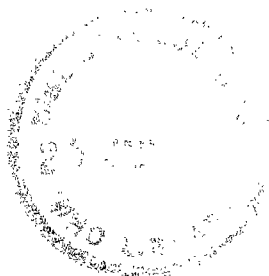


NINETEENTH REPORT

CONTENTS

	<u>Page</u>
1. Introduction	3
2. Revision of the first edition of the International Pharmacopoeia	3
2.1 General methods	3
2.2 Revision of monographs of the first edition and preparation of new monographs	5
3. Reagent specifications	8
4. Centre for Authentic Chemical Substances	9
5. International non-proprietary names	11
6. Information service	12
7. Scientific groups	13
8. List of unpublished working documents	14
Annex 1. Polarography (draft appendix)	16
Annex 2. Cumulative provisional list of additions for the second edition of the International Pharmacopoeia	21



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1. Introduction

The Expert Committee on Specifications for Pharmaceutical Preparations met in Geneva from 27 November - 1 December 1961.

Dr O. V. Baroyan, Assistant Director-General, opened the session on behalf of the Director-General, and stressed the importance of the preparation of the second edition of the International Pharmacopoeia. He expressed the thanks of WHO to those members of the Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations and other specialists whose co-operation is making this work possible. It was expected that, following this session of the Expert Committee and after further work by the Secretariat by correspondence and with consultants, it would be possible to have the complete text of the monographs and appendices for the second edition prepared for printing at the time of the next session. An important part of the work of WHO concerning pharmaceutical preparations referred to the establishment of a collection of authentic chemical substances by the WHO Centre in Stockholm. The Organization was pleased to have received recently samples of the thirteen reference substances for melting-point determination, for national and other laboratories dealing with the quality control of pharmaceutical preparations.

2. Revision of the first edition of the International Pharmacopoeia (Ph.I.)

2.1 General methods

2.1.1 Polarography

During the discussion on the revision of the first edition, the Committee considered the use of polarography as a tool for the assay of certain classes of pharmaceutical preparations. Although so far no monograph calls for the use of polarography, the Committee was of the opinion that a draft appendix which had been received¹ should be further examined by interested experts, and then be introduced into the second edition of the Ph.I. for general guidance. This guidance was considered valuable because of the uniquely selective nature of polarographic analysis, and its imminent official adoption in at least one national pharmacopoeia.

¹ Annex 1: Polarography

2.1.2 Tablet disintegration test

The Committee noted that tests now available for tablet disintegration may not necessarily indicate the rate at which the constituent medicament may be absorbed in the body. It was pointed out that, since absorption may take place only after the medicament has dissolved in the gastro-intestinal fluid, the limiting factor may well be the rate at which dissolution takes place, and this in turn is related to the surface area of the fragments into which tablets break up in the body.

The Committee was informed that, until satisfactory methods are devised for measuring the rate of dissolution, it will not be possible to provide a more meaningful index to physiological availability of the medicament presented in the form of uncoated compressed tablets than the test for disintegration. However, reports showed that the disintegration test described in the monograph on Compressi¹ gave rise to difficulties especially due to the element of personal interpretation called for in carrying out the test. In order to remove this difficulty, it was agreed to replace the test in the second edition of the Ph.I. by the procedure described in the British Pharmacopoeia, but to retain the present requirements for the maximum time of disintegration of the tablets.

2.1.3 Identification tests

At present the identification tests of the International Pharmacopoeia are mostly based on the determination of the melting-point of the substance, the melting-point of a derivative, and on qualitative reactions. Infra-red spectrophotometry or chromatographic procedures such as gas chromatography and paper chromatography are probably the best available methods for identification. In circumstances where these preferred methods cannot be employed, less definitive and simpler methods might be used.

Professor Kuhnert-Brandstätter has agreed to prepare a document dealing with identification of the substances of the Ph.I. along the following lines: (a) determination of the melting-point; - (b) determination of the mixed melting-point of the sample and an authentic specimen; - (c) determination of one eutectic melting-point. The Expert Committee was of the opinion that such a document would be helpful and

¹ Ph.I., first edition, Volume II, p.40

should be considered for inclusion as an appendix to the second edition of the Ph.I.; the Secretariat was asked to make the necessary arrangements for having such a document circulated for comments.

2.1.4 Uniformity of composition of tablets

The Committee considered reports that tablets in which the amounts of medication are quite small, in relation to the amounts of inert diluents, may vary substantially from the declared strength on an individual basis. Such inter-tablet variations can be shown only by an analysis of each individual tablet of a batch, in contrast to the usual practice of conducting a single analysis of a composite sample of a number (usually 20) of tablets. This accounts for the fact, the Committee was informed, that the variation has heretofore escaped general attention. From the standpoint of the Ph.I., however, the Committee concluded that the essential problem is that of deciding whether a limit test on inter-tablet variability should be a requirement for every batch of tablets. It seemed sufficient, therefore, to call attention to the possibility of this variation and await further developments in the programmes of the national pharmacopoeias.

