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Differential Electrolytic Potentiometry with Periodic Polarisation Part XXI. Introduction and Instrumentation

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Analyst, 1973, **98**, 697–711.

Differential Electrolytic Potentiometry with Periodic Polarisation Part XXII. Symmetrical Periodic Current Differential Electrolytic Potentiometry in Oxidation - Reduction Titrimetry

The application of pure, symmetrical, bias-free square, sine and triangular wave periodic polarisation to all types of oxidation - reduction titrations is reported. Electrode configuration and earthing and the destructive effect of bias are examined. Titration curve shapes are the same as those of classical d.c. differential electrolytic potentiometry, but the periodic current densities required are much higher than for d.c. The electrode response speed is greatly accelerated, unpoised potentials are steady, warning is given of the approach of the end-point in type II (*b*) titrations, electrodes retain full activity for very long periods and errors in titrations of iron(II) with dichromate or cerium(IV) are eliminated in the periodic method as against the d.c. method. Discrimination in type II reactions is slightly attenuated in the periodic method, and in titrations at low concentrations it is considerably attenuated. The nature and the conditions of the titration, the speed of the electrode charge-transfer process, the ballast load, the current density, the electrode area, the shape of the applied waveform, the applied frequency, and the deactivation of electrodes, are examined in detail. The benefit of the constant-current mode over the constant-potential mode is demonstrated.

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Analyst, 1973, **98**, 712–724.

The Detection of Light Elements by X-ray Emission Spectroscopy with Use of Low-energy Satellite Peaks

A method for the indirect detection of light elements, L (L = C, N, O or F), by using X-ray emission spectroscopy is described. The technique relies upon the formation of certain low-energy satellite peaks to those X-ray emission peaks which originate from electronic transitions involving the valence shell of an element, A, when A–L bonds are made. The energy difference between the main peak and the satellite peak is characteristic of the ligand (F, 20 $\pm$ 1 eV; O, 14 $\pm$ 2 eV; N, 9 $\pm$ 2 eV; and C, about 5 eV). Applications to compounds that contain more than one type of ligand are described and experimental limitations are discussed.

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Analyst, 1973, **98**, 725–731.

Stability of Dilute Standard Solutions of Antimony, Arsenic, Iron and Rhenium Used in Colorimetry

A study has been made of the stability of dilute standard solutions of antimony (4 μg ml^{-1}), arsenic (20 μg ml^{-1}), iron (50 μg ml^{-1}) and rhenium (5 μg ml^{-1}) used in colorimetry. The standard elements in these solutions were determined over a period of 2 months by using colorimetric procedures developed in this laboratory and reported previously. Tests were carried out on standard solutions stored in soda-glass, in borosilicate glass and in rigid polyethylene containers.

The dilute standard antimony solutions, prepared either by dissolving antimony potassium tartrate in water, or by dissolving elemental antimony in sulphuric acid and diluting the solution with water, were found to be stable (*i.e.*, to deteriorate by less than 2 per cent.) over a period of 50 days. Similar dilute standard antimony solutions containing hydrochloric acid deteriorated rapidly, however.

The dilute standard arsenic solutions prepared either by dissolving arsenic(III) oxide in sodium hydroxide solution and then neutralising the solution with hydrochloric acid, or by dissolving disodium hydrogen arsenate heptahydrate in water, were found to be stable. Arsenic(III) in the former standard solution was oxidised slowly by dissolved oxygen, but the total arsenic present in the solution remained unchanged and could be determined by the molybdenum-blue method.

An iron(III) standard solution, 0·06 M in hydrochloric acid and prepared from ammonium iron(III) sulphate, was stable for at least 2 months, as was a standard potassium perrhenate solution in a buffer solution of pH 6.

Light in the laboratory and the material of the containers did not adversely affect the solutions reported to be stable. Light accelerated the deterioration of the antimony solutions that contained hydrochloric acid, and the material of the containers had a slight effect on the rate of deterioration.

