REVIEW ARTICLE

APPLICATIONS OF POLAROGRAPHY IN PHARMACEUTICAL ANALYSIS

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INTRODUCTION

THE polarographic method of analysis was devised by Professor J. Heyrovský in Prague about 25 years ago, but it is only during the last ten years that polarography has been accepted for pharmaceutical analysis. Nevertheless, it is now agreed that the technique is of unquestionable value and the polarograph is assured of a permanent place among the complex equipment of a modern analytical laboratory.

In the past there has been a tendency among some enthusiasts to be uncritical of polarographic methods and to use a polarographic procedure whether or not some other more satisfactory method exists. Some of the polarographic analyses suggested in the literature need complicated chemical separations and do not compare favourably with simpler colorimetric and volumetric methods. Whenever a new analytical problem is being examined, a polarographic approach should always be considered, but, before any method is adopted for routine use, its merits must be compared with those of methods based on other analytical techniques. For many pharmaceutical problems the polarograph can offer a method that has advantages over all other techniques.

The fundamental principles of polarographic analysis were worked out in Heyrovský's laboratory and are adequately described in Kolthoff and Lingane's[1] and Heyrovský's[2] monographs. These survey polarographic literature up to the beginning of 1941. Stock's[3] and Müller's[4] reviews provide useful introductions to practical polarography and von Stackelberg's[5] book covers the literature until the end of 1949.

CHARACTERISTICS OF POLAROGRAPHY

In polarography the sample to be examined is dissolved in a base solution containing an excess of a base or supporting electrolyte and is placed in a special electrolytic cell, which has a pool of mercury as anode and mercury dropping from a capillary at the rate of one drop every 2 to 4 seconds as cathode. When a gradually increasing voltage is applied to the cell and the corresponding current is measured on a galvanometer, it is possible to determine from the resulting current-voltage curve both the nature and the concentration of the reducible substances in the sample. If the dropping mercury electrode is made the anode, analytical data on oxidisable substances may be obtained. The current-voltage curves, or polarograms, as they are usually called, may be plotted manually or recorded, either photographically or by means of a pen-recorder. A recording instrument is recommended for pharmaceutical investigations.

A typical polarogram obtained with an air-free solution of 0·02 per cent. of cadmium sulphate in 0·1N potassium chloride is shown in Figure 1. Under standard conditions, the diffusion current (i.e., the height of the step or wave) is proportional to the concentration of electroreducible substance present. This relationship is the basis of quantitative polarography. The half-wave potential, which, as its name implies, is the value of the potential of the dropping mercury electrode, measured against a standard external reference electrode, at the point on the current-voltage curve at which the current is one-half of its limiting value, is a special property of the particular electroreducible substance present and is independent of the concentration of the substance and of the characteristics of the dropping mercury electrode used. Qualitative polarography is based on these facts. The saturated calomel electrode is usually accepted as the standard reference electrode and all half-wave potentials mentioned in this review will be referred to it.

The magnitude of the diffusion current is governed by Ilkovič's equation,[6] $i_d = 605nD^{\frac{1}{2}}Cm^{\frac{2}{3}}t^{\frac{1}{6}}$ in which i_d is the diffusion current in microamp., n is the number of electrons involved in the reduction of one molecule of reducible substance, D is the diffusion coefficient of the reducible substance in sq. cm. per sec., C is its concentration in millimoles per l., m is the weight of mercury in mg. flowing out of the capillary per sec. and t is the drop time in sec. Recent work has shown that one of the assumptions made during the mathematical derivation of the Ilkovič equation is of doubtful validity and it is recommended that the equation should be multiplied by the factor $(1 + X.D^{\frac{1}{2}}t^{\frac{1}{6}}m^{-\frac{2}{3}})$. Lingane and Loveridge[7] give a value of 39 for the factor X, while Strehlow and von Stackelberg[8] quote a figure of 17. From the Ilkovič relation, it follows that results obtained with different capillaries can be compared provided that the product, $m^{\frac{2}{3}}t^{\frac{1}{6}}$ is known. Data for m and t should therefore be included in all polarographic reports.

Scope of Polarography

Theoretically every substance that is either electro-reducible or -oxidisable within the potential range of the dropping-mercury electrode (i.e., between +0·6 and −2·6 v.) can be determined polarographically, but for most solutions the range is much smaller. If several electroreducible substances are present together in the same solution, they can all be estimated if their half-wave potentials are more than 0·2 v. apart. Steps that are less than 0·2 v. apart can sometimes be separated by either changing the *p*H value of the base solution or by converting the reactants into complexes from which they deposit at potentials far enough apart for each step to be measured separately.

Under normal conditions, the accuracy of polarographic analysis is about ±2 per cent. in the concentration range 10^{-2} to 10^{-4} molar and ±5 per cent. between 10^{-4} and 10^{-5} molar. By taking special precautions it is sometimes possible to obtain greater accuracy. However, when the small quantity of material needed for an analysis is considered, the degree of accuracy is seen to compare favourably with that of other

micro-analytical techniques. The sample is not destroyed during measurement and can if necessary be recovered for further studies.

Practical Polarography

Current-voltage curves do not always have the ideal shapes shown in Figure 1 and are often surmounted by so-called "maxima." The discharge curve rises sharply, but, instead of gradually developing into a