2.2 Revision of monographs of the first edition of the Ph.I. and preparation of new monographs

The Expert Committee received a report from the Chairman on the meeting of the Secretariat with three consultants earlier in the year to examine a large number of monographs and appendices on the basis of work done by the Secretariat and through correspondence with specialists. This meeting with consultants had made it possible for the Secretariat to prepare 150 monographs for presentation and discussion at this session. The Committee agreed that the other monographs to be revised for inclusion in the second edition should be finalized by the Secretariat by correspondence with consultants and at special meetings. The new monographs to be included in the second edition¹ should be dealt with in the same way. Draft texts for these new pharmaceutical preparations would be obtained from different pharmacopoeia commissions and other suitable sources. The Committee expressed the view

¹ Annex 2: Cumulative provisional list of additions to the second edition of the International Pharmacopoeia (Ph.I.)

that the texts of monographs and appendices for pharmaceutical preparations requiring biological assays should be submitted to experts in biological standardization work.

2.2.1 Complexometric titration

The Committee recognized that the general method for the assay of many metals by means of complexation with suitable reagents is now firmly established in all countries. The concensus of the Committee was that revised or new monographs dealing with certain metallic salts or metallo-organic compounds should prescribe assay for the metal content by means of complexometric titration, wherever this is consistent with generally accepted professional practice. The wide variety of available techniques and end-point indicators will make it necessary to check carefully all proposals in more than one laboratory.

After discussion concerning the selection of indicators for the determination of calcium, it was agreed to recommend the adoption of methylthymol blue, and mixed calcein-thymolphthalein for the second edition of the Ph.I.

It was agreed also to recommend for the assay of bismuth compounds in acid medium direct titration with xylenol orange as the indicator. In some instances where it is preferable to have back titration, other indicators might be found useful.

2.2.2 Paper-chromatographic tests

The difficult situation existing in the purity testing of ergotamine and ergometrine gave rise to extended discussion and consideration of paper-chromatographic tests. In spite of the minor difficulties involved in the application of these tests, the Committee recommended the adoption of certain specified techniques, as representing the most dependable way now available to assess the purity of the substances.

These are the first examples of the use of paper chromatography in the Ph.I., but the Committee took note of statements of possible application to other substances, either of paper chromatography, or of newer similar techniques, such as thin-layer chromatography.

2.2.3 Identification testing by use of infra-red spectrum

For a number of steroids and related substances, the Committee considered and recommended the adoption of infra-red identification tests, generally using the potassium bromide pellet method, with suitable precautions. It may be noted that the determination of the infra-red absorption spectrum of complex organic molecules such as steroids is the principal generally-accepted method for examining the molecular structure and purity of such substances.

2.2.4 The oxygen-flask combustion method

The Committee considered statements on the wide acceptance of the flask-combustion technique for the complete oxidation of organic or metallo-organic compounds, preparatory to assay by an accepted method. It was specifically recommended that this oxidation method be used as the preliminary step for the identification of fluorine in steroids containing that element. In addition, it was recommended that a general appendix on the flask-combustion method be provided, to outline its potential utility in the analysis of pharmaceuticals containing chlorine, bromine, iodine, sulfur, phosphorus, and other elements mentioned in the literature. The Committee took note of a warning to the effect that in the case of substances containing very high percentages of iodine (i.e. about 60 per cent. and more), erratic values might result from the use of a preliminary oxygen-flask combustion.

2.2.5 Specifications for certain ergot alkaloids

The Committee discussed at length reports of problems of standardization of the sterile solutions particularly, and of other forms in which ergotamine tartrate is used. The conventional method of assay of ergotamine tartrate solutions measures both the active alkaloid and inactive forms, such as ergotaminine, which are formed on storage. A proposed procedure, based upon paper chromatography, was provisionally adopted for inclusion in the second edition of the Ph.I. in the monograph on Ergotamine Tartrate Injection. An appropriately revised definition of Ergotamine Tartrate Injection, taking account of these facts, was agreed upon. The Committee was informed of the existence of an alternative method which was said to be simpler in that it represented separation of the epimers by liquid-liquid extraction on a suitable column. Both of these methods, it was noted, require an authentic specimen

of ergotamine tartrate for reference purposes so the Committee adopted a request that such a specimen be provided as a WHO Authentic Chemical Substance.

2.2.6 Additions to the list of monographs

In view of the need for international specifications for those therapeutic agents that have become established since 1959, when the Supplement to the first edition of the Ph.I. appeared, the Committee selected nine titles for which monographs are to be prepared for the second edition, in addition to the new monographs proposed at previous sessions.¹ These nine titles include preparations from the following classes of therapeutic agents: steroids, antibiotics, non-mercurial diuretics and long-acting sulfonamides.

