC membrane which was 0.2 mm in thickness was cured 24 hours later. The colour of the membrane remained as new for one week while the membrane electrode was dipped in 0.01M sodium dihydrogen orthophosphate solution. However it turned a dark green colour when left in the open atmosphere for 3 days.

The characteristic response of the electrodes to quinolinium cation is presented on Fig. 33 and seems to be independent from the colour change of the membrane. The standard 1.0M quinoline(C₆H₄CHCHCHN) solution was prepared according to the method of Lench(319), by dissolving 12 ml of A.R. grade quinoline in 25 ml of 6M hydrochloric acid and diluting to 100 ml with water. More dilute solutions were prepared from this one as required and 6 M hydrochloric acid was added to make 1.5 M hydrochloric acid in the final
Fig. 33. The responses of the quinoline phosphomolybdate PVC membrane electrodes to quinolinium cation.
solution.

The electrodes (Q4, Q5 and Q6) filled with 0.1M potassium chloride solution as an internal electrolyte showed a remarkable response to sodium chloride solution only at a high concentration (0.1M and 1M). The electrodes (Q) were very responsive to proflavine sulphate, \( \text{HH}_2\text{SO}_4\text{NH}_2 \cdot 2\text{H}_2\text{O} \), aqueous solution down to \( 10^{-7}\text{M} \) (see Fig. 34).

The application of these electrodes for the potentiometric titration of some acid dyes with proflavine has shown some useful results (see Fig. 35). The acid dyes which gave S-shaped curves were CI Acid Yellow 199, CI Acid Red 114, CI Acid Blue 62 and CI Acid Red 151. The steepest portion was rather more diffuse. This is likely to occur because of the high solubility of the proflavine-acid dye compound.

PVC electrodes based on Basic Orange 30:1 Reineckate were also prepared according to the procedure described elsewhere in this chapter; 0.15g of PVC powder dissolved in 3ml of tetrahydrofuran was added in the mixture of 0.15g of Basic Orange 30:1 Reineckate, 0.25ml of 2-nitrophenyl-n-butyrate and 0.20ml of di-isooctylphthalate. The final mixture was poured into a glass ring (3.6cm i.d.) sitting on a flat glass plate and left for 24 hours to cure the membrane. The electrode filled with 0.1M potassium chloride as an internal electrolyte showed a response (see Fig. 36) depending on the concentration of either Basic Orange 30:1 or Reineckate. The smaller Nernstian factor of 40mV / decade for Basic Orange 30:1 solution was rather more promising than the one for Reineckate with the elastic
Fig. 34. The responses of several PVC membrane electrodes to proflavine sulphate.
Fig. 35. Potentiometric titration curves of 50 ml of $10^{-3}$ M (I) Acid Blue 62 and (II) Acid Red 151 using Q6 PVC membrane electrode.
Fig. 36. The responses of Basic Orange 30:1 Reineckate PVC electrode to (i) Basic Orange 30:1 and (ii) Reineckate.
The electrode was also found to be responsive to pH. The potential decreased by 35 mV between pH 3 and 9.

The liquid state electrode described before was prepared by soaking the natural rubber discs in the mixture of nitrobenzene and o-dichlorobenzene (1:1 by volume) containing the sensor, tetr phenylphosphonium 12-tungstosilicate with the presumable molecular formula of (PPh₄)₄SiW₁₂O₄₀ and fitting the membrane on the electrode body. Nitrobenzene in the solvent mixture increases the solubility of the sensor while o-dichlorobenzene has its main function in swelling the rubber membrane for this work. This electrode showed a response towards Crystal Violet and tetraphenylborate solution. In particular a super Nernstian response of 62 mV to Crystal Violet was noticed in the concentration range of 10⁻⁴ M and 10⁻⁵ M with the electrode. The response behaviour of the electrode was obviously worse than the PVC electrode using the same sensor in every respect.

Another liquid state electrode based on Crystal Violet tetraphenylborate was prepared in the same way mentioned above except that only a single solvent, o-dichlorobenzene, was used. The response graphs of the electrode to Crystal Violet and tetraphenylborate are shown on Fig. 37. A super Nernstian response of the electrode was often obtained for the low concentration (10⁻⁵ and 10⁻⁶ M) of tetraphenylborate solution. The pH change exerts a slight effect on the electrode as shown on Fig. 38. The electrodes could be more useful as an indicator electrode for the potentiometric
Fig. 37. The responses of the Crystal Violet tetraphenylborate liquid state electrode to (I) Crystal Violet and (II) tetraphenylborate solution.
Fig. 38. The pH effect on the Crystal Violet tetraphenylborate liquid
state electrode in (I) $10^{-4}$M Crystal Violet and (II) $10^{-3}$M sodium
tetraphenylborate solution.
titration of dyes than for direct measurement of the potential corresponding to the dye concentration (see Fig. 39).
Fig. 39. Titration curves of 25ml of $10^{-3}$M Crystal Violet with $10^{-3}$M sodium tetraphenylborate solution using a liquid state electrode:

