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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on Wednesday, October 6th, 1948, at 7 p.m., at Gas Industry House, 1, Grosvenor Place, London, S.W.1. The President, Mr. Lewis Eynon, was in the Chair. The following papers were presented and discussed: "Ether Peroxide as a Possible Source of Error in the Röse - Gottlieb Butterfat Test," by M. M. Muers, Ph.D., F.R.I.C., and Miss M. A. House, B.Sc.; "The Standardisation of Hortvet Thermometers," by R. Aschaffenburg, Ph.D., and J. A. Hall, A.R.C.S., B.Sc., D.I.C.; "The Use of Reichert, Polenske and Kirschner Values in the Determination of Butterfat, Coconut Oil and Palm Kernel Oil," by K. A. Williams, B.Sc., Ph.D., F.R.I.C.

NEW MEMBERS

Victor Morris Bond, B.Pharm. (Lond.), Ph.C., A.R.I.C.; Francis Hereward Burstall, M.Sc. (Birm. and Lond.), A.R.I.C.; John Kenneth Eaton, B.Sc. (Wales), M.Sc. (Manc.), Ph.D. (Lond.), A.R.I.C.; Donald Michael Evans; Alan French, B.Sc. (Lond.), A.R.I.C.; Edward Cecil Harris, M.Sc. (Wales), A.R.I.C.; James Saunders, B.Sc. (Edin.); Herbert Kenneth Southern, M.Sc. (Manc.), A.R.I.C.; Thomas Martin Aitken Tudhope, B.Sc., Ph.D. (St. And.).

DEATH

WE deeply regret to have to report the death of George Rudd Thompson, Past President (1924–25).

NORTH OF ENGLAND SECTION

THE Eleventh Summer Meeting was held at the Balsfield Hotel, Windermere, from May 28th to 31st. The attendance was seventy-seven. The Chairman (Mr. C. H. Manley) presided, and among those present were The President (Mr. L. Eynon), Dr. Roche Lynch and Miss Roche Lynch, Dr. Hughes and Mrs. Hughes.

The Chairman welcomed the members, particularly those from the South.

On Saturday morning a paper was read by Dr. E. B. Hughes, F.R.I.C., on "Chemistry in the Kitchen." The paper was discussed and a cordial vote of thanks to Dr. Hughes was proposed by Mr. J. G. Sherratt and seconded by Mr. J. F. Clark.

A resolution expressing the greetings of the Section and declaring its loyalty to the Council and the Parent Society was proposed by Mr. R. K. Matthews and seconded by Mr. J. R. Walmsley.

On Sunday afternoon a tour of the Lake District was made by motor coach.

BIOLOGICAL METHODS GROUP

A MEETING of the Biological Methods Group was held in the rooms of the Geological Society, Burlington House, Piccadilly, W.1, at 6.30 p.m., on Thursday, October 21st, 1948, with Dr. A. J. Amos in the Chair. The subject of the meeting was "The Assay of Curare and of Curarimimetic Substances," and the following papers were read and discussed: "The Use of the Isolated Diaphragm," by G. A. Mogey, M.B., B.Ch., B.A.O., and J. W. Trevan, M.B., B.S., B.Sc., F.R.C.P.; "A Modification of the above Method," by G. B. West, Ph.D., B.Pharm., Ph.C.; "The Use of Anaesthetised Rats," by J. Raventós, M.D.; "The Use of the Conscious Mouse or Rat," by H. O. J. Collier, B.A., Ph.D.; "The Rabbit Head-Drop Method," by F. C. MacIntosh, M.A., Ph.D.

PHYSICAL METHODS GROUP

THE Sixteenth Ordinary Meeting of the Group was held at 2.0 p.m. on Saturday, October 2nd, 1948, at the Stork Hotel, Queen Square, Liverpool. The meeting was held jointly with the North of England Section.

Mr. C. H. Manley, Chairman of the North of England Section, was in the Chair when the first two papers were read, and Dr. J. G. A. Griffiths, Chairman of the Physical Methods Group, when the last paper was read. About eighty members and visitors were present.

The following papers were read and discussed: "Analysis of Rare Earth Oxides by means of Emission Spectra," by D. M. Smith, B.Sc., D.I.C., F.Inst.P., and G. M. Wiggins; "Determination of the Rare Earths, using the Intermittent Arc," by J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.; "The Chromatographic Estimation of Vitamin A in Whale Liver Oil," by N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson, B.Sc., Ph.D., F.R.I.C., and J. P. Savage, B.Sc., A.R.I.C.

Dr. W. F. Elvidge, Mr. J. Haslam, Mr. B. S. Cooper, Mr. E. van Someren, and Mr. F. J. Woodman contributed to the discussion on the first two papers and Dr. W. F. Elvidge, Prof. R. A. Morton, Mr. C. Daglish, Prof. T. P. Hilditch, Mr. H. E. McGrath, and Mr. J. P. Savage to the discussion on the last paper.

The Chairman thanked the authors of the papers for their contributions and Dr. J. R. Edisbury and Mr. Arnold Lees for making the local arrangements for the meeting.

MICROCHEMISTRY GROUP

A MEETING of the Microchemistry Group was held jointly with the Leeds Area Section of the Royal Institute of Chemistry and the Leeds University Chemical Society at Leeds University on Tuesday, October 26th, 1948. During the afternoon members of the Group visited various departments of the University, including the Brotherton Library, the Department of Coal Gas and Fuel, the Textile Department, the Chemistry Building and the Department of Bimolecular Structure. Members of the Group were entertained to tea in the University Refectory at the invitation of the Leeds Area Section of the Royal Institute of Chemistry.

In the evening a symposium on Microchemical Methods in Forensic Analysis was held in the Large Lecture Theatre of the Department of Chemistry. The Chair was taken by Mr. Norman Strafford, Chairman of the Group, and the following papers were read: "A General Account of Microchemical Methods in Forensic Investigations," by Dr. J. B. Firth, Director of the North-Western Forensic Science Laboratory (Home Office), Preston; "Microchemical Methods in Forensic Toxicology, with particular reference to Barbiturates," by Mr. G. E. Turfitt, Deputy Director, Metropolitan Police Laboratory; "Colorimetric Methods for the Detection and Estimation of Alkaloids and Relative Compounds," by Mr. E. Pedley, Home Office Laboratory, Preston.

Some Observations on Biological Standards

BY A. A. MILES, F.R.C.P.,

National Institute for Medical Research

(*An Address to the Biological Methods Group on April 11th, 1948*)

IN talking to this Society about biological standards and standardisation no one could fail to be conscious that the subject had already been thoroughly ventilated in an address given at the Annual General Meeting of the Society by Sir Henry Dale in 1939,[1] and by Sir Percival Hartley, my predecessor in the Department of Biological Standards at Hampstead, in his address to the Pharmaceutical Society in 1935 and in his Dixon Lecture in 1945.[2] Anything to be said about standards in Britain must be largely reiteration of the principles and practice developed by these two pioneers. However, I am not ashamed to develop a well-worked theme, because a great deal of what they insisted on needs insisting on still. Some of the concepts lying behind a biological standard are perhaps so simple that their implications are never examined by many who use biological standards—and in consequence they are continually flouted in practice. I shall, however, vary the story a little by examining, in some detail and at the risk of being very elementary, what these implications are and how they come to be misunderstood.

Although I am particularly concerned with the thirty-five International Standards that we hold for the World Health Organisation at the National Institute for Medical Research, and with a number of British Standards either derived from the International Standards or existing, in the absence of an International Standard, in their own right, what I wish to say is valid I think for all kinds of standards, from the preparations set aside by the solitary research worker to give himself a base line in his work, to the International Standards accepted for use throughout the world.

THE THREE AGES OF A STANDARD

At some time in the study of a biologically active substance that proves to be important either in research or applied biology, the need for a standard arises; and that standard, which is a reference point in the measurement of a biological activity, provides at this stage the only unequivocal measure of activity. Here we have the first of the three ages of a standard—when it is necessary; the other two are—when it is convenient, and when it becomes superfluous.

The necessary standard—Biology is full of hypothetical substances postulated as the cause of specific biological phenomena. Indeed, the invention of active principles often outruns the evidence and becomes plain word-spinning. Even in 1673 the habit was sufficiently notorious for Molière in his "*Malade Imaginaire*" to introduce a burlesque examination in medicine, where the examiner asks for the mode of action of opium, and the candidate replies: "I am asked by the learned doctor the cause and reason whereby opium puts people to sleep. To which I answer, because it has a soporific quality whose nature is to stifle the senses." And before we flatter ourselves to-day that we do not invent things like soporific principles unless there is a good reason for thinking that there are solid substances behind them, we might recall that something very like the old "vital principle" is still invoked to explain some features of living matter. Nevertheless, when some practical measure of an activity is required, we usually have a real substance to deal with as the vehicle of activity.

Since the biological effect is the *raison d'être* of the unknown substance, the first measure of activity must be in terms of the biological effect, *e.g.*, the paralysis of the neck muscles of the rabbit by curare-like substances, or the death of a culture of *Staphylococcus aureus* in presence of penicillin. Now, when we come to define a unit of activity, by analogy with chemistry and physics, we quite rightly attempt to describe the unknown in terms of the known; but here we come upon the first fallacy in the application of standards, and a major one. For if we decide to define a unit of the paralysing principle in curare as the smallest amount causing head-drop in the rabbit, we are describing the unknown, not in terms of the known, but only in terms of the familiar. Because except in the broadest way, we are quite ignorant of the causes that make one rabbit differ from the next in its response to the curare alkaloids. There is no such thing as a standard animal—a point I shall return to later.

When it is impossible to devise a suitable standard for the substance we must remain in the unsatisfactory state of adopting a biological system as our "known" and try to reduce its variability to a minimum; but since we do not know what variables are important, we must try to hold everything in the system as constant as possible. You will find monuments to this necessary state of affairs in many pharmacopoeias, where besides the desired qualities of the biological substance, there are listed precise and tedious descriptions of the technical minutiae that must be observed if the test is to be reliable. At its best, direct measurement of potency in terms of biological response is exemplified in the minimum requirements for Diphtheria Prophylactic[3]; at its worst, it may be little more than what Mr. Gridgeman has aptly labelled zoomancy, that is, "divination by observing the actions of animals." I am not suggesting that the attempts to standardise the biological reagents in an assay are useless; but they must never obscure the fact that such standardisation is only skirting round the problem of assay. A "standardised" inbred animal colony is often less variable in its response to a given substance than a randomly bred mixed colony, but its chief virtue is to make assay more economical; it can do little to reduce the equivocation in an assay which measures the amount of substance producing a given biological response.

Our first principle then, in seeking for some point of reference in the biological system that exhibits the effect we are interested in, is to choose, not the thing about which we have or think we have the most knowledge, but the thing we can keep stable most easily: and in most cases this will prove to be the substance we are studying.

At this point we may be held up while we find the best means of stabilising a preparation of the active substance. In general, the methods are variants of one theme, the removal as far as possible from the preparation of those environmental factors that are chemically active or promote chemical activity. That is, molecular oxygen, water, light and heat. In other words, dry the stuff, and keep it sealed under an inert gas in the dark and cold. This procedure is partly justified by extrapolation from short-term experiments. No one doubts, without reference to a standard more established than a control solution left on the bench, that vitamin C rapidly oxidises in alkaline solution. But are the slower deteriorations in a standard preparation necessarily slow-motion versions of the more dramatic ones? We can partly answer the question by subjecting the standard preparations to accelerated degradation tests—which consist mainly of exposing the material in a sealed container to light and relatively high temperatures for days or weeks, and then comparing it with the same material preserved in the dark, at −10° C. When successful, such tests are sufficient to justify adopting at least a provisional standard preparation. Another justification comes from analogy: experience of similar substances shows that potency is preserved in such preparations. Of the thirty-five International Standard preparations, potency is assigned to twenty-eight, and, in the experience of the last 20 years, these preparations, twenty-seven of which are held in the dry state, have maintained their potency unimpaired. On this basis we can predict with some confidence the success of preservation by these methods, particularly modern methods of drying from the frozen state at carefully controlled temperatures, which are more efficacious than the cruder methods used for the earlier standards. But the analogy is not enough; it immediately raises the question, how do we know that these preparations have lost no potency? For if a preparation has been preserved by the best method available, any test of maintained potency during storage must be in terms of the preparation itself, which is absurd, or of a similar preparation which may be equally subject to deterioration. Now the proper way out of this impasse is usually taken, but often by mere accident of numbers, and seldom as a clearly recognised necessity. We must at the very least copy the pre-Marconi navigator who, in the absence of a direct astronomical observation, takes his time from chronometers and checks any deterioration in their behaviour by having three of the best that money can buy. Let us be even more certain, and take six preparations; then, if a standard preparation always matches the potency of five other similar but different preparations preserved in the dark and cold at different institutes, it is statistically speaking far more likely that all six have maintained potency than that all six have declined, strictly in parallel, over a number of years. With many International Standards their repeated comparison with national biological standards constitutes a statistical check of this kind.

The convenient standard—Convenient standards may come into being directly, or develop from necessary standards. Here, we actually know enough of the constitution of the active substance to be able to characterise it fully by chemical and physical means, but the available methods are troublesome or prohibitively wasteful of time and money, and the biological tests more convenient. Penicillin is an excellent example of a necessary standard that has just graduated, in theory at least, into the convenient class. To take a crucial case: if we were required to prove in a court of law that a certain liquid contained penicillin, a chemical and physical proof might take months of work and without enormous preparatory labour be possible only in two or three laboratories in the world. Whereas the demonstration in the liquid of the same relative antibacterial activity against a number of bacterial species, and the same susceptibility to the enzyme penicillinase, as the International Standard, would probably constitute, in this country at least, an acceptable biological proof of the identity of penicillin. Most of the B vitamins and the steroid sex hormones for which standards have been established are in the convenient class, though some have already reached the desired end and become largely superfluous.

The superfluous standard—Standards become superfluous for two reasons: either the active principle is purified, and perhaps synthesised, and becomes readily definable by chemical and physical means like some of the B vitamins; or it ceases to be interesting.

Thus, the International Standard for arsphenamine is scarcely used to-day because neoarsphenamine and sulpharsphenamine have largely replaced arsphenamine in medical practice; the pneumococcal antisera and staphylococcal α-antitoxin are in a similar, though perhaps less deserved, eclipse because the sulphonamides and penicillin have for the time being superseded them. I shall say something about criteria of superfluity at a later stage.

THE ESTABLISHMENT AND EXPLOITATION OF STANDARDS

Suppose that we have proved that a reasonably stable preparation of the active substance can be made. The standard may still be unfit for general use. It may be too precious or too scarce to be distributed in the dry state to all users. Thus the crystalline all-*trans*-β-carotene which is the International Standard for vitamin A demands of its users that they have micro-balances for weighing out small quantities and the facilities for dissolving it in oil at low temperatures under a canopy of an inert gas—otherwise the resulting solution will be inaccurate, and, if more brutal methods of solution are used, it will contain other, less active, isomers of β-carotene. To avoid this, the vitamin is dispensed in standard solution. The pre-war standard solutions were made in coconut oil, which had a long period of induction, in that the natural anti-oxidants of the oil, which protected the β-carotene from oxidation, took some time to deteriorate in cold storage. During the war the only coconut oil obtainable proved to have a short induction period, and the standard solutions rapidly deteriorated until new standard solutions in arachis oil were adopted. When difficulties of this kind are solved there is no reason to delay setting up a standard. Nevertheless, the matter is sometimes postponed in the hope that the active principle will be isolated in a still purer form—a form which would clearly be better for a standard preparation. This, though laudable, is sometimes misguided, and ignores the very *raison d'être* of the establishment of biological standards: as a good deed shining in a naughty world of impure substances; it is often better to have a grossly impure standard than none at all.

Several preparations of active material may be incorporated in the standard, provided that they are thoroughly mixed before the pool is distributed into the containers in which it will be finally stored. And, quite apart from any preliminary tests on the ingredients before the final dispensing, the definitive tests of the standard must be made on the final preparation, after all manipulation and processing is finished.

The size of the batch depends on the scarcity of the substance and its purpose. The batches of International Standard penicillin and the British Standard tubocurarine, for example, are relatively small, because at the time of preparation both were expensive substances. The upper limit of size is determined mainly by the mechanical difficulties of preparing a large homogeneous batch and dispensing it in one operation. For example, in setting up standards for the A and B human blood group antisera the size of the batch is limited by the capacity of the centrifugal freeze-drying machine at our disposal, since it would be risking variation within the preparation to dry it piecemeal. Again, for the third International Standard for digitalis, which we are in process of making, we need about 3000 ampoules, each containing 3 g. of powdered digitalis leaf, *i.e.*, 10 kg. . But since this standard will be used by various nations for establishing national standards—a lengthy and in these days of scarce laboratory animals a difficult process—we are blending several samples of leaf to make a batch 10 times this size. All the care expended by seventeen collaborating laboratories in various countries in assaying this new preparation in terms of the second International Standard will characterise 100 kg.; and after 10 kg. has been set aside for the third International Standard, 90 kg. of identical potency will be available for national standards. Since powdered digitalis leaf is cheap and abundant, the limit here is set by the difficulty of making a homogeneous blend of more than 100 kg. of powder.

On an international level this plan of making huge standard preparations could clearly be adopted whenever possible, as a saving of time and a means of increasing precision in biological standardisation throughout the world. With scarcer substances, however, we shall have to abide by the usual practice of requiring that the International Standards are used for determining the potency of sub-standards, which then become current for assays within the nation or the laboratory concerned.

The next step depends on the purpose of the standard. It may be a standard, like our proposed British Standard for British Anti-Lewisite (B.A.L.), for an undesirable activity (*i.e.*, as a standard in a toxicity test) or for a desired activity. Toxicity standards, and sometimes potency standards (*e.g.*, neoarsphenamine and sulpharsphenamine) may be intended only on maximum or minimum levels. Thus, no neoarsphenamine sold under the Therapeutic Substances Act may be more toxic, or less potent in curing an infection of mice with *T. equiperdum*, than the International Standard. No *measure* of potency is required, and in such a case there is no more to be done than issue the standard with recommendations about its use. But where the potency is to be measured, as with an antitoxin, or insulin, or digitalis, a unit is required.

THE USE AND ABUSE OF UNITS

In the jargon of biological standardisation a unit is the specific biological activity contained in a given weight of the standard preparation, and in the biological and particularly the medical field its meaning should be confined to that. Those biological standardisers who use the word in a more dubious fashion seem to imagine, like Humpty Dumpty, that when they use a word "it means just what they choose it to mean—neither more nor less"; and would extend its meaning from the one I have given you to cover measures like "the amount required to bring about such and such a result." But my definition is legitimate by descent, being a justified extension of the eighteenth-century usage which defines it as any determinate quantity, dimension, etc., adopted as a standard of measurement. I have already discussed the logic of inventing such things as cat-units for digitalis, rabbit-units for insulin and, to quote a more modern instance, man-units for the anti-anaemia principle in extract of liver. But whatever the validity of the logic, the use of such units implies an attempt to measure an unknown in terms of a familiar but uncomprehended complex of variables. The practice is to be deprecated for other reasons than that a potency would thereby be imperfectly or equivocally measured. The biological unit of activity has an honourable lineage, being exemplified in over a score of International Standard preparations, and in many other reputable standards in many countries of the world. Consequently, to define a unit that is not properly determinate is to steal prestige for something that at best is a general description or a minimum requirement; to bring the valid units into disrepute; and to hide the real problem of standardisation by pushing it into another field, namely, the problem of the standard cat, or rabbit, or man. Even Plato, though he would have relegated the performance of biological assay to the slaves' quarters, had the root of the matter in him. He would, I think, have said that because individual cats differ so much from one another the only real cat with changeless determinate qualities was one abstracted from the imperfect cats in the sensible world and laid up in heaven as a reference and goal. From the point of view of biological assay, it is precisely thence we should relegate the determinate cat and all other determinate animals, together with any units based directly on them.

Of course, the size of the unit must be related to a particular degree of biological activity. The unit just recommended for international adoption for Old Tuberculin corresponds to 0·1 ml. of the 1/10,000 dilution of the International Standard Old Tuberculin, because that has been a convenient effective minimal dose for testing hypersensitivity in tuberculous persons in Europe and North America. It does not, however, follow that all the persons reported as sensitive to minimally effective doses of tuberculin, wherever and whenever prepared, have been sensitised to the same degree; but the establishment of an international unit will make it possible for all workers to characterise such sensitivity in terms of a defined unit and of fractions and multiples of that unit.

The unit, however, has no *necessary* connection with a useful dose of a biological substance; the assayist is usually first in the field and the unit is usually determined for his convenience. We have, for example, the penicillin unit which, devised in terms of a small volume of a culture of staphylococci, is grossly and irritatingly disproportionate compared with a curative dose in man. The assayist's unit is usually much smaller than the amounts effective in therapy. But since the unit is primarily intended to convey the activity of the standard preparation, there is no good reason for increasing the size of an established small unit to make it commensurate with an average dose of the substance. Either assayist or user has to have a clumsy scale of measures; and because it is less awkward to talk in tens of thousands than in phrases like "point nought nought nought this and that," the range of potency values in common use is best fixed well above the decimal point. If we made the penicillin unit, for clinical convenience, equal to 100,000 of the present international unit, the assayist would have to talk of batches of penicillin with potencies, say, of "0·0096 u./mg." The clinician, though he grumbles, is not disproportionately unhappy with his tens of thousands and his millions; and he gets some, but not much relief, from prefixes like *mega*. Unfortunately, both the international prefixes indicating multiplication, namely, *kilo* and *mega*, by reason of their terminal vowels, combine badly with *unit* by reason of its long initial vowel. *Mega-unit* is an awkward mouthful. It is true that precedent allows us to drop the terminal vowel in *mega*, but *megunit* is not much pleasanter. *Kilo-unit* is also awkward; and even if custom allowed us to omit the "o," many English-speaking doctors would rather let a patient infer that he was seriously in need of heroic dosage by ordering 10,000 units of something, than raise sinister visions of euthanasia in his mind by prescribing 10 "kilunits."

THE ASSUMPTIONS UNDERLYING THE USE OF A STANDARD

I now turn to the assumptions that must be made about a standard preparation if any validity can be assigned to the result of an assay. Some of them may sound dubious and perhaps unjustifiable for preparations whose establishment is based on ignorance rather than knowledge. Nevertheless, I hope you will find them acceptable if I insist at the same time that the assumptions must at all times be subject, *not* to doubt, but to experimental test.

(1) *The standard preparation is preserved so as to maintain its full activity.* This I think is unexceptionable, and I have already discussed the evidence needed to make it acceptable.

(2) *The preparation contains as impurities neither material having specific activity resembling that of the active principle, nor any substance synergic with it.* This is a very tall order, especially when we consider witches' brews like crude pituitary extract as a standard for pituitrin, or 0·1 ml. of lemon juice as the early standard for vitamin C. As an example of a preparation containing similar, active substances, we have the proposed International Standard agglutinating antisera for the A and B blood groups. Here native sera from human donors are pooled. The anti-A sera contain a small amount of antibodies to the B blood group substance, and the anti-B sera a small amount of A antibodies; but both standards will be specified for use in concentrations below the threshold agglutinating power for the minor antibody.

(3) *The biologically active material is homogeneous.* Here again the assumption strains our credulity. The likelihood that either of the assumptions (2) or (3) is true will depend on the sort of substance we are dealing with, but whatever its sort, this likelihood will never on *a priori* grounds be impressively great. Moreover, we know of substances, such as penicillin, which were demonstrated to be heterogeneous after the establishment of international biological standards.

Nevertheless, if the standard is to be useful these two assumptions must be made and can be questioned only on positive evidence. There is no room for that engaging but useless form of argument known as the "appeal to ignorance." We have already met it in the argument for deferring setting up a standard until the active substance has been further purified, on the grounds that there *might* be other substances present that are similarly active or at least synergic. There might, but no one is likely to discover them unless the assumption that there are none is proved *quantitatively* wrong; and that cannot happen until a standard of some sort is established. There may be more things in the standard preparation than are dreamt of in our philosophy, but the speculation, as pure speculation, is best confined to the battlements of Elsinore; it has no place in biological assay.

(4) It follows from (3) that *whatever biological system is used for comparing test substance with standard, the ratio of activities in terms of units determined by the specific response of that system will remain constant.* That is, when test and standard are compared, it does not matter what living organism or part of a living organism is used, the specific potency of the test preparation will always prove to be the same.

This assumption underlies the policy adopted whenever possible in this country in official requirements for biological assay. No method of assay is obligatory; some are recommended, and assayists are expected, if they use others, to devise methods at least as good. Each worker is free to develop his own methods, in which he will clearly be competent and therefore more likely to get a better answer than by the use of an imposed standard method. Indeed, an ideal, universal and sufficient specification for all biological substances for which standards were established would read "its potency shall be measured in terms of the standard preparation in such manner that limits of error of the determination, calculated from the result of the assay, are not more than x per cent." Such latitude in method is not the practice in all countries; and the imposition of an obligatory method as well as a standard is often upheld on the ground that unless methods are specified, grossly discrepant results are obtained. Now this is quite true, and such discrepant results often dictate a return to the next best thing—definition by "minimum requirements." But it must be clearly recognised that if this is proved to be the case, it is the standard, and not the validity of the assumption, that is at fault. The proper remedy for reintroducing the unknown variability of the experimental living organism or tissue into the specification is an early revision of the defective standard.

If, on the other hand, an authority puts the cart before the horse, and retains obligatory methods while it is introducing standards, there will be less chance of discovering heterogeneity of the standard than by allowing a free choice. The substitution of *B. subtilis* spores for *S. aureus* in the assay of penicillin in pursuit of that will-o'-the-wisp, the standardisable

organism, led, through the discovery of variable *aureus/subtilis* ratios, to the recognition of the heterogeneity of penicillin. The discovery that cod-liver oil was much more potent than irradiated ergosterol in promoting the calcification of bone in chickens led to the recognition of a vitamin D in cod-liver oil that differed from calciferol. The difference is practically immaterial in the rat assay of oils for vitamin D for human use, because rats and men use both forms equally well. But for the chick, D_2 is clearly less effective than D_3. The present calciferol standard is therefore inadequate, and we are at present in the preliminary stages of establishing an International Standard for vitamin D_3.

Again, the testing of tetanus antitoxins against different preparations of toxin has revealed a heterogeneity for which at present there is no adequate explanation. In such circumstances we can do little to improve matters except specify things other than the standard. Heterogeneity also mars the digitalis standard. We know there are various mixtures of glycosides in extracts of digitalis leaf, and that frogs, guinea-pigs and cats, to name three orthodox test animals, differ in their response to various preparations of tincture. Here the preference of many physicians for mixed glycosides in therapy, rather than for single glycosides, makes replacement of the standard by a number of glycoside standards impracticable; nor can we, as we do at present with the D vitamins, choose for assay the animals that most nearly represent those for which the stuff is intended, because some patients seem to behave towards digitalis like frogs and some like cats. In any event, the choice of similarly responsive animals for assay is not to be elevated to a principle, for like other violations of my assumptions, it postulates heterogeneity.

(5) My next assumption, which is really a variant of (4), is that *the active substance assayed is the same as the substance in the standard.* If it is not, heterogeneity will be revealed in one way or another, and should be remedied. It is not always: for example, the Rideal-Walker assay of disinfectant action has in the years since its devising been subject to all kinds of revision, devoted mainly to standardising everything but the substances to be assayed. Phenol has had to serve as a standard for all manner of disinfectants and mixtures of disinfectants, each with a different rate of killing the typhoid bacillus, a different concentration coefficient and a different temperature coefficient; potency is then determined from an arbitrarily selected total killing end-point at a fixed temperature. The result of this attempt to fit every disinfectant into the Procrustean bed of phenol is a series of comparisons, some of which are about as valid as giving a numerical value to the relation of chalk and cheese, because about 40 per cent. of their spelling is similar.

(6) My last assumption should have come first, but I deal with it here because it leads conveniently to my next point. *The biological activity cannot be adequately measured by chemical or physical means.* The operative word is "adequately." If chemical and physical methods are grossly inadequate the standard is necessary. When they do become adequate enough the standard may be degraded to one of convenience and later to superfluity.

DISCARDING AND ESTABLISHING STANDARDS

One of the functions of the custodian of biological standards is to fulfil his own natural desire, in the light of their increasing numbers, to discard as many standards as he can as being superfluous, and to combat everybody else's natural desire to do so lest the chemists' and physicists' belief in purity and definability outruns the evidence.

The only dependable criterion of superfluity is that a biological test is no longer necessary in turning out a routine preparation of the substance with full confidence that its biological activity will be as invariable as its chemical and physical properties.

It is not enough that the substance has been prepared in the pure state; for one can make reasonable assumptions of purity without really knowing the precise constitution of the substance. This is at least true of biological substances of large molecular weight; and, as Pirie[4] has pointed out, many of the criteria of purity, such as crystallinity, constant composition after constant techniques of purification, electrophoretic or other physical homogeneity, do not necessarily guarantee purity in the sense that a synthesisable substance of low molecular weight is known to be pure. Moreover, if we consider those biological substances of high molecular weight that are reputed to be pure—some bacterial toxins, some enzymes, proteins like insulin—a high, maximum and constant degree of biological activity is often the most forceful evidence of purity that can be adduced. In the near future it is only standards of substances of low molecular weight, say below 5000, that are likely to become superfluous. Even here we must temper the wind to the shorn lamb,

especially when we deal with International Standards; for in many countries of the world the biological test may remain far more convenient than the chemical or physical tests.