It is expected that draft monographs for these titles will be prepared and distributed for comment well in advance of the next session of the Committee.

3. Reagent specifications

The Committee noted that the text of the specifications for the proposed volume of specifications for reagents required to carry out the tests described in Volumes I, II and the Supplement to the first edition of the International Pharmacopoeia² had now been sent to the WHO Division of Editorial and Reference Services for publication. Galley proofs would be sent to the members of expert committees who had contributed to the formulation of the specifications, and mention of their availability would be made in the WHO Chronicle. Arrangements were being made for the completion of the specifications in French and, later, in Spanish.

The Committee expressed its thanks to Dr J. L. Powers, Washington, and other experts who had made this work possible, and was of the opinion that the volume would be a useful complement to the first edition of the International Pharmacopoeia and of direct help to laboratories dealing with the quality control of pharmaceutical

¹ Annex 2: Cumulative provisional list of additions to the second edition of the International Pharmacopoeia (Ph.I.)

² 17th Report of Expert Committee on Specifications for Pharmaceutical Preparations, item 2 (WHO/Pharm/377)

preparations. For reagents used in monographs for the second edition which would not be described in this volume of reagents, it was suggested that the Secretariat should make texts available for inclusion in the second edition of the Ph.I.

4. Centre for Authentic Chemical Substances

The Committee expressed its deep appreciation of the work done by the WHO Centre for Authentic Chemical Substances at the laboratories of the Swedish Pharmaceutical Association in Stockholm, to establish a collection of thirteen reference substances for use in standardizing melting-point determinations, in accordance with the recommendations of previous expert committees,¹ and in addition to the existing WHO Authentic Chemical Substances.² The collection had been established after collaborative assays undertaken in a number of laboratories in different countries.

At the previous sessions of the Expert Committee on Specifications for Pharmaceutical Preparations it was decided that the Centre should study the possibility of issuing the following steroids as authentic chemical substances: cortisone acetate - hydrocortisone acetate - desoxycorticosterone acetate - hydrocortisone sodium succinate - dienestrol - liothyronine sodium - diethylstilboestrol - methyltestosterone - estradiol benzoate - prednisolone - ethinyl estradiol - prednisone - ethisterone - progesterone - hydrocortisone - testosterone propionate. That programme is now under way in close co-operation with the United States Pharmacopoeia Convention.

A letter has been sent from the Secretariat to members of national pharmacopoeia commissions and members of the Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations asking the following three questions:

¹ 16th Report, item 9 (WHO/Pharm/351), 17th Report, item 5 (WHO/Pharm/377), and 18th Report, item 3 (WHO/Pharm/386) of Expert Committee on Specifications for Pharmaceutical Preparations

² Ph.I., first edition, Supplement, Appendix 17

- "... (a) what chemical reference substances are included in your present national Pharmacopoeia?
- (b) what chemical reference substances are considered for inclusion in your Pharmacopoeia or other official volume of specifications of your country?
- (c) what chemical reference substances should, in your opinion, be added to the collection of the WHO Centre for Authentic Chemical Substances? ..."

The answers received expressed strong support for the proposal to establish on an international basis a greater number of authentic chemical substances; it was also evident that in some countries the basic drug, when assayed by a chemical method, was used as the "reference standard" in the spectrophotometric assay of a dosage-form product such as an injection or tablets. A survey of the replies received indicates that, in addition to steroids, the main interest for authentic chemical substances at present concerns folic acid, cyanocobalamin, digoxin, ergocalciferol and ergotamine tartrate. Suggestions will be invited from national quality control laboratories to determine what other substances should be added to the collection.

The Expert Committee examined the report on melting-point reference substances and recommended that it should be published in the Bulletin of WHO. It was suggested that before publication the following additions should be made to the report: (a) directions issued to the participants in the collaborative assay, (b) the original values obtained from the participating laboratories, and (c) available data on the purity of the substances. The Expert Committee also recommended that when the substances were available a notice should be inserted in the WHO Chronicle and supplied to editors of national pharmaceutical journals.

4.1 Spectrophotometry: chemical reference standard versus standardization of apparatus

The Committee considered statements on the relative convenience, safety, and necessity involved in the use of special chemical reference standards, as against the use of published absolute wave length and absorbancy values; the latter, it was assumed, could be used only with instruments which had been standardized against non-specific permanent standards.

It was noted that the holmium glass filter (which is not yet in general use) and other means exist for standardizing a spectrophotometer as to wave-length, but that no completely dependable tool is available for standardizing in respect of absorbancy throughout the entire useful spectrum. It is precisely in respect of absorbancy that modern instruments show the greatest variability. After consideration, the Committee was of the opinion that for the present, a conservative approach demands that specially prepared reference chemical substances be furnished to aid in the performance of official spectrophotometric assays, wherever no alternative reference is indicated.