(I) Crystal Violet 12-tungstosilicate electrode.
(II) Crystal Violet tetraphenylborate electrode (modified Orion electrode body).
(III) Crystal Violet tetraphenylborate electrode.
CONCLUSION

The PVC membrane electrodes incorporating tetrphenylphosphonium \( \text{P}_{12}\)-tungstosilicate and Crystal Violet tetrphenylborate were shown to give fast and Nernstian responses to tetrphenylphosphonium and tetrphenylborate ions. This property allows the electrodes to be used in good applications for the determination of acid and basic dyes potentiometrically. Potentiometric titration curves for the titration of basic dyes with sodium tetrphenylborate solution give sharper end points than those for the titrations of acid dyes with Crystal Violet solution using either PVC or liquid state electrode based on tetrphenylphosphonium \( \text{P}_{12}\)-tungstosilicate and Crystal Violet tetrphenylborate. Quinoline phosphomolybdate has also been used as a sensor for the determination of dyes using the PVC membrane electrodes. The potentiometric titration curves for acid dyes with proflavine using the electrode(Q) showed reasonably good features, but they were rather diffuse owing to the high solubility of the product.

In general the assay values of the dyes were high because of difficulties in estimating the equivalence point at the inflection point of each potentiometric titration curve. The slow time-response of the electrode and the system will be blamed for one of the sources of error that also include the insufficient understanding of the reaction mechanism for the potentiometric titration.

The present work has provided a useful method for the
determination of dyes using the ion-selective electrodes developed here. The preparation of a complete working curve for every dye titration using the electrode is considered to be absolutely essential for the practical applications of dye determination.

Three kind of plasticizers used in the work made the PVC membranes similarly elastic without any typical change of the response pattern of the electrodes. The solvent mediator improved the response character of the PVC electrode. However, care must be taken not to use too much solvent mediator and plasticizer. Otherwise the material having a high boiling point will delay the curing of the PVC membrane or spoil the membrane. The common drawback of organic solvent mediators may be in their long term instability even in air and the solubility in the sample solution to be measured.

From that point of view the liquid state electrode with a natural rubber membrane which can be easily regenerated by soaking it in the solvent containing sensor is clearly superior than the PVC membrane electrode. The potential drift and fluctuation, however, make it less satisfactory than the PVC electrode for the present work. The sensitivity of the measurement is undoubtedly limited by the potential fluctuation of the electrode. The use of a sodium sulphate salt bridge containing sodium nitrate or potassium chloride connected to a calomel reference electrode was found to be useful in obtaining steady and reproducible measurements. Conditioning the electrode in the solution to be measured or titrant led to a better response behaviour
It is worth mentioning a faster response of an electrode is obtained in a step concentration change produced by the rapid addition with fast mixing of a strong aliquot of determinant to a sample solution than a transfer of the electrode from one solution to another of the same species. If the cell is open circuited, the time taken for the equilibrium on the membrane surface and the liquid junction of the reference electrode is much greater than the case of the cell had not been disrupted.

The selectivity of the electrodes which is one of the most important criteria in their assessment has not been the concern of the present work. The electrodes developed here are mainly based on the principle of precipitate formation reaction between two species. The same kind of dyes tend to react more or less with the same host ion in the membrane of the electrode. Producing a table of selectivity coefficient for a dye would require a great deal of labour and it was decided that this would not be so valuable an approach for practical applications.

The electrodes are rather more useful for assaying a pure dye sample by potentiometric titration than the direct determination of dye impurities in trace amounts. Factors such as temperature, ionic strength of the sample solution, internal electrolyte for the PVC electrode, stirring speed of the solution and the salt bridge had to be carefully adjusted in order to produce comparable results.
AMPEROMETRIC TITRATION OF DYES

INTRODUCTION

One of the well known electroanalytical techniques, amperometric titration, has been tested for the determination of dyes employing differential pulse polarography.