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Analyst, 1973, **98**, 732–738.

OCTOBER, 1973 Vol. 98, No. 1171

THE ANALYST

Differential Electrolytic Potentiometry with Periodic Polarisation

Part XXI.* Introduction and Instrumentation†

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Previous work on periodic polarisation of indicator electrodes is reviewed and discussed, and is faulted on the premise that perfectly symmetrical, bias-free waveforms may not have been used. A statement of intent of the present work is made. The fabrication, activation and testing of electrodes is described, waveform generators are critically discussed, and measuring instruments for periodic and d.c. potentials are both described and evaluated. A simple device for interfacing instruments to recorders, offering a high degree of band spread, is described. High-voltage square-wave generation by use of relays is also discussed. Waveform monitoring was a crucial factor in the work, and the accurate balancing of shape, amplitude and half-cycle duration is described. Frequency measurement and timing by means of a crystal clock, and amplitude and bias detection by integration and by d.c. differential electrolytic potentiometry, are appraised. Finally, the working assembly is described, together with the technique for the elimination of electrical interference.

THE polarisation of a pair of identical, conducting indicator electrodes with a very small, heavily stabilised, direct current affords a very exact method for the location of a titrimetric end-point, *viz.*, d.c. differential electrolytic potentiometry.[1,2] Periodic and biassed periodic polarisation have been extensively examined,[3–7] and have shown certain advantages over the d.c. method.

The first use of periodic polarisation appears to have been made by Franck,[8,9] who employed a low-frequency alternating current (a.c., a term specifically denoting a sine waveform) of constant amplitude. His ballast resistance was too low for adequate current stabilisation. This technique is therefore bipotentiometric and not amperometric, although Franck used a three-electrode configuration that included a rotating disc electrode. Juhasz[10] titrated iodine with thiosulphate solution and used a.c. polarisation of two vibrating platinum electrodes, thus eliminating the need for a stirrer. Schmidt[11] used two amalgamated-silver electrodes in the titration of tetraphenylborate with thallium(I). Kitagawa,[12–14] Morisaka[15,16] and Morisaka and Harada[17–20] have studied the a.c. polarisation of a pair of identical electrodes in oxidation - reduction and ion-combination titrations. The accuracy of the sine waveforms, and the stability of the current, are uncertain and probably dubious. Other waveforms have been used and compared with a.c.

A comprehensive examination of square-wave titrimetry by Laitinen and Hall[21,22] led them to some conclusions, certain of which can now be ascribed to limitations of the instruments. They pointed out that Franck's a.c. method is limited by the relatively large charging current of the double-layer capacitance associated with the electrode, even in the absence of electrode reactions. They state that with a square waveform and proper adjustment of experimental conditions, the charging current can occur as a pulse at the beginning of each half-cycle; if the meter does not respond to the charging current, an improvement over the sine wave method should result. They do not explain what they mean by this "pulse", or how it is to be obtained. It is no more than a requirement that the waveform generator should have a fast rise time, and should have sufficient reserve to intensify rapidly the power on the leading edge of the square wave in order to charge the capacitive load. A rise time

* For Part XX of this series, see reference list, p. 710; for Part XXII, see p. 712.

† Presented at the Second SAC Conference, Nottingham, 1968.