5. International non-proprietary names

The Expert Committee noted that at a meeting of the Sub-Committee on Non-Proprietary Names¹ held on November 8-11, 1961, a further 174 names were selected for early publication as a list of proposed international non-proprietary names. It was noted that the Sub-Committee had revised and extended the General Principles for Guidance in Devising International Non-Proprietary Names with the object of affording a better indication of the style which should be followed by those first concerned with suggesting a non-proprietary name for a new drug. Adherence to these General Principles by national authorities, manufacturers and others who submit names to WHO would facilitate the selection of such names as international non-proprietary names. It was also noted that the Sub-Committee had provided an abbreviated nomenclature for the designation of the inactive parts (acid or base radicals) in salts and esters of therapeutic interest.

The Expert Committee welcomed the arrangements that had been made to ensure that the chemical nomenclature used in defining the substances to which international non-proprietary names were applied would be in accordance with the rules adopted by the International Union of Pure and Applied Chemistry. In those instances where international rules were not available, the systematic nomenclature adopted for the indices of Chemical Abstracts would be used. This systematic nomenclature would be used in the Cumulative List of international non-proprietary names that was now with the

¹ 11th Report of Sub-Committee on Non-Proprietary Names of Expert Committee on Specifications for Pharmaceutical Preparations (WHO/Pharm/394)

printer and which, when available, would greatly facilitate the use of these names by national authorities and others concerned with the nomenclature of pharmaceutical preparations.

The Expert Committee expressed its appreciation of the considerable work undertaken by members of the Sub-Committee throughout the year, by frequent correspondence and by attendance at the session. From the study of basic problems and close co-operation between members of the Sub-Committee it has been possible to issue an increasing number of international non-proprietary names that find ready acceptance and use throughout the world.

6. Information service

The Committee was informed that a programme was proceeding slowly to put into effect certain recommendations made by a Study Group on the Use of Specifications for Pharmaceutical Preparations in December 1956.¹ Considering that the object of this programme is to provide helpful information on new pharmaceutical preparations as early as possible after their commercial introduction, the Committee explored ways of obtaining this information which is available mainly from those firms that produce the drugs. It was agreed that an approach should be made through consultation with as many producers as possible by the Secretariat and by members of the Expert Advisory Panel. In conferences of this kind an effort should be made first to indicate the general utility of the programme from a public health standpoint and second to point out that the information might actually be submitted in as many as three parts over a considerable period of time. The first part, to be made available about the time the drug is introduced into medical practice, might comprise sufficient information to identify the drug, including some physical characteristics, and such additional data as may have been supplied in a request for an international non-proprietary name. The second part, to be made available not later than the time at which the preparation goes into foreign distribution, might include definite information on physical characters, suitable methods for the assay of the various forms in which the drug is issued, and data on stability. Finally, as soon as available, additional information might be offered.

¹ Wld Hlth Org. techn. Rep. Ser., 1957, 138

The Committee considered that, in preparing this information for distribution to the control authorities of the Member States, WHO has an obligation to evaluate its adequacy by judging whether in the hands of a qualified analyst the methods will demonstrate how well the preparation meets all claims for strength and purity. It appeared that this evaluation could best be carried out by a corps of experts with whom standing arrangements existed. It was considered essential that such experts should have had first-hand experience in drug analysis. The evaluation should be concluded promptly, usually within a few weeks. Only by this means, it appeared, could the information be provided when it was most needed.

The Committee was of the opinion that the suggested information service would relieve drug producers of the considerable and increasing burden of meeting requests from various national control authorities for such information as will permit the authorities to test and allow the importation of the drugs into their respective countries. It was envisaged that WHO might assume the role of compiling this information and fulfilling the requests for it. Moreover, the service would enable the quality control authorities to release new drugs more promptly in the countries to which they were exported.

7. Scientific groups

The Committee considered a proposal from the Secretariat that, in view of the increasing specialization in pharmaceutical analysis, there was an urgent need for meetings of small groups of specialists in various fields. The object of the meetings would be to review existing analytical methods and, from the personal knowledge and experience of the specialists, to submit to the Expert Advisory Panel on the International Pharmacopoeia and Pharmaceutical Preparations the recommended procedures for use in the Ph.I. or for general application in pharmaceutical analysis.

The Committee agreed that the proposed meetings would make a valuable contribution to the work of providing specifications for pharmaceutical preparations, and recommended that arrangements should be made for the meetings to commence in 1963. In the view of the Committee some meetings should be concerned with general methods of analysis applicable to many drugs, and other meetings should consider the

specifications and analytical procedures appropriate for certain groups of drugs. Examples of the general methods of analysis that require study include non-aqueous titration, chromatographic methods such as paper chromatography, partition chromatography, thin-layer chromatography, and gas chromatography, and spectrophotometry

Examples of groups of drugs which call for study by specialist experts include the cardiac glycosides, steroids, non-mercurial diuretics, and synthetic penicillin. It was considered desirable that the meeting on synthetic penicillins should be arranged in collaboration with the Biological Standardization Unit of WHO because both microbiology and pharmaceutical analysis are involved.