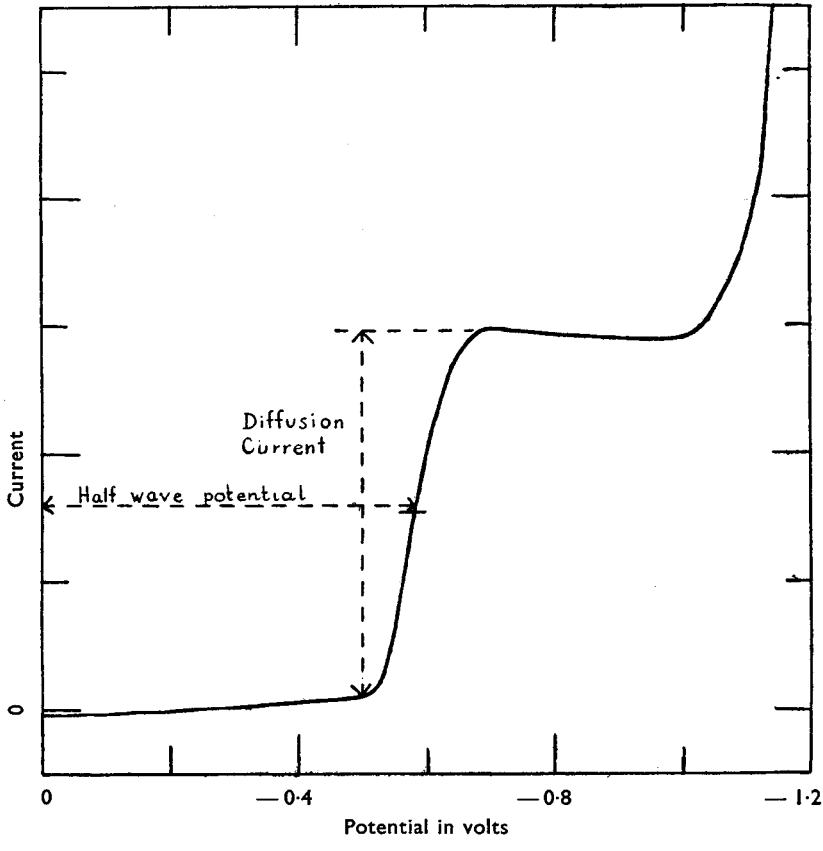

Fig. 1. Typical Polarogram.
Air-free solution of 0·02 per cent. of cadmium sulphate in 0·1N potassium chloride.

limiting current, the current increases abnormally until a critical value is reached and then decreases towards a limiting value corresponding with the normal diffusion current. The more or less sharp maxima shown in polarogram 1 of Figure 2 results. Fortunately maxima of this type can usually be suppressed by adding to the solution small quantities of surface-active substances. Gelatin and methyl red at a concentration of 0·01 to 0·1 per cent. are frequently used for this purpose. Maxima on catalytic waves (*vide infra*) do not normally respond to small amounts of surface-active substances.

Since oxygen is reduced at the dropping mercury electrode and interferes with the polarograms of most substances, it is generally necessary to remove dissolved air from polarographic solutions by bubbling either oxygen-free nitrogen or hydrogen through the cell before electrolysis or, if the solution is neutral or alkaline, adding about 1 per cent. of solid sodium sulphite.

Special care is needed in the selection of base solutions for the examination of organic substances. Since the *p*H value of the medium has considerable effect on half-wave potential, all solutions should be buffered. In unbuffered solutions, *p*H changes at the electrode surface frequently give rise to the development of drawn-out steps and sometimes to the formation of two steps. If the organic compound is insoluble in water, a mixture of water with a miscible organic solvent that will dissolve both buffer mixture and organic compound must be used. All the ingredients of the base solution should be tested polarographically to ensure the absence of impurities that would be reduced at a more positive potential than the substance under examination. Ethanol, *iso*propanol and dioxane need to be freed from aldehydes and peroxides.

Under certain conditions, ions that deposit at more negative potentials than hydrogen and are able to increase greatly the hydrogen over-potential form, in addition to ordinary reduction steps, catalytic waves. These waves can be distinguished from reduction steps by observing the effect of *p*H value on their height. Catalytic waves show big changes in height for small changes in *p*H value and frequently attain a height many hundreds of times greater than that of the corresponding reduction step. The height of a catalytic wave cannot be associated with a normal reduction mechanism.

Special Polarographic Techniques

For certain measurements the dropping mercury electrode may be replaced by either a rotating or a vibrating platinum micro-electrode. So far platinum electrodes have not been used extensively in pharmaceutical analysis, but they are useful for measurements in the positive potential range, in which mercury is oxidised, and for respiration studies on living organisms, when mercury might be toxic.

Amperometric titrations, in which either a dropping mercury electrode or a platinum electrode is employed as indicator electrode, have been developed during the last 15 years. They arise directly from polarography; the alternative name, polarometric titrations, was suggested by Majer to indicate their relationship to polarography. The technique depends on the measurement, at a constant potential, of the change in diffusion current of a reducible or oxidisable substance when the substance is titrated with a suitable reagent. The method is particularly useful for titrating small amounts of metal ions and can frequently be used when reversible indicator electrodes for titration by the classical potentiometric method are not available. Amperometric titrations need relatively simple equipment and sometimes yield more accurate results than ordinary polarography.

Recently, new techniques such as differential, derivative and oscillographic polarography[4] have been introduced. Derivative polarography promises to be of value for determining in the presence of each other substances whose half-wave potentials are close together. Oscillographic polarography should find many applications in the field of reaction kinetics.

Pharmaceutical Applications

The pharmaceutical applications of polarography will be discussed under the following headings:—

(1) Dissolved oxygen and peroxides.
(2) Trace metals and metal-containing drugs.
(3) Antiseptics and insecticides.
(4) Vitamins.
(5) Hormones.
(6) Antibiotics.
(7) Alkaloids.
(8) Miscellaneous pharmaceutical substances.
(9) Blood serum and cancer diagnosis.

Dissolved Oxygen and Peroxides

The determination of dissolved oxygen provides a simple exercise in quantitative polarography. Oxygen dissolved in an electrolyte solution (cf. Fig. 2) is reduced at the dropping mercury electrode and yields two distinct steps. The first step (Kolthoff and Miller[9]), at −0·1 v., is due to the reduction of oxygen to hydrogen peroxide and the second, at −0·9 v., to the reduction of hydrogen peroxide to either water or hydroxyl ion. The second oxygen step coincides with that obtained for the electrolysis of an air-free solution of hydrogen peroxide. These steps have been used for measuring the oxygen content of a wide range of fluids, including whole blood (Weisinger[10]), body fluids (Beecher, Follansbee, Murphy and Craig[11]), fermentation liquors (Hixson and Gaden,[12] Bartholomew, Karow, Sfat and Wilhelm[13] and Wise[14]) and milk (Hartman and Garrett[15]) and for studying the respiration rates of micro-organisms (Winzler[16] and Skerman and Millis[17]).

If the potential applied to the polarograph cell is kept constant at about −0·5 v., the variation of step height (i.e., oxygen level) with time can be recorded. When a suspension of yeast cells in a glucose medium is placed in a special air-tight polarograph cell, the height of the oxygen step falls at a steady rate, proportional to the number of yeast cells in the suspension. As soon as the amount of oxygen in the solution falls below a certain minimum value, respiration ceases and a horizontal trace is obtained. In this way, Petering and Daniels[18] were able to study the respiration of micro-organisms such as green algae, yeast, blood-cells and animal tissues. Winzler[16] examined the effect of cyanide and azide on the respiration of baker's yeast and Skerman and Millis[17] studied oxygen consumption in bacterial culture media. The method is sensitive and compares favourably with the classical manometric methods.