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of 50 to 100 ns is now attainable, and in that context a current reserve of about 10 to 20 mA is adequate for r.m.s. current densities up to 100 μA. They say that the two square-wave modes, constant applied voltage or constant applied current, applied to a pair of identical electrodes, both yield a measure of the slope of the current - voltage curve in the zero-current region, provided that (*a*), the amplitude of the applied signal is sufficiently small that the current - voltage curve can be regarded as linear in the zero-current region, (*b*), the charging pulse is of short duration compared with the frequency of repetition, and (*c*), a steady state is reached in a small fraction of the periodic cycle. These requirements are incompatible; condition (*c*) requires an infinitely fast electrode process, while (*a*) requires an infinitely fast or a very slow reaction, and if applied to the end-point region, where it is needed, it would require so small an input signal that there would be no output. Requirement (*b*) is simply for a high slew rate in the signal generator. Laitinen and Hall drew the dubious conclusion that the constant-current mode, application of a high-voltage signal through a high resistance, could not be used because of requirement (*a*); this conclusion suggests a lack of understanding of this aspect, because the signal applied to the electrodes is still of small amplitude. Despite this conclusion, they conducted several titrations at constant current. However, most of their work concerned a constant-voltage technique, and was therefore biamperometric; the applied signal was between 20 and 50 mV, which hardly meets their requirement (*a*). This is satisfactory for fast electrode processes, which they used, but is likely to render the method unusable for slow processes. In making a comparison with d.c. amperometry with a spinning platinum microelectrode in iodine titrations, they concluded that the square-wave method offered a ten-fold benefit in sensitivity. Hall and Flanigan[23] used square-wave titrimetry in the stepwise determination of iron(III) and iron(II) with EDTA and hexacyanoferrate(III).

By use of the constant-current technique, Kitagawa[12] found little difference between sine and square waveforms. Riolo and Soldi[24] compared square-wave potentiometry, zero-current potentiometry and amperometry in the titration of cadmium with EDTA at twin dropping-mercury electrodes. Square-wave polarographs have also been used for amperometric titrations.[25–29]

The work summarised above has been largely empirical and applicative, with little attempt at comparison with other established techniques, and with little or no examination of the variable parameters or of fundamental matters. Probably the greatest deficiency has been that no serious attempt has been made to produce pure waveforms, accurately shaped and balanced, and free from harmonics, distortion and bias. The importance of these factors will be made clear. Furthermore, little, if any, attention has been given to the mechanism and kinetics of the electrode processes, and only qualitative mention has been made of the influence of the relaxation time of the electrode reactions on perturbation by periodic polarisation.

The primary aim of the present work was to investigate such influential parameters as: the waveform, frequency and amplitude of the applied signal; single and dual electrode configurations; the addition and removal of various kinds of bias; the nature, area, pretreatment and ageing of the electrode; the nature of the electrode reaction; reagent concentrations and volumes and the equilibrium constant of the titrimetric reaction; the precision, accuracy, discrimination and response speed; and to compare the results with those of classical zero-current potentiometry and d.c. differential electrolytic potentiometry. While this work was in progress, the rationalisation of electrode processes in stirred solutions was under development[30] and the titration curve analogue[2] was used to make certain predictions as to the shapes of titration curves to be expected from various input signals and various electrode combinations. It was pointed out[4] that a stable, symmetrical, constant-amplitude periodic current applied to a single indicator electrode in a symmetrical titration would, at the equivalence point, give a pure periodic output potential measured against a reference half-cell such as a saturated calomel electrode, the peak-to-peak magnitude being equal to the peak height of a d.c. differential electrolytic potentiometric curve produced from a pair of electrodes and a current equal to the amplitude of the periodic current. Application of such a periodic current to a pair of indicator electrodes would, at the equivalence point, produce a similar periodic output potential, that at the second electrode being 180° out of phase with that at the first, thus giving a net periodic potential between the two electrodes of twice the amplitude of the single-electrode configuration. At other points in the titration the output periodic potentials would be unsymmetrical. The titration curve analogue must