Amperometric titrations with one indicator electrode, the dropping mercury electrode, was introduced by Heyrovsky and Berezicky (1929). Since that time, the use of rotating platinum electrodes, rotating graphite electrodes and mercury coated platinum electrodes (324) have been recommended as indicator electrodes for amperometric titrations. The earlier name "Polarometric titration" has been firmly replaced by the term "Amperometric titration" suggested by Kolthoff and Pan.

The "Galvanometric titration" described by Salomon in 1897 gave rise to different names like "dead-stop end point" and "kick-off end point". However the term biamperometric titration came into popular use for all of the amperometric titrations with a pair of identical indicator electrodes.

Amperometric titrations can be employed if either reactant gives a diffusion current. The end point of a titration is detected by extrapolation of the linear segments of the plots of current versus volume of titrant added. The technique has proved particularly useful in determining the end points of titrations involving precipitation and complex formation.
Many reviews and articles on both types of amperometric titration are found in the literature (324,325).

The application of polarography for the indication of end point in the amperometric titrations of metal ions (250,255,326) has been studied. The titration curve for a metal ion can be derived by measuring one of the following parameters as a function of the amount of reagent added: (a) the limiting current of the metal ion; (b) the limiting current of the complex; (c) the anodic limiting current of the reagent; (d) the shift of the half-wave potential for the metal ion.

Titrations with polarographic indication of the end point seem to be superior to spectrophotometric titrations normally carried out in the presence of metallochromic indicators and also very advantageous for the study of a complex composition because the polarographic measurements can be done at any hydrogen ion concentration.

Myers and Osteryoung(327) have critically discussed the use of differential pulse polarography for end point detection and stated its superiority over other amperometric methods. The technique has its advantage over the other polarographic methods especially from the point of the potential-time sequence and the current measuring sequence which are chosen to minimize the contribution of capacitive current to the measured current.

Differential pulse polarography which has the higher signal to noise ratio of the two pulse polarographic methods has been used to determine acid and basic dyes by amperometric titration in the present work.
The two substances, acid dyes and Crystal Violet, or basic dyes and sodium tetraphenylborate have been chosen to precipitate one another when their solutions are mixed. The peak potentials for the determinations were adjusted to measure the currents of the determinand while the titrant does not yield a limiting current. The dye solutions \(1 \times 10^{-4} \text{M}\) prepared in 20% Britton-Robinson buffer were titrated with a concentrated solution of titrant \(1 \times 10^{-3} \text{M}\), so that volume change is negligible.

Several amperometric titration curves of acid dyes with Crystal Violet solution and basic dyes with standard sodium tetraphenylborate solution have shown promising features for the practical application of the technique.
EXPERIMENTAL

APPARATUS AND CHEMICALS

A PAR 174 polarographic analyzer was used to apply voltage and monitor currents. A pHM 64 research pH meter, an electromagnetic stirrer unit and a polarographic glass cell with water jacket to control the temperature by means of a thermostatic water bath (type SU6, Grant Instrument Ltd.) were used throughout this work.

A saturated sodium nitrate agar bridge was connected to a saturated calomel reference electrode. All potentials were recorded vs. the SCE.

A 1.0 x 10^{-3} M standard solution of sodium tetraphenylborate was prepared by dissolving 0.171g of A.R. NaB(C_6H_5)_4 (BDH) in 500 ml of double distilled water and standardized with a standard silver nitrate solution by a potentiometric titration method using a sulphide ion-selective electrode (Orion Model 94-16) as shown on Fig. 40. The curve(ii) on the Fig. was the result of a second titration and it was noticed that the shape was different from the first one(i) due to the deposits on the electrode surface(308).

10^{-3} M Crystal Violet and other solutions were prepared from BP grade and ICI samples respectively and used without further purification. More dilute solutions were prepared from these stock solutions to be in 20% (v/v) of pH 5.5 B-R buffer for the amperometric titration.

Catecholsulphonephthalein (Catechol Violet) (1 x 10^{-3} M) was prepared from Fisons L.R. grade sample by dissolving 0.155g
Fig. 40. Potentiometric titration curves of 25ml of $1 \times 10^{-3}$M sodium tetrphenylborate solution using a sulphide ion selective electrode.
into 100 ml of distilled water.  $10^{-3}\text{M}$ aluminium standard solution was prepared by dissolving 0.224g of L.R. grade $\text{Al}_2(\text{SO}_4)\cdot(\text{NH}_4)_2\text{SO}_4\cdot24\text{H}_2\text{O}$ (Fisons) in 250 ml of distilled water.

In general 20 ml of $10^{-4}\text{M}$ dye solutions were pipetted into the polarographic cell and deoxygenated with nitrogen gas for 10 minutes. The peak potential ($E_p$) of differential pulse polarography for a dye was decided by manually scanning the potential on the PAR equipment.