Recently the technique has been used to measure oxygen levels during the submerged culture production of penicillin and streptomycin. During fermentation, oxygen is supplied to the mould by bubbling air or oxygen through the medium. At high rates of penicillin production, the growth of the mould may be limited by the rate at which oxygen dissolves from

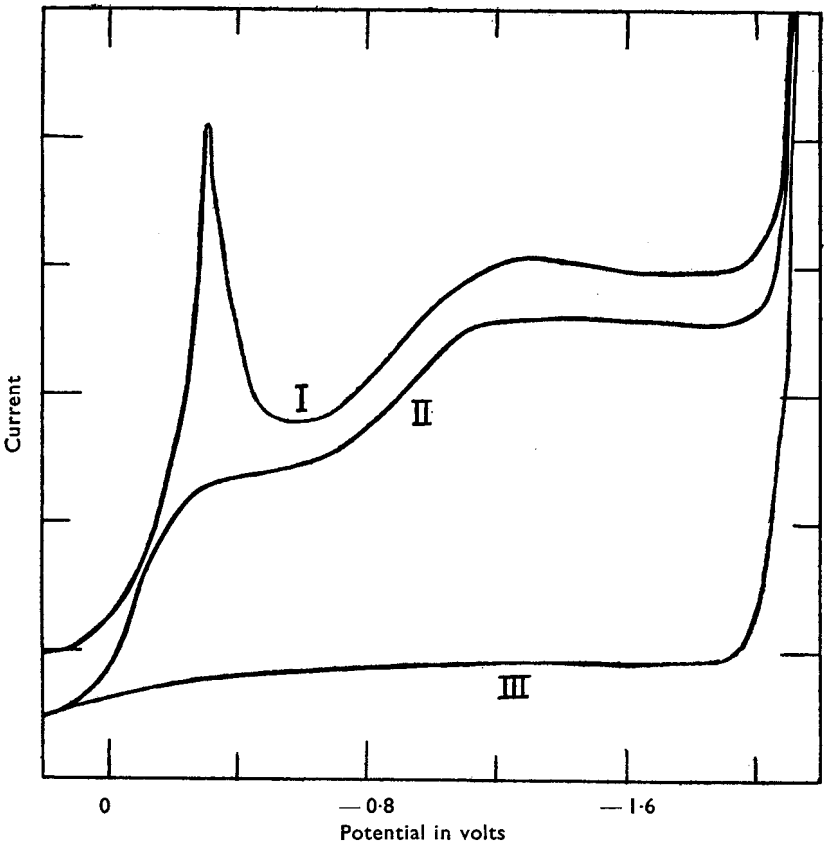

FIG. 2. Polarograms for oxygen.

I. 0·05N potassium chloride solution saturated with air.
II. After addition of a trace of methyl red.
III. After removal of oxygen by nitrogen.

the gas phase. Polarographic measurements on penicillin and streptomycin fermentation liquors have been reported by Hixson and Gaden[12] and by Bartholomew, Karow, Sfat and Wilhelm[13] in America and by Wise[14] in this country.

Peroxides and aldehydes are reduced at the dropping mercury electrode. Reimers[19] has described a method for the determination of peroxides and aldehydes in ether. A mixture of 5·0 ml. of the ether sample, 5·0 ml. of aldehyde-free ethanol and 10 ml. of 0·04N tetramethylammonium hydroxide is polarographed before and after removal of oxygen with

ether-saturated nitrogen. Examination of the first polarogram gives information on the aldehyde content of the sample and of the second on peroxide content. Benzoyl peroxide, lard peroxide, methyl linoleate peroxide andethyl, *iso*propyl and *n*-butyl ether peroxides may be determined in an ethanol-benzene mixture containing lithium chloride as base electrolyte (Lewis, Quackenbush and de Vries[20]); the technique has been used to study changes in lard (Lewis and Quackenbush[21]).

Trace Metals and Metal-containing Drugs

Polarography has been used extensively to determine trace metals in pharmaceutical products and to estimate drugs that contain metals as a constituent. The metals examined include antimony, arsenic, cadmium, copper, iron, lead, magnesium, mercury, vanadium and zinc. Unfortunately the procedures are not always so simple as the corresponding polarographic methods for these metals in metallurgical samples. If the organic matter is reduced at the same or at a lower potential than that of the metal a preliminary chemical separation or ashing procedure must be undertaken. Blood and urine containing traces of arsenic or vanadium need lengthy pre-treatments to remove reducible organic matter before an extract suitable for polarography is obtained. These pre-treatments are probably as complicated as those needed before any other analytical method can be used and it is doubtful whether for these particular analyses anything is to be gained by polarography.

On the other hand, Page and Robinson[22] have shown that the polarograph can offer advantages in special problems such as the estimation of antimony in pharmaceutical preparations and Goodwin and Page[23] have used the technique for studying the metabolism of the antimonial drugs for treatment of bilharzia and kala-azar. It is difficult to distinguish by ordinary chemical methods between ter- and quinquevalent antimony in organic matter. However, tervalent antimony in 1·0N hydrochloric acid forms a good polarographic step with a half-wave potential of −0·15 v., whereas quinquevalent antimony does not. Consequently, tervalent antimony can be determined in the presence of quinquevalent. This technique has been adopted as the British Pharmaceutical Codex (1949) test for tervalent antimony in sodium stibogluconate (sodium antimony$^{(V)}$ gluconate). Gelatin (0·01 per cent.) is included in the polarograph solution as a maximum suppressor.

Since the half-wave potential of tervalent antimony in 1·0N hydrochloric acid is relatively low, −0·15 v., the step appears before those due to most biological substances and tervalent antimony can therefore be determined directly in urine; blood samples need relatively little additional pre-treatment. Quinquevalent antimony can be measured after reduction with sodium sulphite. In this way, the valency state and the quantity of antimony appearing in the organs and body fluids of animals that had received stibophen (sodium antimony$^{(III)}$ biscatechol 3 : 5-disulphonate), sodium stibogluconate and other antimonial drugs could be ascertained.