Not only standards, but units also need defence against premature discard. Nowadays we have the "milligram-equivalent," which presumably is intended to be more accurate or informative than the word unit, while not claiming the definitive status of plain "milligram," and to indicate that the active substance so designated has been isolated in the pure state. No one objects to designating activity in milligrams, if the active substance is single (*i.e.*, not a member of a family of similar substances) and its constitution is known. But the milligram-equivalent makes the worst of it, both scientifically and verbally. Verbally, because it substitutes seven syllables for two, and has no valid meaning that is not expressed by the term "unit"; and scientifically because it introduces a complication into the use of standard preparations of varying degrees of purity. For to each standard or sub-standard preparation must be assigned its own milligram-equivalent and the results of all assays are therefore subject to an appropriate conversion factor. Whereas, with units, a given weight of the standard preparation as a whole is the unit, and all assays in terms of different standard preparations are directly comparable.

In this ramble through the subject of biological standards I have left out many important things. I have not discussed the problems, which are instructive and urgent, of standardising antigens, and particularly virus antigens that depend for their efficacy on the maintenance of the virus in a state of suspended animation; in any event, there is at present little that can be said about them. I must also neglect many interesting statistical problems; in any event there is little profitable that I am qualified to say about them.

Most of my observations have been trite and familiar, but they are I think worth making again; and particularly those on the simple assumptions that underlie the use of a biological standard. These are fundamental, and I hope I have put them with the necessary conviction; in spite of the fact that, as some of you will realise, they are violated in one way or another in our current practice with many of the biological standards established to-day.

As a custodian of standards, and as one of the midwives who attend the birth of standards on a national and international level, I should perhaps sum up in the form of advice to those about to make standards. I cannot achieve the brevity of Mr. Punch on an analogous occasion in his "Advice to Those about to Marry," which was "don't." It would not be good advice; for in the biological field, standards should be set up whenever possible to avoid the pitfalls of attempting unnecessarily imperfect assays directly in terms of biological effects.

(*a*) Start early; even pioneer research is the better for a point of reference for the activity of the substance investigated.

(*b*) Preserve the preparation by the best means you know, and establish its stability as soon as possible by comparison with other preparations similarly preserved.

(*c*) However pure you think the standard preparation is, if you do not *know* the chemical constitution of the substance or if biological activity is still an essential part of its definition, devise a provisional unit, and stick to it, remembering that a unit is the activity of a given weight or volume of a standard preparation.

(*d*) Set aside sufficient standard preparation to ensure continuity of the standard when the first preparation runs out: "sufficient" means enough for an extensive test to establish the unitage of a new preparation in terms of the old within reasonable limits of error.

(*e*) Lastly, think of your standard as a possible candidate for national or international recognition, and follow the precepts, upon which I have in fact based this talk, laid down in the past by the Permanent Commission on Biological Standardisation of the League of Nations Health Organisation, and by those who have worked with it.[5,6] By the time a standard comes up for international recognition, very often all that international agreement can do is to recognise a *fait accompli*; and it is a kindness to these harrassed international bodies, and to all subsequent users of the standard, to see that your accomplished facts are from the beginning fit for universal adoption.

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Discussion

Dr. J. O. C. Collier asked whether Dr. Miles thought that there ought to be a standard of pyrogenicity.

Dr. Miles replied that while it was desirable to have such a standard, so many different types of material were pyrogenic that it was difficult to say what kind of standard should be set up.

Mr. A. L. Bacharach suggested that each laboratory should set up its own internal standard of pyrogenicity using, if necessary, a different substance for each class of material tested. For example, in the testing of penicillin, the standard should itself be a penicillin, and so on. He went on to point out that there was another method for checking the stability of a standard preparation besides the device described by Dr. Miles of having half-a-dozen separate standard preparations and assaying them against each other from time to time. If the mean response of the experimental animals to a given dose of the standard, averaged over a long period of successive assays, remained the same year by year both within the same laboratory and between different laboratories, then this was strong presumptive evidence that the potency of the standard had not varied.

Mr. H. C. H. Graves emphasised the danger of abandoning prematurely the international unit, based on biological assay, in favour of units of weight. In the Labelling of Food Order, 1944, manufacturers were called upon to declare the vitamin B_1 content of a food, not in i.u. but in mg. This might sometimes lead to the consumer being seriously misled if the amount declared was in a form that was not available when ingested. Certain live yeasts, for instance, although containing appreciable amounts of vitamin B_1, nevertheless acted as depletors of this vitamin when consumed by mouth. Biological assay of such yeast would indicate the true position—that they were actually negative sources of the vitamin.

Dr. E. C. Wood pointed out that another aspect of the same question was to be found in the use of an International Standard of carotene when assaying vitamin A. One i.u. of carotene was by definition equivalent to 0·6 mg., but what weight of crystalline vitamin A acetate should be regarded as equivalent to 1 i.u. was at present uncertain. What did Dr. Miles think about this?

Dr. Miles replied that this difficulty would be met in due course by the provision of two International Standards, one of carotene, as at present, and the other of vitamin A acetate.

Mr. N. T. Gridgeman asked if there was any point in maintaining a carotene standard once a vitamin A standard had been established. Carotene could be determined by physico-chemical methods without recourse to biological assay at all.

Dr. Miles replied that there was some demand for a standard carotene preparation for use in calibrating such instruments as spectrophotometers, and it was also required by agricultural analysts.

Mr. Bacharach deprecated the practice in some pharmacopoeias of making it compulsory for manufactures to assay certain materials biologically and to lay down the method by which this should be done. The practice of the British Pharmacopoeia was far better; the potency of biologically active substances is defined, and it is therefore implied that *if* a biological assay is carried out on the material it will be found to have the potency laid down in the monograph. It is left for the manufacturer to decide whether he will in fact standardise the materials he sells by biological assay, or whether he will use other methods.

Dr. Miles said that the principle of demanding that a substance would have a certain potency *if* tested, instead of making a potency test obligatory, might be usefully applied, but that it might be difficult to reconcile it with the principles at present governing regulations in this country.

Mr. S. A. Price asked Dr. Miles's opinion on the question raised by Mr. Graves, that there might well be a large difference between the total amount of a vitamin in a certain material and the available amount.

Dr. Miles replied that comparative assays in terms of a standard were not primarily concerned with the availability of the active substance in other conditions; and that maximum availability was presumably assumed in a statement of potency.

Dr. Wood pointed out, however, that the method of assay and the species of animal to be used were not laid down, and it might well happen, therefore, that in assaying a specimen of yeast against the International Standard of vitamin B_1, the result obtained if rats were used might be very different from the result if, for example, dogs were used. It would appear that there might thus be some ground in certain cases for specifying at least the species to be used for the biological assay, if not the technique of the assay itself.

A somewhat lengthy discussion followed on this matter, and it was generally agreed that the difference between total and available content involved some difficult problems.

Mr. K. A. Brownlee referred to the report that minute traces of cobalt had a marked effect on the potency of penicillin, and enquired what effect this would have on the International Standard for penicillin.

Dr. Miles replied that if the report was confirmed it might be necessary to modify the standard. It would not be justifiable to add cobalt to the standard, since there did not appear to be any fixed chemical relations between the penicillin and the cobalt.

The Use of *Neurospora Crassa*, Mutant 9185, for the Assay of Aneurine

BY J. S. HARRISON AND E. J. MILLER

(*Read at the Annual General Meeting of the Biological Methods Group, December 16th*, 1947)

TATUM AND BELL[1] have published details of a biochemical mutant strain of the mould *Neurospora crassa*, which requires aneurine for growth. This mutant, number 9185, appeared to be a suitable organism for the microbiological assay of aneurine, as the authors showed that it responded specifically to the complete molecule of aneurine, the thiazole and pyrimidine moieties, alone or together, being incapable of replacing aneurine. We have confirmed this point but the organism has behaved erratically. An account of attempts to evolve a satisfactory assay is given below.

EXPERIMENTAL

Method—The assay method was basically that described by Horowitz and Beadle[2] for the determination of choline by means of *N. crassa*, mutant 34,486. The mould was maintained by weekly transfers on malt - agar slopes and allowed to sporulate. The spores (conidia) were suspended in sterile saline and 1 drop of the suspension was used to inoculate 10-ml. quantities of medium containing the necessary concentrations of standard aneurine or the test substances in 50-ml. Erlenmeyer flasks. The solutions were steamed for 30 minutes before inoculation. In apparatus cleaned with chromic acid, this form of sterilisation was found in practice to be adequate, excessive heat treatment being undesirable at the *p*H of the medium (*p*H 4·9) on account of the relative instability of aneurine at high temperatures. The flasks were incubated for 5 days at 28° C., and the mycelium was then filtered off, with suction, on to small filter paper circles in Gooch crucibles, rolled into small pellets, dried at 105° C. for 2 hours, cooled and weighed.

The composition of the medium used in the original experiments was exactly as used by Horowitz and Beadle[2] for the "cholineless" mutant. The medium was made in concentrated form, five times the final strength, to allow the addition of standard and test solutions.

Standard curve—The levels of aneurine used to prepare the standard curve were 0·05, 0·1, 0·2 and 0·4 μg. per flask, and a linear relationship was found to hold between the logarithm of the response and the logarithm of the dose of aneurine. Before test samples were examined, the reproducibility of the standard curve was investigated and several difficulties were encountered. (1) There was poor agreement between replicate flasks at the same aneurine level, often more than 10 per cent. spread. (2) When the doses of aneurine were plotted against responses the points did not lie on a regular curve. (3) About one in seven of the flasks containing low levels of aneurine, including the controls without aneurine, showed considerably more growth than the other similar flasks, in some cases approaching maximal. These exceptional growths are referred to below as "high blanks." It is interesting that the original curves shown by Tatum and Bell[1] to illustrate the growth response of four *N. crassa* mutants, have similar erratic points which do not fall on a regular curve; it is possible that the bad agreement is attributable to the same cause as the "high blanks."

Various factors were examined in an attempt to overcome these difficulties. The three main factors considered were the inoculum, the medium and the stability of aneurine on heat sterilisation. In case the original culture was not a pure strain, and the "high blanks" were caused by contamination with another organism, single conidia were isolated by means of the micro-manipulator and cultures were grown from them on malt agar slopes. After sporulation, conidia from these cultures were used to inoculate an aneurine-free medium, but the same proportion of "high blanks" was obtained, showing that the property was an inherent characteristic of the individual conidium. No attempt was made to isolate ascospores in order to study the segregation of the mutant gene. This would involve crossing with a strain of opposite "sex." Variation of the number of conidia used for the inoculum from 3 to 40,000 did not significantly affect the proportion of "high blanks," nor did it matter whether conidia or vegetative mycelial cells were used. When the inoculum introduced into aneurine-free medium was reduced to one conidium by using conidia selected by micro-manipulation, the proportion of "high blanks" remained the same although the effect took several days longer to develop. When good growth had taken place in medium containing

no aneurine, conidia taken from this growth and used as inocula for further tests in aneurine-free medium all grew well, indicating a persistence of the adaptation. As it was thought that adaptation might be responsible for the condition that produced the "high blanks," the agar medium on which the inoculum was grown was modified by reinforcing it with aneurine, using 3 μg. per ml. This amount, being additional to that in the malt extract already in the medium, represents a considerable excess of aneurine over the usual needs of the organism. "High blanks" persisted with similar frequency when inocula were prepared from cultures grown on this medium.

In case the liquid medium was not suitable for stable growth the *p*H was varied, standard curves being prepared over a *p*H range 3·5 to 4·9. The more acid *p*H suppressed growth but did not eliminate erratic figures. The medium was also modified, without effect on the "high blanks," by addition of inositol, calcium pantothenate, nicotinamide, casein hydrolysate, cystine and tryptophane and by replacing the sucrose by glucose.

A further possibility was that the conditions of sterilisation might affect the aneurine in different flasks unevenly. To test this a homogeneous sterile solution of aneurine was in some experiments added aseptically after sterilisation of the medium, but the agreement of the replicates was not improved.

A study of the results shows that in 200 flasks containing no aneurine the incidence of "high blanks" is 15 per cent., and the percentage does not differ significantly from this at levels of 0·02 and 0·05 μg. of aneurine per flask, that is, on about the first quarter of the standard response curve. At 0·1 μg. the percentage has fallen to 4, and at higher levels than 0·05 μg. the occurrence is less frequent until, at the highest levels, a high value cannot be distinguished from a bad replicate. At the same time, as the levels of aneurine increase, the excess of the higher figure over the normal decreases, and it is reasonable to suppose that with optimal or excess aneurine the occurrence of high values is practically nil, otherwise it would be impossible to maintain a culture of the mutant which retains its original properties. It is indeed remarkable that the culture has retained its properties through many generations, as it seems quite possible that one or more cells may develop the capability of growing without aneurine. In such event the culture could not be used for aneurine assay, as the modified cells would outgrow the others.

Assay of test samples—In spite of the rather discouraging erratic behaviour described, some assays were carried out on natural products, concentrating on one batch of dried bakers' yeast of which the aneurine content had been determined by other methods. The yeast was first extracted by standard techniques as used for other aneurine assays, using mild acid treatment followed by enzymatic digestion with takadiastase. Dilutions from the treated yeast were tested at approximately the same levels as those used for the standard curve. In view of the vagaries of the organism, five or six replicate flasks were used at each level. The number of micrograms of aneurine corresponding to the average weight of mycelium at each level, neglecting obvious "high blanks," was read off from the standard curve and the aneurine content of the sample was calculated. The mean of the values at the various levels was taken as the final result. No consistent drifts were observed, and the average aneurine content obtained by this method agreed well with those determined by the thiochrome and yeast fermentation[3] methods. As a linear "log to log" relationship has been shown to hold between response and aneurine level, a method of computation by potency ratios, based on the parallel linear responses of test sample and standard when their logarithms are plotted against the logarithms of the doses can be used for routine assays.

Results on the dried yeast test sample were 15·7 μg./g. for the *N. crassa* assay, 14·6 μg./g. for the thiochrome method and 14·7 μg./g. for the yeast fermentation method. These values were the means of 9, 6 and 7 separate assays respectively. The 95 per cent. limits of error of the three methods were $\pm$4·9, $\pm$1·7 and $\pm$7·0 μg./g. respectively, that is to say, there is a nineteen out of twenty chance that any single assay will have an error less than these figures. There is no significant difference between the mean values obtained by the three methods of assay.

Comparison between the N. crassa *assay for aneurine and the* N. sitophila *assay for vitamin* B_6—To determine whether poor replication was caused by faulty technique, results obtained by the *N. crassa* assay for aneurine were compared with results of vitamin B_6 assays using *N. sitophila*, where the technique employed was almost identical. The *N. sitophila* test showed much better internal agreement between flasks. The standard error in the weight of mycelium in individual flasks in the aneurine assay was 2·3 mg. compared with 0·4 mg.

for the B_6 assay. Thus the test with *N. sitophila* gives much better internal agreement than the *N. crassa* method. On the other hand, the agreement between separate assays is of the same order for both assays. This comparison between similar tests for vitamins B_1 and B_6 is an example of our general experience that microbiological assays for aneurine behave much more erratically than similar tests for other vitamins. This fact is reflected in the large number of suggested ways of testing microbiologically for aneurine, none of which appears to be entirely satisfactory, at any rate if a specific test for aneurine is required in contrast to an assay that also includes as aneurine the thiazole and pyrimidine moieties.

Conclusion

It is concluded that the assay described, although unsatisfactory as regards internal agreement, provides a useful confirmatory test for determining vitamin B_1 in the absence, as far as we are aware, of a more satisfactory specific microbiological technique. Providing certain precautions are taken, the precision of the method is comparable with that of other microbiological assays.

Summary

An aneurine-requiring mutant of the mould *Neurospora crassa* has been used for the microbiological determination of aneurine. Internal agreement within the assay is poor, but results on a sample of dried bakers' yeast are close to those obtained by other methods.

The authors thank the Directors of the Distillers Company Ltd. for permission to publish this paper.

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The Rapid Micro-Analytical Determination of Carbon and Hydrogen in Organic Compounds*

By A. F. COLSON

Introduction

In routine micro-analytical work there is a growing demand for a more rapid method of determining carbon and hydrogen, and other elements, in organic compounds than is provided by the classical method developed by F. Pregl.[1] Before the development of the procedure described in this paper, the author had made repeated and unsuccessful attempts to produce a more rapid method by modification of the Pregl apparatus and procedure. These attempts were abandoned on the appearance in the literature of a rapid method devised by R. Belcher and C. E. Spooner,[2] based on the combustion of the organic sample in a rapid stream of oxygen (50 ml. per min.) in an empty silica tube at 800° C. In this method halogens and sulphur are retained on heated silver gauze, and oxides of nitrogen are absorbed in a sulphuric acid solution of potassium permanganate or potassium dichromate. The time required to burn the sample and sweep out the combustion products is only 10 minutes, compared with the 40 minutes required by the Pregl method. This attractive method was subjected to careful and prolonged trial and the conclusion was reached that its range of application was too narrow for the requirements of general routine analysis. The rapid method subsequently developed by the present writer appears to be applicable to the analysis of a wide range of organic compounds containing nitrogen, halogens and sulphur in addition to carbon and hydrogen. The method depends upon combustion of the sample in a rapid stream of oxygen (40 ml. per min.) in a silica helix heated at 800° C. Oxides of nitrogen are absorbed by heated lead peroxide, and halogens or sulphur are retained on heated granular

* This paper will be read and discussed at the Annual General Meeting of the Microchemistry Group on January 28th, 1949, and a report of the discussion will be published afterwards.

silver. The time taken to burn the sample and sweep out the products of combustion varies between 10 and 15 minutes according to the nature of the compound.

The method has been tested by analysis of various pure organic compounds and has been satisfactorily used as a routine procedure for at least one year. Since the rapid method is still regarded as being on trial, the standard Pregl procedure is retained as a referee method.

The apparatus

The complete combustion train, depicted in the figure, comprises the following components.

1. *A White - Wright*[3] *flowmeter.*

2. *A pre-heater* supported in an electrically heated tube-furnace, A. It is constructed from clear fused silica tubing and is packed with wire-form copper oxide held in position by asbestos plugs. The body of this apparatus is 20·0 cm. in length and 14·0 mm. in external diameter. The external diameter of the inlet and outlet tubes is approximately 5·0 mm.

3. *A purifying tube* containing Carbosorb between two columns of Anhydrone (magnesium perchlorate). The external diameter of each limb is about 12·0 mm. and the length about 11·0 cm. The length of the inlet and outlet tubes is 4·0 cm. and the external diameter 5·0 mm. The stoppers are secured with Krönig's cement and the inlet tube is attached to the preheater by "aged" rubber tubing.[4]

4. *A helical silica combustion tube,* constructed from clear fused silica tubing; this component comprises the following three parts:

(*a*) A straight portion 38·0 cm. long and of 11·0 mm. external diameter, provided with a side-arm (external diameter 5·0 mm.), connected by "aged" rubber tubing to the purifying tube 3. This section contains a column of silica chips (22–30 B.S.S. sieve), 7·0 cm. long, held in position by asbestos plugs, followed by platinum wire (diameter 0·005 inch) plugs and enclosed in a small "split" furnace, B.

(*b*) A helical portion constructed from clear fused silica tubing of internal diameter 8·0 mm. The length of this section is 24·0 cm., its external diameter is 4·0 cm., and it contains twelve "turns." The helix is enclosed in an electrically heated furnace, C.

(*c*) A straight portion 23·0 cm. long, drawn out at the end to form a neck 3·5 cm. long and of 3·0 mm. external diameter. This portion of the combustion tube, containing a 15·0-cm. length of granular silver held between plugs of silver wire, is packed before being sealed on to the helix. The silver packing is heated by the electric furnace D.

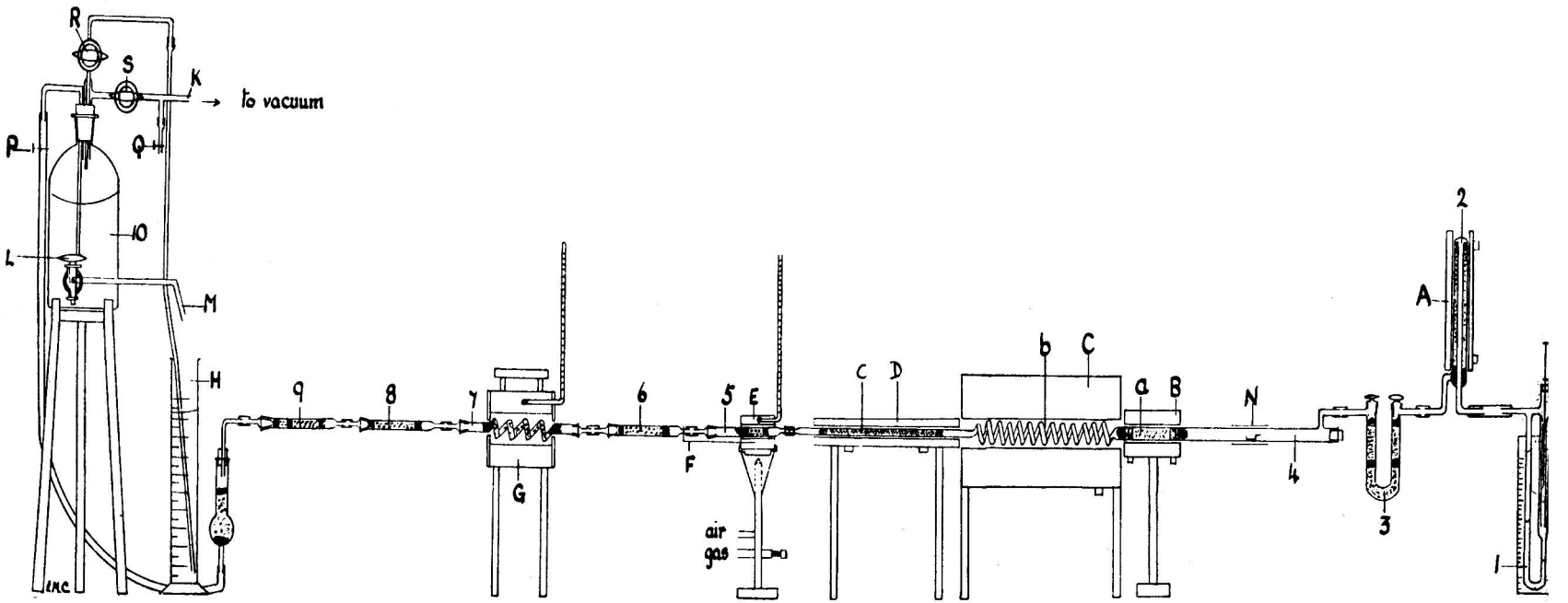
5. *A tube containing lead peroxide between plugs of glass wool.* The length of the column of lead peroxide is about 1·0 cm., and the tube itself is 10·0 cm. long and 8·0 mm. in external diameter. The portion of the tube containing the reagent and about 3·0 cm. of the empty section are enclosed by a gas-heated cylindrical copper block, E, provided with a thermometer and a copper heating-hook, F. The copper block is 6·0 cm. long and its diameter is 4·0 cm.

6. *A Pregl-type absorption tube containing Anhydrone,* for the retention and weighing of the water produced in the combustion of the sample.

7. *A Pyrex helical tube* (made from tubing of 8·0 mm. external diameter) containing about 34·0 cm. of lead peroxide held between glass beads and plugs of glass wool. The inlet and outlet ends of the helix are 5·5 cm. in length and are provided with ground-in stoppers secured with Krönig's cement. These stoppers carry connecting tubes 3·0 cm. long and of 3·0 mm. external diameter. There are three coils in the helix, which is 8·0 cm. long and 4·0 cm. in external diameter. It is enclosed in the aluminium block G, the upper half of which is detachable. The block is 11·0 cm. by 9·0 cm. by 9·0 cm., and is provided with a thermometer.

8. *A Pregl-type absorption tube.* This contains Anhydrone and serves to retain traces of moisture that may have been driven out of the lead peroxide in the helical tube, 7.

9. *A Pregl-type absorption tube containing Carbosorb,* attached by impregnated rubber tubing to the Anhydrone tube, 8. This Carbosorb tube is used for the retention and weighing of the carbon dioxide produced in the combustion analysis.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10. *A Mariotte bottle* connected through the usual guard tube to the exit end of the Carbosorb tube, 9. It differs from the usual type[5] in that provision is made for the return of water from the cylinder H to the bottle, by applying suction at the point K. Except where otherwise indicated, the various components are connected together by thick-walled impregnated rubber tubing.[6]

Procedure

Conditioning of the apparatus—

The following preliminary operations must be carried out before a newly assembled combustion train can be used for accurate analyses. It is also recommended that the same preliminary treatment should be adopted when a combustion train has been lying idle for some time.

(1) Switch on the current to the furnaces A, B, C and D, which have been previously adjusted to give temperatures of 650°, 860°, 800° and 600° C. respectively. At the same time adjust the burners under the metal blocks E and G to heat these components to 180° C., and regulate the flow of oxygen through the completely assembled apparatus until a gas rate of 40 ml. per minute is indicated by the flowmeter, 1. The weighed absorption tubes and the guard tube, 8, need not be connected at this stage.

(2) When the various furnaces and heating blocks have attained the required temperatures (after about 1 hour), heat the straight portion of the combustion tube with a bunsen flame, beginning at a point about 5·0 cm. from the mouth of the tube and finishing at the entry to the small furnace, B. Continue the passage of oxygen through the train for 3 or 4 hours.

(3) Connect the absorption tube 6, the guard tube 8 and the tube 9 to the train as in the figure. Reduce the oxygen rate to about 10 ml. per minute and introduce into the combustion tube about 5·0 mg. of an organic compound (*e.g.*, dinitrobenzoic acid) contained in a small platinum boat. Shut off the oxygen supply, attach the guard tube of the Mariotte bottle, 10, to the Carbosorb tube, 9, open tap L and when the flow of water from tube M ceases, turn on the oxygen supply and adjust the rate to 40 ml. per minute. Finally decompose the sample by careful heating with a bunsen flame, gradually advancing the burner and the stainless steel gauze sheath, N, until they reach the furnace B. Repeat the heating of the combustion tube, and when 500 ml. of water have collected in the cylinder H, reduce the oxygen rate to about 10 ml. per minute. After a few seconds close tap L and detach the guard tube from the absorption tube 9.

The apparatus should now be in proper condition for carrying out combustion analyses.

The actual determination—

(1) Detach the oxygen-filled tubes 6 and 9, connect the lead peroxide helix, 7, to the tube 5, and return the water in cylinder H to the Mariotte bottle by applying suction at K after closing taps P and Q, and opening R and S. Clean the absorption tubes 6 and 9 in the usual manner and weigh them after weighing out accurately about 5·0 mg. of the sample.

(2) Connect the weighed tubes to the combustion train, introduce the sample into the combustion tube at a point at least 5·0 cm. from furnace B, close taps R and S, turn off the oxygen supply and open tap L.

When the flow of water from tube M has stopped (indicating the absence of serious leaks in the system), turn on the oxygen supply and adjust the rate to 40 ml. per minute. Burn the sample as already directed and continue the passage of oxygen for 5 minutes after the bunsen burner has reached the furnace B. Complete the determination as already indicated.

Experience has shown that the time required to burn the sample and sweep out the products of combustion is never more than 15 minutes and may be only 10 minutes, since very often the sample can be burned, and the burner advanced to the furnace B, in about 5 minutes.

Analytical results—

The rapid method described in this paper has been applied to the analysis of solid and liquid compounds containing carbon and hydrogen alone, or in combination with oxygen, nitrogen, sulphur and halogens.

The results presented in Tables I to VI illustrate the wide application of the method, and the accuracy attainable. The analytical results have been checked by the standard Pregl method where the purity of the sample was in doubt or its composition was unknown.