The amperometric titration has been carried out recording the maximum currents corresponding to the peak potential of the dye. The solution was mixed using a magnetic stirrer unit after every addition of an aliquot of a standard solution for one minute while the deaeration was going on. The manual scan of the potential was essential with the PAR 174 because of shifts in the peak potential. The differential pulse peak current was measured after 35 drops of mercury in order to fully charge the memory circuits (327).
RESULTS AND DISCUSSION

Acid Blue 62 with an anthraquinonyl group and another acid dye, H\textsuperscript{87034} with an azo group gave very well defined single waves at $-0.55\text{V}$ and $-0.75\text{V}$ respectively in acidic buffer condition. The effect of the temperature, the height of mercury column and pH on the polarogram of a dye have been discussed previously. These factors should be kept constant within an error range during the titration. The acid dyes mentioned above were readily precipitated by the titrant, Crystal Violet. The diffusion currents of those dyes were gradually decreased by the addition of the titrant as shown on Fig. 41 for their typical amperometric titration curves. Almost ideal titration curves resulted for both dyes. However, the calculated assay values were slightly different from the one proposed by the manufacturer (ICI) as 82\% for Acid Blue62(79.6\%) and 108\% for H\textsuperscript{87034}(99\%).

The peak of titrant, Crystal Violet which appeared after the end point was moved towards more negative direction, i.e. from $-0.81\text{V}$ to $-0.86\text{V}$ by increasing the concentration.

Acid Yellow 199 with nitro and azo group gives a polarographic peak at $-0.41\text{V}$ in pH 5.5 B-R buffer. In diluting the concentration of the dye by gradual addition of the Crystal Violet during the titration the acid dye peak was split into two. The peak current of the first one at $-0.33\text{V}$ was lower than the second one at $-0.41\text{V}$ which was used for the amperometric titration of this dye. The titration curve did not exhibit a linear decrease at the
Fig. 41. Amperometric titration curves of 20ml of 1 x 10^{-3} M Crystul Violet/ml titrant added, 1 x 10^{-3} M Hydrogen (E_p = -0.75V) and (ii) Acid Blue 62 (E_p = -0.55V) in pH 5.5 Britton & Robinson buffer.
beginning of the titration and this could be due to the aggregation of the acid dye molecules. The purity of the dye was calculated from the titration graph as 98%. The positive error (2%) would be mainly caused by the slow equilibration between the reactants at low concentration.

For basic dyes the amperometric titrations were carried out with a standard sodium tetraphenylborate solution in pH 5.5 B-R buffer. A typical titration curve for a basic dye is shown on Fig. 42. The end point could be easily determined by the simple graphic method on the figure. The dye, Basic Yellow 59 produces two well defined polarographic peaks at -0.26V and -0.52V as mentioned previously. The first one was chosen for the amperometric titration under these conditions because the baseline of this peak remained flat throughout the titration. The amperometric titration curve shows marked rounding indicating the high solubility of the precipitate formed. Naturally the more rounded titration curve produces the more positive result.

Basic Yellow 28 shows a reasonably good titration curve (I) (Fig. 43). However, the curve (II) indicates that too weak a concentration of the titrant when compared with the concentration of the substance being titrated has been used for the titration. Positive error has resulted from the titration curves as expected.

The amperometric titration curve of Basic Orange 30:1 with sodium tetraphenylborate solution exhibits different phenomena as can be seen on Fig. 44. The adsorption and aggregation of the dye molecules seems to occur together.
Fig. 42. Amperometric titration of 20 ml of $10^{-4}$ M CI Basic Yellow 59 employing differential pulse polarography at $E_p = -0.26$ V.
Fig. 43.
Amperometric titration of (I) 20ml and (II) 60ml of $10^{-4}$M CI Basic Yellow 28 employing differential pulse polarography.
(at $E_p = -0.86V$)
Fig. 44.
Amperometric titration curve of 20 ml of $10^{-4}$M CI Basic Orange 30:1 in pH 5.5 B-R buffer employing differential pulse polarography.

Peak current / µA

Volume of $1 \times 10^{-3}$M NaBph$_4$ / ml
As a result of the dye aggregation the peak current of the dye decreases gradually at the beginning of the titration. The other effect starts to dominate the shape of the curve increasing the slope of the titration curve more rapidly than the expected rate. A similar titration curve was obtained for the $10^{-3}\text{M}$ Crystal Violet solution under the same condition.