Methods for determining a wide range of metals in materials of pharmaceutical interest are described in the literature. These include copperin

powdered and liquid milk (Cranston and Thompson[24]), milk fat (Coleman, Thompson and Branum[25]) and lard (Lupton, Mitchell, Oemler and Woolaver[26]), lead in lard[26] and urine (Cholak and Bambach,[27] Reed and Gant,[28] Weber[29] and Baker[30]), arsenic in biological material (Bambach[31]) and iron in pharmaceutical preparations (Bingenheimer and Christian[32]). The three earlier polarographic methods for lead in urine involved dry ashing at high temperatures; one method[27] also included a preliminary electrolytic separation. By using an entrainment technique, Baker[30] has been able to simplify the procedure considerably.

In Bambach's method[31] for arsenic, the arsenic is evolved as arsine and absorbed in mercuric chloride solution; the mercury arsenides are converted to arsenious oxide and mercurous chloride and the mercury is precipitated with hydroxylamine. The residual solution is treated with hydrochloric acid and examined polarographically. The method covers the range, 1 μg. to 1 mg. of arsenic and has an accuracy of 3 to 5 per cent. for quantities above 10 μg. Brdička[32] has used polarography to determine quinquevalent arsenic in arsphenamine, 0·01N lithium chloride being used as base electrolyte.

In Bingenheimer and Christian's method[33] for iron in American pharmaceutical preparations, a solution containing about 0·1 per cent. of ferric iron in 0·5M sodium citrate, containing 0·005 per cent. of gelatin as maximum suppressor, is polarographed. The iron must be dissolved completely in the ferric state.

Antiseptics and Insecticides

Several mercury-containing antiseptics and the new insecticides, gammexane and parathion, can be determined polarographically.

Page and Waller[34] have shown that thiomersalate (sodium ethyl mercurithiosalicylate) in 1·0N hydrochloric acid gives well-defined steps. The steps occur at a relatively low potential, −0·48 v., and are suitable for the routine determination of thiomersalate in vaccines and other pharmaceutical preparations. Phenylmercuric acetate and nitrate, but not mercurochrome or mercuric chloride, can be determined in this way. Benesch and Benesch[35] have used the method to determine the mercurial diuretics and Osborn[36] has adopted it for the routine determination of phenylmercuric acetate in a gelatin base. Owing to the large amounts of gelatin in Osborn's preparation, it was necessary to carry out the measurments in a special cell maintained at 58° C.

Commercial gammexane, which is the γ-isomer of 1 : 2 : 3 : 4 : 5 : 6-hexachlorobenzene, may occur mixed with at least four other isomers, of which only gammexane itself is both biologically active and polarographically reducible. It is unusual for only one isomer of a substance to be reducible; the reducibility of the γ-isomer is attributed to its polar structure. Ingram and Southern[37] used an aqueous-ethanolic solution containing 1·0 per cent. of potassium chloride as base solution. Dragt[38] preferred a potassium chloride-sodium acetate buffer mixture in aqueous acetone and Schwabe[39] 0·1 tetraethylammonium iodide in 80 per cent. aqueous ethanol. Heptachlorocyclohexane (Monnier, Roesgen and

Monnier[40]) is also reduced polarographically, but it is unstable at *p*H 11·5 while the γ-isomer is stable at *p*H 13·0 so that gammexane may be determined in the presence of heptachlorocyclohexane in aqueous acetone buffered at *p*H 11·5.

Bowen and Edwards[41] have determined parathion (O : O-diethyl-O-*p*-nitrophenyl thiophosphate) with an accuracy of ±1 per cent. in commercial preparations. The procedure depends on the reduction of the nitro group and is carried out in aqueous acetone with 0·05N potassium chloride as base electrolyte.

VITAMINS

Most water-soluble and some oil-soluble vitamins are either oxidised or reduced at the dropping mercury electrode and can be estimated in pure solution. Unfortunately most of the assays are unsuitable for foods and natural products. Methods have been reported for the following vitamins:—aneurine, riboflavine, nicotinic acid, pantothenic acid, pyridoxine, folic acid, vitamin B_{12}, ascorbic acid, α-tocopherol and vitamin K.

Lingane and Davis[42] determined riboflavine in the presence of aneurine and nicotinic acid and in pharmaceutical products such as liver extracts. The steps formed by aneurine and nicotinic acid appear at higher potentials than the riboflavine step and are therefore masked by steps due to more easily reducible organic matter. The mechanism of the riboflavine step has been studied by Brdička and Knobloch[43] and that of the nicotinic acid wave by Tompkins and Schmidt.[44] Knobloch[45] has determined nicotinamide in solutions intended for injection; 0·1N potassium chloride was used as base solution.

Wollenberger[46] has shown that under certain *p*H conditions aneurine forms a catalytic wave about 4,000 times higher than the normal aneurine reduction step. This catalytic wave can be used to detect traces of aneurine but is unsuitable for quantitative work. Pyridoxine and pantothenic acid[42] are reduced only at relatively high potentials.

Folic acid in 0·1M lithium borate gives a step at −0·8 v.; xanthopterin, rhizopterin and aporhizopterin give similar steps at the same potential. When Rickes, Trenner, Conn and Keresztesy[47] made the original observation, the structure of vitamin Bc, as folic acid was then called, was unknown, but the similarity in the steps suggested that the four substances had the same pterin structure. For routine estimations of folic acid in tablets, Mader and Frediani[48] recommend 1 per cent. tetramethylammonium chloride (*p*H 9·5) as base electrolyte with methyl red as maximum suppressor. Duncan and Christian[49] prefer a mixture of boric acid, potassium chloride and sodium hydroxide (*p*H 9).

Diehl, Sealock and Morrison[50] have shown that 9×10^{-4}M vitamin B_{12} in acid, neutral and alkaline solution gives a well-defined step at −1·12 v., which in 0·1M potassium cyanide shifts to −1·33 v. owing to the formation of a cyanide complex.[51] In neutral solution vitamin B_{12} also forms a catalytic wave with a half-wave potential of −1·53 v. (Fantes, Page, Parker and Smith[52]). The wave can be used to detect small amounts

($1{\cdot}0 \times 10^{-6}$ molar) of vitamin B_{12}, but is unsuitable for quantitative purposes.