TABLE I

C H [O] COMPOUNDS

Compound analysed	Calculated C %	Calculated H %	Found C %	Found H %
Anthracene	94·4	5·6	94·3	5·6
			94·2	5·7
Vanillin	63·2	5·26	63·3	5·2
			63·1	5·2
Dextrose	40·0	6·7	39·9	6·5
			40·1	6·6
Benzoic acid	68·9	4·9	68·8	5·1
			68·7	5·0
β-Naphthol	83·3	5·6	83·6	5·7
			83·4	5·8
Benzene	92·3	7·7	92·5	7·7
			92·5	7·7

TABLE II

C H [O] S COMPOUNDS

Compound analysed	Calculated C %	Calculated H %	Found C %	Found H %
Sulphonal	36·8	7·0	36·6	7·1
			36·7	7·0
Sulphosalicylic acid	33·1	4·0	33·2	4·1
			33·0	4·0
Diphenyl sulphone	66·0	4·6	66·3	4·7
			66·2	4·6
α-Anthraquinonesulphone	70·3	2·95	70·3	2·8
			70·0	3·0

TABLE III

C H [O] HALOGEN COMPOUNDS

Compound analysed	Calculated C %	Calculated H %	Rapid method C %	Rapid method H %	Pregl method C %	Pregl method H %
Hexachlorobenzene	25·3	0·0	25·4	—	—	—
			25·6	—	—	—
β-Chloronaphthalene	73·84	4·3	74·4	4·2	74·6	4·0
			74·6	4·1		
Phenacylchloride	62·1	4·6	62·3	4·6	—	—
			61·9	4·5	—	—
o-Chlorotoluene	66·4	5·5	66·7	5·3	66·9	5·4
			66·9	5·6		
3 : 9-Dibromo-benzanthrone ..	52·6	2·1	52·5	2·3	52·4	2·2
			52·7	2·1		
Carbon tetrachloride	7·8	0·0	8·0	—	—	—
			7·9	—		
Chloroform	10·5	0·8	10·4	0·9		
			10·2	0·8		

TABLE IV

C H [O] N COMPOUNDS

Compound analysed	Calculated		Rapid method		Pregl method	
	C	H	C	H	C	H
	%	%	%	%	%	%
m-Dinitrobenzene	42·8	2·4	43·0 42·9	2·2 2·5	—	—
3 : 5-Dinitrobenzoic acid ..	39·6	1·9	39·5 39·7	1·8 1·9	—	—
p-Nitraniline	52·2	4·3	52·3	4·2	—	—
Acetanilide	71·1	6·7	71·2 71·3	6·9 6·8	— —	— —
Phenacetin	67·0	7·3	67·2 67·3	7·1 7·2	—	—
Dicyano-diamide (N = 66·6%)..	28·5	4·8	28·9 28·8	5·0 4·7	28·7	4·7
Guanazole-1-carboxylic-hydrazide (N = 62·4%)	22·9	4·5	23·3 23·0	4·8 4·5	23·1	4·5
Guanazo-guanazole (N = 67·5%)	28·9	3·6	28·9 29·1	3·7 3·4	28·8	3·6

TABLE V

C H [O] N S, C H [O] N Br AND C H [O] N S Cl COMPOUNDS

Compound analysed	Calculated		Rapid method		Pregl method	
	C	H	C	H	C	H
	%	%	%	%	%	%
Thiourea	15·8	5·3	16·1 15·9	5·2 5·0	16·1	5·1
C-*p*-methylthiophenoxyacetamide	59·7	6·0	59·9 59·8	5·8 5·9	—	—
p-Bromoacetanilide	44·9	3·7	44·9 44·8	3·8 3·7	44·8	3·6
4-Chloro-3-nitrobenzene sulphonamide	30·4	2·1	30·4 30·3	2·0 2·2	—	—
N-*p*-chlorophenyl-N′-dimethyl-guanylthiourea	46·75	5·05	46·8 46·6	4·9 5·1	46·5	5·2
Benzylthiuronium chloride ..	47·4	5·5	47·5 47·6	5·7 5·6	—	—

TABLE VI

MISCELLANEOUS RESEARCH COMPOUNDS

Sample	Calculated		Rapid method		Pregl method	
	C	H	C	H	C	H
	%	%	%	%	%	%
No. 1 (nitrogenous)	—	—	90·2 90·3	6·0 5·9	89·9	5·9
No. 2 (nitrogenous)			84·4 84·3	5·5 5·3	84·6	5·3
No. 3 (hydrocarbon)	93·2	6·8	93·3 93·0	6·7 6·7	—	—
No. 4	—	—	70·8 70·6	6·3 6·5	70·9 70·8	6·3 6·4

Discussion

THE REAGENTS—

Anhydrone and Carbosorb (14 *to* 20 *mesh*)—These reagents should be freed from fine particles by sieving before use. The Anhydrone should be renewed when it becomes visibly moist, and the Carbosorb when it has absorbed about 300 mg. of carbon dioxide. Tests carried out with Caroxite,[7] the recently described indicating absorbent for carbon dioxide, have shown this American product to be at least as efficient as Carbosorb and easier to remove from the absorption tube when exhausted.

Granular silver—This is prepared as directed by Walden, Hammeth and Edmonds,[8] and is graded to give particles passing No. 14 and held on No. 22 B.S. sieve. It contracts on heating and should therefore be "pre-shrunk" by heating at 650° C. in a silica dish for about 1 hour, before use in the combustion train. About 30·0 g. of "pre-shrunk" silver is sufficient to give the requisite column length.

Lead peroxide (14 *to* 20 *mesh*)—A suitable product may be prepared from analytical reagent grade lead peroxide, by digestion with a mixture of concentrated nitric acid (sp.gr. 1·42) and fuming nitric acid (sp.gr. 1·5), evaporation of the bulk of the acid, and washing the product with (*a*) water until most of the remaining acid is removed, and (*b*) dilute (0·5 per cent.) hydrogen peroxide until free from acid. The partially dried lead peroxide may then be cut into cubes (1·0-mm. edge) and "polished" by rotation in a glass bottle.

THE APPARATUS—

The White - Wright flowmeter, 1—The flowmeter shown in the figure has recently been slightly modified in this laboratory to facilitate the introduction of the manometer liquid. The modification consists in the provision of a short neck on the horizontal outlet tube. This neck is situated immediately above the manometer limb and is closed by a rubber bung.

The helical combustion tube (*silica*)—The exit end of this tube was originally provided with a ground joint to facilitate the introduction and withdrawal of the silver packing. This arrangement was used for many of the analyses reported in this paper, but was later abandoned in favour of a separate tube containing silver and connected by impregnated rubber tubing to the exit end of the helix. This separate tube possessed the advantage of being easily detachable for renewal of the silver or of the tube itself.

The attention of the author has recently been drawn[9] to the fact that while this form of apparatus is satisfactory in the analysis of compounds containing nitrogen or halogen, it may give rise to low values for hydrogen in the analysis of sulphur compounds. This error has been shown to be due to condensation of traces of sulphuric acid on the cooler portion of the combustion tube lying between the furnaces C and D. The one-piece tube shown in the figure is therefore now employed, and the two furnaces C, D are brought close together as indicated.

Helical combustion tubes of glass—A considerable amount of work has been carried out to test the suitability of glass combustion tubing for the construction of the helix. Experience suggests that the best of such tubing is satisfactory and may indeed prove to be preferable to the more expensive silica. One such glass helix operated at 750° C. has given satisfactory service in over two hundred combustion analyses and is still in use. This performance equals that of any silica helix so far employed.

The heating blocks, E, G (*see figure*)—The thermostatically controlled electric heater devised by H. Kirby,[10] may with advantage be used in place of the gas heated block E. A similar device to replace the large block G, is under consideration.

THE METHOD—

The "blank" value of the combustion train—This has not been found to exceed 0·1 mg. for the water absorption tube and 0·05 mg. for the carbon dioxide absorption tube, by any significant amount.

The oxygen rate—The gas rate of 40·0 ml. per minute, specified in the directions for carrying out rapid combustion analyses, has been found to be satisfactory for a wide range of compounds. For many, however, a speed of 50 ml. per minute may be used without loss of accuracy.

NOTES ON THE RETENTION OF OXIDES OF NITROGEN—

Liquid absorbents such as potassium dichromate in concentrated sulphuric acid were not adopted in the final form of the rapid combustion method because (1) the reagent is

rapidly exhausted, and (2) it was desired to avoid the increase of pressure in the apparatus arising from the inclusion of the necessary "bubblers."

A few experiments were carried out with finely divided copper as an absorbent for oxides of nitrogen. It was found, however, that although some retention of oxides of nitrogen did occur, it was not quantitative and decreased rapidly. In the earlier stages of the investigation experiments were carried out with lead peroxide placed immediately before the weighed Anhydrone tube, but it was found that the water produced in the combustion could not be quantitatively driven out of the peroxide in a short enough time. The lead peroxide had therefore to be placed in the position shown in the figure (see 7), although the possible retention of some oxides of nitrogen by the Anhydrone in the weighed tube 6 had then to be considered. On the latter point opinion appears to be divided,[2,11,12,13] but the writer's experience suggests that some retention of nitrogen oxides does in fact occur. It was found, however, that this effect may be avoided by the intervention of a short column of lead peroxide between the tube c and the Anhydrone tube 6. The effect of this lead peroxide is illustrated by the results given in Table VII. It is recommended that until some water absorbent that does not absorb oxides of nitrogen can be found, the short column of lead peroxide should be retained.

TABLE VII

Substance analysed	Calculated value H %	Lead peroxide present H %	Lead peroxide absent H %
3 : 5-Dinitrobenzoic acid	1·89	1·85	2·20
		1·90	1·90
		2·00	2·30
		1·70	1·80

The length of life of the granular silver and the lead peroxide—No definite information is yet available, but the silver packing now in use has served for the analysis of over fifty halogen compounds and is still effective. The short column of lead peroxide should be renewed after forty to sixty analyses depending on the amount and mode of combination of the nitrogen in the samples. The long column of lead peroxide has remained effective for more than sixty analyses of nitrogenous compounds.

SUMMARY

A rapid combustion method for the micro-determination of carbon and hydrogen in organic compounds is described. The sample is burned in an "unpacked" helical combustion tube of silica or glass (heated to 800° C. or 750° C. respectively), through which a stream of oxygen is passed at the rate of 40·0 ml. per minute. Halogen and sulphur, if present, are retained on granular silver heated to 600° C., and oxides of nitrogen are removed by passage through lead peroxide heated to 180° C.

A single analysis may be completed in about 40 minutes, and for a series of analyses the average time for each determination is about 30 minutes. The method gives accurate results over a wide range of compounds and is suitable for routine work.

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NOTE—

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A Critical Examination of the Empty Tube Combustion Method

By G. INGRAM

ALTHOUGH the Pregl micro-combustion methods offer an appreciable saving of time compared with the corresponding macro-methods, the need for more rapid and simpler procedures has resulted in the development of new techniques and in some cases has led also to the simplification of the apparatus normally required. For the micro-determination of carbon and hydrogen in organic compounds, although some important changes in both technique and apparatus have been made,[1,2,3,4] the conventional method of Pregl is generally preferred. In their paper on "A New Technique for the Ultimate Micro-Analysis of Organic Compounds," Belcher and Spooner[5] have described an adaptation, for micro-analysis, of the empty-tube combustion process developed at Sheffield[6,7,8] and applied successfully to the macro-analysis of coals, cokes, oils and other organic materials. They found that the combustion of organic compounds can be accelerated without loss of accuracy and with the use of simpler apparatus. They applied the combustion process to the determination of carbon, hydrogen, sulphur and halogens but, owing to pressure of other work,[9] confined their investigation to compounds not particularly volatile or explosive and it remained to confirm their findings, to extend the process to the analysis of more volatile or difficult compounds and to adapt the method, if necessary, to the routine analysis of research samples.

Combustion of the sample is effected with a rapid stream of oxygen in an empty combustion tube, which is heated by a furnace 25 cm. long and maintained at a temperature of 800° C. With an oxygen flow-rate of 50 ml. per minute both combustion and sweeping out is accomplished in about 10 minutes so that an analysis, including the weighings, may be completed in about 30 minutes.

As this technique appeared to offer outstanding advantages it was decided by the present author to compare it with the standard Pregl method for the determination of carbon and hydrogen with a view to using it for routine analysis. From these studies it was found that errors are less than those found with Pregl's universal filling. Satisfactory results were obtained with the range of compounds examined by Belcher and Spooner and also with some other compounds, but difficulties were experienced with certain types of research compounds frequently analysed in these laboratories. By including certain minor modifications, however, these compounds were analysed successfully and from the present author's experience it appears that the modified method may be confidently used with all types of organic compounds.

Experimental

In the preliminary tests on the method the original method of Belcher and Spooner was followed as closely as possible. After some practice had been acquired with a number of samples, satisfactory results could be obtained with a range of compounds similar to those examined by Belcher and Spooner. In addition, more explosive substances, such as picric acid, yielded satisfactory results. With some compounds, however, incomplete combustion occurred, volatile liquids and hydrocarbons being prone to behave in this way. With the research samples normally subjected to routine examination in these laboratories, low carbon and slightly high hydrogen values were obtained. These were traced to the formation, by these particular compounds, of a fine carbon fog which passes right through the combustion tube and is retained in the water absorbent.

Several factors were varied to overcome these difficulties. A slower stream of oxygen was employed, the temperature of the furnace was raised and the length of the heated zone was altered. When the flow of oxygen was decreased to less than 20 ml. per minute, using the recommended hot zone of 25 cm., it was found that even a simple compound such as benzoic acid was not completely burnt even when combustion was carried out slowly, although it had responded satisfactorily to the original conditions. Likewise, raising the temperature of the furnace to 950° C. had little effect. On the other hand, however, some surprisingly good results were obtained when some simple compounds were combusted, with the original flow-rate of 50 ml. per minute, when the heated zone of the combustion tube was reduced to 15 cm. in length, which is 10 cm. shorter than that originally recommended. In these

particular experiments, however, the internal diameter of the combustion tube was somewhat wider, being 12 mm. instead of 9 mm. as used in the preliminary experiments. Some typical results, obtained under the preceding conditions with 3 to 5 mg. of sample, are given in Table I.

TABLE I

Substance	Oxygen flow-rate ml.	Found C %	Found H %	Required C %	Required H %
Benzoic acid	20	66·75	4·68	68·8	4·9
	50	68·75	4·98		
		68·97	5·01		
Succinic acid	20	39·81	5·23	40·68	5·08
	50	40·86	5·06		
		40·58	5·15		
Phenacetin	50	67·13	7·32	67·04	7·23
Naphthalene	20	92·7	5·9	93·7	6·3
	50	93·43	6·23		
		93·68	6·42		

Length of heated zone 15 cm. with oxygen flow of 50 ml. and 25 cm. with oxygen flow of 20 ml. per minute.

Other modifications were then tried in order to effect satisfactory combustion of difficult substances. A longer heating zone, 30 cm. in length, was used and the temperature of the furnace was raised to 950° C. The wider tube of 12 mm. internal diameter was retained in the assembly. Under these conditions satisfactory results were obtained for all types of compounds apart from those producing the carbon fog. It was found, however, that even these could be analysed successfully by placing a 5-cm. length of copper oxide gauze in the form of a roll in the combustion tube at the front end of the furnace (Fig. 1A). This roll held back the carbon fog and effected its complete oxidation. Later it was found that a plug of quartz wool was as efficient as the copper oxide and more convenient, because the gauze broke inside the tube and attacked it to some extent, causing frequent breakages.

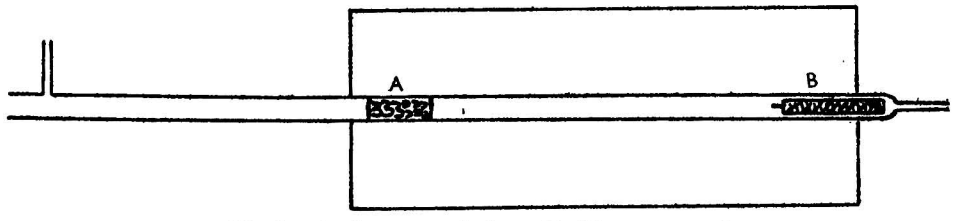

Fig. 1. A. Quartz wool plug. B. Silver gauze roll.

Interfering gases arising from the combustion of substances containing sulphur, halogens and nitrogen were removed from the gas stream with the same absorbents as were used by Belcher and Spooner. They found the potassium permanganate - sulphuric acid solution for absorbing nitrogen oxides recommended earlier by Elving and McElroy,[10] and used externally, gave satisfactory results, but in the present investigation it was found that the reagent must be used with caution. The solution deteriorates rapidly and must be renewed after every two or (at the most) three combustions. If this precaution is taken, absorption of nitrogen oxides is complete, but after three combustions with the same portion of reagent some of the gases pass through the bubbler.

Some difference of opinion exists as to whether or not nitrogen oxides in amounts sufficient to vitiate the hydrogen figure can be retained by the desiccant. It was found in the present investigation that no such absorption occurs unless a high concentration of water collects at the inlet end of the absorption tube. The use of the rapid technique is an advantage in this respect, as the rapid stream of gas entering the absorption tube drives the water vapour well into the desiccant layer.

The results recorded below (Table II) and those from combustion of research samples (Table III) were obtained with the modified combustion train using a quartz tube of 12 mm. internal diameter and heating-zone 30 cm. in length, of which 25 cm. was maintained at 950° C. and the remaining portion, containing the silver gauze, at 700° C. A potassium

permanganate - sulphuric acid solution was used to absorb the nitrogen oxides and was contained in a bubbler connected between the water and carbon dioxide absorption tubes. Sulphur oxides and halogen were retained in the combustion tube by means of a silver gauze roll situated at the beak end of the tube. The combustion tube also contained a 5-cm. plug of quartz wool at the front end of the combustion furnace zone (Fig. 1) and an oxygen flow-rate of 50 ml. per minute was used for the combustion. Belcher and Spooner also used this technique for the determination of sulphur and halogens, the analysis being completed titrimetrically. Since the need for such determinations arises only infrequently in these laboratories this phase of the work was not fully investigated. However, a few analyses of a halogen-containing compound were carried out by their original technique. Hydrogen peroxide was used as the absorbent and the hydrochloric acid produced was titrated with 0·01 *N* sodium hydroxide. Results obtained, which were within 0·3 per cent. of the theoretical values, showed that the technique should be applicable to this type of determination.

TABLE II

Substance	Found C %	Found H %	Required C %	Required H %
Benzoic acid	68·7	4·87	68·8	4·9
	68·67	5·07		
Benzene	92·18	7·75	92·3	7·7
	92·24	7·75		
Phenacetin	67·06	7·34	67·04	7·23
	67·13	7·24		
Picric acid	31·58	1·34	31·44	1·31
	31·6	1·3		
	31·33	1·39		
Dimethylglyoxime	41·54	6·93	41·4	6·9
	41·46	6·93		
p-Diacetoxyazobenzene	64·53	4·8	64·42	4·69
Chlorbenzoic acid	53·52	3·2	53·7	3·19
	53·78	3·4		
	53·8	3·0		
Dichloroacetanilide	47·18	3·52	47·1	3·43
	47·05	3·5		
DDT	47·36	2·6	47·4	2·6
Tetra-bromo-diphenylamine	29·62	1·47	29·7	1·44
Sulphonal	36·95	7·15	36·8	7·06
	36·87	6·94		
N-Acetyl-sulphanilamide	44·97	4·82	44·9	4·67
	44·95	4·89		
	44·73	4·86		

TABLE III

Empirical formula	Found C %	Found H %	Required C %	Required H %
$C_{18}H_{14}O_3N_2.2H_2O$	63·2	5·1	63·1	5·3
$C_{22}H_{16}N_2$	85·3	5·2	85·7	5·2
$C_{11}H_9ON$	77·2	5·2	76·9	5·3
$C_{18}H_{12}H_2$	84·4	4·7	84·3	4·7
$C_{18}H_{13}O_4N_3$	64·3	4·0	64·5	3·9
$C_{12}H_9ON$	78·8	5·1	78·7	5·0
$C_{27}H_{17}N_3$	84·45	4·6	84·6	4·5
$C_{24}H_{17}O_2N_3$	76·1	4·3	76·0	4·5
$C_{15}H_{11}O_6N_5$	50·6	3·2	50·4	3·1
$C_{24}H_{15}O_2N_3$	76·6	4·2	76·4	4·0
$C_{24}H_{17}N_3$	82·8	5·15	83·0	4·9
$C_{36}H_{22}N_4$	84·5	4·3	84·7	4·3
$C_{36}H_{24}N_4$	84·0	4·9	84·3	4·7

DISCUSSION

The investigation has shown that the Belcher and Spooner empty-tube method for the micro-determination of carbon and hydrogen is as efficient as the standard Pregl method. Complete oxidation of the sample is effected in the empty combustion tube in the presence

of a large excess of oxygen, provided that the hot zone is sufficiently long. The experiments carried out indicated that although compounds of simple type are readily oxidised with a hot zone of 15 cm., a length of 30 cm. as distinct from the 25 cm. originally recommended is normally required to cope with all types of organic compounds. Further, it was found necessary to place a quartz wool plug inside the combustion tube to trap and burn any carbon fog formed, when substances that readily form such fog are combusted. The above modified assembly has now been in use on a routine basis for six months and has given satisfactory results for all types of compounds.

In addition, evidence has been obtained which has confirmed the findings of other investigators, namely, that the moist desiccant does not retain nitrogen oxides, so that it is possible to use external absorbents. It should be stated that potassium permanganate - sulphuric acid solution is by no means a satisfactory absorbent unless changed after a few combustions, which means that the conditioning process frequently has to be carried out during a run of combustions. There is obviously a need for further investigation to find more suitable reagents for the absorption of nitrogen oxides. Such an investigation has been proceeding for some time in these laboratories and promising results have been obtained. These results will be the subject of a further communication at a later date.

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COURTAULDS LTD.
MAIDENHEAD, BERKS.

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A Method for the Determination of Some Nitrophenolic Proofing Agents in Felt

BY J. G. NORVALL AND C. KENYON

THERE is often a requirement for felt to be proofed with mixtures of

(*a*) *p*-nitrophenol and 2 : 4-dinitro-α-naphthol

or (*b*) *p*-nitrophenol and 4 : 6-dinitro-*o*-cresol

in the proportions of at least 0·25 per cent. by weight of each constituent, to afford protection against bacterial and fungicidal action.

The estimation of these proofing agents by the more orthodox chemical methods is difficult and not very reliable. It was considered that the chromatographic technique might offer a means for the quantitative separation of these nitro-bodies, which could then be determined colorimetrically.

Experiments carried out on these lines have led to the method now proposed, which involves the following stages.

(i) Treatment of the sample with hot water to extract the nitrophenolic bodies.

(ii) Removal of wool acids from the extract by means of lead acetate.

(iii) Extraction of the nitro-bodies from the solution with ethyl ether.

(iv) Separation of the nitro-bodies by chromatography on alumina and elution with hot water.

(v) Colorimetric determination of *p*-nitrophenol by reduction and application of the indophenol-blue reaction.[1]

(vi) Colorimetric determination of the dinitro compound by reduction and reaction of the amino product with ferric chloride solution.

Method

PRELIMINARY TREATMENT OF SAMPLE—

Extract 5 g. of shredded felt with hot water until all nitrophenolic compounds are extracted—as shown by testing a portion of the extract with sodium hydroxide solution, which gives yellow colorations with the nitro-bodies concerned at concentrations as low as 0·4 mg. per 100 ml.

Add 5 ml. of 10 per cent. aqueous sodium hydroxide solution to the aqueous extract and concentrate to a volume of about 100 ml. Neutralise with acetic acid (to litmus paper), add 10 ml. of 10 per cent. lead acetate solution and allow to stand for 1 hour. Filter and wash the precipitate with water. Make the combined filtrate and washings slightly acid with hydrochloric acid and extract the solution with successive 50 ml. quantities of ethyl ether until all nitro-bodies have been removed. Three extractions are usually sufficient but if in doubt continue the extraction until the aqueous layer remains colourless when tested with excess of sodium hydroxide solution.

Combine the extracts and concentrate to a volume of about 20 ml.

CHROMATOGRAPHIC PROCEDURE—

Adsorbent—Activated alumina, type H, 100 to 200 mesh, obtained from Messrs. Peter Spence and Sons Ltd.; used as received without further activation.

Developing solvent—30 per cent. solution of 95 per cent. ethyl alcohol in ethyl ether.

Erect a simple chromatographic column of internal diameter 15 mm. for use without suction. Pour in sufficient adsorbent to give a column 200 mm. long and consolidate by tapping. Pour the ethereal solution of the nitro-bodies on to the column and wash in with further small quantities of ether. Develop the chromatogram until the pale yellow zone of *p*-nitrophenol has moved about halfway down the column; 100 ml. of developing solvent is usually sufficient.

At this stage, both the dinitro compounds considered would be adsorbed at the top of the column. An orange zone indicates dinitro-α-naphthol and a deep yellow zone indicates dinitro-*o*-cresol.

Add about 10 ml. of ethyl ether to the column and allow the column to become almost dry. Detach the tube and remove the adsorbent gradually from the top end by rotating and tapping the tube in an almost horizontal position. By this means, the adsorbent may be detached in small portions and poured out into beakers. Pour the top part of the column, containing all the dinitro compound, into one beaker and the central part of the column, containing all the *p*-nitrophenol, into a second beaker, discarding as much as possible of the colourless portions of the column. This simple means of separation is made possible by the wide space existing between the two zones after proper development.

Elute each section separately by extraction with hot water. For this operation it is essential to stir the adsorbent well with hot water, allow to settle and pour off the solution through a filter. Repeat this extraction until the supernatant liquid is colourless. Finally, transfer the adsorbent to the filter and wash well.

Evaporate each solution to a volume of about 40 ml.; sufficient alkalinity to prevent loss of nitro-bodies on evaporation is attained by contact with the adsorbent.

Determine the amounts of the separated nitro compounds as described below.

COLORIMETRIC PROCEDURE—

Note—The dilutions employed here have been chosen to give depths of colour suitable for measurement with the Spekker absorptiometer.

(*a*) *Determination of* p-*nitrophenol*—Filter the solution into a 100-ml. standard flask, wash the filter free from *p*-nitrophenol with water and make up to volume at 20° C.

Pipette 20 ml. of this solution into a dry small beaker. Add exactly 5 ml. of 2 *N* acetic acid and about 1 g. of zinc dust. Allow to stand for 1 hour with occasional stirring. Filter through a dry paper.

Pipette 2 ml. of the filtrate into a 100-ml. standard flask and dilute to about 75 ml. with water. Add 10 ml. of 1 per cent. sodium-*o*-cresate solution and then 5 ml. of 2 *N* sodium hydroxide solution and make up to volume at 20° C. with water. Mix well and allow to stand for 30 minutes to ensure full colour development.

Compare the intensity of colour of the solution with standards prepared from known amounts of *p*-nitrophenol in the same way.

If the Spekker absorptiometer is used for the colorimetric measurement, Ilford yellow filters, No. 606, are suitable. The calibration graph is a straight line over the whole range.

(*b*) *Determination of dinitro-α-naphthol or dinitro-o-cresol*—Filter the solution if necessary, then add 2 ml. of concentrated hydrochloric acid and about 1·0 g. of zinc dust and heat on a water-bath for 15 minutes, stirring occasionally.

Filter into a 250-ml. standard flask and wash the zinc residues well with water. Add 2·5 ml. of 10 per cent. aqueous ferric chloride solution and make up to volume at 20° C. with water. Mix well.

Under these conditions, 2 : 4-dinitro-α-naphthol and 4 : 6-dinitro-*o*-cresol give orange and wine-red coloured solutions respectively, and the maximum colour development is immediate and stable for several hours.

Compare the intensity of colour of the solution with standards prepared from the appropriate dinitro compound in the same way.

If the Spekker absorptiometer is used for the colorimetric measurement, Ilford violet filters, No. 601, are suitable for use with dinitro-α-naphthol, and Ilford yellow-green filters, No. 605, for dinitro-*o*-cresol. The calibration graph for each compound is a straight line over the whole range.

Results obtained

The method was applied to synthetic mixtures of compositions unknown to the operator and the results obtained are tabulated below.

Table I

Analysis of mixtures of *p*-nitrophenol and dinitro-α-naphthol

Test No.	Composition of mixtures		*p*-Nitrophenol mg. found	Dinitro-α-naphthol mg. found
	p-Nitrophenol mg.	Dinitro-α-naphthol mg.		
1	8·0	20·0	7·8	20·1
2	10·0	15·0	9·8	15·2
3	12·0	16·0	12·2	16·3
4	15·0	7·0	15·0	7·0
5	16·0	12·0	15·7	12·3
6	20·0	8·0	19·8	8·2

Table II

Analysis of mixtures of *p*-nitrophenol and dinitro-*o*-cresol

Test No.	Composition of mixture		*p*-Nitrophenol mg. found	Dinitro-*o*-cresol mg. found
	p-Nitrophenol mg.	Dinitro-*o*-cresol mg.		
7	6·0	18·0	6·2	18·0
8	8·0	15·0	8·4	15·1
9	10·0	14·0	10·1	14·2
10	14·0	10·0	13·6	9·9
11	16·0	9·0	16·3	9·1
12	18·0	6·0	17·6	5·8

The Spekker absorptiometer was employed for all the colorimetric measurements in this test.

These results indicate that figures obtained by this method would not be expected to be in error to an extent exceeding 3 per cent.

Summary

A new method for the determination of mixtures of (*a*) *p*-nitrophenol and 2 : 4-dinitro-α-naphthol, or (*b*) *p*-nitrophenol and 4 : 6-dinitro-*o*-cresol, in proofed felt is described; it enables these constituents to be determined to within 3 per cent. of the amounts present.

The nitro-bodies, extracted from the felt with hot water, are dissolved in ethyl ether and separation is effected by chromatography. Determination of the separate substances is made by colorimetric analysis.

The chromatographic step is very simple and the method is suitable for the routine examination of proofed felt.

We wish to express our thanks to the Admiralty for permission to publish this paper; the experimental work was carried out at the former Naval Ordnance Inspection Laboratory, Holton Heath, Dorset.

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NAVAL ORDNANCE INSPECTION LABORATORY
CAERWENT, CHEPSTOW, MON.

March, 1948

The Determination of Uronic Acids in Soil

BY M. V. TRACEY

URONIC acids are commonly determined colorimetrically by means of naphthoresorcinol,[1] a method that is not suited for their determination in soils. A more satisfactory method is that of Lefèvre and Tollens[2] in which a sample of material is boiled with 12 per cent. hydrochloric acid for 3·5 hours. The carbon dioxide evolved is carried through an absorption train by a current of air free from carbon dioxide and estimated subsequently by gravimetric or titrimetric methods. There have been many modifications of this method in which the strength of acid, the time of boiling and the design of apparatus have been varied. All agree in using a relatively cumbersome apparatus requiring attention for 4 to 5 hours for a single determination. Perhaps this is why few workers have applied it to soil analysis[3–9] although it has been shown that uronic acids may exceed 30 per cent. of the soil organic matter.[6] The recent identification by Forsyth[10] of glucuronic acid in fractions isolated from a number of soils will probably lead to further interest in soil uronic acids.

In the course of work on leaf fibre it became desirable to develop a rapid method for the determination of uronic acids. It was found[13] that the method of Lefevre and Tollens could be modified by carrying out the decomposition in small sealed tubes heated at 111° C. in boiling toluene. The carbon dioxide evolved was introduced into a Van Slyke apparatus by breaking the tube while enclosed in pressure tubing attached to the gas chamber. Subsequent operations were those of Van Slyke and Folch.[11] Since a large number of tubes can be boiled at one time with no attention, the time occupied by the analysis of each sample is reduced to that required for the Van Slyke carbon dioxide determination—about 15 minutes. The presence of carbonate in the soil can readily be allowed for by separate determination.