not be pushed too far, and can be applied only to fast electrode processes, but it is worth noting that the predictions shown in Fig. 1 were confirmed experimentally so far as the shape of the curve is concerned. As the first phase of this work was drawing to a close,[3] the rationalisation mentioned[30] was completed, and by combining the computer program VOLTAMMETRY 9[30] with the program COMBSYMTIT, DEP 9 was produced and was used to simulate the output waveform at points near to the equivalence point in a symmetrical oxidation - reduction titration wherein the electrode processes are fast,[31] and for a sine waveform input $I_\Delta = 20 \sin \omega t\ \mu A$, with the result shown in Fig. 2. The input waveform is a pure sine wave with a peak current density of 20 μA. One complete cycle of the output waveform at each annotated stage in the titration is shown. The output waveform is a low-amplitude, distorted sine wave (approximating to a pulsed harmonic) until the titration reaches 24·82 ml in a 25·00-ml titration. Within 0·01 ml, at 24·83 ml, the pulse suddenly increases by about 300 mV. As the equivalence point is approached, this pulse increases logarithmically in height and linearly in breadth until it consumes the whole waveform at 25·00 ml. The sudden appearance of the pulse gives warning of the approach to the equivalence point. The progression reverses precisely after the equivalence point, and the pulse disappears abruptly between 25·17 and 25·18 ml. The waveforms shown in Fig. 2 are for electrodically fast reactions; for electrodically slow reactions, a similar pattern is displayed but the potentials are increased by the amount of charge-transfer overpotential. It may be noted that the difference between triangular, sine and square waveforms is small, especially with slower electrode processes.

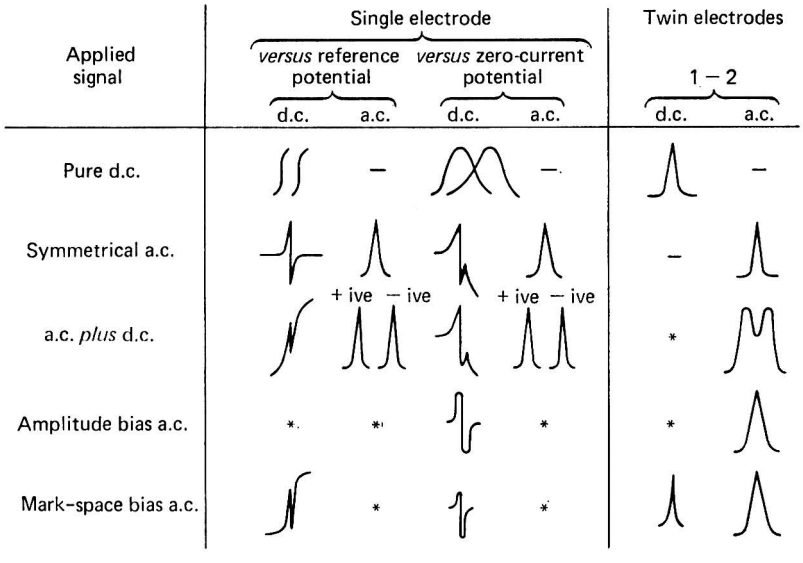

Fig. 1. Analogue predictions of titration curve forms. * Indicates possible presence of fine structure

EXPERIMENTAL

INDICATOR ELECTRODES—

Platinum—Platinum wire of 22 s.w.g., spot-welded to a tinned copper connecting lead, was sealed directly into lead-glass tubing. The assembly was very carefully annealed so as to eliminate the possibility of large and erratic residual potentials at the metal - glass junction[32]; annealing is also said[33] to give a more active electrode in respect of the reaction of surface oxide with hydrogen, although it is without effect on simple electrode processes. Finally, the electrodes were rigorously tested for leakage through the metal - glass seal.

Silver and silver halide—Electrodes were made from 22 s.w.g. mint-silver wire by a modification of the earlier method[34] aimed at reducing the number of failures caused by the blue-glass not properly wetting the metal, and by cracking the blue-glass - soda-glass seal,

both of which result in leakage. The first fault was mitigated by drawing out blue-glass tubing (supplied by Plowden and Thomson) to a fine capillary that was just wide enough for it to slide over the silver wire. A 5-mm length of this capillary was threaded on to the silver wire, already spot-welded to a tinned copper connecting wire, and the glass sealed on, thus wetting the silver wire over the corresponding length. The second fault was eliminated by blowing a blue-glass bulb on the end of the sheath of soda-glass tubing and thoroughly annealing the joint. A very small hole was made in the blue-glass bulb, the glass-coated wire inserted through the hole from within, and the glass sealed to the sheath. This process reduced the initial failure rate of over 80 per cent. to less than 5 per cent. Halide coatings were formed as previously described.[34]