By virtue of its reducing properties ascorbic acid yields an anodic step (cf. Fig. 3), a fact that was first discovered by Kodiček and Wenig.[53]

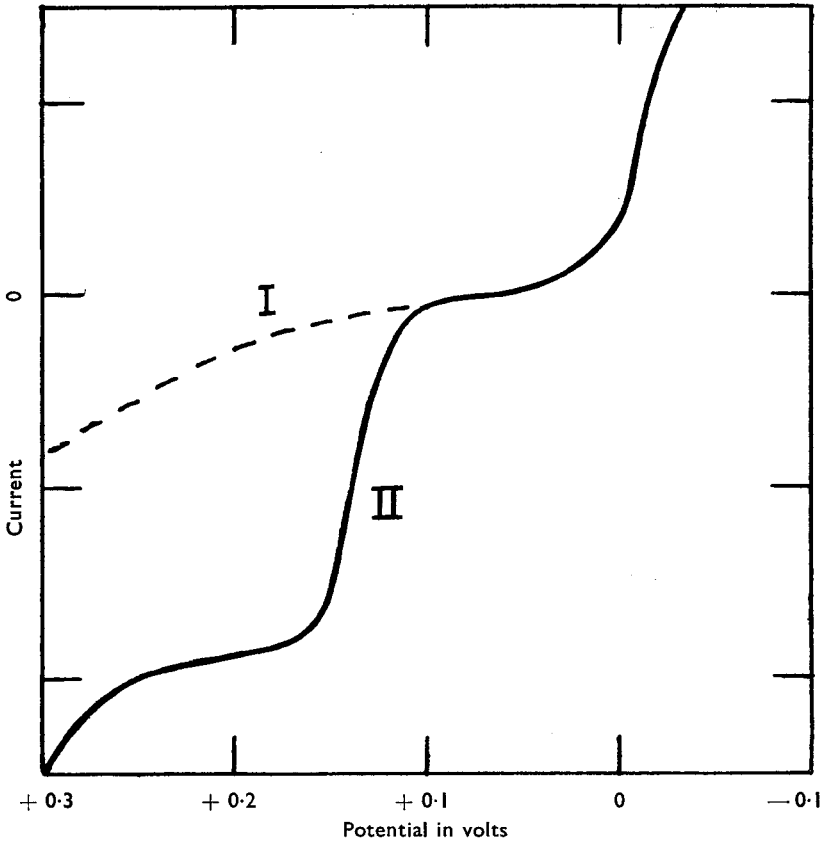

FIG. 3. Polarograms for ascorbic acid.

I. 0·05M phthalate buffer solution (*p*H 4·0).

II. 0·0002M ascorbic acid in 0·05M phthalate buffer solution.

These authors used a phosphate buffer solution (*p*H 7) for estimating ascorbic acid in fruit juices. Gillam[54] obtained better results by using 0·05M potassium hydrogen phthalate (*p*H 4·0) as base electrolyte. If the *p*H value is too low, the anodic step is shifted to such a high positive potential that the limiting current cannot develop. At high *p*H values the vitamin is unstable. Steps that are easier to measure (Page and Waller[55]) may be obtained by expanding the potential range of the instrument so that the voltage range of the potentiometer corresponds to 0 to $-1{\cdot}0$ v. instead of 0 to $-3{\cdot}0$ v. In this way, the steps are spread out and their height can be measured more accurately. Polarography has been used

to estimate ascorbic acid in fruit, vegetables[54] and milk (Perrin and Perrin[56]) and in tablet and ampoule preparations (Mata[57]).

Smith, Spillane and Kolthoff[58] showed that α-tocopherol in an aniline-anilinium perchlorate buffer solution gives an anodic step that can be used within certain limits for the determination of α-tocopherol in the presence of β- and γ-tocopherol. Unfortunately 2·5 to 5·0 per cent. of either sesame oil or fish oil or 0·15 per cent. of cholesterol will interfere with the determination and in the presence of these substances α-tocopherol cannot be determined at concentrations of less than 10^{-3} molar (Beaver and Kaunitz[59]).

McCawley and Gurchot[60] have shown that synthetic vitamin K_1, menaphthone (2-methyl-1 : 4-naphthoquinone), and phthiocol give well-defined polarographic steps in a 60 per cent. ethanolic acetate buffer solution (*p*H 6·56). The polarographic behaviour of vitamin K_1 in 50 per cent. aqueous *iso*propanolic 0·05N potassium chloride and of menaphthone in a 75 per cent. ethanolic acetate buffer solution (*p*H 6·24) has been reported by Hershberg, Wolfe and Fieser[61] and by Page and Robinson[62] respectively. Recently Onrust and Wöstmann[63] have described a polarographic procedure for determining menaphthone in mixtures with ground wheat germ and maize meal; Knobloch[64] has determined vitamin K_5 (4-amino-2-methyl-naphthol) in a phosphate buffer solution (*p*H 7·0).

Hormones

Thyroxine, insulin, adrenaline and several sex hormones give characteristic polarograms, that under certain conditions can be used for their determination.

The polarographic reduction of thyroxine was first reported by Simpson and Traill[65] who showed that thyroxine in 40 per cent. ethanolic 0·5N sodium carbonate containing 1 per cent. of tetramethylammonium bromide gave three steps, the first step having a half-wave potential of −1·24 v. In the same medium, 3 : 5-diiodotyrosine gave a double step with a half-wave potential of −1·72 v., thus making it possible to determine thyroxine in the presence of diiodotyrosine (cf. Fig. 4). Borrows, Hems and Page[66] studied the effect of various substances chemically related to thyroxine and obtained better polarograms by using a base solution containing 20 per cent. of *iso*propanol instead of 40 per cent. of ethanol. The technique has been extended to the measurement of thyroxine in iodinated proteins[66,67] and has been used by Clayton, Free, Page, Somers and Woollett[68] to estimate the chemical purity of thyroxine labelled with radioactive iodine.

The polarographic behaviour of adrenaline has been discussed by Kockelmeyer and Hauss[69] and by Sartori and Cattaneo.[70] Wielle and Gerlich[71] used polarography to determine the purity of an insulin sample; the colorimetric and polarographic methods for measuring the cystine content of insulin have been compared by Sullivan, Hess and Smith.[72]

Eisenbrand and Picher[73] first showed that certain sex hormones can be determined polarographically. 4 : 5-Unsaturated 3-ketosteroids, such as testosterone, progesterone, corticosterone and desoxycorticosterone, are

reducible at the dropping mercury electrode, but unsaturated 17-keto-steroids (e.g., androsterone) are not. However Wolfe, Hershberg and Fieser[74] condensed the latter with Girard T reagent (tetramethylammonium acethydrazide) to give water-soluble hydrazones that are electro-reducible.