Bulbs of about 15-mm. diameter are blown at the ends of pieces of clean soda glass tubing of wall thickness 1 mm. and length about 10 cm. The tubes are numbered with glass ink, dried in an oven and stored in a desiccator. A maximum of 50 mg. of sample is introduced into a tube by means of a small spatula that will reach the bulb so that no contamination of the tube walls can occur, and weighed. Liquids are introduced by means of a capillary pipette. Samples must be free from water, and solutions should be dried off in the bulb. 0·25 Ml. of 12 per cent. hydrochloric acid is now added and the tube is drawn out in a flame and sealed, so that a narrow prolongation of about 2 cm. is produced. The sealed tubes, held upright in a small rack, are immersed in toluene contained in the bottom of a tall beaker. The upper portion of the beaker contains a water condenser. The tubes are then heated in the boiling toluene for 5 hours. After cooling, a tube is inserted as far as the bulb in a piece of pressure tubing about 8 cm. long. The other end of the tubing is attached to the bent tube leading from the gas chamber of the Van Slyke - Neill manometric apparatus. The pressure tubing is exhausted in the usual manner and after the introduction of 2 ml. of 0·5 *N* sodium hydroxide the mercury is lowered to about halfway down the large tube. The tap is turned to connect the pressure tubing with the chamber and the sealed tip of the bulb is broken by bending the pressure tubing. Subsequent operations are those of Van Slyke and Folch.[11] If carbonate is to be determined, a side bulb is blown on the wall of a tube just above the bulb and the hydrochloric acid is retained there until sealing is completed. After tipping the acid on to the sample, one such tube is heated in boiling toluene and the other is kept at about 40° C. for the same time. The value obtained at 40° C. is the carbonate value and is subtracted from the 111° C. value to obtain a corrected uronic acid value.

The work of Nickerson[12] on the increased rate of evolution of carbon dioxide by sugars boiled with hydrochloric acid in presence of iron indicated that the determination might be inaccurate when applied to soils rich in iron and low in organic matter that contain cellulose or other polysaccharides. Similarly, high results might be expected on high-manganese soils. A report[8] that additions of stannous chloride, ferrous sulphate or zinc dust equal to about 3 per cent. of the soil weight depress the yield of carbon dioxide, and that manganese dioxide leads to the production of chlorine when determinations are made by the method of Lefevre and Tollens also indicated that inaccurate results might be obtained in presence of iron.

Estimations of yields of uronic carbon dioxide were accordingly made on a high-iron soil to which was added a pectin preparation containing about 50 per cent. by weight of reducing sugars, and manganese dioxide. A red laterite soil from Wollong Bar, New South Wales, provided by Dr. Swaby, was used; it had organic carbon 4·75 per cent. and iron extractable by 2 *N* hydrochloric acid 0·32 per cent. The results obtained are given in Table I

TABLE I

RECOVERY OF PECTIN ADDED TO SOIL WITH HIGH IRON AND MANGANESE CONTENTS

			CO_2 evolved per 100 g. dry weight g.	Mean g.	Uronic anhydride per 100 g. dry weight g.		% Pectin calculated from (1), (2) and (3)
(*a*)	Soil		1·49; 1·54; 1·59	1·54	6·16		
(*b*)	Soil ..	98%	1·64; 1·66; 1·77	1·69	6·76	6·46 (1)	
	MnO_2 ..	2%					
(*c*)	Soil ..	67%	5·46; 5·50; 5·53	5·50	22·00		37%
	Pectin	33%				21·96 (2)	
(*d*)	Soil ..	65%					
	Pectin	32%	5·30; 5·39; 5·76	5·48	21·92		
	MnO_2 ..	3%					
(*e*)	Pectin ..		12·0; 12·1; 12·2	12·1	48·4	48·4 (3)	

NOTE. Figures are corrected for moisture and MnO_2 contents. The percentage of uronic anhydride was calculated by assuming a 25 per cent. yield of carbon dioxide from pure ash-free polyuronide (formula weight 176_n). Hence uronic anhydride in g. = carbon dioxide in g. × 4·00. The figure in the last column was calculated from

$$(100 - x)\ 6{\cdot}46 + 48{\cdot}4\,x = 100 \times 21{\cdot}96,$$

where x = percentage of pectin in the mixture of soil and pectin.

and indicate that satisfactory determinations are possible in the presence of iron and manganese in large amounts. Whilst the effect of iron and manganese in causing increased oxidation of other organic matter appears to be excluded, there is no evidence that all the carbon dioxide evolved does in fact arise from uronic acids. The fact that substantial yields of carbon dioxide may be obtained from allantoin, alloxan, *p*-aminobenzoic acid, and hypoxanthine,[13] some of which have been isolated from soils in traces, must be taken into account. Final decisions on the merits of uronic acid determinations made in the manner described above must await isolation of uronic acids from soils in quantities sufficient to account for the observed yields of carbon dioxide. Until this has been done the method described may be of value by virtue of its ease and rapidity.

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January, 1948

The Determination of Aluminium in Aluminium Bronze

By W. T. EDWARDS

For an adequate control of aluminium bronzes made to Specifications DTD 197, 164, etc.,[1] *i.e.*, copper - aluminium alloys containing approximately 10 per cent. of aluminium with possible additions of iron, nickel and manganese, it is essential that the aluminium content be determined both rapidly and accurately if the requirements of the specifications are to be met.

On account, mainly, of the distinctly hygroscopic and adsorbent nature of the classical aluminium precipitates the methods at present in use fulfil neither of these conditions. A considerable amount of investigatory work aimed at their improvement and a review of the current literature[2] indicated that precipitation by 8-hydroxyquinoline (oxine) would offer advantages. This paper describes the procedure finally adopted, in which aluminium is precipitated by 8-hydroxyquinoline in an ammoniacal solution from which interfering elements have been removed, or in which their effect has been suppressed.

The procedure first tried was that outlined by the British Aluminium Co.[3] and although satisfactory generally it uses hydrogen sulphide for the precipitation of copper, manganese and zinc. This not only makes the method objectionable but also involves two lengthy filtrations of metallic sulphide precipitates. Copper can be removed by electrolysis and as this also provides a ready method for its determination it is the method most often used; where, however, a copper figure is not required, this metal can be readily suppressed by means of cyanide.

Manganese and zinc in an ammoniacal solution buffered by an excess of ammonium chloride can be precipitated as ferrocyanides and removed by filtering. In the procedure described, the ferrocyanide is formed from the iron content of the alloy, or by added ferric chloride during the acid stage, the cyanide being added as potassium cyanide in the alkaline stage under reducing conditions. The iron and nickel in the alloy both form complex anions with cyanide, and are thus rendered incapable of reaction with the oxine.

In this ammoniacal solution, from which all interfering elements have been removed or in which they have been suppressed, the aluminium is precipitated by oxine solution. By keeping the solution hot and adding the reagent very slowly at first, a condition is reached whereby many precipitating nuclei are formed in the solution and the precipitate is thus built up slowly without occluding any foreign element. It has been found[4] that, after precipitation, coagulation takes place better in a large excess of ammonia, so ammonia solution is added just prior to precipitation. The complete method is as follows.

Method

Reagents—

"*Electro-acid mixture,*" consists of 3 parts each of nitric and sulphuric acids by volume, together with 10 parts of water.

2 *Per cent. 8-hydroxyquinoline solution,* made by dissolving the solid in acetic acid and neutralising the hot solution with dilute aqueous ammonia; it should be filtered before use.

N/2·248 *Bromate - bromide solution*—Dissolve 12·34 g. of potassium bromate and 50 g. of potassium bromide in water, and make up to 1 litre. This solution may be taken as standard.

N/4·5 *Thiosulphate*—Dissolve 55·16 g. of sodium thiosulphate in water and make up to 1 litre. Standardise against the bromate - bromide solution.

Procedure—

Weigh 0·5 g. of alloy into a 150-ml. tall type beaker and dissolve it in 15 ml. of "electro-acid mixture," boil off excess of nitrous fumes and remove copper by electro-deposition in the usual way. Transfer the electrolyte to a 500-ml. conical flask. Where the copper content is not required weigh the sample direct into the flask, dissolve in the acid mixture and without electro-deposition. Add 5 g. of tartaric acid, 2 or 3 drops of 10 per cent. ferric chloride solution if no iron is present in the alloy and 10 g. of ammonium chloride and make just alkaline with diluted aqueous ammonia (1 + 1). Add 5 g. of potassium cyanide (10 g. if the copper has not been removed) and 0·5 g. of sodium sulphite crystals, and boil for approximately 2 minutes. Allow the precipitated ferrocyanides of manganese and zinc, if any, to settle,

filter them on a fairly thick pulp pad and wash with hot 10 per cent. ammonium chloride solution. To the filtrate add 20 ml. of diluted aqueous ammonia (1 + 1), heat just to boiling and at almost boiling temperature precipitate the aluminium by adding 75 ml. of the 2 per cent. oxine reagent, dropwise at first, with constant stirring.

For the gravimetric finish, allow the precipitate to settle in the warm and then filter on a dried and weighed sintered glass crucible, grade 3 porosity, and wash with hot water. Dry in an oven at 140° to 150° C. for about 1 hour and weigh. Weight of aluminium oxinate × 0·0587 = Weight of aluminium.

For the volumetric finish, after the precipitate has settled, filter on to a No. 4 Whatman fluted paper and wash well with boiling water. Dissolve the precipitate back into the original beaker with hot diluted hydrochloric acid (1 + 1), adjust the volume to about 200 ml., with the acid concentration about one-fifth of that of concentrated hydrochloric acid, and cool. Titrate slowly with $N/2{\cdot}248$ bromate - bromide solution, using methyl red as an indicator and add approximately 1 ml. in excess. Add about 0·5 g. of potassium iodide crystals and titrate the liberated iodine with $N/4{\cdot}5$ thiosulphate, using starch as an indicator.

$$\text{ml. of bromate - bromide soln. added} - \frac{\text{ml. of thiosulphate soln. required}}{2} = \text{mg. of aluminium}$$

or 1 ml. of bromate - bromide soln. consumed ≡ 1 mg. of aluminium

DISCUSSION—

In the volumetric finish a large excess of bromate - bromide solution should be avoided, as bromine may be evolved if its solubility is exceeded or on addition of the potassium iodide an insoluble iodide may be formed which redissolves with difficulty. In either event an error is caused.

Some typical results obtained by this method on samples of a DTD 197 alloy containing nickel and iron are reproduced in Table I together with check results obtained by an outside laboratory using the classical referee methods.

TABLE I

DTD 197 ALLOY

Sample No.	1	2	3	4	5	6
Al% by method described	9·80	9·71	10·07	10·02	10·17	9·23
Al% found by outside laboratory	9·86	9·80	10·07	10·02	10·19	9·26

For a 10 per cent. aluminium bronze results can be reproduced within ±0·07 per cent., *i.e.*, giving a standard deviation of ±1 per cent. of the amount of aluminium present.

SUMMARY—

A method has been described for the determination of aluminium in aluminium bronze. Interfering metals are either removed or suppressed, and the aluminium is precipitated with 8-hydroxyquinoline solution under carefully controlled conditions. The method has been found suitable for use both for routine and reference work; a single determination takes 2 to 3 hours.

Thanks are due to Langley Alloys Ltd., in whose laboratories most of this work was carried out, also to Mr. H. F. Hourigan, B.Sc., F.R.I.C., Principal Scientific Officer, for very helpful advice and criticism, and to the Engineer-in-Chief, Post Office, for permission to publish.

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CHEMICAL SECTION
POST OFFICE ENGINEERING DEPARTMENT
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December, 1947

Notes

NOTE ON TETRASODIUM 4 : 4′-BIS-(γ-PHENYLPROPYLAMINO)-DIPHENYLSULPHONE-α : γ : α′ : γ′-TETRASULPHONATE ("SULPHETRONE")

TETRASODIUM 4 : 4′-bis-(γ-phenylpropylamino)-diphenylsulphone-α : γ : α′ : γ′-tetrasulphonate, first described[1] in 1938, has been the subject of almost continuous pharmacological and clinical research during the past ten years and under the name of Sulphetrone has been used in extensive trials in the treatment of tuberculosis.[2] The drug is about to be released for general use in medicine and, on this account, analysts should possess some knowledge of its chemical composition and analysis.

Sulphetrone may be prepared by the action of an aqueous solution of sodium bisulphite upon 4 : 4′-bis-(cinnamylidene-amino)-diphenylsulphone and subsequent precipitation with alcohol. The commercial product, although yielding analytical figures in approximate agreement with formula (I), is not of constant

$$[C_6H_5.\underset{\displaystyle SO_3Na}{CH}.CH_2.\underset{\displaystyle SO_3Na}{CH}.NH.C_6H_4]_2SO_2$$

(I)

composition and it is therefore desirable that the sulphetrone content of each batch should be determined. We have attempted to do this in several ways and our results are summarised below.

On addition of acid, sulphetrone yields diazotisable material and, although the hydrolysis is not quantitative even with chemically pure material, good specimens yield about 85 per cent. of the nitrogen in diazotisable form. This is the basis of the method which has been used for the estimation of the drug in body fluids and tissues of experimental animals and man, the diazo-compound being coupled with *N*-(1-naphthyl)-ethylenediamine and the specific absorption measured with a sensitive photo-electric absorptiometer.[3]

For the routine assay of the drug, however, it was found that a more satisfactory determination could be carried out if the acid hydrolysate were treated with sodium nitrite and coupled with dimethyl-α-naphthylamine, the specific absorption of the reaction mixture being measured with a Spekker absorptiometer, using a No. 5 green filter. Alternatively, the acid hydrolysate could be titrated with standard sodium nitrite solution, using starch iodide paper as external indicator.

In aqueous solution sulphetrone exhibits a well defined ultra-violet absorption curve, having $E^{1\%}_{1\,cm.}$ max. 306 mμ. approximately 380 calculated with respect to the anhydrous salt. Measurement of the ultra-violet absorption may be used for the assay of dilute aqueous solutions of sulphetrone in absence of interfering substances.

EXPERIMENTAL

COLORIMETRIC ASSAY—

Prepare a series of standard solutions containing 1 mg. to 0·5 mg. of 4 : 4′-diaminodiphenylsulphone in 100 ml. by suitably diluting with distilled water a 1 per cent. solution in dilute hydrochloric acid. Treat successively 10 ml. of each standard solution with 0·4 ml. of concentrated hydrochloric acid, 1 ml. of 0·1 per cent. sodium nitrite solution, 10 ml. of alcohol and 1 ml. of a 1 per cent. alcoholic solution of dimethyl-α-naphthylamine. Mix thoroughly and after 10 minutes measure the specific absorption with a Spekker absorptiometer, using a No. 5 green filter. Construct a calibration curve from the drum readings obtained.

Prepare a solution of the sulphetrone under examination, so that it will possess a content of 4 : 4′-diaminodiphenylsulphone within the range covered by the calibration curve, making due allowance for moisture (7 to 16 per cent.) and for the fact that only 80 per cent. recovery is usual. Mix 10 ml. of the solution with 0·4 ml. of concentrated hydrochloric acid and heat in a boiling water-bath for 20 minutes. Cool, make up the volume to 10 ml. with distilled water and treat by the process described above for the standard solutions. After taking the absorptiometer reading use the calibration curve for obtaining the result of the assay, which may be expressed in terms of anhydrous sulphetrone (M.W. 892·5) or 4 : 4′-diaminodiphenylsulphone (M.W. 248·2).

The same procedure is followed if measurements are made with the Klett colorimeter or Lovibond tintometer.

TITRATION WITH SODIUM NITRITE—

Dissolve about 5 g. of sulphetrone, accurately weighed, in water and adjust the volume to 100 ml. in a standard flask. Place 10 ml. of the solution in a wide-necked 100-ml. flask and add 20 ml. of 10 per cent. hydrochloric acid. Boil the mixture gently for 1 hour, making up from time to time the loss due to evaporation by addition of distilled water. Cool the flask and contents thoroughly by placing in ice and, after washing down the solid material from the sides of the flask, titrate the liquid with standard sodium nitrite solution, using starch iodide paper as external indicator.

A little care is needed in judging the end-point, as the strongly acid mixture affords a positive reaction with starch iodide paper. In this case, however, the colour develops at the edge of the drop, but when the correct end-point is reached an immediate blue colour shows at the centre. The sodium nitrite solution is adjusted so that 1 ml. is approximately equivalent to 0·0446 g. of anhydrous sulphetrone (6·9 g. of sodium nitrite per litre). Sodium nitrite solutions are unstable and must be standardised before use against a

solution of 4 : 4′-diaminodiphenylsulphone in 5 per cent. hydrochloric acid (1 ml. of 0·1 *M* sodium nitrite is equivalent to 0·0124 g. of 4 : 4′-diaminodiphenylsulphone).

Most batches of the commercial drug afford assay figures corresponding to 80 to 90 per cent. of $C_{30}H_{28}O_{14}N_2S_5Na_4$, calculated with respect to the material dried at 110° C. *in vacuo*. The two methods of assay give results in close agreement.

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Feb., 1948

THE ASSAY OF METHYL SALICYLATE OINTMENTS

PRELIMINARY experiments confirmed the observation by Garratt that direct steam distillation yielded low results.[1] It was observed, however, that if the steam distillation was carried out in presence of a mineral acid methyl salicylate could be recovered rapidly and quantitatively from ointments and estimated in the distillate by the usual methods. The method proposed is as follows.

Transfer 2 g. of a strong ointment or 8 g. of a dilute ointment to a 500-ml. distilling flask fitted for steam distillation (weigh on glazed paper and transfer paper and ointment to the flask). Add about 50 ml. of water and 5 ml. of 6 *N* sulphuric acid and steam distil until oily drops are no longer visible in the distillate —about 100 ml. Turn off the condenser water and steam out the condenser.

Then distil a further 100 ml. under the same conditions and steaming out the condenser as before.

To each distillate, add 20 ml. 0·5 *N* sodium hydroxide, boil under reflux for 1 hour, cool and titrate with 0·5 *N* hydrochloric acid to phenolphthalein end-point; 1 ml. of 0·5 *N* ≡ 0·07603 g. of methyl salicylate.

The following results have been obtained by the method outlined, ointment No. 3 was prepared in the laboratory and contained 50 per cent. of methyl salicylate, assaying 100 per cent.

No.	Description	Methyl salicylate: Stated %	Methyl salicylate: Found %
1	Ung. Methyl Salicyl. B.P.C.	50	50·0
2	" "	50	52·3
3	" "	50	49·8; 50·0
4	Wintergreen Cream	50	49·6
5	" "	12·5	12·3
6	Wintergreen Ointment	—	48·6
7	Menthol and Wintergreen Cream ..	—	50·4

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March, 1948

Ministry of Fuel and Power

RP 1/8/7/3

SPECIAL TEST FOR COMMERCIAL PETROL

REFERENCE is made to the Ministry's circular of June 1st, 1948, P.R.527, MFP/P/139, on the above subject. As a result of many helpful criticisms and suggestions received from Public Analysts and Police Forces the Ministry's technical advisers have worked out, in collaboration with the Society of Public Analysts, a modified test which would appear to give a more sensitive and more stable reaction. This is set out below.

METHOD OF TEST

Chemicals required

1. Hydrochloric acid, A.R.
2. 2 *N* Caustic soda solution.
3. Petroleum ether (light petroleum, b.p. 40° to 60° C.).
4. Vanadate reagent (prepared by dissolving 0·1 g. of ammonium vanadate in 70 ml. of concentrated hydrochloric acid and making up to 100 ml. with distilled water).

Procedure

Place 30 ml. of the sample in a separator of approximately 100-ml. capacity. Add 2 ml. of 2 *N* caustic soda solution and shake for ½ minute. Allow to separate and reject the lower, aqueous layer. Add a

further 2 ml. of 2 *N* caustic soda solution, shake for ½ minute and, after separating, again reject the lower, aqueous layer. Wash the upper layer by shaking for ½ minute with 2 ml. of water and after separating reject the lower, aqueous layer. Add 2 ml. of hydrochloric acid, shake for ½ minute, allow to separate and run the lower, acid layer into a clean separator of approximately 100-ml. capacity.

Make the acid extract alkaline by addition of 15 ml. of 2 *N* caustic soda solution and cool if necessary; add 15 ml. of petroleum ether and shake for ½ minute. Allow to separate and reject the lower, aqueous layer; allow the separator to stand for 1 minute and reject any further aqueous layer that may separate. Add 2 ml. of vanadate reagent, shake and allow to separate.

If no blue-violet colour is developed in the lower layer the test shall be deemed to be negative.

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October 20th, 1948

The Fiducial Limits of Assays of Vitamin D

A JOINT COMMITTEE set up by the Nutrition Panel of the Food Group of the Society of Chemical Industry and the Biological Methods Group of the Society of Public Analysts and Other Analytical Chemists has been considering the question of the fiducial limits that might reasonably be expected in biological assays of vitamin D using rats. The British Standards Institution has issued a specification for the assay of vitamin D_3 by the chick method (B.S.S. 911) in which maximum fiducial limits of 75 to 133 per cent. (at a probability level of 95 per cent.) are laid down. There is, however, no official specification for the rat assay of vitamin D, and the Committees of the two bodies mentioned came to the conclusion that an enquiry into this matter would serve a useful purpose.

The Committees of the two bodies, therefore, each appointed three representatives to an *ad hoc* Committee, the representatives of the Nutrition Panel being Dr. M. A. Pyke, Mr. L. C. Dutton and Dr. J. I. M. Jones, and those of the Biological Methods Group Dr. A. J. Amos, Dr. E. C. Wood and Mr. N. T. Gridgeman. This Committee drew up a questionnaire which was sent to all organisations believed to be competent to supply useful data. The questionnaire asked for details of the average fiducial limits obtained by the organisations in their own assays, and for their opinion as to the *maximum* fiducial limits to which all assays might be expected to conform in commercial practice. A notice was also inserted in the June 1948 issue of THE ANALYST and in July 10th, 1948, issue of *Chemistry and Industry* asking anyone who could supply information but did not receive a questionnaire to get into touch with the Committee.

The Committee was very gratified to find that all the organisations in Great Britain known to be conducting regular rat assays of vitamin D sent in completed questionnaires, many of which were accompanied by letters commenting on the answers and providing much additional useful information. The Committee wishes to record its thanks to these organisations for their helpful and willing co-operation. It is confidently believed that what follows is based on the statements and opinions of all British organisations having reasonable experience of the rat assay of vitamin D.

Two main facts emerge from the enquiry. First, all the organisations concerned use at least two dose levels both of the Standard Preparation and of the Test Preparation. This is, of course, the minimum number permitting the validity of the assay to be examined. Secondly, the average fiducial limits obtained by these organisations in their own assays vary from 54 to 170 per cent. to 87 to 115 per cent. at a probability level of 95 per cent.

It must be remembered that the fiducial limits obtained in a given laboratory will depend on several variables, one of which is the number of animals used per assay. Some of the variation from organisation to organisation in the average fiducial limits obtained is due to this factor.

Another important source of variation is the fact that some of the organisations are assaying preparations of which the expected potency is known within reasonable limits, whilst others are dealing with materials whose potency can be predicted only very roughly if at all. Other things being equal, closer fiducial limits can always be obtained if some information regarding the expected potency of the Test Preparation is available. This enables the assayist to ensure that the mean responses to the doses of Test Preparation approach equality with the mean responses to the corresponding doses of the Standard Preparation.

After considering the replies to the questionnaire in the light of these facts, the Committee is of the unanimous opinion that, in the absence of any special circumstances, it is reasonable to expect that assays of vitamin D by the rat method in normal commercial practice should have fiducial limits (calculated to a probability level of 95 per cent.) not wider than 60 to 170 per cent. The maximum fiducial limits suggested by all organisations answering the questionnaire fall within this range. The result of any assay conforming to these limits should be a commercially acceptable estimate of the true potency of the material assayed.

International Congress on Analytical Chemistry

UNDER the aegis of the Netherlands Chemical Society an International Congress on Analytical Chemistry was held at Utrecht during the first week of June, 1948. The Congress was presided over by Professor C. J. van Nieuwenberg of the Technical University, Delft, with Dr. H. A. J. Pieters of the Netherlands State Mines as General Secretary. The total attendance of members was about 350, including some 40 chemists from Great Britain. Lectures and papers were contributed by the following British members: Messrs. W. F. J. Cuthbertson (Glaxo Laboratories Ltd.), W. J. Gooderham (The Gas Light and Coke Co.), A. C. Menzies (Hilger and Watts Ltd.), M. Milbourne (I.C.I. Ltd., Metals Division), D. H. Smith (Johnson, Matthey and Co. Ltd.), H. W. Thompson (Oxford University), T. I. Williams (I.C.I. Ltd.), E. C. Wood (Virol Ltd.). Others present included Messrs. R. C. Chirnside and B. S. Cooper (Research Laboratories of the General Electric Co. Ltd.), W. F. Elvidge (Boots Pure Drug Co.), J. G. A. Griffiths (Air Ministry), J. E. Page (Glaxo Laboratories Ltd.), N. Strafford (I.C.I. Ltd., Dyestuffs Division) and H. N. Wilson (I.C.I. Ltd., Billingham Division).

There were five sections of the Congress: (1) General methods, standardisation; (2) Electrical methods; (3) Emission spectrography; (4) Optical measurements and physical methods of separation; (5) Microbiological methods and detection of traces.

The lectures and papers of Sections (1), (2), (4) and (5) will be published as a special number of *Analytica Chimica Acta*. The subscribers to this periodical will receive a copy free of charge and other persons will be able to order copies from Elsevier's Publishing Co., Spuistraat 118, Amsterdam-C. The papers of Section (3) (Emission spectrography) will be published in *Spectrochimica Acta* and can be ordered from *Spectrochimica Acta*, Specola Vaticana, Castelgandolfo, Italy.

Société Suisse de Chimie Analytique et Appliqué

THE 60th Anniversary Meeting of the Société was held at Sion, Valais, Switzerland, on September 10th and 11th, 1948. This Society was represented by Mr. R. C. Chirnside, F.R.I.C., who attended by invitation and to whom we are indebted for the following account.

The chief papers read at the two-day meeting were on analytical methods using inactive isotopes and on the toxicology of mercury. Other papers dealt with coffee and coffee extracts, olive oil, the influence of fatty foods on the development of tumours in choline-deficient rats, the phosphatase reaction in milk, and metallurgical analysis by means of high vacuum. The last-mentioned paper dealt exclusively with aluminium and its alloys, the constituents of which were determined by fractional distillation under high vacuum.

In the discussion that followed the paper on the toxicology of mercury, the author and the speakers were in unanimous agreement that, although the methods developed by A. Stock for the determination of small quantities of mercury were excellent, his published work on its toxicity was not in accord with common experience but must relate to hypersensitive subjects.

It is anticipated that these papers will be published in full.

Our representative was much impressed by the hospitality accorded to him by the Société and by the excellence of the social functions that he had the honour of attending as a guest.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Detection of Caramel Colouring in Wine and other Alcoholic Liquids. P. Valaer (*J. Assoc. Off. Agric. Chem.*, **1947, 30, 331–337**)—If the Milos test ("*Methods of Analysis of the A.O.A.C.*," 6th Ed., 1945, 15·38–9) is followed carefully and any coloured residue that may be obtained is subjected to the confirmatory procedure, the presence of caramel in wine will be demonstrated, and no reaction will be given in its absence. While collaborative work on Milos's test was in progress, Mallory and Love (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 631; ANALYST, 1946, **71**, 186) developed a method for determining caramel which is approximately quantitative and, at the same time, Mathers developed another method, hitherto unpublished, which combines speed with accuracy. These two methods are alike in that the caramel is co-precipitated with certain precipitation agents. The Mallory - Love procedure is the longest method recorded, whereas that of Mathers is the simplest and shortest yet devised. The method of Mathers is as follows.

Procedure—To 10 ml. of filtered wine in a Babcock cream bottle or ordinary centrifuge bottle, add 1 ml. of pectin solution (1 g. of pectin dissolved in 100 ml. of 25 per cent. alcohol), 3 drops of concentrated hydrochloric acid, and 50 ml. of alcohol, mix thoroughly, centrifuge, and decant the supernatant liquid. Dissolve the residue in 5 ml. of water, add 3 drops of concentrated hydrochloric acid and 50 ml. of alcohol, and again mix, centrifuge, and decant. Repeat this step (adding only acid and alcohol) until the supernatant liquid is colourless. Dissolve the residue in water; a brown solution indicates the presence of caramel. Confirm the result of the test by adding to this solution in the Babcock bottle 1 ml. of 2 : 4-dinitrophenylhydrazine reagent (1 g. of 2 : 4-dinitrophenylhydrazine, 7·5 ml. of sulphuric acid, and 67·5 ml.

of 95 per cent. alcohol). Place the bottle in a beaker of boiling water for 30 min., and then set it aside to cool for 30 min. If caramel is present, a precipitate will form during the first 15 min. of the heating period, and if a precipitate has not appeared by the end of the cooling period, caramel is absent. Disregard any precipitate that forms thereafter.

Experiments were made applying all the accepted methods for the detection of caramel to one representative of each of four troublesome classes of products encountered during a long experience of the analysis of wines for caramel, *viz.*, (1) very old elderberry, plum or dried fruit wines, or similar products which, through age, exposure, oxidation and perhaps some heating, have become very dark; (2) heated carbohydrate material that may have been treated in a number of ways and sometimes heated to about 150° C., a temperature too low for the production of true caramel; (3) solutions of the darkest raw Cuban sugar and of other forms of commercial brown sugar that are exceedingly dark but have not been heated above the temperature attained in the normal production of cane sugar; (4) all forms of commercial molasses (blackstrap) such as are used for the production of industrial alcohol. Wines consisting wholly or partly of one or more members of these four classes, but which do not contain any caramel colouring matter, may give coloured solutions in the Milos procedure when the final residues are dissolved in water. Although these solutions do not look exactly like caramel solutions, workers unfamiliar with them might be deceived at this stage. If these solutions are now submitted to the tentative confirmatory test, the colour will be eliminated if it is not due to caramel. Similarly, the coloured aqueous liquid may be submitted to the Mathers procedure, when the absence of caramel will again be demonstrated. The Mathers test is also applicable to the dark solutions sometimes obtained at the end of the Mallory - Love procedure, and the final aqueous liquids from the Mallory - Love test, the A.O.A.C. confirmatory test, and the Mathers test may all be subjected to the dinitrophenylhydrazine test already described.