Myers and Osteryoung used Triton X-100 to improve the accuracy of the amperometric determination of copper employing differential pulse polarography. They explained the result as the preferential adsorption of the surfactant onto the glass walls of the cell, which decreases cation adsorption or desorption.

The amperometric titration result of Crystal Violet ($10^{-4}\text{M}$) with sodium tetraphenylborate solution ($10^{-3}\text{M}$) also showed rounded titration curves. The peak current of Crystal Violet at $-0.82\text{V}$ in pH 5.5 B-R buffer continuously increased for 15 minutes and then gave a steady value. The same thing also occurred even for $5 \times 10^{-6}\text{M}$ Crystal Violet titrated with $5 \times 10^{-5}\text{M}$ sodium tetraphenylborate solution. The reason seems to be the adsorption phenomena of the Crystal Violet molecule on the precipitate. The aggregation effect would not support this reason because the monomeric dye molecule would be dominated at lower concentration than $4 \times 10^{-5}\text{M}$ Crystal Violet as reported by Hillson and McKay(26).

A well known metal indicator, Catechol Violet was titrated with standard aluminium solution at the peak potential of the dye, $-0.85\text{V}$ in pH 10.0 B-R buffer (see Fig. 45).
Fig. 45. Amperometric titration curve 50 ml of Catechol Violet (1 x 10^{-4} M) employing differential pulse polarography.
The binary complex between aluminium and Catechol Violet was calculated to be a 1:1 complex from the amperometric titration curve. The dye gives a very well defined single peak under these conditions. The second peak with broad peak width appeared at about \(-1.1\) V after long standing of the solution in contact with the air. The peak could possibly occur in alkaline solution by the oxidation of Catechol Violet as already mentioned by Chester, Dagnall and West (328).
CONCLUSION

The amperometric titration of dyes employing differential pulse polarography (DPP) has given a bright prospect as a useful method for the determination of dyes. The technique affords a particularly useful means for the titration of very dilute solutions owing to the inherent high sensitivity.

Either DC or pulse mode can also be applied for the end point detection of amperometric titrations. However, erroneous results would occur with these modes with polarograms not having flat plateaus.

The end point for the amperometric titration of acid dyes, Acid Blue 62, H-87034 and Acid Yellow 199 with the titrant, Crystal Violet comes slightly later than the equivalence point due to the high solubility of the precipitate and also the adsorption of the titrant on the surface of the precipitate formed.

The basic dye titrations with a standard tetraphenylborate solution showed similar results to the acid dyes. Therefore the purities of the dyes, Basic Yellow 28 and Basic Yellow 59 have been assessed as 22% both of which are 2% higher than the expected values.

Basic Orange 30:1 and Crystal Violet, however, turned out to be 78% and 94% for their assay values respectively. The low results are likely caused by the adsorption of the substances to be measured. The aggregation of dye molecules at the initial stage of the titration made the linear line to be curved.
diffusion current of the dyes by the time went by up to 15 minutes indicated the desorption of the dye molecule from the precipitate. The effect was so serious for Basic Orange 30:1 that the end point came too early.

A further study has to be done in order to preclude these effects before the application of the method for their determination. The accuracy of the determination could be improved by using higher concentration for both substances, determinand and titrant if possible.

The amperometric titration curve of Catechol Violet with standard aluminium solution showed the binary complex ratio to be 1:1 even though the ratio of Catechol Violet: aluminium was determined as 2:1 by the method of continuous variations. The amperometric studies of the other complexes formed between Catechol Violet and other metal ions are considered to be useful for further development as dye determination methods.
GENERAL DISCUSSION

The present work has been mainly concerned with the determination of dyes by electroanalytical methods, i.e. amperometry, polarography and potentiometry.

A revolution in the technique of dye manufacture has been made since the second world war although the history of dyeing fabrics started from the date of Egyptian and Chinese as early as 3000 BC (329); the materials requiring to be dyed are much more diverse than formerly. Natural organic dyes extracted from plant materials (eg, indigo), and occasionally from animals such as insects (cochineal) or molluscs (Tyrian purple) were used to dye textiles for many centuries.

Quantitative analysis of dyes is required for quality control in the dyeing industry and to control the dye concentration in a commercial product in the process of manufacture. In addition, all colours in foods, drugs, and cosmetics have to be set within safe limits or tolerances.