It was strongly recommended that arrangements should be made for meetings of two groups of experts during 1963, one to consider non-aqueous titration (especially of weak acids) or thin-layer chromatography, and the other the cardiac glycosides or synthetic penicillins. The importance of the preparatory arrangements for these meetings was emphasized and it was recommended that this work should start early in 1962.

8. List of unpublished working documents

1. Bervenmark, H. Centre for Authentic Chemical Substances:
Diding, N. A. WHO Melting-Point Reference Substances
Ohrner, B. (WHO/Pharm/391 and Add.1)
2. Diding, N. Centre for Authentic Chemical Substances:
Report on the work in 1961 (WHO/Pharm/392)
3. Hofmann, A. Ergotamine Tartrate: Specifications for the substance
and its preparations (WHO/Pharm/Ed.Sec/107)
4. Jakubczak, D. M. Evaluation of Calcein-Thymolphthalein mixed indicator
Morecambe, F. A. for calcium titrations (WHO/Pharm/Ed.Sec/101 Add.2)
5. Maurina, F. A. Edetate Titration: Determination of Calcium
(WHO/Pharm/Ed.Sec/65)
6. Roushdi, I. M. Assay of some official iron and mercury salts
Abdine, H. complexometrically (WHO/Pharm/Ed.Sec/103)
Sadek, W.
7. Schöniger, W. Determination of halogens and other elements:
the oxygen flask method (WHO/Pharm/Ed.Sec/105)

Polarography

Polarography is an electrochemical method of analysis based on the measurement of the current flow resulting from the electrolysis of a solution at a micro-electrode as a function of an applied voltage. From the polarogram obtained in this fashion it is possible to obtain qualitative and quantitative information about electro-reducible and oxidizable substances. The normal concentration range for substances being analysed is 10^{-2} to 10^{-4} molar.

In conventional polarography the microelectrode is a dropping mercury electrode (D. M. E.) consisting of small reproducible drops of mercury flowing from the orifice of a capillary tube connected to a mercury reservoir. A saturated calomel electrode (S. C. E.) with a large surface area is the most widely adopted reference electrode. As the voltage applied to the cell increases only a very small residual current flows until reduction or oxidation of the sample occurs. At this point the current increases, at first gradually, then almost linearly with voltage and then gradually reaches a limiting value as shown in the accompanying figure. On the initial rising portion of the polarographic wave the increased flow of current results in a decrease in the concentration of the electroactive species at the electrode surface. As the voltage and current increase the concentration of the reactive species decreases further to a minimal value at the electrode surface. The current is then limited by the rate at which the reacting species can thermally diffuse from the bulk of the solution to the surface of the microelectrode. The final current rise is caused by the reaction of the supporting electrolyte. This large concentration of electrolyte is inert within the potential range under investigation. It prevents the reactive species from reaching the electrode by electrostatic attraction thus assuring that the limiting current is diffusion controlled.

Diffusion current. The limiting current is the sum of the residual current and the diffusion current (i_d). The residual current must be subtracted from the limiting current to give the wave height, a value that is a measure of the rate of diffusion of the electroactive species. The diffusion current is directly proportional to the concentration of the electroactive species in the sample as is shown by the Ilkovic equation

$$i_d = 708nD^{1/2}C_m^{2/3}t^{1/6}$$

Annex I

where i_d is the maximum current in microamperes obtained just before the drop falls, n the number of electrons required per molecule of electroactive substance, D its diffusion coefficient in square centimeters per second, C the concentration in millimoles per liter, m the rate of mercury flow from the dropping mercury electrode in mg. per second and t the drop time in seconds.

According to the Ilkovic equation the diffusion current should increase from a small value at the beginning of a drop to a maximum value as the drop falls. The diffusion current would then drop abruptly to the low value and again increase in the same manner. By employing a suitable recorder to measure the current, saw-toothed waves are obtained. Pen-and-ink recorders are sufficiently rapid to follow the current during the second half of the drop life but are too slow to respond to rapid current changes and will not show the current falling to a value near zero. The average of the oscillations of the recorder pen is not equal to the average current during the life of the drop. The maximum of the oscillations should be determined as a measure of the current. For instruments using galvanometers to measure the current or pen recorders adjusted to simulate galvanometer recording, the saw-toothed waves correspond to oscillations about the average current. In this case the average of the oscillations should be determined as a measure of the current. For polarograms obtained in this fashion the i_d of the Ilkovic equation refers to the average current in microamperes during the life of the drop and the coefficient 708 is replaced by 607.