Occasionally in testing for caramel in spirits with the Marsh reagent and with the *cyclo*hexanol reagent, positive indications will be obtained with both reagents when no caramel is present. *Cyclo*hexanol is an alternative reagent in the official method because it indicates the absence of caramel when the spirit has been stored for a long time with charred wood, or when certain coal tar dyes are present, or when the spirit has been quickly aged with raw, uncharred or untoasted white oak chips (*J. Assoc. Off. Agric. Chem.*, 1944, **28**, 467). Sometimes both reagents give indications of caramel when the spirit contains an infusion of peat-dried barley malt or an unusually heavy infusion of both toasted and untoasted white oak chips or very dark coloured wine solids or certain dyes, but no caramel. If it is necessary to prove the presence of caramel in the spirits, the sample should be analysed by the tentative confirmatory test followed by the Mathers test. All the above-mentioned substances that give a coloured liquid are quickly eliminated by these two tests, the final solution being practically or completely colourless. Should the final solutions be brown at the end of the Mallory - Love, the Mathers, or the A.O.A.C. tentative confirmatory tests, the dinitrophenylhydrazine test should be applied to them in the manner already described. A. O. JONES

Determination of Ascorbic Acid in Presence of Interfering Substances by the "Continuous Flow" Method. L. J. Harris and L. W. Mapson (*Brit. J. Nutrition*, 1947, **1**, 7–38)—By measuring the rates of reaction between certain reducing substances and 2 : 6-dichlorophenolindophenol, the authors have shown (i) that a rapid qualitative test can determine whether reducing substances present in a foodstuff have the properties of ascorbic acid or whether significant amounts of interfering substances are present, and (ii) that ascorbic acid can be quantitatively estimated in caramelised dried vegetables, which contain reducing substances resembling gluco-reductones. The reaction rates of a number of reducing substances with indophenol are given; of these only hydroxytetronic acid has a reaction rate approximating to that of ascorbic acid.

The method used was to force a mixture of equal quantities of a solution of indophenol and a dilution of the unknown, exactly equivalent to it in total reducing power, through a long uniform glass tube, the density of colour of the solution at various distances along the tube being measured by a movable photo-electric colorimeter. With constant flow of the liquid, the distance along the tube from the point of mixing was proportional to the duration of the reaction.

The apparatus consisted of two identical graduated reservoirs, capacity 200 to 400 ml.; from the bottom of each ran a tube through which the reacting liquids were forced by similar pressures on the surface of the liquids. The two tubes met at the commencement of the reaction tube, the tubing at the junction being narrowed to ensure instantaneous mixing. Manometers were fitted to each bottle in order that the pressure in each might be kept identical and at any desired figure. The rates of flow of the solutions under equal pressure were influenced by their densities. This could be counteracted when the difference in densities was small, by interposing tubing of smaller diameter between the reservoir and the reaction tube. With greater differences, up to 10 per cent. of sucrose could be added to the dye solution to equalise the densities without appreciable effect on the rates of reaction of the substances studied. The authors used a pressure producing a rate of flow of 430 ml. per min.; with the conditions obtaining in their apparatus, a distance of 40 cm. along the reaction tube represented a time of 1·1 sec.

Procedure—The concentration of the unknown extract or solution was determined against the standard indophenol solution (2 : 6-dichlorophenol-indophenol solution adjusted to *p*H 3·5 and equivalent to 0·04 mg. of ascorbic acid per ml.) by static photo-electric colorimetry. It was necessary to allow the reaction to proceed for

5 min. and to ensure at least 1·5 ml. of indophenol solution in excess. The extract was then diluted to be exactly equivalent to the indophenol solution.

Calibration of galvanometer—The standard dye solution was passed along the reaction tube together with a 2 per cent. solution of metaphosphoric acid adjusted with sodium citrate to *p*H 3·5. The amount of light passing through a small rectangular section of the tube was measured photo-electrically, a Wratten 61N filter being used. The galvanometer was of the moving coil suspension type with optical lever, with a sensitivity of 125 mm. per micro-amp. at 1 metre distance. The galvanometer reading was noted for different dilutions of the dye down to 10 per cent. of the standard concentration. It was found that the concentration of dye (as per cent. standard) $= K (\log G_w - \log G_d)$, where G_d was the galvanometer reading for the tube filled with water, and G_w was the reading for the solution of given strength.

Examination of unknown solutions—The unknown solution at *p*H 3·5 was passed through the tube along with the dye, and readings were taken at various positions representing various times of reaction. The percentage of dye remaining unreduced was given by $K (\log G_b - \log G_f)$. The galvanometer blank reading G_b was obtained by measuring the colour of the mixed solutions after reaction had proceeded to completion: G_f was the reading given by the solutions during flow. The constant K had been previously determined during the calibration. From this, the amount of dye reduced in a given time could be determined. The authors found that in 1·1 sec. the reaction with ascorbic acid (0·04 mg. per ml., temperature 20° C., *p*H 3·5) proceeded to 88 per cent. of completion, and with gluco-reductones, under the same conditions, to 38·5 per cent. With mixtures of the two substances, the resultant rate was proportional to the relative amounts present and this made possible a determination of both when present together. V. M. BOND

Biochemical

Determination of Tocopherol Content during the Commercial Processing of Soya Bean Oil. H. W. Rawlings, N. H. Kuhrt, and J. G. Baxter (*J. Amer. Oil Chem. Soc.*, 1948, **25**, 24–26)—A mixture (1 mg.) of α-, γ-, and δ-tocopherols in the same approximate ratio as they occur in soya bean oil, *viz.*, 10 : 60 : 30, reduces about 10 per cent. more ferric chloride than does 1 mg. of α-tocopherol. The assay devised by Rawlings (*Oil and Soap*, 1944, **21**, 257; ANALYST, 1945, **70**, 58) has accordingly been modified by introducing the factor 0·91 to correct the result obtained for the enhanced colour due to δ-tocopherol.

Procedure—Dissolve sufficient of the sample in redistilled absolute alcohol to produce a solution containing from 50 to 300 μg. of tocopherol per ml. To 1 ml. of this solution add 1 ml. each of 0·1 per cent. ferric chloride ($FeCl_3.6H_2O$) and 0·25 per cent. α,α′-dipyridyl solutions in ethyl alcohol. Add 22 ml. of redistilled absolute alcohol, mix, set aside, and, 10 min. after the addition of the ferric chloride reagent, read the colour density in an Evelyn colorimeter (1·9-cm. cell) using a 520-mμ. filter. For colorimeters fitted with a 1-cm. cell use a 2-ml. aliquot of the sample and 1 ml. each of 0·2 per cent. ferric chloride and 0·5 per cent. α,α′-dipyridyl reagents.

Determine the apparent tocopherol concentration in the sample from a curve prepared from pure α-tocopherol and multiply it by the correction factor 0·91 to obtain the correct assay of mixed tocopherols. The method is estimated to be accurate to within 10 per cent. A. H. A. ABBOTT

Biological Estimation of Vitamin D. J. I. M. Jones (*Quart. J. Pharm.*, 1945, **18**, 92–107)—The response data obtained with rats in the routine biological assay of vitamin D over a period of 4 years has been examined. These data include responses to 3 graded doses of the International Standard irradiated ergosterol, measured both by the line test, data for periods 2 and 3 were similar periods are covered in which the rat colony had a different home; responses by the X-ray technique increased over each successive period, but with the line test, data for periods 2 and 3 were similar and higher than for period 1. The curve obtained by plotting the logarithms of the doses against the responses is linear, although the slope shows variation. The number of cases not satisfying the statistical criteria of linearity is not greater than would be expected from sampling errors. There is, however, a lack of homogeneity of variance making each assay, of necessity, "self-contained." The fiducial limits of different assays show considerable variation and there is a tendency for this to decrease in consecutive periods, this being possibly related to the increase in mean response. The limits may be reduced by suitably designing the assays to compensate for inter-litter differences.

Agreement between the results of the two methods is good, the results by the line test being, on the average, 99 per cent. of those by the X-ray technique when applied to preparations of potencies ranging from 20 to 50,000 i.u. per g. The design of assays should be such as to minimise difference in initial weight of the rats since, on the whole, evidence indicates that such differences have an effect on the assay. J. ALLEN

Determination of Aneurine in Potatoes. J. Bouman (*International Review of Vitamin Research*, 1948, **19**, 386–393)—Methods for determining aneurine in potatoes are reviewed, and a new procedure that is claimed to have important advantages is described.

Procedure—The potatoes having been cleaned, steamed in their skins, ground, and mixed, weigh 20-g. lots into two 100-ml. flasks. To both, add 40 ml. of a 25 per cent. sodium chloride solution in 0·25 per cent. acetic acid, and to one, 45 μg. of aneurine. Add 25 mg. of papain, 12 mg. of diastase, and 1 ml. of toluene to each and incubate at 40° C. overnight. Centrifuge the suspensions, and to 20 ml. of each liquid add 4 drops of concentrated hydrochloric acid and 20 ml. of *iso*butanol. After shaking for 2 min. and centrifuging, place 2 ml. of

the aqueous solutions in centrifuge tubes and add 2 ml. of methanol to each. Pass a stream of air through the solution and add 1 ml. of 30 per cent. sodium hydroxide solution. After 45 sec., add 0·25 ml. of 5 per cent. potassium ferricyanide solution, and after 1·5 min., 13 ml. of *iso*butanol. Maintain the air stream for 4 min. Carry out blank determinations without adding the ferricyanide. After centrifuging, pipette 10 ml. of the *iso*butanol layer into a tube containing 0·5 ml. of 96 per cent. ethanol, and measure the fluorescence.

This procedure gives higher results than the earlier method and the percentage of recovered aneurine is greater (95 per cent. as against 75 per cent.).

Blighted potatoes contain a substance that, in alkaline solution, gives a fluorescence that is diminished by the addition of ferricyanide. As the interfering substance is extracted by the *iso*butanol from acid solution, correct values are obtained in analyses of blighted potatoes by the above method. V. BOND

Microchemical Spot Test for Mammalian Urine Contamination on Fabrics. N. H. Ishler, K. Sloman, and M. E. Walker (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 670–672)—Attempts were made to develop a drop reaction for well-known constituents of urine, such as creatinine and urea. Direct chemical methods such as the biuret test for urea and the Weyl nitroprusside test for creatinine were unsuccessful when applied directly to contaminated fabric. Evolution of ammonia from urea by urease and its detection by means of filter paper impregnated with manganese nitrate and silver nitrate provided a rapid and sensitive test for urea.

METHOD—To prepare the urease solution allow a 10 per cent. aqueous suspension of Jack Bean meal to settle for a few minutes and pour off the supernatant liquid for use as the urease solution. The suspension can be stored for a week in a refrigerator without apparent loss of enzymic activity. Dilute 14 ml. of a 50 per cent. solution of manganese nitrate to 100 ml. and add it to 4 g. of silver nitrate dissolved in 100 ml. of water. Treat the combined solutions with 0·1 *N* sodium hydroxide solution until a black precipitate begins to form, filter, and store the filtrate in a dark-coloured reagent bottle. Soak coarse filter paper in the reagent and dry it quickly on the metal top of a steam-bath heated to about 100° C. The paper may develop brown stains even when stored in the dark, but these do not interfere with the test. It is desirable, however, to prepare fresh papers weekly.

Procedure—If the suspected stain is not visible in ordinary light, outline it with pencil while observing it under ultra-violet light. Apply 2 to 4 drops of the urease solution to the stained area and allow it to soak for 5 to 10 sec. Place the fabric on the surface of a heated steam-bath and immediately place over it a piece of the impregnated paper, so that the paper is wetted by the damp area of the fabric. Lay a piece of glass rod across the paper and press firmly. If the stain is due to urine, a black spot will appear on the paper in about 30 sec. Although there is little likelihood of a false indication from ammonia vapour in the laboratory atmosphere, a sensitised paper placed beside the specimen under examination will serve as a blank test to avoid error from this source. If the urease solution fails to wet and penetrate effectively some heavily sized fabrics, 0·1 per cent. of a wetting agent such as Naconol (National Aniline) may be added to the urease solution. This will neither give a false indication nor impede the reaction. The test gives satisfactory results with fabric dusted with grain products (oatmeal, corn meal, and flour) and these do not give a false reaction in the absence of urine. Sulphides produce a black stain when in direct contact with the paper. Sucrose and dextrose do not interfere, but molasses contains reducing substances that give a false positive reaction. In presence of either sulphides or organic reducing materials, a false indication may be avoided by supporting the impregnated paper above the specimen on two horizontal glass rods placed about an inch apart on the fabric. The time required for development of the stain is then doubled. Wetting the sensitised paper retards the reaction still further.

In order to form an estimate of the relative advantages of any one test, the A.O.A.C. methods ("*Methods of Analysis*," 6th Ed., 1945) were examined. The urease test (42·98) depends upon the same principle as the test described, but the ammonia is detected by formation of ammonium chloroplatinate. This test was found to be valid in presence of grain materials likely to be encountered in flour sacking. The xanthydrol test for urea (42·99) depends upon formation and microscopical examination of xanthydrol-urea but, since false positive results were obtained with the test, it was not examined further. The method "Extraction of Urea and Crystallisation of Urea Nitrate" (42·100) gave characteristic needle-shaped crystals of urea by extraction with alcohol and acetone, but it was found difficult to convert these to a recognisable form of urea nitrate. By the official urease test (*supra*), 0·05 per cent. of urea can be detected in 12 min.; by the new test 0·01 per cent. of urea can be detected in 30 sec., and the rapidity of this test reduces the likelihood of extraneous contamination. A. O. JONES

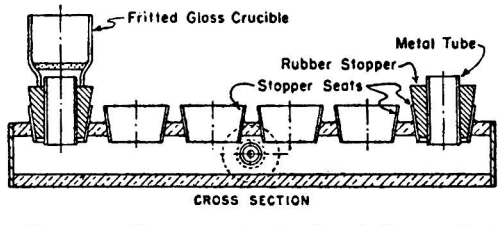
Agricultural

Determination of Moisture in Fertilisers. W. H. Ross and K. S. Love (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 617–623)—In an examination of methods for the determination of moisture in fertilisers, some of which may contain water of hydration and unstable organic matter, the following conclusions were reached. The official method ("*Methods of Analysis of the A.O.A.C.*," 6th Ed., 1945, p. 20) usually gives higher values for moisture in fertilisers containing water of hydration than does the air-flow method (see the following abstract) or the method depending upon desiccation of the sample *in vacuo* over magnesium perchlorate ("Anhydrone"). The air-flow method gives lower

results for moisture in materials that tend to lose water of hydration *in vacuo* over anhydrone than either the official or the vacuum-drying methods. The presence of free acid (Hill and Jacob, *J. Assoc. Off. Agric. Chem.*, 1934, **17**, 487) increases the rate at which monocalcium phosphate monohydrate loses water of hydration in the official method. The air-flow method gives higher results than does the vacuum-drying method in a material such as ammonium nitrate, which contains occluded water, and although ammonium nitrate undergoes decomposition at 100° C., the rate is too slow to interfere with the official method. All three methods give essentially the same results with stable materials that do not contain occluded water or water of crystallisation. One or two hours' drying by the air-flow method at 60° C. appears to suffice for the fertiliser materials tested, with the possible exception of crystalline ammonium nitrate containing occluded water. The official method is not suitable for the determination of free moisture in presence of easily oxidisable organic matter.

A. O. JONES

Air-Flow Method for Determining Moisture in Fertilisers. J. O. Hardesty, C. W. Whittaker, and W. H. Ross (*J. Assoc. Off. Agric. Chem.*, **1947, 30, 640–648**)—The decomposition of organic matter and of crystalline hydrates and the liberation of water of constitution that may occur when fertilisers are dried at 100° C. can be largely avoided if the moisture is removed rapidly at a low temperature, as by passing a current of air, heated to about 60° C., directly through the sample. Although the drying of the material over a suitable desiccant for at least 2 hr. in a well-evacuated desiccator approaches the ideal procedure more closely than most of the other methods examined, some powerful drying agents (*e.g.*, magnesium perchlorate) can reduce the partial vapour pressure of the moisture surrounding the sample to such an extent that some of the crystalline hydrates (*e.g.*, magnesium ammonium phosphate hexahydrate) may lose water of crystallisation.



METHOD—The apparatus for the air-flow method consists of a metal manifold vessel of dimensions 10·5 × 2·5 × 1·25 in., provided with a 0·25-in. nipple centrally fixed in one of the longer sides for attachment to a suction apparatus, and six 1·25-in. tapered stopper seats evenly spaced along the top to accommodate rubber stoppers. A metal tube of length 1·25 in. and diameter 0·5 in. extends through each stopper to a height of about 0·25 in. above the surface and serves to centre a sintered-glass crucible over the hole in the stopper. Since the crucible is held in place only by suction, it is necessary to grind a smooth surface on the lower edge of the crucible and on the surface of the stopper to ensure air-tight contact when air is being drawn through the sample in the crucible. The crucibles, which are of the ordinary type used for filtration, should have a sintered-glass plate of fine porosity, and, for accurate work, a set of crucibles of matched porosity should be used. The rates of flow of air through the crucibles may be compared by means of a gas meter or by fitting the crucibles to the tightly-fitting rubber stopper of a separating funnel filled with water, the time taken by the funnel to empty being measured. Since the resistance to air flow afforded by the disc is large relatively to that afforded by the sample, the rate of flow is almost independent of the amount and particle size of the material.

Procedure—Spread a 2-g. sample of the fertiliser evenly over the disc of the tared crucible, apply suction to the apparatus and fit the crucible to its stopper, making sure that the contact is air-tight as shown by resistance of the crucible to removal. Close any unused seatings in the apparatus with plain rubber stoppers. Adjust the suction to about 0·5 atm. and place the apparatus in an oven fitted to receive it and previously adjusted to 60° C. Maintain the air flow for 2 hr. or longer. The oven thermometer should have its bulb near the top of the manifold vessel to indicate the temperature of the air entering the crucibles. After cooling the crucibles in a desiccator, ascertain the loss in weight.

It has been shown (Hardesty and Davis, *Ind. Eng. Chem.*, 1946, **38**, 1298) that fertiliser mixtures containing superphosphate, inorganic nitrates, and organic matter may undergo decomposition at temperatures above 85° C., the organic matter being oxidised by nitric acid formed by interaction of the nitrate and acid calcium phosphate or free phosphoric acid. No appreciable loss of volatile matter other than free moisture was observed at temperatures below 85° C.

In experiments with cocoa shell meal, one of a class of organic fertiliser conditioners that retain moisture tenaciously, oven-drying at 100° C. gave considerably higher values than either the vacuum-drying or the air-flow method. This is attributed to slow decomposition of organic matter at that temperature. Drying *in vacuo* over magnesium perchlorate for 21 to 26 hr. also gave somewhat higher results than those of the air-flow method, and when the material dried over the desiccant was further treated by the air-flow method, it regained an amount of moisture equal to the difference between the original results obtained by these two methods. This indicates that the moisture in the air drawn through the highly adsorptive material prevented as complete drying as was obtained by means of the desiccant. When extremely small changes in moisture content are of interest (*e.g.*, in the control analysis of ammonium nitrate) it may be expedient to dry the air passed through the sample or to maintain it at a selected constant relative humidity.

Samples of mixed fertilisers of differing composition and having a free moisture content of

about 7 per cent. were subjected to moisture determination (1) by the official A.O.A.C. method ("*Methods of Analysis*," 6th Ed., 1945, p. 20); (2) by vacuum desiccation over magnesium perchlorate; (3) by the air-flow method. The results show that the official method is not adapted to the determination of free moisture in samples containing easily oxidisable organic matter. Such decomposition does not occur during the determination either by the vacuum desiccation method or by the air-flow method. A. O. JONES

Water Analysis

Determination of Small Concentrations of Calcium and Magnesium by Titration with Standard Soap Solution. J. W. McCoy (*Anal. Chem.*, **1947, 19**, 1002–1003; *cf.* Polsky and Feddern, *Ind. Eng. Chem., Anal. Ed.*, **1942, 14**, 644)—Total hardness and calcium contents between 5 and 50 p.p.m. are determined by titration with standard soap solution at two suitably adjusted *p*H values; the magnesium content is obtained by difference. Standardisations must be carried out at the same *p*H as the determinations, *i.e.*, *p*H 8·3 for total hardness and *p*H 11·7 for calcium content. To avoid a "ghost point" or "false lather," a foam lasting 5 min. is taken to indicate the end-point.

Procedure—Calcium content—To a 50-ml. sample in an 8-oz. stoppered bottle add 1 ml. of 10 per cent. ammonium chloride solution and 2·7 ml. of *N* sodium hydroxide. Add small increments of standard soap solution, and shake vigorously after each addition until a foam lasting 5 min. is obtained.

Total hardness—Adjust the *p*H of a second 50-ml. sample to *p*H 8·3 (the first pink colour of phenolphthalein) and then titrate with soap solution.

Equivalent p.p.m. of calcium carbonate are read off from the standardisation curves obtained at the appropriate *p*H and the difference gives the magnesium content.

An application of the method to the control of lime and magnesia feeds for silica removal units in high-pressure water-treatment plants is detailed. A sample of the flocculent suspension of calcium and magnesium oxides is divided into two. One part is filtered, and titration of the filtrate, after the necessary *p*H adjustments, gives the quantities of the dissolved oxides. The other is dissolved in acid and titration gives the composition of the combined suspended and dissolved oxides. The amount and composition of the suspended floc can thus be determined; also, by comparison with a titration on the raw water, the increase in hardness due to dissolution of the oxides can be found.

C. F. HERBERT

Organic

Volumetric Determination of Carbon-Bonded Halogen with Sodium Naphthalene. F. L. Benton and W. H. Hamill (*Anal. Chem.*, **1948, 20**, 269–270)—*Special reagent*—Prepare solutions of "sodium naphthalene" in ethylene glycol dimethyl or diethyl ether according to the directions of Scott *et al.* (*J. Amer. Chem. Soc.*, **1936, 58**, 2442) and use at approximately 0·5 *M* concentration. Store the reagent in an automatic burette and transfer the solution from the preparation flask to the burette under an atmosphere of nitrogen.*

Procedure—Displace the air from a separating funnel by dry nitrogen and introduce the sample, contained in a thin-walled, sealed glass bulb. If the sample be solid, introduce it directly into the separating funnel and add 5 ml. of toluene to dissolve it. Add 10 to 20 ml. of sodium naphthalene reagent, stopper the separating funnel, shake vigorously to break the bulb, and continue shaking for 2 to 3 min. longer. Add 2 or 3 consecutive volumes of water, up to a total of approximately 100 ml., to destroy the excess of the reagent and dissolve the sodium halide. Acidulate the combined aqueous layers with nitric acid and titrate potentiometrically with 0·1 *N* silver nitrate, using a silver-plated platinum wire and a saturated calomel electrode with an ammonium nitrate-agar gel bridge.

The nitro-group causes interference in both aliphatic and aromatic compounds, and hexabromobenzene and 2 : 2-difluoroheptane do not react at all. Compounds containing active hydrogen will probably form an insoluble sodium salt.

The authors believe that potentiometric titration can be successfully replaced by the Volhard or Mohr methods of titration.

[* ABSTRACTOR'S NOTE—To 1000 ml. of a molar solution of naphthalene in pure, dry ethylene glycol diethyl ether contained in a 2-litre flask equipped with a mercury-sealed stirrer and from which all air has been displaced by nitrogen, add about 25 g. of sodium. Stir mechanically for about 2 hr. Do not allow the temperature to rise above 20° C.; cool the mixture, if necessary, by immersing the flask in a bath of methanol cooled by addition of solid carbon dioxide.

If the dimethyl ether is used as a solvent, the reaction must be carried out at −30° C.]

A. H. A. ABBOTT

Colorimetric Micro-determination of Formic Acid based on Reduction to Formaldehyde. W. M. Grant (*Anal. Chem.*, **1948, 20**, 267–269)—The method described for the determination of 0·25 to 15 μg. of formic acid in 0·5 ml. of solution is based on reduction of formic acid to formaldehyde by means of magnesium, and subsequent measurement of the formaldehyde by means of chromotropic acid. For determination in blood, formic acid is first separated from interfering substances, such as protein and carbohydrates, by quantitative vacuum micro-distillation at low temperature. In applying the method to mixtures of formic acid and formaldehyde, the formaldehyde is first removed by treatment with phenylhydrazine.

The sensitivity of the procedure for determining formic acid, whilst superior to that of existing methods, is slightly less than that which would be attained if the formic acid was quantitatively converted to formaldehyde. At present, a net

yield of approximately 29 per cent. of the theoretical is obtained, corresponding to a total yield of 34 per cent. with a coincident loss of 15 per cent. of the formaldehyde formed. Among possible interfering substances is carbonic acid, which can be reduced to formaldehyde by magnesium, but it is easily eliminated by preliminary acidification of samples. Interference by acetic acid or acetaldehyde occurs only when there is a high proportion of these substances.

METHOD—*Special reagents—Chromotropic acid*—Dissolve 0·6 g. of chromotropic acid (1 : 8-dihydroxynaphthalene-3 : 6-disulphonic acid) in 20 ml. of water and add the solution to 180 ml. of concentrated sulphuric acid. *Magnesium ribbon*—Roll strips 10 cm. long and 3 mm. wide into coils 1 cm. in diameter. The coils should weigh approximately 80 mg. and must be stored in a desiccator over sodium hydroxide.

Procedure—Transfer a 0·5-ml. sample, containing not more than 15 μg. of formic acid, to a test tube containing an 80-mg. coil of magnesium ribbon. Immerse the test tube in ice and add a total of 0·5 ml. of concentrated hydrochloric acid in ten separate portions of 0·05 ml. at intervals of at least 1 min. Allow to stand for 1 min., add 1·5 ml. of chromotropic acid reagent, and heat the tube in boiling water for 30 min., taking precautions against absorption of water vapour and uneven illumination. Centrifuge to remove the white precipitate and measure the colour of the supernatant liquid in a photo-electric colorimeter. Establish the relation between the colorimeter reading and the quantity of formic acid by measurement of the colours produced by submitting a series of standards containing from 0 to 15 μg. of formic acid in 0·5 ml. of 0·01 *N* hydrochloric acid to the procedure described above.

If the sample is suspected to contain carbonates, acidify to *p*H 2 by adding hydrochloric acid before the magnesium reduction.

To determine the formic acid in blood, mix 0·5 to 1 ml. of blood with 2 volumes of a solution containing 5 per cent. of sulphosalicylic acid in 0·25 *M* sulphuric acid. Centrifuge the mixture and separate the formic acid from carbohydrates by low temperature vacuum distillation of the supernatant liquid. This distillation can conveniently be made in an appropriate, evacuated and sealed tube (Grant, *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 729) with the condenser cooled in a bath of "dry ice." The distillate is treated in the manner described for pure solutions and a correction made in the calculation for the change in volume resulting from removal of non-volatile substances. For average rabbit blood, the mixture of blood and sulphosalicylic acid solution contains 93 per cent. by volume of volatile materials.

If formaldehyde is present in the sample, measure the amount directly and make a correction for the colour produced by it in the determination for formic acid. To remove formaldehyde from blood samples, add phenylhydrazine to the supernatant liquid obtained from the mixture of blood with sulphosalicylic acid and sulphuric acid in the proportion of 10 mg. of phenylhydrazine hydrochloride per ml. of supernatant liquid, and allow the mixture to stand for 5 min. at room temperature before transferring it to the distillation apparatus.

A. H. A. ABBOTT

Further Studies on Ofner's Method for the Determination of Invert Sugar. E. J. McDonald and A. L. Turcotte (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 124–130)—Copper solutions of low alkalinity are better suited for the analysis of small amounts of invert sugar in presence of sucrose than are those of higher alkalinity. In a critical study by Jackson and McDonald (*Ibid.*, 1943, **26**, 462), Ofner's method (*Z. Zuckerind. Czechoslov. Rep.*, 1928–29, **53**, 733; 1931–32, **56**, 249; 1934–35 **59**, 52) for the determination of invert sugar in refined sugars is recommended, but with some modifications, including the substitution of an ordinary asbestos gauze plate for the metal gauze to ensure more uniform heating and avoid the "flame spot." The precision of the method is greatly increased by acidification with acetic acid before the addition of iodine and the subsequent addition of hydrochloric acid, rapid oxidation of cuprous chloride in acid solution being thus avoided. Jackson and McDonald confined their experiments to sugar samples containing from 5 to 25 mg. of invert sugar. In the present investigation the precision of the method was determined both with invert sugar alone and with sugar samples containing less than 0·05 per cent. of invert sugar, and the results confirm the validity of the modified Ofner method.

Invert sugar can be determined more accurately alone than in presence of sucrose, and it therefore appears advisable to base results on invert sugar values and to apply a correction for the increased reduction due to sucrose. Invert sugar used in the experiments was prepared by weighing equal amounts of pure laevulose and dextrose and by hydrolysis of pure sucrose, and identical analytical results were obtained in the two procedures. The normality of the iodine solution was 0·0323, but, when 5 mg. or less of invert sugar were being determined, 0·00323 *N* iodine was used, the results being expressed in terms of the former solution. The reagents and procedure were those of Jackson and McDonald ("*Methods of Analysis of the A.O.A.C.*," 1945 Ed., 34·47–8), but the sodium thiosulphate solutions, against which the iodine solutions were standardised, were repeatedly standardised against pure copper or a copper sulphate solution in which the copper had been determined electrolytically; and, during boiling, the mouth of the flask was closed by a glass bulb or by a small inverted beaker.