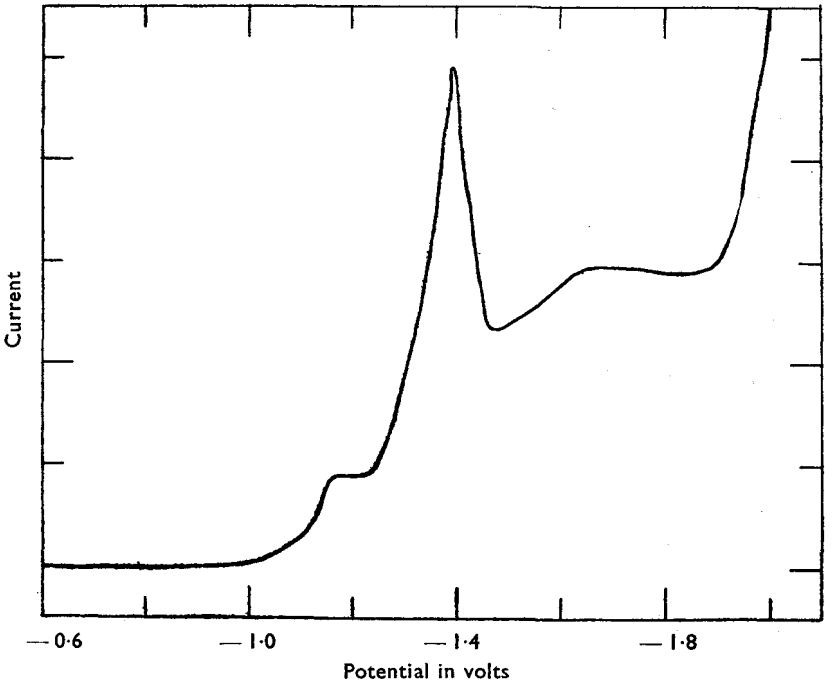

FIG. 4. Polarogram for 0·05 per cent. of thyroxine in 20 per cent. *iso*propanol containing 1·0 per cent. of tetramethyl ammonium bromide and 2·65 per cent. of sodium carbonate.

The condensation is undertaken in the presence of glacial acetic acid and the product examined in a base solution containing sodium hydroxide and chloride.

Girard T derivatives of 4 : 5-unsaturated ketonic steroids also give well-defined steps that can be used for analytical purposes. Certain non-ketonic steroids such as cholesterol can be determined after oxidation with aluminium *tert*-butoxide to the corresponding ketone and subsequent condensation with Girard's reagent (Hershberg, Wolfe and Fieser[75]).

Barnett, Henly and Morris[76] have used polarography to determine total 17-ketosteroids in urine. By employing a special preliminary separation, they[77] were able to differentiate between 3(α)- and 3(β)-hydroxy-17-ketosteroids. Some separation of the steps for individual ketosteroids may be achieved by using Werthessen and Baker's[78] expanded voltage scale technique, but otherwise the steps are too close together for individual ketosteroids to be estimated. A comparison of results for urinary 17-ketosteroids obtained by the polarographic and colorimetric methods

has been reported by Barnett, Henly, Morris and Warren.[79] Sartori and Bianchi[80] used polarography to determine methyltestosterone in pharmaceutical preparations.

Björnson and Ottesen[81] have shown that œstrone forms a polarographically-reducible Girard T derivative and have used the technique to estimate œstrone in pregnant mare urine. The method is suitable for analytical control of the large-scale production of œstrone. Œstrone, œstradiol and œstriol (Heusghem[82]) and the synthetic œstrogens, stilbœstrol, hexœstrol and dienœstrol (Gry[83]) may be determined after conversion to the corresponding nitroso derivatives. It is claimed that 5 to 20 μg. of the œstrogen can be detected.

Bingenheimer and Christian[84] discovered that stilbœstrol suppresses the oxygen maximum in the polarogram for 10 per cent. ethanolic 0·001N potassium chloride and used the property to determine stilbœstrol in tablets. These were extracted with ether, the ether was removed and the residue dissolved in the base solution. The solution was saturated with oxygen and polarographed. The quantity of stilbœstrol in the tablets was obtained by comparing the height of the oxygen maximum with that for a standard stilbœstrol solution.

Antibiotics

Polarographic methods for the determination of antibiotics such as penicillin, streptomycin and chloramphenicol have been described. Although the procedure for penicillin (Page,[85] cf. Clarke, Johnson and Robinson[86]) has been superseded by other methods, the procedure is of some interest as being the first non-biological assay method to be developed for penicillin. The technique depends on the observation that penicillamine (β:β-dimethylcysteine), a hydrolytic product of penicillin, in an ammoniacal cobalt buffer solution yields a catalytic wave similar to that given by cysteine.

Brdička[87] noticed that either cysteine or cystine reduced in buffered cobalt or nickel solution gave a single large catalytic wave (cf. Fig. 5). The height of the catalytic wave formed by cystine was twice that given by the same molar quantity of cysteine, but was 500 times as great as that of the step for the normal reduction of cystine to cysteine. These waves can be used to determine cysteine and penicillamine in the concentration range, 0·025 to 0·125 mg. per 100 ml. At higher concentrations, the catalytic wave height becomes independent of further increases in cysteine concentration. For analytical work on penicillin, the sample is inactivated with 0·1N sodium hydroxide, hydrolysed by warming with N hydrochloric acid, dissolved in the ammoniacal cobalt buffer mixture and polarographed immediately. The method can only be used for relatively pure penicillin samples and is unsuitable for metabolism solutions.

Levy, Schwed and Sackett[88] found that streptomycin in 3·0 per cent. tetramethylammonium hydroxide solution gives at $-1{\cdot}45$ v. well-defined steps due to reduction of the maltol group and used the procedure to estimate streptomycin in simple solutions. The technique cannot

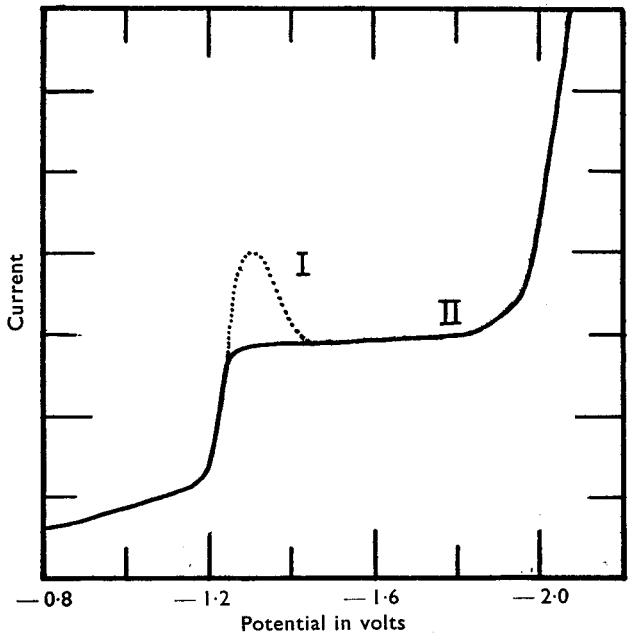

FIG. 5A.