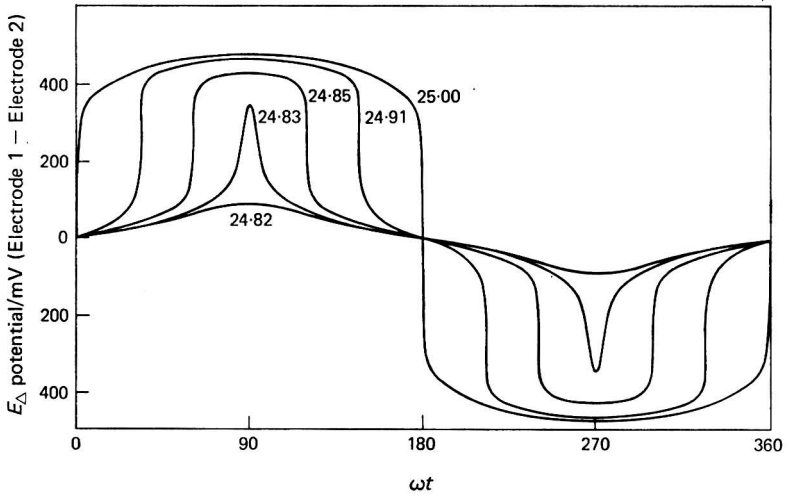

Fig. 2. Theoretical calculation of waveform at points in the end-point region of a titration, for fast electrode processes. Annotations indicate volume of titrant added. $\Delta V = 0{\cdot}01$ ml; $\delta x = 3 \times 10^{-3}$ cm; $A = 1$ cm^2; [H+] = 1·0 M; $I_{\Delta max.} = 20$ μA; $I_\Delta = I_{\Delta max.} \sin \omega t$.

	Titrant	Titrand
E'_0	1·01	0·29 V
n	1	1
$k_{mass\ ox}$	$3{\cdot}3 \times 10^{-6}$	$3{\cdot}3 \times 10^{-6}$ l cm^{-2} s^{-1}
$k_{mass\ red}$	$3{\cdot}3 \times 10^{-6}$	$3{\cdot}3 \times 10^{-6}$ l cm^{-2} s^{-1}
[X]	0·1	0·01 mol l^{-1}
V	25·00	250·00 ml

Antimony - antimony oxide electrodes—These electrodes are the best conducting hydrogen ion sensitive electrodes,[35] and are best made by a casting technique[36] in a Pyrex glass sheath, electrical connection being made by melting resin-cored solder above the antimony and inserting a tinned copper connecting wire. The glass tubing is pre-heated, clamped above a crucible containing molten Specpure antimony, and a 5-cm column of metal sucked into the tube by means of a pipette filler. Considerable difficulty was encountered in securing a good glass - metal seal, and leakage was revealed by the sluggish behaviour of the electrode and highly deficient response to change in pH. An empirical investigation was conducted in order to define the optimum casting conditions. A partial or complete antimony mirror was formed on the inside of the tube, and in order to make a complete mirror with a good seal to both glass and core metal the following conditions were found to be necessary. Firstly, the temperature of the antimony had to be very little above the melting-point; if it was too close the antimony solidified on contact with the relatively cool glass and if the temperature was too high, the core formed was loose and could easily be pushed out. Secondly, the glass tube was heated to a uniform temperature of 120 °C in an oven, quickly clamped in position, lowered and the molten antimony drawn up. Higher or lower temperatures caused the partial or complete loss of the mirror and immediate or eventual leakage between the metal

core and the glass sheath. Thirdly, variation of the gauge and wall thickness of the glass tubing had no effect, but it proved to be necessary always to use fresh Specpure antimony; antimony recovered from electrode failures gave poor results, probably because of the presence of trace amounts of copper.