Control of the diffusion current. The Ilkovic equation suggests variables that must be controlled to ensure that the diffusion current is directly proportional to the concentration of electroactive material. At 25° the diffusion coefficients for aqueous solutions of many ions and organic molecules increase 1 to 2 per cent. per degree rise in temperature. Thus the temperature of the polarographic cell must be controlled to 0.5° at the generally accepted standard temperature of 25°. The quantities m and t depend upon the dimensions of the capillary and the height of the mercury column above the electrode. Although results obtained with different capillaries can be compared if the product $m^{2/3}t^{1/6}$ is known, it is advisable to use the same capillary with a constant head of mercury during a series of analyses. The

Annex I

diffusion current is proportional to the square root of the height of the mercury column. A mercury reservoir with a diameter above 4 cm. will prevent any significant drop in the mercury level during a series of runs.

The capillary for the dropping mercury electrode has a bore of approximately 0.03 to 0.05 mm. and a length of 6 to 8 cm. The height of the mercury column, measured from the tip of the capillary to the top of the mercury pool, will vary from 40 to 80 cm. The exact length of the capillary and the height of the mercury are determined by trial-and-error to give a drop-time between 3 and 5 seconds at open circuit while immersed in the test solution.

The current flowing through the sample during the recording of a polarogram is in the microampere range. Consequently concentration changes in the bulk of the sample are negligible and several polarograms can be run on the same test sample without any significant differences.

Half-wave potential. The half-wave potential ($E_{1/2}$) occurs at the point on the polarogram one half the distance between the residual current and the limiting current plateau. This potential is characteristic of the electroactive species and is independent of its concentration or capillary used to obtain the wave. It is dependent upon the solution composition and may change with variations in the pH, solvent system or the addition of complexing agents. The half-wave potential thus serves as one criterion for the qualitative identification of a sample.

The potential of the dropping mercury electrode is equal to the applied voltage versus the reference electrode after correction for the iR drop through the test solution (this is especially important for non-aqueous solutions which generally possess a high resistance). The expression "versus S. C. E." should follow potentials measured with the saturated calomel electrode to avoid confusion with potentials referred to other reference electrodes.

Removal of dissolved oxygen. Oxygen must be removed from the sample if polarograms are to be made at potentials more negative than about 0 volt versus S. C. E. Oxygen is reduced at the D. M. E. in two steps, first to hydrogen peroxide and then to water. Bubbling pure nitrogen through the sample for 10 to 15 minutes

Annex I

immediately before recording the wave will reduce the oxygen concentration to a negligible level. To prevent losses from evaporation the nitrogen should be bubbled through a solution similar to the sample before entering the cell. Sufficiently pure nitrogen can be obtained commercially and no further purification is usually necessary.

Before recording a polarogram the flow of nitrogen must be switched to flow over the solution. It is necessary for the solution to be quiet and vibration-free during the time the wave is recorded to ensure that the current is diffusion-controlled.

Measurement of the wave height. To use a polarogram quantitatively it is necessary to measure the height of the wave. Since this is a measure of the magnitude of the diffusion current it must be measured vertically. To compensate for the residual current the segment of the curve preceding the wave is extrapolated beyond the rise in the wave. For a well formed wave where this extrapolation parallels the limiting current plateau, the measurement is unambiguous. For less well defined waves the following procedure may be used unless otherwise directed in a monograph. Both the residual current and the limiting current are extrapolated with straight lines as shown in the figure. The wave height is taken as the vertical distance between these lines measured at the half-wave potential.

Procedure. **MERCURY IS POISONOUS.** Work in a well ventilated laboratory and clean up any spilled mercury.

Transfer a portion of the final sample dilution to a suitable polarographic cell immersed in a water bath regulated to 24.5 to 25.5°. Pass a stream of nitrogen through the solution for 10 to 15 minutes to remove dissolved oxygen. Start the mercury dropping from the capillary, insert it into the sample solution and adjust the height of the mercury reservoir. Switch the flow of nitrogen to pass over the surface of the solution and record the polarogram over the potential range given in the monograph using the proper recorder or galvanometer sensitivity to give a suitable wave. Measure the height of the wave and unless directed otherwise in the monograph compare this with a calibration curve obtained under the same conditions as the sample in solutions of the same composition.

Annex I

Selected references

Elving, P. J., "Application of Polarography to Organic Analysis" in Organ Analysis, Interscience Publishers, Inc., New York, 1954, Vol. II, p.195.