Although spectrophotometric analysis is usually considered to be the most convenient method, it cannot distinguish between the fibre-reactive dye and the unreactive hydrolyzed dye. Furthermore, it can hardly afford a satisfactory result from a system mixtures more than one dye which normally give overlapping absorption spectra. Classical chemical methods, such as the reduction of azo dyes with titanium(II) or chromium(II) chloride, oxidation of solubilized vat dyes or other oxidizable compounds with
cerium(IV) salts, or a precipitation titration of dyes can be used only for determining a functional group or estimating a dye capable of reacting with the same reagent. Differential pulse polarography, however, could overcome a defect in spectrophotometric methods for the determination of food colours in a drink. The two differential pulse polarographic peaks of Sunset Yellow FCF and Tartrazine have been separated by using a quaternary ammonium salt, tetraphenylphosphonium chloride and the peak currents measured accurately by one of the most sensitive techniques. The use of Triton X-100, tetraethylammonium chloride decreased the height of the differential pulse polarographic peak of the food colours studied. Methyltriphenylphosphonium chloride and tetraphenylphosphonium chloride enhanced the peak height of Tartrazine but suppressed that of Sunset Yellow. This would be the special case in which the increase in the peak current results from the presence of a surfactant with a charge opposite to that of the depolarizer. Otherwise the phenomenon could be explained as the formation of a more stable depolarizer than Tartrazine itself in the presence of the onium compound. No effect has been observed with tetramethylammonium chloride on the peak height of the colours. The suspended solid material in the solution did not interfere with the polarographic measurement.

These remarkable characteristics make the method superior to spectrometry. Therefore the technique could be directly applied to any dye solutions containing a suitable supporting electrolyte for the simultaneous
determination of dyes without further separations of them from the basic matrices. The parameters such as pH and temperature affect the diffusion current of the dye to be measured. The polarographic method for dyes could be grouped as functional group analysis and would be an easier and simpler method than the classical method used widely for the determination of dyes.

The main disadvantage of the method exists on the requirement of a dye sample whose purity is known. The aggregation and adsorption of dye molecules could also be a source of error for the determination of dyes at high concentration. At low concentration of dyes such as the $10^{-5}$M range, however, the monomeric entity of a dye would be the only species present in the solution. The adsorption peak appeared prior to the main peak can be diminished either by using a shorter mercury drop time, 0.5s or running the polarogram at high temperature, e.g., 35°C.

Classical DC polarography has been applied to study the electrochemical mechanism of some food colours. At an early stage a transfer of four electrons was reported for Amaranth by McKeown and Thomson\(^{32}\) substituting their experimental figures, $i_d = 1.2$, $C' = 0.1$, $D = 6.9 \times 10^{-6}$, and $m^{2/3}t^{1/6} = 1.90$ into the Ilkovic equation, $i_d = 607 nCD^{1/2}m^{2/3}t^{1/6}$. They concluded that the azo group in the colour is completely reduced to yield the corresponding amines.

Other workers\(^{90}\) calculated the number of electrons involved in the reaction using the relation between $E_1$ and $\log(i/(i_d - i))$. The same colour, Amaranth as well as
six other azo artificial food colours have been found to correspond to two electron reduction in acidic medium. Florence(182) divided azo compounds into four kinetic groups, depending on the disproportionation behaviour of their hydrazo derivatives: Group 1; Hydrazo derivative stable, Group 2; Rate = r_o + K_H(H^+), where r_o is the spontaneous rate due to water catalysis, Group 3; Rate = r_o + K_OH(OH^-), Group 4; Rate = r_o + K_H(H^+) + K_OH(OH^-).

An earlier study of a large number of aromatic azo compounds showed that the hydrazo disproportionation reactions were all acid-catalysed, with the exception of Orange I(4-(phenylazo-4-sulphonate)-1-naphthol) which exhibited both acid and base catalysis. The effect of pH on the number of electrons involved in the electrode reactions of several hydroxyazo compounds has been tabulated in the paper(182). The table indicates that most of the compounds are reduced with four electron transfer reaction at pH 2.0 except 3,3'-dihydroxyazo benzene which is reduced with two electrons at all pH values. However the higher the pH the smaller the number of electrons which are involved for most of the compounds except phenylazonaphthol dyes which produced well-formed waves with n = 4 in both acid and alkaline solution.

Different number of electrons seem to be involved for the food colours studied in the present work judging from the peak heights at different conditions.

Jacobsen and Lindseth(245) studied the effects of surfactants in differential pulse polarography. The
peaks of reducible substances are much more sensitive to the presence of non-ionic surfactants than the classical DC waves. Surfactants with the same charge as the depolarizer act as electrochemical masking agents, whereas peak currents may be enhanced by oppositely charged surfactants because of increasing the rate of the electron transfer.