REFERENCE ELECTRODES—

Glass electrodes—The glass electrodes used in silver - halide titrations were E.I.L. GHS23 lithium - barium glass or GG23 MacInnes-Dole glass. High-capacity, large-area saturated calomel electrodes (S.C.E.) in potassium chloride, or saturated mercury(I) sulphate electrodes (S.M.S.E.) in potassium sulphate, electrodes that had a working area of 35 cm^2 in order to minimise any risk of polarisation, were used as appropriate. Remote junctions, terminating in low-leakage ceramic plugs, were used to connect the reference electrodes to the titration solution. The potentials of these electrodes, measured *versus* the standard hydrogen electrode, were +0·615 V for the S.M.S.E. and +0·244 V for the S.C.E. at 20 °C.

WATER—

The water used for the preparation of solutions, supporting electrolytes, washing and storage of electrodes, and all other purposes in this work, was sterile, grease and surfactant free, less than 10^{-12} M in dissolved solids, and was prepared in special stills.[37] Whenever water is mentioned in the papers in this series, use of the above is implied unless otherwise specified. This water is in equilibrium with atmospheric carbon dioxide and oxygen.

Carbon dioxide free water—This water was prepared from the above water by methods previously described.[36]

Oxygen-free water—This water was prepared by boiling water as mentioned above and allowing it to cool under a continuous purge of white-spot nitrogen scrubbed with chromium(II) chloride and water. Deoxygenation *in situ* was achieved by prolonged purging of the solution in the cell with purified nitrogen; a 20-minute purge gave a solution 10^{-6} M in oxygen, while a 2-hour purge gave a solution 1 to 5 $\times$ 10^{-7} M in oxygen.

ACTIVATION OR PRE-TREATMENT OF ELECTRODES—

As is evident on inspection of the titration curves shown in earlier papers in this series and as will be further demonstrated, electrodes used in zero-current potentiometry or d.c. differential electrolytic potentiometry become more or less quickly deactivated by adsorption of impurities or formation of films on, or oxidation of, the electrode surface. The subject has been reviewed with particular reference to platinum electrodes.[38] Ion-combination electrodes can be checked by measuring the slope factor and response speed, while platinum electrodes can be checked by measuring the response speed in known enviroments and by the amount of charge-transfer overpotential, which manifests itself in an increase in the differential potential, E_Δ, and a change in the shape of the titration curve; type I[39] curves can change into type II curves when the electrodes become very dirty. After fabrication, or when deactivation became apparent, the following activation procedures were used.

Platinum electrodes—First, the electrodes were subjected to immersion in freshly prepared aqua regia at 60 °C for 1 to 2 minutes so as to remove grease or gross contamination, secondly, anodisation in 11·6 M hydrochloric acid at a current density of 200 mA cm^{-2} for 60 s followed by washing, and thirdly, cathodisation in 0·05 M sulphuric acid at an applied cell potential of 4 to 6 V for 10 minutes with a final thorough wash with water.

Silver electrodes—These were subjected to immersion in 6·0 M nitric acid for a sufficient time to produce a clear, matt surface, and to a thorough wash.

Silver halide electrodes—These cannot be re-activated, except by stripping the film in cyanide and preparing a fresh film.

Antimony electrodes—De-activation usually arises from a thickening of the oxide film on the surface of the electrode, and a fresh surface is exposed by cutting a thin slice of sheath and core off the end of the electrode with a diamond saw.

STORAGE OF ELECTRODES—

If any electrode is allowed to dry out, it will become deactivated and, although soaking in water or the appropriate electrolyte for several days will restore the activity to some electrodes, in particular glass membrane electrodes, it is usually more convenient to activate

the electrode again by more certain means. Electrodes used in this work are stored in water when not in use.