I. Ammoniacal cobalt buffer solution.
II. Ammoniacal cobalt buffer solution containing 0·01 per cent. of gelatin.

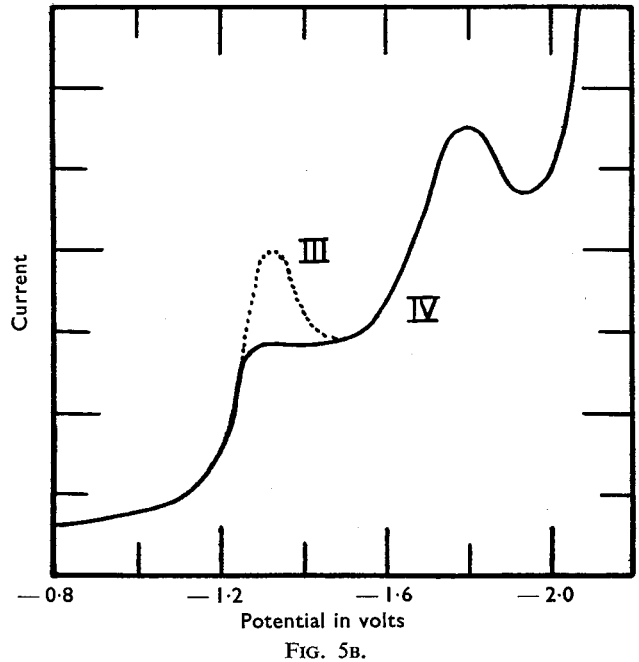

FIG. 5B.

III. Ammoniacal cobalt buffer solution containing 0·00005 per cent. of cystine.
IV. Ammoniacal cobalt buffer solution containing 0·00005 per cent. of cystine and 0·01 per cent. of gelatin.

differentiate between streptomycin and mannosidostreptomycin (Bricker and Vail[89]) and has been largely replaced by colorimetric methods.

Hess[90] has described an interesting polarographic method for chloramphenicol. The method, which depends on the reduction of the nitro group to hydroxylamine, is suitable for the routine analysis of broths. A potassium hydrogen phthalate buffer solution is used as base electrolyte.

Alkaloids

Most alkaloids yield catalytic waves and a few give reduction steps. Pech[91] in a preliminary survey of the quinoline and *iso*quinoline alkaloids found that quinine and quinidine in ammonium chloride solution yield identical catalytic waves, whose heights are proportional to the concentration of the alkaloids. The waves could be used to detect quinine and quinidine at concentrations as low as 10^{-7} molar. Cinchonine and cinchonidine behaved qualitatively in the same way as quinine, but the *iso*quinoline alkaloids, codeine, morphine, narcotine and hydrastine, gave much smaller catalytic waves than quinine. Reimers[92] observed that strychnine in 0·1N sodium sulphite gives a wave suitable for the rough determination of strychnine in the presence of quinine.

The only systematic polarographic investigation of the alkaloids has been conducted by Kirkpatrick.[93] In a study of over 30 alkaloids, he noticed that the catalytic waves formed by most alkaloids are in their height, formation, types of maxima and variation with *p*H value characteristic of the individual alkaloid and, provided the examination is conducted under standard conditions, can help in the identification and estimation of an unknown alkaloid. Preliminary purification is as necessary for the polarographic method as for the application of chemical tests, but polarography has the advantage that the alkaloid may be recovered unchanged.

The effect of *p*H value on the catalytic wave formed by morphine is shown in Figure 6. The wave at *p*H 8·0 has been selected for quantitative work, the relationship between diffusion current and concentration being linear over the range, $0{\cdot}3 \times 10^{-4}$ to $1{\cdot}3 \times 10^{-4}$ molar.

Kirkpatrick recommends polarography for the routine determination of morphine, diamorphine, emetine, strychnine and atropine in injection solutions. Single alkaloids may be extracted with chloroform from tablets and dissolved, after solvent removal, in 0·001N hydrochloric acid, mixed with the appropriate buffer solution and examined polarographically. Strychnine may be determined in mixtures with brucine and in nux vomica preparations. Mixtures containing 0·05 to 1·75 mg. of strychnine gave results that agreed within 3 per cent. of those obtained by the British Pharmacopœia 1932 procedure.

The polarographic behaviour of morphine has also been studied by Rasmussen, Hahn and Ilver.[94] If the nitroso derivative is formed, morphine can be determined in the presence of narcotine, papaverine and codeine. The technique has been extended to the determination of morphine in poppy capsules.[95] Further observations on the polarography of thujaplicin, berberine, hydrastine and cotarnine have been reported by

Šantavý,[96] who has also described a method for determining colchicine in seeds and in tincture of colchicum.[97] A polarographic procedure for lobeline in lobelia was published by Nyman and Reimers[98] but it gives lower results than those obtained by the Swiss Pharmacopœia method.