The applicability of the polarography is growing in organic analysis, especially in the pharmaceutical industry and in environmental trace analysis. In general, the polarography may be very much superior in speed and convenience to any other technique particularly in the situations where several substances have to be determined in each sample. Meites, Campbell and Zuman\(^{(249)}\) have stated that polarographic methods have become, in many cases, comparable or even superior to atomic-absorption spectroscopy in sensitivity, selectivity and reliability.

The degradation studies of food colours would be one of the most promising approach using the admirable technique.

The use of PVC membrane electrodes has become popular because a solid construction is convenient for practical purposes. The addition of plasticizer and solvent mediator to the PVC matrix membranes was found to be necessary to obtain Nernstian behaviour. By increasing the viscosity of the organic liquid the pore size of the PVC membrane is diminished to macromolecular dimensions\(^{(330)}\) and convection is avoided.

The PVC membrane electrodes based on a basic dye-
12-tungstosilicate and tetraphenylphosphonium 12-tungsto-
silicate were responsive to their own host cation as well as tetraphenylborate anion. In the absence of plasticizer and solvent mediator, negligible responses were observed from the electrodes. The tetraphenylphosphonium 12-tungstosilicate PVC electrodes with 2-nitrophenyl-n-butyrate as a solvent mediator showed fast response to the onium cation in Nernstian manner. The slope factor of the electrode was gradually decreased by the time went by as described elsewhere. The more important role of a PVC electrode therefore seems to rely upon the solvent mediator.

The criteria of choosing a solvent mediator is considered to be on the easier solubility of a sensing material in it, viscosity and stability in working conditions. The PVC electrodes developed here have short life time due to the decomposition of the solvent mediator. Because of the short term stability of the electrodes the direct measurements of the potential corresponding to a dye concentration was not suitable for the purpose of the determination of a dye. The potentiometric titrations of acid dyes with Crystal Violet solution and basic dyes with sodium tetraphenylborate using the ion-selective electrode developed produced satisfactory results with a big potential jump in the end point area. The end point could easily be determined by taking the inflection point of the titration curve even if one electrode was used over and over again for several titrations. In that case the potential jump of the curve was decreased but the similar shape of the titration curves remained up to 6 weeks.
The titration curves of basic dyes with sodium tetraphenylborate solution showed a steeper shape than the ones for acid dyes with Crystal Violet solution because of the bigger selectivity coefficient of the PVC membrane electrodes to a basic dye cations and tetraphenylborate anion than that of acid dye anions. In fact the selectivity coefficients of the electrodes have not been studied here because the electrodes are more or less equally responsive to dye cations.

The responses of the Crystal Violet or tetraphenylphosphonium 12-tungstosilicate PVC membrane electrodes were sufficiently fast to trace the change in concentration of basic dye or tetraphenylborate anion that satisfactory titration curves could be attained using an automatic titrator. When the titration of 25ml of $10^{-3}$M Crystal Violet solution was carried out with $10^{-3}$M tetraphenylborate solution within 5 minutes using the automatic titrator, the end point was observed approximately up to 5% earlier than in the case of a slow titration taking around 10 minutes. The difference is probably caused by the slow reaction between the determinand and the titrant and by the adsorption of the determinand on the surface of the precipitate formed. Thus the electrode can easily respond to the titrant which is not in excess theoretically.

Vytras (305) has titrated several univalent nitrogen-containing organic compounds including Crystal Violet with sodium tetraphenylborate using $\text{aq}$ potassium ion-selective PVC membrane electrode with valinomycin sensor. He described that the titrations of some bulkier ions such as Crystal Violet have a drawback due to the equilibrium.
voltage established slowly especially near the inflection point, which makes the titration longer up to 45 minutes. Therefore the titration curves for the Crystal Violet reported have a rather diffused shape.

The PVC electrode based on quinolinephosphomolybdate gave a good response curve to proflavine sulphate and therefore was applied to the potentiometric titration of acid dyes with proflavine solution. Reasonably good shapes of titration curve were obtained from those titrations. The end point area with small tangent is probably caused by the high solubility of the precipitate as well as the slow response time of the electrode to reacting species in low concentration.

Liquid state electrodes with natural rubber membranes could be used for the potentiometric titration of basic dyes. The response time of the electrode was slower than that of the PVC electrode. The potential drift was another drawback of the liquid state electrode. The easy regeneration of the membrane by soaking it in an organic solvent containing a sensing material makes it valuable to use routinely.