It was found that when not more than 5 mg. of invert sugar was present the following linear relation existed, $S = 0{\cdot}177 + 1{\cdot}01241\,I$, where S = mg. of invert sugar and I = ml. of 0·0323 *N* iodine consumed. Above this range, the equation $S = 0{\cdot}187 + 0{\cdot}855\,I + 0{\cdot}005117\,I^2$ was applicable.

Since sucrose, even after careful purification, reduces alkaline copper solutions, the question arises whether to make a correction for the reduction of pure sucrose or to express the results on the basis of invert sugar alone. Values reported in the

literature for the apparent invert sugar content of pure sucrose range from 0·003 to 0·013 per cent. In the present work, the value found was 0·013 per cent.

If the sucrose hydrolyses during the determination, it is to be expected that methods using different boiling times and solutions varying in *p*H would give varying results. Analyses in which 1 mg. of invert sugar and a sample of sucrose containing 1·3 mg. of apparent invert sugar were used and in which the boiling period ranged from 1 to 20 min. showed that the reducing effect of sucrose is caused, at least in part, by substances formed during the analysis rather than by any constituent of the original sample. Furthermore, when sucrose is present, small variations in the boiling period were found to exert a greater effect on the amount of copper reduced than when invert sugar alone is present. When the total invert sugar content of 10 g. of sucrose was not more than about 5 mg., the effect of the sucrose and added invert sugar seemed to be additive. It is therefore recommended that for sucrose samples containing 0·05 per cent. or less of invert sugar the iodine used should be calculated to invert sugar by means of the equation (*supra*) and the results reported as apparent invert sugar. Alternatively, a correction amounting to 1·1 ml. of 0·0323 *N* iodine for each 10 g. of sucrose may be subtracted from the titration to compensate for the reducing action of pure sucrose. The result would not then include the apparent invert sugar due to the reducing action of the sucrose. For amounts of invert sugar above 5 mg. the table published by Jackson and McDonald (*loc. cit.*) is more convenient and accurate. A. O. JONES

Chemical and Microbiological Differentiation of Enantiomorphs of Galactose and Xylose. J. W. Appling, E. K. Ratcliff, and L. E. Wise (*Anal. Chem.*, 1947, **19**, 496–497)—It was shown by Richtmyer and Hudson (*J. Amer. Chem. Soc.*, 1936, **58**, 2540) that D- and L-altrose possess markedly different reducing powers towards optically active reagents such as alkaline copper tartrate prepared from *dextro*-tartaric acid; and differences of a similar kind were obtained with D- and L-arabinose. It is now shown that the same type of asymmetric oxidation occurs with D- and L-galactose and D- and L-xylose, respectively. The Munson - Walker method was used and the Fehling's solution was prepared with L-(*dextro*)-tartaric acid. As examples from a number of results quoted, 75 mg. of L-galactose yielded 144·7 mg. of cuprous oxide, and 75 mg. of L-xylose yielded 151·7 mg. of cuprous oxide, whilst the corresponding values for the D-isomerides (Wise and McCammon, *J. Assoc. Off. Agric. Chem.*, 1945, **28**, 167) were 154·8 mg. and 168·4 mg., respectively. The reducing values of L-galactose are especially sensitive to slight variations in the Munson - Walker technique.

Using the methods previously described (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 28), the D-component of a racemic galactose mixture was destroyed by *Saccharomyces carlsbergensis* at 30° C. within 48 hr., or within 24 hr. with continuous shaking, whilst the L-isomeride was not attacked. Neither isomeride was fermented by *S. bayanus*. *Hansenula suaveolens* ferments D-xylose quantitatively (*Idem, ibid.*, 1945, **17**, 182), but has no action on L-xylose or L-galactose. It should be possible, therefore, to ferment D-xylose selectively in the mixture of this sugar with L-galactose which is obtained by the acid hydrolysis of flaxseed mucilage.

W. C. JOHNSON

2 : 4-Dinitrophenylhydrazine for the Determination of Essential Oils. E. Hucknall (*Quart. J. Pharm.*, 1945, **18**, 84–85)—A general technique has been developed for the preparation of the 2 : 4-dinitrophenylhydrazones of those aldehydes and ketones that may be regarded as characteristic constituents of certain essential oils, thus enabling oils isolated from medicinal and other preparations to be unequivocally identified.

Method—Separate the oil from the preparation under examination either by steam distillation in an apparatus of the type described by Cocking and Middleton (*Ibid.*, 1935, **8**, 435) or by distillation with ethylene glycol *in vacuo* (Sabetay, *Ann. Chim. analyt.*, 1939, **21**, 173). Dissolve 0·05 to 0·5 ml. of the separated oil in about five times its volume of ethyl alcohol and add the solution dropwise, with vigorous shaking, to 2 to 5 ml. of a saturated solution of 2 : 4-dinitrophenylhydrazine in 2 *N* hydrochloric acid at 0° C. Filter, wash with 2 *N* hydrochloric acid, and then with water until the

	Oil	Characteristic constituent	2 : 4-Dinitrophenylhydrazone		
			Colour	Crystallising solvent	m.p. ° C.
1.	Bitter almond	Benzaldehyde	Orange	Acetic acid	237
2.	Caraway	D-Carvone	Red	" "	190
3.	Cassia	Cinnamic aldehyde	"	" "	255
4.	Cinnamon	" "	"	" "	255
5.	Citronella	D-Citronellal	Orange	Ethyl alcohol	77
6.	Cummin	Cuminic aldehyde	Red	Acetic acid	243
7.	Dill	D-Carvone	"	" "	190
8.	Lemon	Citral	"	" "	110
9.	Lemongrass	"	"	" "	110
10.	Pennyroyal	Pulegone	"	Ethyl alcohol	147
11.	Peppermint	Menthone	Orange	" "	146
12.	Rue	Methylnonylketone	Yellow	" "	63
13.	Spearmint	L-Carvone	Red	Acetic acid	193

precipitate is free from chloride, and recrystallise from the appropriate solvent. Note the colour (in bulk), crystalline habit, and melting-point of the 2 : 4-dinitrophenylhydrazone. The table gives the relevant information for those oils with which the reagent has been found to be satisfactory. Peppermint oil is included because, although menthol is the characteristic constituent, it is often necessary to distinguish this oil from other mint oils.

J. ALLEN

The Wijs Method of Determining Iodine Numbers. Proposed Modification. A. Vossgard and E. Björsvik (*Z. anal. Chem.*, 1938–39, **115**, 195–204)—The effect of varying the time of standing, the excess of halogen, and the composition of the solvent has been investigated. A solution of iodine monochloride in a mixture of glacial acetic acid and carbon tetrachloride is more stable than a solution in glacial acetic acid, and no crystallisation takes place at room temperature.

Procedure—Weigh a sample of the oil, depending on its iodine number, such that an excess of 65 to 400 per cent. of halogen is later established. Add 25 ml. of 0·2 *N* iodine monochloride in a mixture of 45 parts by volume of glacial acetic acid and 55 parts of carbon tetrachloride, and allow the whole to stand for 30 min. at about 20° C. Add 10 ml. of 10 per cent. potassium iodide solution and titrate back the excess of iodine with 0·1 *N* sodium thiosulphate.

Results obtained agree with the Wijs method on fish and vegetable oils with iodine numbers ranging from 8 to 190, the maximum deviation being ±0·3 unit.

M. E. DALZIEL

Colorimetric Determination of Certain Primary and Tertiary Amines. F. L. English (*Anal. Chem.*, 1947, **19**, 457–459)—Procedures are described for the determination of certain primary amines in the presence of their *N*-alkyl derivatives and of certain *N*-dialkyl amines in the presence of the corresponding primary and secondary amines. In both types of determination the mixture of amines is treated in acid solution with sodium nitrite. Tertiary amines are then determined by the intensity of the yellow colour of their *p*-nitroso compounds. When primary amines are to be determined the nitrite treatment is followed by addition of Chicago acid (1-amino-8-naphthol-2 : 4-disulphonic acid) to form a red azo dye, the colour intensity of which is measured with a similar solution, without Chicago acid, in the comparison cell to compensate for any yellow colour contributed by tertiary amines. Nitrosamines derived from secondary amines are substantially colourless. The Cenco - Sheard - Sanford Photelometer is used with 1-cm. flat absorption cells. The Cenco Photelometer Type B–2 and the Fisher Electrophotometer are unsuitable.

A calibration curve is prepared for each method and, to avoid certain small errors due to extraneous colours, suitable known mixtures, rather than single amines, are employed for purposes of calibration. For this purpose specially purified amines are employed.

METHOD—*Reagents*—(1) Dissolve 3 g. of sodium nitrite and 5 g. of sodium bromide in water and dilute to 100 ml. The bromide increases the velocity of diazotisation. (2) Dilute 10 ml. of 37 per cent. hydrochloric acid to 100 ml. (3) Dissolve 10 g. of sulphamic acid in water and dilute to 100 ml. (4) Sodium acetate solution, 50 per cent. by weight in water. (5) Dissolve 1 g. of purified Chicago acid in water containing 2 ml. of concentrated hydrochloric acid and dilute to 100 ml. To purify the Chicago acid dissolve 100 g. (100 per cent. basis) in 800 ml. of water, add 20 ml. of concentrated hydrochloric acid and 40 g. of decolorising carbon, heat to 70° C., filter, cool to 10° C., add 200 ml. of concentrated hydrochloric acid, cool to 0° to 5° C., filter with suction, wash successively with 150 ml. each of 1 : 1 hydrochloric acid and methanol, and acetone, and dry in a vacuum desiccator. To test for purity, dissolve 1 g. in 100 ml. of water containing 2 ml. of concentrated hydrochloric acid. The solution should be coloured a light greenish-yellow and should give a reading not lower than 70 against distilled water when the Photelometer is used with the green filter.

Procedure for aniline in its N-methyl or ethyl derivatives—Dissolve 1 ml. of the sample in water with 2 ml. of concentrated hydrochloric acid and dilute to 100 ml. To 1 ml. of this solution add 1 ml. of dilute hydrochloric acid and 10 ml. of water, cool to 0° to 5° C., add 1 ml. of sodium nitrite - bromide solution, allow to stand for 1 min. in ice, add 1 ml. of sulphamic acid solution, mix thoroughly, and continue agitation for 30 sec. in the ice-bath. Add 2 ml. of Chicago acid solution, 10 ml. of sodium acetate solution, and 25 ml. of methanol, and dilute to 100 ml. with water. Read the colour intensity against a solution prepared similarly but omitting the Chicago acid. The green filter is used in the colorimeter.

α-Naphthylamine in ethylnaphthylamines—Proceed as for aniline up to and including the addition of sulphamic acid, then add 5 ml. of Chicago acid solution and 10 ml. of sodium acetate solution and heat to 30° C. in warm water. The comparison solution contains no Chicago acid and must not be heated.

m-Toluidine in methyltoluidines—Proceed as for aniline but, after the addition of Chicago acid and sodium acetate, heat on a steam-bath for 5 min., cool, add 50 ml. of methanol, and dilute with water to 100 ml. Prepare the comparison solution similarly, but heat on the steam-bath for 5 min. *before* the addition of the Chicago acid.

o-Toluidine in its ethyl derivatives—Follow the usual procedure of diazotisation and treatment with sulphamic acid, then add 5 ml. of Chicago acid solution and 10 ml. of sodium acetate solution, warm to 30° C., and dilute to 100 ml. Prepare the comparison solution similarly but omit from it the sample aliquot.

Dimethyl- and diethylaniline in the presence of corresponding primary and secondary amines—Take a 1-ml. aliquot of the sample solution, prepared as for the aniline determination, add 1 ml. of glacial acetic acid and 10 ml. of water, cool to 0° to 5° C., and add 5 ml. of a solution of 75 g. of sodium

acetate in 100 ml. of water. Allow to stand in ice for 2 min. (for dimethylaniline) or 10 min. (for diethylaniline), add 25 ml. of methanol, and dilute with water to 100 ml. Remove gas bubbles by vigorous shaking. Prepare a blank with 1 ml. of dilute hydrochloric acid in place of the sample aliquot. Use a blue filter in the colorimeter.

Diethyl-m-toluidine in monoethyl and/or m-toluidine—To 2 ml. of the 1 per cent. v/v sample solution, add 1 ml. of glacial acetic acid, 50 ml. of water, and 5 ml. of a solution of 75 g. of sodium nitrite in 100 ml. of water, allow to stand at room temperature for 1 min., add 25 ml. of methanol, dilute with water to 100 ml., mix, allow to stand for 10 min., and proceed as for dimethylaniline. Concentrations of *m*-toluidine above about 40 per cent. interfere, giving high results through decomposition of the diazonium compound.

Diethyl-*o*-toluidine and diethyl-α-naphthylamine are not determinable by similar procedures since their nitroso compounds are not sufficiently coloured. The methods described will detect 0·05 per cent. of the compound sought and cover the range up to about 6 per cent. for primary and 10 per cent. for tertiary amines. W. C. JOHNSON

Photo-colorimetric Method for the Determination of Quaternary Ammonium Salts. E. L. Colichman (*Anal. Chem.*, 1947, **19**, 430–431)—Auerbach's method (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 492) for all quaternary ammonium salts offers great sensitivity and selectivity in presence of common inorganic and organic impurities and permits the determination of very small concentrations. Difficulties in clarifying the extracted solution led Auerbach to substitute benzene for ethylene dichloride in a revised, but somewhat longer and less widely applicable method (*Ibid.*, 1944, **16**, 739; ANALYST, 1945, **70**, 143). In order to preserve the high selectivity of the method and eliminate the somewhat tedious operations, not always subject to precise control, a modification has been developed which, although less sensitive and not applicable to determinations of concentrations of 0 to 20 p.p.m., is entirely adequate for higher ranges in routine commercial analyses.

The method consists of forming the quaternary ammonium bromophenol blue salt in carbonate solution in presence of a critical excess of the dye. The colour density of the solution is measured directly by means of a photo-electric colorimeter without extracting the colour complex with an organic solvent.

Procedure—Prepare a calibration curve for the quaternary salt in the range 0 to 500 p.p.m. by the following procedure. To 50·00 ml. of water, 1·00 ml. of 10 per cent. sodium carbonate solution, and 1·0 ml. of the quaternary salt solution (0 to 500 p.p.m.), add exactly 2·00 ml. of 0·040 per cent. bromophenol blue solution. Record the colour densities of the resulting solutions after exactly 5 min. with a photo-electric colorimeter and a red filter transmitting at 645 mμ. Prepare the reference solution for setting at 100 per cent. transmission and thus compensating for any colour from highly coloured quaternary ammonium salt in the same way, but replace the 2.00 ml. of dye solution by water. Plot p.p.m. of quaternary salt against colour density semi-logarithmically.

In the range 0 to 100 p.p.m., the calibration curves thus prepared have excessively steep slopes and will not yield results as accurate as those in the range of 100 to 500 p.p.m. To avoid this difficulty use the following proportions of reactants in the lower range of concentrations. To 50·00 ml. of water, 1·00 ml. of 10 per cent. sodium carbonate solution, and 4·00 ml. of standard quaternary salt solution (0 to 100 p.p.m.), add exactly 1·00 ml. of 0·040 per cent. bromophenol blue solution. Otherwise proceed as already described.

Once calibration curves have been established for a given quaternary salt, the same procedure can be used to determine unknown concentrations, and with concentrations above 500 p.p.m. simple aliquot dilutions will yield solutions to which the procedure is applicable. In the range 100 to 500 p.p.m., results are reproducible to within about ±2 per cent. In the range 50 to 100 p.p.m., reproducibility of 5 to 7 per cent. is possible and in the range 25 to 50 p.p.m., 7 to 10 per cent. Concentrations below this amount should not be determined by the method without first deriving a third calibration curve in which only a very small excess of dye is used. Sensitivity is thus increased, so that concentrations of 10 to 25 p.p.m. can be determined with a little more than semi-quantitative accuracy.

Addition of very small amounts of triethylamine, triethanolamine, ethylamine, phenylenediamine, diethanolamine, or oleyldimethylamine had no effect on the light transmission or on the results of the quantitative determination of the two salts used experimentally, *viz.*, octadecenyldimethylethylammonium bromide and octadecenyldimethylbenzylammonium chloride, and it seems probable that the selectivity of the method is similar to that of Auerbach's method. Tinted quaternary antiseptic solutions when diluted to within the range 100 to 500 p.p.m. (*e.g.*, the yellow commercial product "Cepacol" containing cetylpyridinium chloride) produced only a slight deviation in the amount of light transmitted, and, with the compensation of the blank solution, the deviation was within the limits of error of the method.

A. O. JONES

Determination of Methylene Blue. G. F. Davidson (*J. Text. Inst.*, 1947, **38**, T408–T418)—In the widely used method of Knecht (*J. Soc. Dyers and Col.*, 1905, **21**, 9), in which the dye is reduced to its leuco-compound by a titanous chloride solution standardised against a ferric iron solution, a careful experimental technique is necessary to avoid atmospheric oxidation of the titanous chloride solution, and, consequently, simpler methods less subject to this source of error have been sought, although their validity has generally been tested by reference to Knecht's method. In the method now to be described, reduction of the methylene blue is effected by chromous sulphate and the leuco-compound is titrated potentiometrically with standard potassium

dichromate solution in an atmosphere of carbon dioxide. Since the rises of potential at the beginning and the end of the oxidation are of the order of 400 and 200 millivolts, respectively, the end-points of the titration are sharp and the general technique of potentiometric redox titrations affords a close control of errors due to accidental access of air.

Potentiometric titration with potassium dichromate—The cylindrical titration vessel of 250-ml. capacity is closed by a rubber stopper through which pass a mercury-sealed, glass, motor-driven stirrer, an electrode of 1·5 cm. of bright platinum wire (S.W.G.24) sealed into the end of a narrow glass tube, a thermometer, gas inlet and outlet tubes, the tip of a 25-ml. burette, the side-tube of a calomel electrode, and a tube for the introduction of chromous sulphate solution. The potential measurements are made with a Cambridge electrometer-valve potentiometer. Carbon dioxide is introduced from a cylinder and is freed from oxygen by rolls of freshly reduced copper gauze in a silica tube heated to about 600° C. in an electric furnace. Chromous sulphate solution is made by reducing a solution of chrome alum in 0·1 *N* sulphuric acid with amalgamated zinc prepared by the method of Stone and Beeson (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 188).

Procedure—Prepare a solution containing about 8 mg.-mol. per litre (0·016 *N*) and dilute 10 ml. in the titration vessel with 90 ml. of 2·2 *N* sulphuric acid to give an acid concentration of 2 *N*. Displace the air in the apparatus by means of a current of carbon dioxide, heat the solution to 50° C. by means of a small Bunsen burner, and reduce the dye by addition of a slight excess of chromous sulphate solution. Maintain a slow current of carbon dioxide, stir the solution for about 30 min. to ensure complete removal of air, and titrate with 0·01 *N* potassium dichromate solution. From the titration curve the volume of titrant equivalent to the reduced methylene blue can be obtained with an error of not more than 0·5 per cent.

Precipitation with dichromate—When potassium dichromate solution is added to a neutral solution of methylene blue, a precipitate, presumably of $MbCr_2O_7$ (where Mb is the methylene blue cation), is formed, but, when methylene blue is titrated with potassium dichromate solution, complete precipitation is effected only after addition of a considerable excess of the dichromate solution. The following method was tried. To a measured volume of the dye solution add a known volume of standard potassium dichromate solution, filter through a sintered-glass filter, and titrate a measured aliquot of the filtrate with ferrous ammonium sulphate solution using *N*-phenylanthranilic acid as indicator. With 20 ml. of 8 millimolar methylene blue solution and 50 ml. of 0·02 *N* potassium dichromate, this method gave results about 2 per cent. lower than those of the redox titration method. After this preliminary work had been done, two procedures were reported by Ferrey (*Quart. J. Pharm.*, 1943, **16**, 208; ANALYST, 1944, **69**, 54) in the first of which the mixture of methylene blue and potassium dichromate solutions, made up to a known volume, is filtered through paper and an aliquot is titrated iodimetrically and, in the second, the mixture is heated to 75° C. for 5 min., the cooled mixture is filtered through sintered glass, the precipitate is washed with a small amount of cold water, and the whole of the filtrate is titrated. Investigation now shows that the heating to 75° C. does not significantly affect the results, but that the method in which only a fraction of the filtrate is titrated gives lower results than that in which the precipitate is washed and the whole of the filtrate is titrated.

Determination by visual colorimetry—In the application of visual colorimetry to the determination of methylene blue in a colorimeter of the Duboscq type it is advisable, for maximum accuracy, to compare the solution of unknown concentration with the standard solution in the same cup of the instrument. Fill the left-hand cup with the standard solution and adjust its position relative to the plunger so that the length of the column traversed by the light is 20 or 30 mm., and maintain this setting unchanged during subsequent operations. Fill the right-hand cup with standard solution and find the position at which the two fields show equal colour intensity. Repeat the balancing ten times and take the mean of the readings. Replace the standard solution in the right-hand cup by the unknown solution and take readings in the same way. Birtwell *et al.* (*Shirley Inst. Mem.*, 1923, **2**, 227; *J. Text. Inst.*, 1923, **14**, T297) found that Beer's law does not hold for aqueous solutions of methylene blue. It is necessary therefore to correct the results found by this method. To do this a number of solutions of known concentration are treated by the procedure described and a correction curve is constructed for that range of concentrations. Satisfactory determinations can then be made with an error of about 1 per cent. The standards used in establishing the method were either a 0·1 millimolar solution set at a reading of 20 mm. or a 0·05 millimolar solution set at 30 mm. The unknown solutions should be diluted so that they do not differ by more than 10 per cent. from the concentration of the standard solution. Acetic acid at a concentration of 0·1 *N* should be introduced into all solutions during dilution to prevent absorption of methylene blue by the glass.

Determination by the Spekker absorptiometer—Although there is some disagreement about the data of the absorption curves of methylene blue, all workers agree that there is a main peak at about 660 mμ. and a secondary peak at 600 to 620 mμ., these two peaks being generally ascribed to monomeric and dimeric cations, respectively. Examination of the curves showing the relation between the molecular extinction and the wavelength at various concentrations showed that at 665 mμ. the extinction coefficient falls with increasing concentration, *i.e.*, Beer's law is not obeyed. The curves also show that at wavelengths in the neighbourhood of 620 mμ. the extinction curves cross each other owing to the development of the dimeric band with increasing concentration, and the molar extinction coefficient is here almost independent of the concentration in the range of 0·5 to $1·5 \times 10^{-5}$ *M*. With the Ilford filter

Spectrum Orange 607, which has a fairly narrow transmission band at about 600 mμ., the optical density is proportional to the concentration up to a concentration of $1{\cdot}75 \times 10^{-5}$ *M*. Solutions for measurement should have a concentration of 1·0 to $1{\cdot}5 \times 10^{-5}$ *M*, and it is advisable to include a standard solution in each set of measurements. The Spekker instrument used was a "Sensitive Model" with an absorption cell of 1-cm. thickness. The solutions used were 0·1 *N* with respect to acetic acid, but even at this concentration there was some absorption of dye by the glass and the cell should be rinsed with *N* hydrochloric acid before introducing a fresh solution.

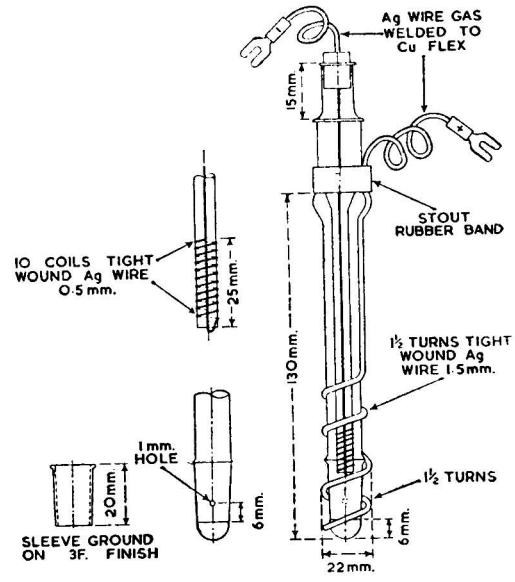
Heating methylene blue at 105° C. for 4 to 22 hr. gives a dry weight in good agreement with that obtained by prolonged drying over phosphorus pentoxide. A. O. JONES

Determination of 1 : 2 : 3 : 4-Tetrahydro-2-Naphthol in Impregnated Clothing. J. Goldenson and S. Sass (*Anal. Chem.*, 1947, **19**, 322–324)—In an investigation of the value of this compound as an insect repellant when incorporated in cloth an analytical method was developed involving extraction of the tetrahydronaphthol from the cloth with ether followed by acetylation with acetic anhydride in pyridine (Peterson and West, *J. Biol. Chem.*, 1927, **74**, 379; ANALYST, 1927, **52**, 607).

Method—Cut the cloth sample into 1-cm. squares, mix, and dry in a desiccator over sulphuric acid for 12 hr. Extract a weighed portion containing 50 to 350 mg. of 1 : 2 : 3 : 4-tetrahydro-2-naphthol for 1·5 hr. with absolute ether in a Soxhlet extractor. Remove the ether carefully from the extract in the flask of the extractor, add $10 \pm 0{\cdot}1$ ml. of an acetylating reagent, prepared by adding 6 parts of acetic anhydride to 94 parts of pyridine, and heat over boiling water under refluxing conditions for not less than 2 hr. Cool the flask in ice-water, add 100 ml. of cooled water, and titrate the solution at 0° to 10° C. with 0·3 *N* sodium hydroxide, preferably electrometrically to *p*H 8·9 with a *p*H meter with external electrodes, or, alternatively, by using a mixed indicator containing 0·05 per cent. of thymol blue and 0·15 per cent. of phenolphthalein in 50 per cent. ethanol. Make a blank determination with unimpregnated cloth containing the same binders as the sample. The difference between the titration volume of the blank and that of the sample after acetylation, when multiplied by the normality of the sodium hydroxide solution and by 14·821 and divided by the weight of the sample, is the percentage of the tetrahydronaphthol in the sample. A. O. JONES

Inorganic

Determination of Chlorine in Resins obtained from Polyvinyl Chloride Compositions. J. Haslam and W. W. Soppett (*J. Soc. Chem. Ind.*, 1948, **67**, 33–35)—The method is based on fusion of the resin sample in a Parr bomb after removal of the plasticiser by ether extraction. Chloride is then determined potentiometrically in a solution of the fused mass. Alternative electrode systems are compared and a special silver-wire electrode is recommended.



Preparation of sample—Immerse strips of suitable size in a freezing mixture of methanol and solid carbon dioxide for about 15 min. and then grind to small size. A rotary pencil sharpener is convenient for this. Place 2 or 3 g. of the ground material in a Soxhlet thimble and, after the material has soaked overnight in the liquid to be used for the extraction, extract with ether for 6 or 7 hr. Dry the extracted resin at 100° C. for 1 hr.

Procedure—Weigh 0·3 g. of the prepared resin into a Parr bomb with 1 g. of catalyst (2 parts by weight of potassium nitrate mixed in a mortar with 1 part of dextrose) and 13 to 14 g. of sodium peroxide. Fire the bomb electrically, and, after cooling, leach out with 70 ml. of boiling water in a 500-ml. beaker. Boil the alkaline solution obtained for 10 min., cool, and make just acid with nitric acid. Determine the chloride electrometrically as described below.

Determination of chloride—The authors investigated three methods of determining the chloride. (*a*) The "bottled end-point" method (Haslam and Sweeney, ANALYST, 1945, **70**, 413). (*b*) A standard method with the Cambridge Instrument Company's potentiometric apparatus. (*c*) A method employing a reference electrode and an indicator electrode, both of clean silver-wire.

The electrode, incorporating both the reference and the indicator electrodes, is shown in the diagram, and the reference electrode (inside) is immersed in an excess of silver ions by filling with a solution made up to contain 64 ml. of a saturated solution of sodium sulphate, 6·0 ml. of 2 *N* nitric acid, 30 ml. of 0·1 *N* sodium chloride, and 30·1 ml. of 0·1 *N* silver nitrate, the solution being decanted after the precipitate has been allowed to settle. To use the electrode, ease the sleeve so that the surfaces

are moistened, and connect the indicator electrode to the positive terminal and the reference electrode to the negative terminal of the Cambridge potentiometric titration apparatus. This modified electrode system is claimed to be more sensitive, to give more trustworthy blank values, and to obviate the use of large volumes of buffer solutions.

W. C. WAKE

Potentiometric Determination of Oxygen with the Dropping Mercury Electrode. H. A. Laitinen, T. Higuchi, and M. Czuha (*J. Amer. Chem. Soc.*, 1948, **70**, 561–565)—The potential assumed by a dropping mercury electrode in a solution containing no capillarily active substances is related to the oxygen concentration of the solution. If the solution is equilibrated with a gas mixture containing oxygen, the percentage of oxygen in the mixture is given by the expression $(E - E_{max.})/217t^{\frac{1}{3}}$ at 25° C., where E is the potential of the electrode, $E_{max.}$ the potential corresponding to the capillary maximum of mercury (both in millivolts), and t is the drop-time in sec. The voltage fluctuation can be eliminated without affecting the result by including in the circuit a high-capacity condenser.