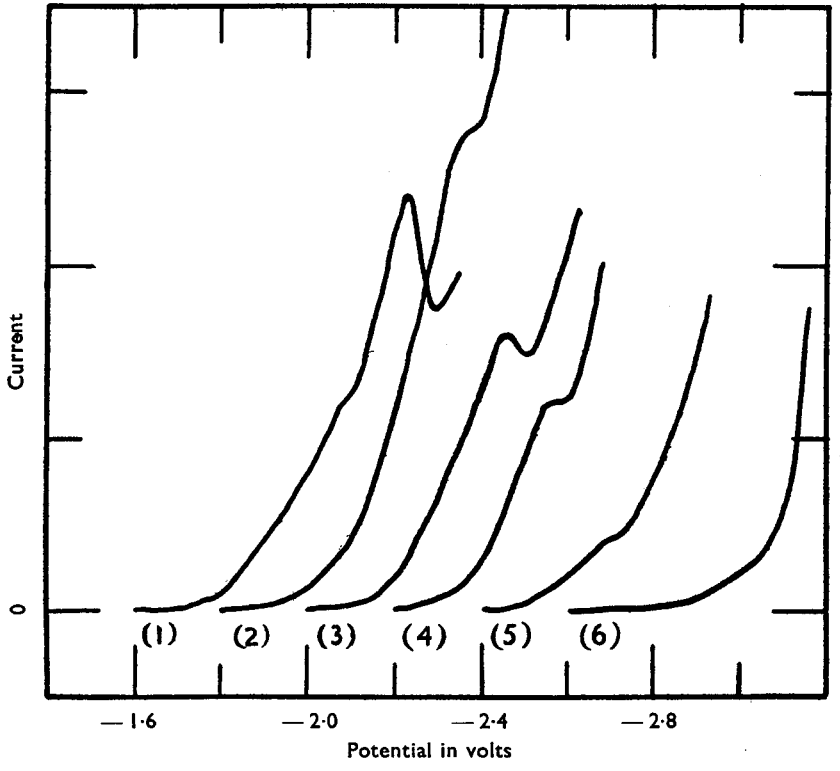

FIG. 6. 1·00M × 10^{-4} morphine in buffer solutions of *p*H value (1) 5·0, (2) 6·0, (3) 7·0, (4) 8·0, (5) 9·0, (6) 10·0. Each polarogram starts at −1·6 v.

MISCELLANEOUS PHARMACEUTICAL SUBSTANCES

Many miscellaneous substances of pharmaceutical interest, such as sugars, saccharin and the iodine-containing contrast agents, can be determined polarographically. Aldoses, such as glucose, rhamnose arabinose, mannose, galactose and lyxose, and disaccharides, such as maltose, lactose and sucrose, give small steps that are attributed to the aldehydo form of the sugar in the equilibrated solutions. Cantor and Peniston[99] used polarography to determine the amount of the aldehydo form present in sugar solutions under different *p*H, concentration and temperature conditions. The sugar solutions were buffered and 5-hydroxymethylfurfural, an aldehyde with approximately the same molecular weight as the sugar, was used as standard (cf., however, Weisner[100]). Ketoses, such as fructose and sorbose, in 0·02N lithium chloride give larger steps than were used by Heyrovský and Smöler[101] to determine

fructose in honey and in urine containing sucrose and glucose. Williams, McComb and Potter[102] recommend calcium chloride as base electrolyte and gelatin as maximum suppressor for the determination of fructose in fruit. Saccharin yields well-defined steps in acid, neutral and alkaline media; the steps have been used by Pech[103] to determine saccharin in tablets and by Momose[104] to determine saccharin in the presence of sugar. A base solution consisting of 0·05N hydrochloric acid and 0·05N potassium chloride is used in quantitative measurements.

The iodine-containing contrast agents that are used for the X-ray examination of various body organs yield characteristic polarograms (Page[105]), which can be used to identify and determine the contrast agents in simple solution. Diodone (diethanolamine salt of 3 : 5-di-iodo-4-pyridone-*N*-acetic acid) and iodoxyl (*N*-methyl-3 : 5-di-iodo-4-pyridone-2 : 6-di-carboxylic acid) in either acid or alkaline base solution given two well-defined steps. The first diodone step is surmounted by a maximum which may be suppressed by including a trace of gelatin in the base solution. Pheniodol (α-phenyl-β-(4-hydroxy-3 : 5-di-iodophenyl)-propionic acid) in sodium carbonate solution forms a double step that was utilised by Free, Page and Woollett[106] to determine the chemical purity of pheniodol labelled with radioactive iodine: a mixture of 1 per cent. of tetramethylammonium bromide and 2·65 per cent. of sodium carbonate in 20 per cent. *iso*propanol was the base solution.

Other applications of polarography include the measurement of formaldehyde in pharmaceutical products (Portillo and Mosquera[107]), digitoxin and gitoxin in tincture of digitalis (Hilton[108]), aloin in aloes, soap, gums, podophyllin and belladonna (Stone[109]) and santonin in *Flores cinae* and in tablets (Šantavý[110]). Knobloch and Schraufstätter[111] have listed the half-wave potentials of many pharmaceutical substances, including barbituric acid, chloramine, 8-hydroxyquinoline and phenolphthalein.

Blood Serum and Cancer Diagnosis

Polarography is of some importance in the study of pathological sera. In 1936, Brdička[112] found that blood serum from cancer patients contained fewer sulphydryl groups than that from normal persons. Direct comparison of untreated serum showed little difference, but significant differences between the two types of serum were observed when the serum was partially hydrolysed with dilute alkali to unmask disulphide groups inside the protein molecule. Still greater differences were noticed if the alkali-treated serum was precipitated with sulphosalicylic acid and the filtrate polarographed in an ammoniacal cobalt buffer solution. Under these conditions the difference was reversed and the cancer serum showed higher waves. Further work has shown that a high filtrate wave is not specific for cancer serum. Most foreign proteins, whether from the break-down of cancer tumour tissue or from centres of infection or inflammation, cause large filtrate waves when they are introduced into the blood stream. Since 1939, over 15,000 serum samples have been examined in Prague by these methods. Brdička's[113] general conclusions are that there

is a 20 per cent. overlap between the upper limit for normal serum and the lower limit for early cancer, but that there is a statistical correlation between wave height and the degree of advancement of the cancer. New growths are indicated by a gradual increase in wave size: after successful treatment of the cancer, whether by surgery or X-rays, the wave reverts to normal size. Robinson,[114] working in London, has largely confirmed the Czech work and has shown that the wave heights of serum filtrates from patients with prostatic cancer decreased when the patients were treated with synthetic œstrogens. The decrease in wave-height could be correlated with an improvement in the patient's condition.

The term "protein index" was introduced by Müller and Davis[115] to characterise and compare results obtained from the polarographic examination of blood serum. The protein index is a function of the ratio of the wave-heights obtained by the digest and filtrate tests. Both tests are conducted under standard conditions on different portions of the same blood sample. The technique is particularly suitable for routine analysis, since it is independent of small temperature changes and is unaffected by relatively large changes in mercury drop-time and drop-size. The protein index for normal persons is fairly constant,[116] but the values for patients with different diseases differ sufficiently to make the test useful in clinical diagnosis.[117]

Conclusion

Many of the applications described in this review are of limited scope, but they are all of some significance in their own particular field and contribute to the general importance of polarography in modern pharmaceutical analysis. This is a rapidly developing subject and we can anticipate that many new applications of polarography will be found in the near future.

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