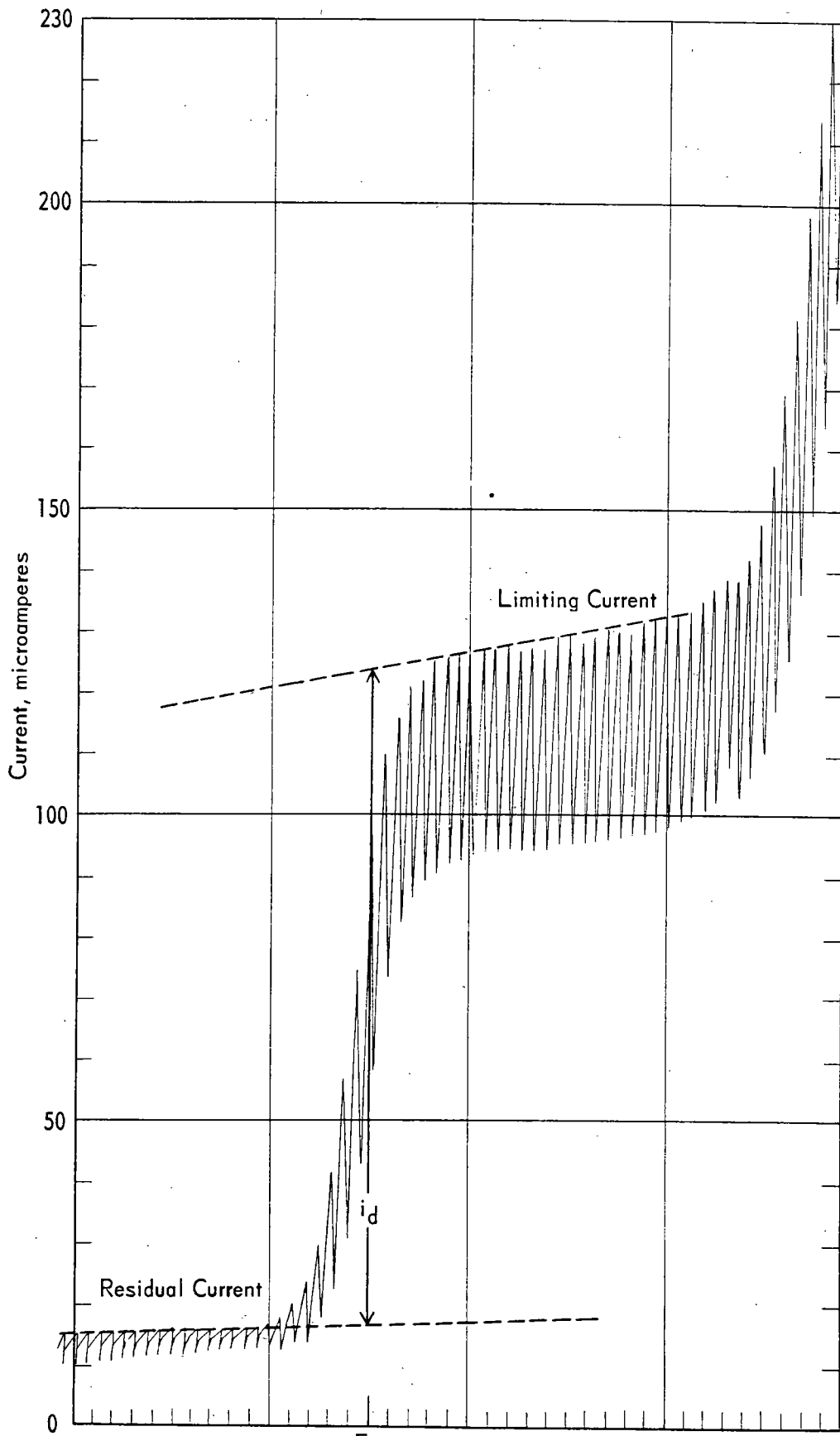
Kolthoff, I. M. & Lingane, J. J. Polarography, 2nd ed., Interscience Publishers, Inc., New York, 1952.

Müller, O. H., "Polarography" in A. Weissberger (ed.), Physical Methods of Organic Chemistry, 2nd ed., Interscience Publishers, Inc., New York, 1949, Part II, p. 1785.

Note: Müller's article in the 3rd ed. should be used. Exact reference is not on hand here.

NOTE ON ATTACHED GRAPH:

A graph similar to this, but not exactly this one, will accompany the monograph.



Potential, E versus S. C. E. volts
 Characteristic Wave Produced by a Reducible Species

Cumulative provisional list of additions for the
second edition of the International Pharmacopoeia (Ph.I.)