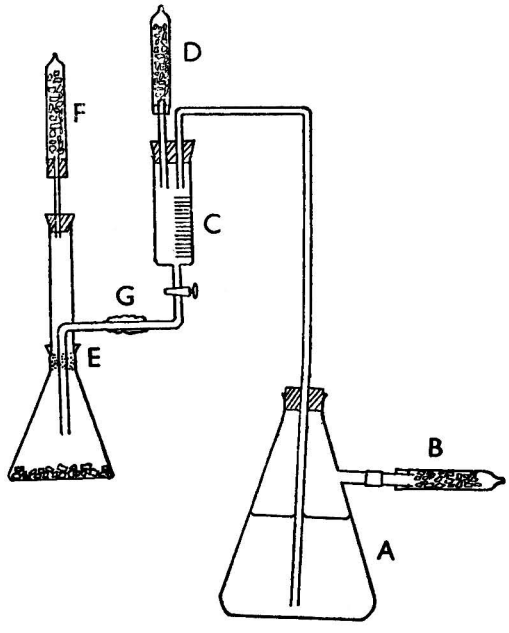
An apparatus has been designed in which the gas can be rapidly equilibrated with the electrolyte; the dropping mercury electrode is protected from gas bubbles by a shield, which also serves to remove the mercury and any reaction products from the bulk of the solution. The electrolyte is made to flow in a direction that carries the solution surrounding the silver - silver chloride electrode away from the bulk of the solution.

Using 0·1 *M* potassium chloride, containing a trace of methyl red to suppress maxima, as electrolyte, the method is sensitive to 0·01 per cent. of oxygen in a gas, and a linear relationship is obtained for the range from zero to 1·0 per cent. of oxygen.

J. G. WALLER

Rapid Determination of Moisture in Liquid Sulphur Dioxide. B. R. DiCaprio (*Anal. Chem.*, 1947, **19**, 1010–1011)—Since the presence of water in sulphur dioxide results in attack of metal equipment, its determination is important. Absorption on phosphorus pentoxide is difficult and slow, but the Karl Fischer method (*Angew. Chem.*, 1935, **48**, 394) is accurate if suitable precautions are taken.

METHOD—*Sampling technique*—Rinse *A*, a 500-ml. suction flask, with absolute methanol, and dry it in an oven at 110° C. Cool it in a desiccator and draw off a 200-ml. sample of sulphur dioxide from the storage cylinder; replace the line from the cylinder on the side-arm by *B*, which contains activated alumina. Fit a two-holed rubber stopper into the top of a 50-ml. calibrated dropping funnel, *C*, with a 24/40 ground-glass joint in the centre section, and pass through one hole a drying tube containing activated alumina, *D*, and through the other a U-tube connecting with the sampling bottle *A* and extending to 0·25 in. from the bottom. Place a 300-ml. Erlenmeyer flask containing 25 g. of Drierite on the ground-glass dropping funnel joint, *E*. Attach the drying tube, *F*, containing Drierite, on the neck of the dropping funnel, and



wrap a cloth round the side-arm at *G* to retain atmospheric moisture.

Procedure—Close the end of *B*, so forcing liquid sulphur dioxide into the dropping funnel. When 50 ml. are collected, release the pressure and immediately drain the sample into the Drierite-containing flask, so flushing the system. Replace the Erlenmeyer flask with a dry one containing 25 ml. of standardised methanol (Smith *et al.*, *J. Amer. Chem. Soc.*, 1939, **61**, 2407). Run up exactly 30 ml. of the sample into the dropping funnel and drain into the flask; then replace it immediately with a flask containing Drierite. Titrate the sulphur dioxide - methanol mixture with Karl Fischer reagent (Seaman *et al.*, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 517) in a Karl Fischer burette in the usual manner.

Calculation—Let *E* represent the titre for the sample, *F* that for the methanol blank, *D* the water equivalent of the reagent as grams per ml., and *G* the specific gravity (1·460) of sulphur dioxide at its boiling point, then water content in p.p.m. = $10^6 (E - F) D/(\text{ml. of sample} \times G)$. Agreement with the more tedious phosphorus pentoxide method is satisfactory, but the reproducibility depends on the analyst and on the equipment used.

M. E. DALZIEL

Congo Red as an Adsorption Indicator. R. C. Mehrotra (*Analyt. Chim. Acta*, 1948, **2**, 36–44)—The utilisation of Congo red as an adsorption indicator for argentometric titrations is described; it is applicable not only in the titration of halide ions by silver solutions, but also in the reverse determination of silver ions by halide solutions.

When a 0·2 per cent. aqueous solution of Congo red is used as an adsorption indicator in neutral solution the dye is wholly transferred to the precipitate at the end-point, but the end-point is not reversible. If, however, the titration be conducted in the *p*H range of 3 to 5 (attained by addition of 0·002 *N* nitric acid) the end-point is accurate and reversible. The reversibility and sharpness of the end-point is further improved by addition of 1 ml. of 1 per cent. chloride-free dextrin solution per 5 ml. of sample taken. In all reverse titrations, *i.e.*, the determination of silver ions by halide solutions, no end-point is possible unless the indicator is added near the end of the titration.

The accompanying table sets out the colour changes and gives notes on the conditions in some typical titrations for which Congo red may be used.

Sample	Titrated with	Colour change	Remarks
0·1 *N* KCl	0·1 *N* $AgNO_3$	Pinkish violet soln. → Violet precipitate	Coagulation at end-point. End-point sharp, but not reversible
0·1 *N* KCl + 0·002 *N* HNO_3	0·1 *N* $AgNO_3$	Dirty blue precipitate → Pinkish-violet precipitate	Coagulation just before end-point. Colour change occurs on coagulated precipitate. End-point sharp and reversible
0·1 *N* KCl + 0·002 *N* HNO_3 + 1 per cent. dextrin	0·1 *N* $AgNO_3$	Blue suspension → Pink suspension	No coagulation. Colour change sharp, reversible, and occurs in suspension only
0·1 *N* KBr or 0·1 *N* KI + 0·002 *N* HNO_3	0·1 *N* $AgNO_3$	Green suspension → Pink precipitate	Sharp, reversible end-point
0·1 *N* KBr or 0·1 *N* KI + 0·002 *N* HNO_3 + 1 per cent. dextrin	0·1 *N* $AgNO_3$	Green suspension → Pink suspension	Sharp, reversible end-point. Colour change in suspension without coagulation
0·1 *N* KCNS + 0·002 *N* HNO_3	0·1 *N* $AgNO_3$	Blue precipitate → Pink precipitate	Coagulation much before end-point. Colour change on coagulated particles
0·1 *N* $AgNO_3$ + 0·002 *N* HNO_3	0·1 *N* KCl	Violet precipitate → Blue precipitate	Indicator added near end-point. Colour change on coagulated particles. Not so sharp for 0·02 *N* $AgNO_3$
0·1 *N* $AgNO_3$ + 0·002 *N* HNO_3 + 1 per cent. dextrin	0·1 *N* KCl	Violet suspension → Blue suspension	Indicator added towards end-point
0·1 *N* $AgNO_3$ + 0·002 *N* HNO_3	0·1 *N* KBr or 0·1 *N* KI	Violet precipitate → Green precipitate	Coagulation before end-point. Colour change on coagulated particles
0·1 *N* $AgNO_3$ + 0·002 *N* HNO_3 + 1 per cent. dextrin	0·1 *N* KBr or 0·1 *N* KI	Violet suspension → Green suspension	
0·1 *N* $AgNO_3$ + 0·002 *N* HNO_3	0·1 *N* KCNS	Violet precipitate → Blue precipitate	Coagulation before end-point. Colour change on coagulated particles

A. H. A. Abbott

Preparation of Silver for Use in the Walden Silver Reductor. G. F. Smith and F. W. Cagle, jun. (*Anal. Chem.*, **1948, 20,** 183)—Silver deposited by displacement from a slightly acid, concentrated silver nitrate solution by the action of copper metal is found to be too finely divided; flow is retarded and clogging easily results when the silver becomes coated with silver chloride. "Tree silver" prepared as is described is granular, brilliantly-reflecting and crystalline, of low apparent density, with a high ratio of surface to mass.

Electro-deposition of silver—In a 4-litre beaker dissolve 500 g. of silver nitrate in 2500 ml. of water slightly acidified with nitric acid. For the electrolysis use a current of 60 to 70 amp. at a potential of 5 to 6 v.; the cathode consists of two 10-cm. square platinum plates with heavy "lead-in" conductor extensions, and suspended by heavy copper connections to direct current; the anode is a 200-mm. rod of silver 10 to 25 mm. in diameter or heavy-gauge silver rectangular sheet of similar weight, and is suspended between the cathodes, which are placed at the edges of the beaker. Stirring and cooling are unnecessary and the deposit is easily dislodged.

About 30 g. of silver so obtained occupy 50 ml., and 400 ml. of 0·1 *M* ferric chloride in *M* hydrochloric acid are reduced during 5 min. of flow.

Regeneration—After reduction of this amount of iron, the silver should be rinsed with water and the reductor filled with diluted aqueous ammonia (1 + 3). Leave for 5 to 10 min., then rinse the solution out and replace with *M* hydrochloric acid. As a precautionary measure, at once acidify or discard the ammoniacal solution of silver chloride.

M. E. DALZIEL

Conductometric Analysis of Thallous Salts with Potassium Chloride. J. Kamecki (*Roczniki Chem.*, **1947, 21**, 124–130)—The possibility of using thallous chloride in conductometric analysis has been investigated.

METHOD—*Apparatus*—The type of conductivity meter used was identical with that described by Jander and Schornstein (*Z. angew. Chem.*, 1932, **45**, 701), its main advantage being the use of a sensitive ammeter instead of a telephone. Very large electrodes, covered with platinum black, were used to reduce polarisation (50 cycles A.C. was used). The resistance capacity of the conductometric cell (0·1 *M* potassium chloride at 20° C.) was 0·0818 ohm^{-1}. The experiments were carried out at a temperature of 20 ± 0·1° C.

Procedure—Introduce a known volume of 0·1 *M* thallous nitrate into a conductometric cell and dilute to 25 ml. with water. Titrate with 0·1 *M* potassium chloride. Satisfactory results are obtained only in rather concentrated solutions (0·04 *M* or higher) owing to the solubility of the thallous chloride.

To reduce the solubility of thallous chloride, titration was carried out in aqueous methanol solution.

Introduce 2 ml. of 0·1 *M* thallous nitrate into a conductometric cell, dilute to 25 ml. with methanol (0·08 *M* solution), titrate with 0·1 *M* potassium chloride. Under these conditions, the deposition of thallous chloride is immediate. Equilibrium sets in rather slowly and after each addition of potassium chloride a few minutes will elapse before conductivity readings become constant. There is no apparent change of conductivity until the end of the titration. The accuracy of titration is to within ±1 per cent.

A. SPORZYNSKI

New Method for the Direct Precipitation of Aluminium in Presence of Iron. H. N. Wilson (*Analyt. Chim. Acta*, **1947, 1**, 330–336)—Precipitation with ammonium benzoate effects separation of aluminium from many bivalent metals, but separation from iron by first reducing this with sulphurous acid did not prove satisfactory, the aluminium benzoate precipitate being often badly contaminated with iron. Thioglycollic acid reduces ferric iron and forms a soluble complex in neutral or alkaline solution. The method described utilises thioglycollic acid and ammonium benzoate.

Procedure—Dissolve the sample and remove the silica in the usual way. Dilute the filtered solution to 200 or 300 ml., nearly neutralise it with aqueous ammonia solution, ammonium chloride being added, if necessary, to give an ammonium salt content of, at least, 5 g. Add 1 ml. of 90 per cent. thioglyollic acid solution, 20 ml. of 10 per cent. ammonium acetate solution prepared from AnalaR acetic acid and AnalaR aqueous ammonia suitably diluted, 20 ml. of 10 per cent. ammonium benzoate solution, and 2 ml. of bromophenol blue indicator. Heat to about 80° C., whereupon any precipitated benzoic acid should dissolve, and add dilute hydrochloric acid, dropwise, to dissolve any aluminium benzoate precipitated. Add slowly diluted aqueous ammonia (1 + 4) with continuous stirring until the indicator begins to change colour and precipitation commences. Boil for 2 to 3 min. and add more ammonia solution if the liquid becomes acid. Then add ammonia solution to the boiling liquid until the solution is red-blue in colour (*p*H 3·5 to 4·0); boil gently for 2 or 3 min. then heat over boiling water for 0·5 hr. without stirring.

Filter on a No. 40 Whatman paper and wash well with a hot, 1 per cent. solution of ammonium benzoate in 2 per cent. acetic acid. If much iron is present wash only 2 or 3 times, redissolve the precipitate in hot, dilute hydrochloric acid, add a few grams of ammonium chloride, and re-precipitate as above. Ignite the paper and precipitate in platinum at about 1000° C.

Zinc, calcium, and magnesium do not interfere with the alumina determination, but the presence of thioglycollic acid and the accumulation of salts result in inaccurate values for these metals when determined by the usual methods. Up to 1 g. of iron does not affect the determination of 20 mg. of aluminium. Chromium, titanium, and vanadium interfere, but tungstates and molybdates do not. After ignition, the alumina from the benzoate precipitation is usually more readily soluble than that precipitated by aqueous ammonia solution.

M. E. DALZIEL

Phosphoric Acid Attack Method for Determination of Silicon in Aluminium Alloys. G. Norwitz (*Anal. Chem.*, 1948, **20**, 182)—The accuracy of the method as described by Lisan and Katz (*Ibid.*, 1947, **19**, 252; Abst., ANALYST, 1948, **73**, 239) is improved by applying the following modifications to the procedure.

(1) A 500-ml. porcelain casserole is preferable to an 800-ml. beaker for the reaction, as the glass is attacked by phosphoric acid if the beaker is kept on the hot-plate more than 2 min. after a clear solution is obtained. The casserole may be heated indefinitely at this stage.

(2) To complete the removal of volatile acids, the boiling for 8 to 10 min. should be replaced by vigorous fuming for a similar period.

(3) After treating the ignited precipitate with hydrofluoric acid, it should be ignited for only 10 min. at 900° C. and not at 1100° C., at which temperature phosphoric acid is slowly volatilised, thus leading to high silica results. M. E. DALZIEL

Thermo-electric Method for Determining Silicon in Carbon Steel. P. D. Korzh (*Zavod. Lab.*, 1945, **11**, 319–323)—Thermo-electric methods have been used for determinations of the thickness

of platings (Nifonov, *Ibid.*, 1934, **3**, 57), for establishing the α-γ transformation in steel (Akimov and Tomaschev, *J. Tech. Phys., U.S.S.R.*, 1936, **6**, 99), and for the sorting of steels, including rough evaluation of silicon content (Akimov and Pevzner, *Zavod. Lab.*, 1939, **8**, 1273). Within certain groups of steels there is a linear relation between the thermo-e.m.f. and the silicon content. Deviations occurring with samples that have been subjected to similar heat and mechanical treatments are caused mainly by considerable differences in the carbon content. It is now shown that, if allowance be made for the effect of carbon, determinations of silicon, with errors not exceeding 0·02 on 0·25 per cent., can be made without difficulty. The time required for a determination is only 3 to 4 min.

Empirically, the thermo-e.m.f., with the hot junction at 200° C. or less, for steels containing from 0·01 to 2 per cent. of carbon and silicon, is given by the equation $E = A/C + B/Si$, where A and B are the thermo-e.m.fs. of carbon and silicon, respectively, and C and Si are the percentage contents of these elements. Under working conditions, an absolutely constant temperature difference between the hot junction at t_2 and the cold junction at t_1 is difficult to maintain, but it is convenient to use the relation $E/(t_2 - t_1) = \phi$, which with small temperature variations of t_1 and t_2 may be taken as constant (ϕ is called the thermo-electric capacity), and to form the equation $\phi = a/C + b/Si$, where a and b are the thermo-electric capacities of C and Si and are quadratic functions of the temperature. The values of a and b may be obtained from measurements of the thermo-e.m.f., in the same temperature range, of two standard samples of steel, similarly heat-treated and containing different amounts of carbon and silicon, and from calculations from the equations $a/C_1 + b/Si_1 = \phi_1$ and $a/C_2 + b/Si_2 = \phi_2$. The silicon content of a steel is then calculated from the equation $Si = b/(\phi - a/C)$, where C, the carbon content, has to be determined by other means. Since the effect of carbon on the thermo-e.m.f. is about one-fifth that of silicon, it is possible to obtain a sufficiently accurate figure for the silicon content by reading from the appropriate graph (ϕ plotted against percentage of Si) in a series of calibration curves covering narrow and adjoining ranges of carbon content.

Apparatus—The stand, 1 (Fig. 1), is fitted with supports, 2, for the hot, 3, and cold, 4 (needle-shaped), electrodes which can be arranged to make contact with the sample, 5. The needle, 4, is insulated from the body of the apparatus by means of an ebonite washer, 10, and carries a terminal, 11, for electrical connection; it is pressed down onto the sample by means of the spring, 6. The hot contact is made when required by forcing the hemispherical end, 8, of the rod, 3, on to the sample against the pressure of the spring, 9, by means of the movable arm, 7. The end, 8, carries a copper-constantan couple and the hot, brass electrode is jacketed with a low-power furnace fed from a low-voltage transformer. The electrical circuit (Fig. 2) contains a rheostat R and an ammeter A for regulating the current to the heating oven, a galvanometer G for registering the temperature in the copper-constantan circuit, and a galvanometer G_1 for measuring the thermo-e.m.f.

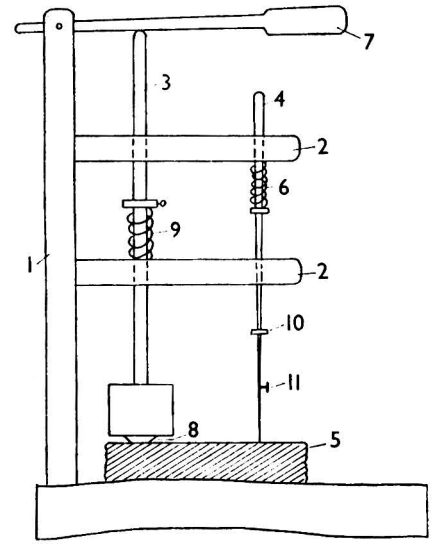


Fig. 1

Calibration—Preliminary work is required to establish suitable working conditions of temperature so that the thermo-electric capacity shall vary linearly over the whole concentration range.

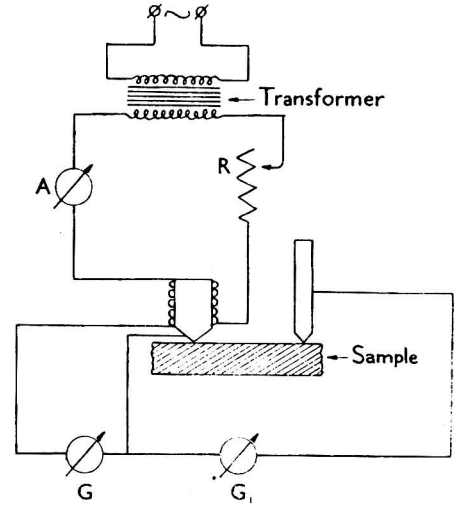


Fig. 2

The author used approximately 15° and 100° C. as the temperatures of the cold and hot junctions, respectively. The coefficients a and b in the formula given above have then to be calculated from the results of tests on a group of samples containing different percentages of carbon and silicon as shown by chemical analysis. The surface of the sample must always be ground on an emery wheel to remove

rust, etc., immediately before the test. Results for samples that have been cleaned a few hours before the test are untrustworthy. In routine work, two samples with silicon contents at the limits of the concentration range are used as controls with each daily batch. G. S. SMITH

Potentiometric Determination of Lead. L. Farkas and N. Uri (*Anal. Chem.*, 1948, **20**, 236–237)—Solutions containing lead ions can be titrated with alkali fluoride in presence of alkali chloride, the end-point being indicated by a drop in the ferric-ferrous oxidation-reduction potential. The lead is precipitated finally as the chlorofluoride.

Procedure—The solution to be titrated should be 0·05 to 0·10 *M* with respect to lead. Add 1 g. of potassium chloride for each gram of lead, and 40 mg. of ferrous chloride containing 0·8 mg. of ferric chloride for each 100 ml. of solution. Titrate the solution with a standard sodium (or potassium) fluoride solution, plotting the volume added against the potential difference between a platinum electrode and a saturated calomel electrode dipping in the solution. The end-point occurs where the rate of change of potential with addition of fluoride solution is a maximum. J. G. WALLER

Amperometric Titration of Manganese. G. Goffart, G. Michel, and T. Pitance (*Analyt. Chim. Acta*, 1947, **1**, 393–407)—Manganous ions can be determined by titration with potassium permanganate using a "dead-stop" end-point (*cf.*, Foulk and Bawden, *J. Amer. Chem. Soc.*, 1926, **48**, 2045). A silver wire and a platinum wire are immersed in the solution and are connected to the terminals of a galvanometer. The end-point is indicated by a permanent displacement of the galvanometer needle.

Procedure—Dissolve an amount of sample containing from 2 to 100 mg. of manganese in an appropriate acid and, when the reaction subsides, dilute to 50 to 100 ml. Boil to remove any gaseous reaction products, and if nitric acid is used, add sufficient urea or sulphamic acid to destroy nitrous acid. Pour the solution into 200 to 300 ml. of a saturated solution of sodium pyrophosphate, stirring to dissolve any precipitate formed, and adjust to *p*H 6·0 to 8·0. Lower a clean silver wire and a platinum wire, each 10 cm. long and 0·5 mm. in diameter, into the solution, and connect the wires to a galvanometer. The galvanometer should have a sensitivity of 0·25 to 1·0 microamp. per mm., a resistance of 250 to 2000 ohms, and a period of less than 3·5 sec. Titrate the manganous solution with 0·002 to 0·02 *M* potassium permanganate, the concentration depending on the amount of manganese present, and stir the solution to prevent any local excess of permanganate at the electrode surfaces. After each addition, the needle is displaced slightly and then returns to its original position, but towards the end of the titration the return is slower, and the permanganate should be added drop by drop until the needle shows a permanent displacement. The manganese content can be calculated assuming one permanganate ion oxidises four manganous ions. There is practically no interference from foreign ions, and the manganese is determined with errors less than ±0·1 per cent. in alloys and steels containing from about 0·5 to 2 per cent. of manganese. The method has also been applied accurately to manganese minerals and to ferromanganese. J. G. WALLER

Titration of Fluoride Ion with Aluminium Chloride. J. H. Saylor and M. E. Larkin (*Anal. Chem.*, 1948, **20**, 194–196)—Titration in nearly neutral solution gives rise to $AlF_6^{'''}$, the sodium salt of which is only slightly soluble in presence of an excess of sodium ions or in alcoholic solution. Various indicators have been employed, but that described is eriochromcyanine-R, which forms a red-violet lake with aluminium ions, the colour varying with *p*H. The colour of an aqueous solution of the indicator varies from yellowish-orange at *p*H 5·4 to 6·0 to yellow at higher *p*H values, and reddish-orange at lower ones; age also affects the colour. The indicator solution should be yellow before addition of the aluminium. A salt error is introduced by sulphate ions, which interfere with the lake formation, but sodium chloride has little effect.

METHOD—*Standardisation of aluminium chloride solution*—Add 2 drops of phenolphthalein solution to 20·00 ml. of standard sodium fluoride solution containing 1·000 g. of fluoride ion per litre and adjust the *p*H with 0·1 *N* sodium hydroxide and 0·1 *N* hydrochloric acid until the solution is just colourless. Add 10 g. of sulphate-free sodium chloride and 4 drops of 0·1 per cent. aqueous eriochromcyanine-R solution. Heat just to boiling and, if necessary, adjust the *p*H to give a solution that is yellow; it should be saturated with sodium chloride. Add, with thorough mixing, aluminium chloride solution from a micro-burette to the almost-boiling solution at a rate of about 1 drop every 2 or 3 sec. and more slowly near the end-point. Just before the end-point the solution darkens; it becomes pink at the end-point, which is sharp above 90° C., but if the solution is boiling vigorously the lake precipitates. Fresh or aged indicator solutions may be used and the indicator blank is about 0·002 to 0·004 ml. under these conditions.

Results for 0·2330 and 0·2421 *N* solutions by this and the 8-hydroxyquinoline method differ by 4 parts per 1000. It is therefore advisable to standardise the solution against pure sodium fluoride.

Determination of fluoride—The titration gives high results when lead, nickel, chromium, carbonate, silicate, sulphide, or sulphate is present, so that distillation is often necessary.

Distil 50 ml. of the sodium fluoride solution according to the method of Willard and Winter (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7), after adding 20 ml. of 18 *M* sulphuric acid, and collect 250 ml. of the distillate in a volumetric flask. If further distillation shows incomplete recovery of fluorine, a larger volume must be collected. Titrate aliquot portions of the solution as described above, using 2 drops of indicator and 5 g. of sodium chloride for each 10 ml. of solution. The indicator

blank is then about 0·015 ml. for 50 ml. of solution, and 0·03 ml. for 100 ml.

Results—High values are obtained if temperatures above 145° C. are used in the distillation, or if the neutralised distillate is concentrated in glass, probably owing to the introduction of dissolved silica. With five distillations, and titrating three portions of each distillate, the mean value of all titrations was within 1 in 450 of the theoretical value, but the maximum deviations of separate titrations were much greater than this. M. E. DALZIEL

Rapid Method for Titration of Chlorides. R. B. Dean and R. L. Harley (*Anal. Chem.*, 1947, **19**, 841–842)—An electrometric method can be used to detect the end-point of the titration of a chloride solution with silver nitrate. A potential is applied from a potentiometer to a silver and a copper wire dipping into the chloride solution, and the current flowing is indicated by a micro-ammeter connected across the electrodes. The potential applied is adjusted before each addition of silver nitrate so that no current flows, and the current produced by a standard addition of silver nitrate is measured. At the end-point this increase is a maximum.

Procedure—A blank determination is first carried out on the reagents. Titrate 10 ml. of a saturated copper sulphate solution containing 1·5 per cent. of 6·0 *N* sulphuric acid, with a standard silver nitrate solution added from a micrometer syringe burette, and record the burette reading at the end-point. Add 0·2 ml. of the solution to be examined, and titrate the chloride in the same way. The difference between the two readings gives the chloride content of the solution.

Of the 44 ions tested, 14 interfere with the method; two of these, silver and mercurous, are incompatible with chloride ions; of the twelve remaining ions, *viz.*, NO_2', MnO_4', CN', S'', S_2O_3'', CrO_4'', Cr_2O_7'', $Fe(CN)_6'''$, $Fe(CN)_6''''$, Br', I', and CNS', the first seven can easily be removed by warming the test solution with sulphuric and oxalic acids.

J. G. WALLER

Determination of Iodide in Developers. G. I. P. Levenson (*J. Soc. Chem. Ind.*, 1947, **66**, 198–200)—The disadvantage of the polarographic method is that expensive and intricate equipment is necessary. The colorimetric method described covers the range 0 to 10 mg. of potassium iodide per litre with an accuracy to 0·5 mg., and is suitable for photographic processing.

Procedure—If the sample gives a clear solution on filtering, add 25 ml. of 8 *N* sulphuric acid to 100 ml. of the filtered solution in a 250-ml. Pyrex conical flask. Otherwise, place 100 ml. of the sample in a similar flask and add cautiously 25 ml. of 8 *N* sulphuric acid. To destroy the froth in a carbonate developer, heat gently to boiling and agitate to release the gas. Add 1 g. of kieselguhr, close the flask with a rubber stopper, and shake it. Open the flask carefully and filter the contents through a close paper on a 2-in. Buchner funnel. Wash the flask and run the rinsings through the precipitate. Return the clear filtrate and washings to the flask.

To the acid solution add 5 ml. of 0·1 *N* silver nitrate and 1 g. of kieselguhr, stopper the flask, and shake it well. Filter the contents through the Buchner funnel and wash with boiling distilled water to remove the gelatin. Return the washed paper and precipitate to the flask, and add 10 ml. of aqueous ammonia (sp.gr. 0·880); stopper the flask, heat gently to 50° or 60° C., and shake thoroughly for about 3 min. Cool in water, open the flask, and add 10 ml. of fresh 1 per cent. sodium sulphide solution; re-stopper and shake. Remove the stopper, rinse any adhering solution back into the flask, and boil until the odour of ammonia is almost gone. Cool the solution, dilute, if necessary, to 25 ml., and add 2 g. of boric acid, which will make the solution acid if the ammonia has been adequately removed. Add 1 g. of powdered potash alum, stopper, and shake again to coagulate the silver sulphide. Filter the suspension through two thicknesses of Whatman No. 5 filter paper on a Buchner funnel or a Gooch crucible; wash the flask twice, pouring the washings through the filter, and transfer the water-clear filtrate to a 200-ml. separating funnel. Add 10 ml. of 8 *N* sulphuric acid and 10 ml. of 0·1 *N* potassium dichromate. Extract with 5-ml. portions of *cyclo*hexane, shaking vigorously for 1 min. at each extraction. Combine the extracts in a test tube and compare with the standard tubes.

For 3 to 5 mg. of potassium iodide, extract three times and, when matched, multiply the concentration of the standard by 3/2. For 5 to 10 mg., extract four times and multiply the matching concentration by 2.

Preparation of standards—Prepare a stock solution containing 15·3 mg. of iodine in 100 ml. of *cyclo*hexane, so that 0·5 ml. is equivalent to 0·10 mg. of potassium iodide. Take suitable volumes of this solution and dilute each to 10 ml. with *cyclo*hexane in tubes of 0·75-in. diameter and compare in a Lovibond - Schofield colorimeter.

Results—Over the range 0 to 10 mg. of potassium iodide per litre, a loss of potassium iodide equivalent to 0·25 mg. per litre is noticeable at concentrations lower than 2 mg. per litre. Results are reproducible to 0·5 mg. per litre. M. E. DALZIEL

Rapid Preparation of a Solution of Sodium Hydrogen Sulphide. H. H. Hodgson and E. R. Ward (*J. Chem. Soc.*, 1948, 242)—A method for the preparation of a comparatively concentrated solution of almost pure sodium hydrogen sulphide (96 to 98 per cent. of the calculated amount of NaHS) in aqueous methanol is described. The solution, which is a powerful reducing agent, is only slightly alkaline and can be kept in a stoppered bottle for at least a week.