The most successful PVC membrane electrodes with tetraphenylphosphonium or Crystal Violet 12-tungstosilicate as a sensor could be worth applying generally for the determination of the dye content in commercial products preferably after lengthening the life time by using a more ideal solvent mediator

Differential pulse polarographic end point detection
has been performed for the amperometric titration of a sample containing surfactants in order to remove the contribution of a capacitive current to be found in the ordinary polarographic method.

Most of the dyes would have a certain amount of surfactant characteristic due to their high molecular weight particularly at high concentrations. At $10^{-3}$M level of reactants the amperometric titration curves of Crystal Violet with sodium tetrphenylborate solution using differential pulse polarography were reproducible within a range of 3% for ten titrations. The deviation of the titration curves at the beginning of the titration from linearity was remarkable and was caused by the aggregation of the dye molecules. Diluting the dye solution with water the relationship between the peak current and the concentration of the dye becomes to show a linear to the end point area. The end point which is normally rounded due to the high solubility of the precipitate is easily determined by a simple graphical method.

The supporting electrolyte system decreasing the solubility product of the precipitate and the back titration technique could also be another useful approach to developing the method for the titrimetric determination of dye. The study of a complex system between dye-metal ion employing the sensitive differential pulse polarographic technique could also lead to a new method of dye determination.
REFERENCES

6. Y. Ishida, Senryo To Yakuhin, 21(10), 223 (1976).
21. ibid, 36, 2482 (1962).
24. ibid, 186, 31 (1960).
30. E. Braswell, ibid, 72, 2477 (1968).
34. F. E. Powell and C. J. Snowden, Analyst, 100, 503 (1975).
48. Y. Ohba, Japan Kokai 7763,346(C1. B41M5/20);
   3231 (1952).
50. ibid, 27 (1951).
51. ibid, 2638 (1951).
   81, 181 (1973).
58. R.D. Lillie, Histopathologic Technic and Practical
   p454 (1965).
59. H. Schweppe, Thin Layer Chromatogr., A laboratory
   Handbook, E. Stahl( Editor), New York, 2nd ed.,
61. ibid, 63, 442 (1971).
63. H. Kutt, D. Lockwood and F. McDowell, Stain Technol.,
   34, 203 (1959).
64. S. I. Rosenthal, H. Puchtler and F. Sweat, Arch.
   Pathol., 80, 190 (1965).
65. O. Schmidiger, Parfuem Kosmet, 57(10), 283 (1976).
66. H. A. Kues and C.E. Teague, J. Chromatogr., 135,
96. O. Yagi, Yosui To Haisui, 19(8), 949 (1977).
5, 84 (1977) ; CA, 88, 106739e (1978).


158. Z. Gregorowicz, P. Gorka and S. Kowalski, Przegl. Wlok,
30(10), 495 (1976).

173. O. Guertler, D. Fuertig and J. Von Grossman, Z Chem.,
13, 18 (1973).


180. M. Mitsuishi, Seni Kako, 23(8), 547(1971).


220. Anon, ibid, 9, 646 (1975).


233. p534 in ref. 11.


270. J. Koryta, Ion-Selective Electrodes, Cambridge Univ.


279. p 60 in Ref. 268.


282. R. P. Buck, p21 in Ref. 274.

283. p 126 in Ref. 268.


289. E. A Materova, S. A. Ovchinnikova and S. A. Smekalova, 
291. A. D. Hirst, p345 in Ref 290.
293. G. S. Craven, M. C. Griffith, Aust. J. Dairy Technol., 
32(2), 75 (1977).
294. B. P. Nikolskii, E. A. Materova and A. L. Grekovich, 
297. K. L. Cheng, E. E. K. Chao, US 4,071,427 (Cl. 204-195M; 
GOIN27/46), 31 Jan (1978), Appl. 742,597, 17 Nov. 1976; 
5pp; CA, 38, 130421r (1978).
299. V. A. Zarinskii, L. K. Shpigun, V. M. Trepalina and 
300. C. J. Battaglia, J. C. M. Chang, D. S. Daniel, D. P. 
Hamblen, C. P. Glover and S. H. Kim (Eastman Kodak Co.), 
301. V. V. Cosofret, P. G. Zugravescu and G. E. Baiulescu, 
302. V. A. Mirkin, V. G. Goncharuk and V. V. Banaquina,
Khimiya i khim. Tekhnol., 20,64 (1976); CA, 88, 57830h (1978).


314. J. Senkyr and J. Petr, p 559 in Ref. 274.


316. A. G. Fogg and K. S. Yoo, p 369 in Ref. 274.


335. A Hulanicki, M. Maj-Zurawska and R. Lewandowski,