Acetazolamide
Acetazolamide tablets
Acetazolamide solium for injection
Adonis Vernalis herb
Aloe
Benzathine penicillin
Bendrofluazide
Bacitracin
Bemegrade
Benoxinate hydrochloride ophthalmic solution
Betazole hydrochloride
Betazole hydrochloride injection
Bethanechol chloride
Bethanechol chloride injection
Bethanechol chloride tablets
Busulfan
Busulfan tablets
Calcium disodium edetate
Calcium disodium edetate injection
Cetyl pyridinum chloride
Chloramphenicol palmitate
Chlormerodrin tablets
Chlormethin hydrochloride
Chlormethine injection
Chlorothiazide
Chlorothiazide tablets
Chlorpheniramine maleate
Chlorpheniramine maleate injection
Chlorpheniramine maleate tablets

Annex 2

Corticotrophine
Cyclizine hydrochloride
Cyclizine hydrochloride tablets
Cyclobarbital calcium
Cyclobarbital calcium tablets
Dextromoramide
Diaphenylsulfone
Diaphenylsulfone tablets
Diatrizoate sodium
Diatrizoate sodium injection
Dimenhydrinate
Diprophylline
Doxylamine succinate
Doxylamine succinate tablets
Edrophonium chloride
Edrophonium chloride injection
Ferrous gluconate
Fludrocortisone acetate
Fludrocortisone tablets
Gluthetimide
Glycobiarsol
Glycobiarsol tablets
Griseofulvin
Helium
Hydrochlorothiazide
Hydralazine hydrochloride
Hydralazine tablets
Hydrocortisone hydrogen succinate for injection
Hydrocortisone sodium succinate for injection
Iodipamide methylglucamine injection
Isoflurophate
Isoflurophate ophthalmic solution

Annex 2

Levallorphan tartrate
Levallorphan tartrate injection
Lucanthone hydrochloride
Lucanthone tablets
Mecamylamine hydrochloride
Mecamylamine hydrochloride tablets
Mechlorethamine hydrochloride for injection
Meclizine hydrochloride
Meclizine hydrochloride tablets
Mephentermine sulfate
Mephentermine sulfate injection
Mephobarbital
Mephobarbital tablets
Meprobamate
Meprobamate tablets
Meralluride
Mercaptomerin
Mercaptopurine
Mercaptopurine tablets
Methylene blue
Methylene blue injection
Methylergometrine maleate
Methylergometrine injection
Neomycin
Nitrofurantoin
Nitrofurantoin tablets
Noscapine
Novobiocin calcium
Novobiocin sodium
Novobiocin sodium tablets
Nystatin
Nystatin tablets

Annex 2

Papaverine sulfate
Paramethadione
Paramethadione capsules
Pentamidine isothionate
Pentamidine injection
Peppermint leaves (to give a generally applicable
method for the determination of volatile oil in
vegetable drugs)
Phenindamine tartrate
Phenindamine tablets
Phenoxymethylpenicillin
Phenoxymethylpenicillin calcium
Phenoxymethylpenicillin potassium
Phenoxymethylpenicillin tablets
Phenylbutazone
Phentolamine methanesulfonate
Phentolamine methanesulfonate for injection
Pholcodine
Phytonadione
Phytonadione tablets
Piperazine adipate
Piperazine adipate tablets
Piperazine citrate
Piperazine phosphate
Piperazine phosphate tablets
Piperocaine hydrochloride
Piperocaine hydrochloride injection
Polyethylene glycol 400 (suitable for injection)
Polyoxyl 40 stearate
Polysorbate 80
Pralidoxime iodide
Prednisolone

Annex 2

Prednisolone tablets
Prednisolone acetate
Prednisone
Prednisone tablets
Prednisone acetate
Probenecid
Probenecid tablets
Prochlorperazine dimaleate
Prochlorperazine dimaleate injection
Prochlorperazine dimaleate tablets
Procyclidine hydrochloride
Procyclidine tablets
Propantheline bromide
Propantheline bromide injection
Propantheline bromide tablets
Propylene glycol
Propylhexedrine
Psyllium seed (to give a generally applicable method
for the determination of the swelling factor of
vegetable drugs)
Pyridostigmin bromide
Pyridostigmin bromide tablets
Pyridostigmin bromide injection
Rhatany root (to give a generally applicable method
for the determination of tannins in vegetable drugs)
Reserpine
Senega root (to give a generally applicable method for
the determination of saponins in vegetable drugs)
Senna leaves
Senna pods
Sodium fluoride
Sodium levothyroxine
Sodium levothyroxine tablets

Annex 2

Sodium liothyronine
Sodium liothyronine tablets
Solasulfone (Solapsone)
Sulfacetamide sodium
Sulfamethoxypyridazine
Sulfamethoxypyridazine tablets
Sulfisoxazole
Sulfisoxazole tablets
Sulfisoxazole, Acetyl
Sulfisoxazole diethanolamine
Sulfisoxazole diethanolamine injection
Sulfoxone sodium
Sulfoxone sodium tablets
Testosterone cyclopentylpropionate
Testosterone cyclopentylpropionate injection
Testosterone enanthate
Testosterone enanthate injection
Thiamylal sodium for injection
Tolbutamide
Tolbutamide tablets
Trimethaphan camphorsulfonate
Trimethaphan camphorsulfonate injection
Warfarin sodium
Warfarin sodium injection
Warfarin sodium tablets
Zoxazolamine