Procedure—Prepare a concentrated solution of sodium sulphide ($Na_2S.9H_2O$ of not less than 95 per cent. purity) in freshly distilled water and dilute with water until the solution contains 13 per cent. w/v of Na_2S. Cool 100 ml. of this solution to below 20° C. and add, with constant stirring, 14 g. of 99·8 per cent. sodium hydrogen carbonate. When

dissolution is complete, cool again to below 20° C., and add 100 ml. of methyl alcohol. Set aside for 30 mins., filter off the crystalline sodium carbonate and wash the residue with successive quantities of methyl alcohol, using 50 ml. in all. The filtrate contains about 3·5 per cent. of sodium hydrogen sulphide and about 0·2 per cent. of sodium carbonate.

For ordinary work, dissolve equivalent amounts of sodium sulphide and sodium hydrogen carbonate in water, add methyl alcohol, and filter off the precipitated sodium carbonate: use the filtrate at once for reduction. A. H. A. ABBOTT

Gravimetric Determination of Sulphites in Presence of Thiosulphates. M. S. Romero and M. S. Gijón (*Inform. Quim. Analitica.*, 1947, **1**, 69–71)—The determination of sulphite in presence of thiosulphate is based on the insolubility of hemihydrated calcium sulphite in a solution containing 70 volume per cent. of 96 per cent. alcohol.

Procedure—To a known volume of the solution containing both sulphite and thiosulphate add sufficient alcohol to give a concentration of 70 volume per cent. of 96 per cent. alcohol. Add a solution of calcium chloride (300 g. of calcium chloride in 300 ml. of water and sufficient 96 per cent. alcohol to give a 70 volume per cent. solution) at room temperature or just above, until precipitation of the sulphite is complete. Filter the hemihydrated calcium sulphite on a G.4 filter-plate, wash with water - alcohol solution of the same alcoholic concentration until free from chloride, dry at 50° C. to constant weight, cool in a vacuum desiccator containing calcium chloride, and weigh.

To determine the thiosulphate dilute the filtrate to approximately 1 litre with distilled water, add a solution containing 4 to 6 g. of potassium iodide and titrate with 0·1 *N* iodine solution, starch solution being used as indicator.

As the presence of alcohol makes the end-point difficult to determine, it is better to precipitate the thiosulphate as the monohydrated barium salt. Add a saturated solution of barium chloride in aqueous alcohol (70 ml. of 96 per cent. alcohol in 100 ml.) to the filtrate at room temperature, stirring well to facilitate the formation of the monohydrate which, as silky crystals, filter well. Allow this copious precipitate to settle and check the supernatant liquid for complete precipitation. Filter, wash the precipitate into a suitable vessel with distilled water, dissolve in acid and determine the thiosulphate iodimetrically, thus obtaining a clearer end-point. A. F. STURGESS

Physical Methods, Apparatus, etc.

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 274–277)—Papers to be published monthly will increase the number of dependable crystal structures available for reference, and crystallographic data are invited from individuals or laboratories. In general, only compounds of importance to research and analysis will be investigated and the relevant data published. Data may be presented as photostat copies, drawings or photomicrographs, and names of workers in individual fields should be included for purposes of acknowledgement. Well-formed crystals will be acceptable.

Final descriptions will include an introduction covering crystallisation procedure, and solubilities; crystal morphology, including crystal system, form, and habit, axial ratios, and interfacial angles; X-ray data, including space-group, cell dimensions, formula weights per cell, formula weight, density, and principal lines; optical properties, including refractive indices, optical axial angles, dispersion, optic axial plane, acute bisectrix, sign of double refraction, and molecular refraction; and thermal data describing behaviour before, during, and after crystallisation from the melt on a microscope slide. Laboratories willing and able to check data under these classifications are invited to co-operate.

Conventions of nomenclature to be adopted are:

Axial ratios—$a : b : c$—0·61 : 1 : 1·10.

Bisectrices—Unless written out, Bx_a and Bx_o denote the acute and obtuse bisectrices, respectively.

Beta angle—In accordance with X-ray crystallographic conventions the beta crystallographic angle will be expressed as the obtuse angle. Also, in defining extinction angles, confusion will be avoided by retaining the word "obtuse." The term "acute" is necessary occasionally for brevity.

Crystallographic axes—For orthorhombic and triclinic crystals $c < a < b$, and for monoclinic crystals $c < a$. The choice of axes will not depend on crystal habit, and, in absence of X-ray data, calculations should be made from the interfacial angles and the crystal will then be set up so that $c < a < b$, or, for monoclinic crystals, $c < a$ with b having any possible value. Results will then agree with X-ray data unless the crystals show a secondary form, when a and b axes could be reversed.

Dispersion—$v > r$ or $r > v$ will be used.

Interfacial angle—$hkl \wedge h'k'l' = \theta°$. Polar angles will be used unless otherwise designated by parenthetical phrases such as "(true)" or "(dihedral)."

Miller indices—Unenclosed indices will be used on drawings. In descriptions, parentheses around the indices indicate a single face, braces indicate a form, and square brackets a zone axis. Lower case italics will not be used as face designations.

Optic axial angle—As usual, $2E$ signifies the angle in air, $2V$ the corresponding angle in the crystal, and $2H$ the corresponding angle in an immersion medium of refractive index 1·515; at different indices optic axial angles can be represented thus—$2H_{1·656}$ The variations $2V_\alpha$ and $2E_\gamma$ are occasionally used. Observe the wavelength at which observations are made.

Optic axial plane—When the plane is normal to 010, the orientation must be expressed as the extinction angle.

Pleochroism—For qualitative expression, X is yellow, Y is orange, Z is red. In the rare case of crossed axial plane dispersion, it must be specified that X, Y, and Z are chosen as they exist at some definite wavelengths, as their position varies with wavelength.

Polymorphism—Roman numerals will be used, I indicating the stable polymorph at room temperature, others in order of apparent stability although, unless transition temperatures are given, the range will not be known.

Profile or terminal angle—This is usually the projection of an interfacial angle on a plane parallel to a common face, and occasionally it is an interfacial angle: it should be noted.

Refractive indices—For uniaxial crystals, ϵ parallel to c and ω perpendicular to c. For biaxial crystals α is the lowest refractive index, β the intermediate, and γ the highest. Prime values will indicate intermediate indices.

Sign of double refraction—The expressions "optically positive," "double refraction, positive," and "uniaxial, positive" will be used.

Vibration directions—The terms X, Y, and Z will be used to express the vibration directions corresponding to the lowest, intermediate, and highest refractive indices, respectively.

In this, the first of the series, data for *p,p'*-DDT, Form I, and *p,p'*-DDT, Form II, are given under the above headings. M. E. DALZIEL

[EDITORIAL NOTE—Future abstracts concerning this project will record only the names of substances for which data are given, and a reference to this abstract.]

Control of Fractional Distillation by Means of Refractive Index Measurements. R. Raw (*J. Soc. Chem. Ind.*, 1947, **66**, 451–453)—The problem in many industrial fractionations is one separating members of homologous series where only very small differences in physical and chemical properties exist. The refractive indices of members of a homologous series often show no greater relative differences than, say, the densities, but they can be measured with an Abbé-type direct-reading refractometer in about 1 min., accuracy to 1 or 2 points in the fourth decimal place. Two factors that influence the accuracy of refractive index measurements are temperature control of the refractometer to ±0·1° C. and recognition of the mixture law for liquids that have no chemical action on each other.

For the control of fractional distillation it is not necessary to know the absolute values of the refractive indices, but it is important that the instrument should give consistent readings throughout the distillation, and it is desirable that all readings for a given distillation should be made by one operator. Procedure in applying refractive index control can vary; either the whole distillation may be controlled by means of determinations on spot samples of the distillate and collection in appropriate receivers, or else testing may be delayed until an arbitrary series of fractions has been obtained.

The accompanying table shows the refractive indices of a number of alkylanilines. Where the variation of refractive index with temperature has been examined, it has been found to be linear. It is thus possible to correct refractive indices from any given temperature to another without incurring an error. A. H. A. ABBOTT

REFRACTIVE INDICES OF SOME LIQUID AROMATIC AMINES

Amine	Sp.gr. at 15°	Ref. index n_D^{15}	Ref. index n_D^{20}	Temp. coeff. of n
Aniline	1·026	1·5870	1·5850	$4·0 \times 10^{-4}$
o-Toluidine	1·005	1·5738	1·5716	4·4 "
m-Toluidine	0·996	1·5697	1·5676	4·2 "
Methylaniline	0·992	1·5727	1·5702	5·0 "
Ethylaniline	0·964	1·5560	1·5535	5·0 "
Ethyl-*o*-toluidine	0·954	1·5483	1·5456	5·4 "
Dimethylaniline	0·961	1·5604	1·5579	5·0 "
Diethylaniline	0·937	1·5437	1·5413	4·8 "
Diethyl-*o*-toluidine	0·912	1·5088	1·5065	4·6 "
Diethyl-*m*-toluidine	0·930	1·5383	1·5362	4·2 "
Benzylethylaniline	1·034	1·5970	1·5950	4·0 "

Determination of Particle Size by Light Scattering. J. Bardwell and C. Sivertz (*Canadian J. Res.*, 1947, **25**, [B], 255–265)—The method developed for the determination of the particle size in suspensions of transparent particles less than 1000 A. (0·1 μ.) in diameter is based on the following modification of the Debye - Einstein equation:

$$\text{mass of particle} = \frac{6·909}{32\pi^3}\frac{\lambda^4}{\mu_0^2}\left(\frac{dD}{dc}\right)_{c=0}\Big/\left(\frac{d\mu}{dc}\right)^2$$

where μ_0 and μ are the refractive indices of the medium and suspension respectively, D is the optical density, and c the concentration in grams per ml. The limiting turbidity gradient dD/dc was evaluated from measurements at a fixed wavelength (5893 A.) over a range of concentration, using a Beckman spectrophotometer, and extrapolating the ratio D/c to zero concentration. An ordinary turbidimeter or nephelometer is sufficient for routine measurements. The specific refractive index $d\mu/dc$ was determined from measurements at the same wavelength on the pure solvent and one suspension of convenient concentration, by means of a Zeiss dipping refractometer. It may also be calculated from the difference between the refractive indices of the suspended particle and the medium by dividing that difference by the density of the suspended phase.

The method is rapid and highly reproducible, and the results obtained for rubber latex are in good agreement with independent measurements.

B. A. SCOTT

Electronic Timing Device and Reflux Ratio Controller. G. O. Thacker and B. Y. Walker (*J. Soc. Chem. Ind.*, **1946**, **65**, 259–261)—The device described is constructed of readily obtainable radio components and is designed to operate a magnetically controlled reflux dividing head for stills using a total condenser. The dividing head is of glass and is suitable for operation at any pressure, whilst its calibration is virtually independent of the properties of the liquid.

The timing device—The circuit is shown in Fig. 1. The instrument is assembled on a steel chassis with a steel panel on which are mounted the reflux time selector switch, the product time selector switch, and a mains on-off switch. Only two external cables are required, the mains (230 v., 50-cycle A.C.) supply and a three-core cable to the electro-magnets. The working of the instrument is as follows. Power from the mains is transformed by the transformer T, and the main high-tension supply is rectified by the valve V_2 and smoothed by the choke L and the condensers C_2 and C_3. The high grid-bias necessary to bias the valve V_1 to cut-off is delivered from an extra winding on the mains transformer, rectified by the Westinghouse rectifier W, smoothed by condenser C_4, and stabilised by the load resistance R_1. Alternatively, a half-wave valve (6C5) rectifier may be used. The anode current passing through the valve V_1 operates the relay, R_b being the normal bias resistor of the valve. The condenser C_1 connected to the grid of the valve V_1 is charged to a high voltage through the resistors on the switch S_2 and discharged through those on switch S_1, these two sets of resistors being connected to C_1 alternately by the contacts 1, 2, and 3 of the relay. The small resistor R is to prevent sparking at the contacts of S_1 when passing from zero to infinity positions. The live side of the mains is connected alternately to each electro-magnet through contacts 4, 5, and 6 of the relay. The two positions on S_1 marked O and ∞ cause the bucket to be held in the "total reflux" and "total product" positions, respectively. The actual value of the resistors required is determined by timing the effect of known resistances with a stop watch. Careful selection of components is essential for satisfactory working. The relay used was the Londex Type LQA fitted with three single-pole change-over switches. Any other type may be used, then minor modifications in the circuit design may be necessary (see the original paper). The valve V_1 was a pentode, 6F6G; the condenser C_1 must be of good quality and of the paper type.

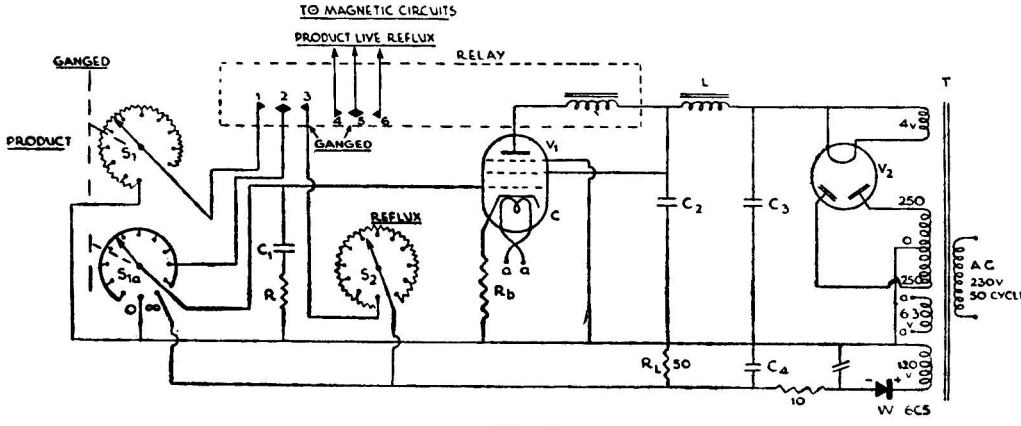


Fig. 1

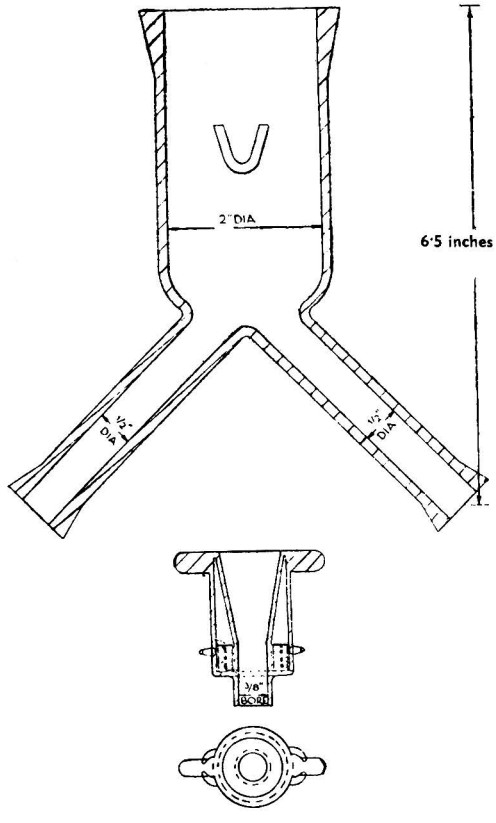


Fig. 2

The dividing head and bucket (Fig. 2)—The dividing head consists of a glass tube about 4 in. long and internal diameter 2 in. with two exit tubes at the base each set at 45° to the vertical. The tilting bucket consists of a laminated annular armature of soft iron through the centre of which passes the glass tube down which the liquid flows. This tube widens out upwards above the metal armature and the whole is enclosed in a wide glass tube sealed to the top and to the bottom of the inner tube. The bucket is suspended in the dividing head by two glass projections at the top of the bucket which fit into two V-shaped supports fused on to the inner wall of the dividing head. At the base of the bucket, four small glass hooks are provided for fixing pads of asbestos string to minimise the possibility of fracture when the bucket is pulled over to each side of the head by the magnets.

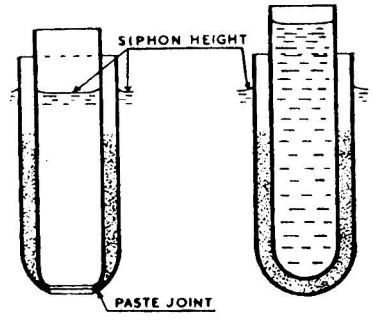
The electro-magnets—These were made from standard laminations (Sankey's Stalloy) and fitted with pole pieces of the same material shaped to fit the outside of the head, thereby keeping the air gap at a minimum. The coil winding, giving a consumption of 60 to 80 watts, consists of 3500 turns of 30 s.w.g. wire wound on a suitable bobbin.

J. ALLEN

Annular Thimble for Soxhlet Extractor. I. C. P. Smith and M. M. Neustadt (*Anal. Chem.*, 1947, **19**, 618)—The new form of thimble, in which the solvent path is much shortened, is designed for the more rapid extraction of flour. The diagram on the left shows the earlier form, in which two thimbles of selected sizes were cut open at the bottom and fastened together with paste, the annular space being filled with the sample and the thimble being then used in the ordinary way. The liquid levels inside and outside rise and fall together, making good contact with the comparatively thin layer of flour.

On the right, a modification is shown. Some of the flour is placed in the bottom of the outer thimble, the inner thimble is inserted, and the remainder of the sample is run in so that it takes up the position shown. A pencilled reference mark may be placed on the inner thimble to ensure an even layer of the sample round the bottom and sides. The solvent runs from the condenser into the inner thimble and through the sample to the outside, then returning to the flask in the usual way. The speed of reflux can be adjusted to maintain the liquid level shown in the inner thimble, or the liquid can be allowed to overflow into the outer thimble, but it must not overflow in such a way as to carry flour down into the flask.

Stock sizes of thimble should be chosen, the inner one being 90 mm. high with an internal diameter of 19 mm., and the outer one 80 × 30 mm. It may be necessary to use a larger size of extractor and outer thimble to accommodate some samples.



Whatman thimbles of 80 × 22 mm. and 80 × 33 mm. can also be used; the latter have walls of double thickness for extracting very fine flours. This arrangement is suitable for 10 g. of sample.

To fill the extractor, gently drop the material into the larger thimble, insert the smaller thimble, place a cottonwool plug between the two thimbles so that the system can be inverted and shaken without loss of material, and by this means depress the inner thimble so that the sample is evenly distributed in the annular space. Leave the plug of cottonwool in position to prevent the washing over of light material during the extraction, especially if the overflow siphon of the Soxhlet extractor is at the same level as the top of the outer thimble. The plug serves also to maintain the inner thimble upright as the material becomes more compact.

This annular system will extract, as a rule, over 90 per cent. of the total fat in a sample in the first half hour, comparing very favourably in performance with the elevated thimble previously described (Neustadt, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 431).

A. O. JONES

Review

THE BRITISH PHARMACOPOEIA 1948. Published under the direction of the BRITISH MEDICAL COUNCIL, with effect from September 1st, 1948. Pp. xl + 914. London: Constable & Co., Ltd., 10–12, Orange Street, Leicester Square, W.C.2. Price 45s.

SIXTEEN years have elapsed since the publication of the Sixth Pharmacopoeia, the first issued under the auspices of the Pharmacopoeia Commission. It was intended to publish the Pharmacopoeia next in 1941 and thereafter at intervals of ten years, and much preparatory work had been done when the outbreak of war made early publication impracticable. An Addendum to the B.P. 1932 was issued in 1936 and six more were produced during the period covered by the war. The present book, therefore, includes the matter retained from the previous Pharmacopoeia with its seven Addenda, together with new matter required to keep official requirements abreast of modern knowledge and practice. The Preface states that "the preparation of the seventh British Pharmacopoeia has, it may be said with confidence, been a more complex and laborious task than the preparation of any of its predecessors, not excluding the original British Pharmacopoeia

of 1864." The truth of this statement becomes more apparent with each examination of the contents. In view of the many interests affected by the replacement of one pharmacopoeia by another, it is customary to issue a new pharmacopoeia some months later than the completion of the work and to that extent the book is unavoidably out of date. The interval has been much increased this time owing to the difficulties and delays of publication, the Preface having been written in September, 1947. Should the intervals between successive pharmacopoeias become five years, as it is understood is the intention of the General Council, it is imperative that a much shorter time should elapse between the preparation of the book and its publication.

The production of the Pharmacopoeia is under the direction of the British Pharmacopoeia Commission, which appoints Committees and Sub-Committees dealing with every aspect of the matter included in the monographs and appendixes. The names of the members who have served on the Commission and Committees are given on pp. xi and xiii to xv: lists of reports and papers prepared for the Commission are given on pp. xx to xxiii and other sources of help and advice from Government and other bodies and from correspondents are acknowledged. The Commission acknowledges, too, the assistance derived from collaboration with the Committee of Revision of the United States Pharmacopoeia.

It is natural to compare the new Pharmacopoeia with its predecessor, to note what is retained, expunged, added or changed, and indeed it is necessary to do so for those who use the book. The comparison is of some difficulty inasmuch as the complete B.P. 1932 consists of eight volumes with contents of different values. The monographs in the Addenda include a number on articles and preparations added during the war years to take the place of others that became unprocurable or in short supply, and these have not been retained in the new book. Other products have had a short official life, having been replaced by more satisfactory substances or preparations. On pp. xxvi to xxxii there are tables listing the additions to, and deletions of, articles and preparations numbering 345 and 145 respectively, a gain of 200: also articles and preparations of which the name, composition or strength has been altered. The page of "Contents" apart from the lists referred to is identical with that in the B.P. 1932. The number of pages dealing with the monographs is increased by 139 and as the margins have been very much lessened the amount of print is increased by about two-thirds. The style of the monographs has been improved by sub-dividing the former "Characters" and "Tests" under a number of side-headings printed in heavier type. Each paragraph is devoted to a single test stated with precision and permitting of no ambiguity. This is the more necessary inasmuch as, to quote from the General Notices, "All the statements contained in the monographs with the exception of chemical formulae given at the beginning of monographs constitute standards for the official substances. A chemical, crude drug or preparation is not of pharmacopoeial quality unless it complies with all the requirements stated." It is further stated, "the standards of purity and strength of the Pharmacopoeia apply to articles which are intended for medicinal use but not necessarily to articles which may be sold under the same name for other purposes." The monographs dealing with crude drugs have been recast. The description comprises paragraphs giving macroscopical and microscopical details. If the drug is used in powder form, a separate description of microscopical characters is given. Finally, a preparation of the powder standardised chemically or biologically may be included as a separate monograph. There is a reference in the General Notices that vegetable drugs are required to be free from insects and other animal matter, and from animal excreta, but no method of examination is given. The paper by Kent-Jones *et al.* (ANALYST, 1948, **73**, 128) will doubtless provide material for an official method later.

There are one or two innovations referred to in the General Notices that are of interest to analysts. Glassware is now standardised at 20° C., "millilitres" is contracted to "ml." and "grammes" to "g." For doses involving grams down to one place of decimals the capital "G" is used, and prescribers are urged to use the same contraction. Smaller doses are given in milligrams (mg.). The expression "weight per ml. at 20°" replaces specific gravity. In a number of instances the Pharmacopoeia gives the dose to be supplied on a prescription if omitted by the prescriber.

The additions and deletions reflect the rapid changes in treatment that have come to pass partly through the growth of chemotherapy during the century and the pressure of events arising from two world wars. Crude vegetable drugs have suffered most. An enormous number, together with their galenical preparations, were jettisoned by the first Pharmacopoeia Commission in preparing the B.P. 1932. The residue has been further lessened by the removal of a dozen more, including cinchona, jalap, buchu, asafetida and copaiba. Cinchona bark, its preparations, and several preparations containing quinine (ammoniated tincture of quinine, Easton's syrup, iron and quinine citrate) have been deleted in order to conserve quinine for the treatment of malaria. There is no statutory prohibition of the use of quinine in making "tonics" and similar proprietary articles. No new vegetable drugs have been included; as none were added in the B.P. 1932 it may be inferred that the discovery of vegetable drugs of therapeutic value has come to an end. *Per contra*, this is the Penicillin Pharmacopoeia, and the isolation of penicillin from a mould growth is only an instance of the tendency to replace crude drugs by their active principles wherever possible. The galenicals prepared directly from drugs are, on the whole, little changed, though there are several deletions. Two infusions only are retained, though there are fourteen other preparations listed as infusions which are nothing of the sort. Plasters and confections have disappeared as well as aromatic waters prepared by distillation. Additions to the active principles isolated from crude drugs include five alkaloids, colchicine, ephedrine, ergotamine tartrate, ergometrine maleate and papaverine hydrochloride, the glycosides digoxin and ouabain (strophanthin-G) and picrotoxin used as an antidote to poisoning by barbiturates.

Inorganic chemicals have been used in therapeutics since the age of Paracelsus and their changes in the new Pharmacopoeia are neither numerous nor important. Calamine and calamine lotion, which have caused trouble to analysts for want of descriptions and standards, become official. Ammonium bicarbonate replaces "Ammon. Carb.", a compound which as sal volatile has a long history. The strength of hydrogen peroxide solution has been doubled. Changes have been made in the content of several chemicals and in the permitted amount of lead and arsenic.

The synthetic organic chemicals added since 1932 are numerous and important and cover a wide therapeutic range. They include a number of articles controlled by foreign patents at the outbreak of war. Three organic arsenicals, acetarsol, carbarsone and tryparsamide, and one preparation of antimony, stibophen, have been added: all are used in tropical parasitic diseases. Chiniofon and suramin used in amoebic dysentery and trypanosomiasis, together with the antimalarials mepacrine and pamaquin, are new. Sulphanilamide, the first of the sulphonamides, appeared in the Fourth Addendum, and others were introduced in the Seventh Addendum. Succinylsulphathiazole, included in the B.P. 1948, completes the list.

Penicillin is represented by the sodium or calcium salt. It is standardised biologically and there are seven preparations, including a lozenge. "The dose is determined by the physician in accordance with the needs of the patient." The monograph is naturally already somewhat out of date. Antiseptics and disinfectants include three aminoacridines, acriflavine, aminacrine hydrochloride and proflavine hemisulphate, two phenol derivatives, chlorocresol and chloroxylenol, and two dyestuffs, crystal violet and brilliant green. There are also a number of new hypnotics and local anaesthetics. The group of barbiturates has been fortified by the addition of two members derived from methyl-urea, hexobarbitone and methylphenobarbitone. The latter has the unfortunate synonym phemitonum, and should not be confused with phenytoinum sodium. Both are used in the treatment of epilepsy and the doses are similar.

Injections have had a chequered history. Hypodermic injections first appeared in the B.P. 1887, and the three given were increased to five in the B.P. 1898 and Addendum. These were retained in the B.P. 1914 but were all withdrawn from the B.P. 1932 which, however, included five new injections for intramuscular administration. Three of these have disappeared from the B.P. 1948, which has no less than 75 monographs dealing with all types of injection. A monograph on water for injection is also included with a biological test to ensure freedom from pyrogens. Tablets, which have been prescribed in enormous numbers for over half a century, have not hitherto received official recognition, except in the solitary instance of nitroglycerin tablet. The B.P. 1948 contains 48 tablets, almost all of which are assayed, and limits of tolerance are given. A general monograph gives methods for determining uniformity of weight of tablets, of weight of drug per tablet and of time for disintegration. In contrast, the number of pills, or rather of pill-masses, has been reduced to five. Uniformity in the size of pills and in the weight of drug per pill has recently been the subject of attention in the Courts, and it will be of interest to learn whether in future the Pharmacopoeia Commission will institute standards for a range of pills or allow pills to lapse as official medicaments. Though a Sub-Committee of the Pharmacy and Pharmacognosy Committee was appointed to deal with capsules, there are no monographs on capsules in the B.P. Presumably the report was not available for preparing the monographs in time for inclusion. The section on ointments has been improved by the addition of new types of ointment-bases and addition of assay processes for articles capable of quantitative estimation. Attention should be drawn to the two ointments described as ointment of mercury and dilute ointment of mercury, containing respectively 30 per cent. and 10 per cent. of mercury. The Pharmacopoeia directs that when ointment of mercury is prescribed or demanded, dilute ointment of mercury shall be supplied unless it is ascertained that ointment of mercury is required.

There are considerable additions to the monographs on biological products. The importance of the sex hormones is recognised by the inclusion of nine members. Vitamins are represented by aneurine hydrochloride, nicotinamide, nicotinic acid and riboflavine covering the B complex, ascorbic acid, calciferol, menaphthone and acetomenaphthone, the last two having the actions of vitamin K.

The appendixes, which occupy about one-quarter of the book, have been increased in conformity with the new matter of the monographs. Appendix I, Materials and Solutions employed in Tests, is increased fourfold, partly by the inclusion of new tests and partly by the tests on the reagents themselves. There are no substantial alterations in the directions for arsenic and lead tests. Scattered through the other appendixes are a few additional methods and tests, including a note on the spectrophotometric determination of vitamins A and D. There is a new appendix describing the fluorimetric assay of aneurine hydrochloride.

The B.P. 1948 is a handsome volume, strongly bound, and should withstand the rough usage of constant reference in dispensaries and laboratories to which it will be subject. Although the book has not been tested for misprints, considerable sections have been closely read in preparing this review and the proof reading appears to have been well done. The reviewer suggests that in preparing the next pharmacopoeia space could be saved by omitting the space formulae of organic compounds; those persons to whom the pharmacopoeia is indispensable have ready access to them in numerous textbooks. J. E. WOODHEAD

New Zealand Government

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