CHECKING ELECTRODES FOR ACTIVITY—

Platinum electrodes—The behaviour during a titration offers a sensitive indication of the degree of deactivation. It is convenient here to mention the behaviour of platinum under large-perturbation periodic polarisation, because this has been advocated as a method of activation.[17,20] A pair of 1-inch bright platinum electrodes were immersed in 1·0 M sulphuric acid and 50 Hz "a.c." was applied from a transformer, the peak-to-peak voltage being adjusted so as to prevent appreciable evolution of gas. The applied cell voltage was 0·3 V and the current density was 25 mA cm^{-2}. Initially (after about 5 minutes), a light grey film formed on both electrodes and gradually darkened to a grey - black film that did not resemble platinum black deposited from a hexachloroplatinate(IV) solution. The film was not adherent and was easily removed with a paper tissue, leaving a dulled surface. This lack of adherence accords with Hoare's theory that the surface of the metal is broken up by alternate dissolution and removal of hydrogen, and not with Anson and King's theory of oxide formation and reduction.[38] The applied signal from an iron-cored inductor is neither pure nor symmetrical.

Application of a carefully balanced sine waveform from the Feedback generator (see below), at a cell voltage of 0·3 V r.m.s. and a cell current of 12·5 mA r.m.s., resulted in one electrode becoming coated with a gold-coloured film, observable within 60 s, while the other electrode remained bright. The gold film was very adherent, scratch-proof, insoluble in 7·0 M sulphuric acid, but soluble in 0·1 M iron(II) sulphate solution. The gold film was formed only within the frequency range 30 to 80 Hz, with either sine or square waveforms. Close examination of the cell signal with a measuring oscilloscope showed that the pure symmetrical waveform became distorted and off-set when the applied voltage was increased so that a periodic signal with a d.c. bias was being applied to the electrodes, presumably because of overloading of the waveform generator. Periodic polarisation of platinum has received a little attention[40,41]; Hoare,[40] when using a.c. polarisation with a d.c. bias, obtained a golden yellow film similar to that described above. He supposed this to be a thin, strongly adherent film of oxide, probably of platinum(IV) oxide, but he did not investigate the frequency range over which the film is formed.

Ion-combination electrodes—These were checked for leakage and deactivation by observation of their relaxation time after a concentration perturbation and by measurement of their slope factor. Active silver electrodes show a slope factor of 55 mV pAg^{-1}. Antimony electrodes were checked by plotting the hydrogen-ion response over the pH range 3 to 7 by immersion in 300 ml of carbon dioxide free water containing 0·75 g of potassium chloride at 20 °C and addition of 10^{-3} M (or stronger) perchloric acid from a micrometer-syringe burette. The process was conducted in pure oxygen.[35] A slope of 55 mV pH^{-1} in the pH range 3 to 6 should be obtained, with a sharp decrease in slope from pH 6 to pH 7.

THE TITRATION CELL—

The vessel comprised a 400-ml beaker, from which the lip had been removed with a diamond saw, fitted with a lid machined from 1-cm thick Perspex in order to give a firm fit, and to act as a stout support for the electrodes, which allowed a precisely reproducible geometry of location. For the latter purpose, holes were drilled as shown in Fig. 3, and included one for admission of purge or blanket gas, which escaped through the hole provided for admission of the burette jet, this hole being large enough (1·5 cm in diameter) to permit split drops of titrant to be removed from the tip of the burette. The solution was magnetically stirred with a PTFE-coated magnetic follower, the motor being controlled by a Variac transformer so that reproducible stirring speeds could be obtained. The electrodes were so positioned that mass transfer would be the same at each current-carrying electrode, while the zero-current indicator and the reference electrode junction were out of the current field. Zero-current, d.c. differential electrolytic potentiometric and periodic differential electrolytic potentiometric titrations could be conducted simultaneously and potentials between two polarised electrodes, one polarised and one zero-current electrode, and one electrode, either polarised or zero-current, and the reference electrode could be monitored. In addition,

both d.c. and periodic potentials of the periodically polarised electrodes could be measured against each other or *versus* a zero-current or reference electrode.

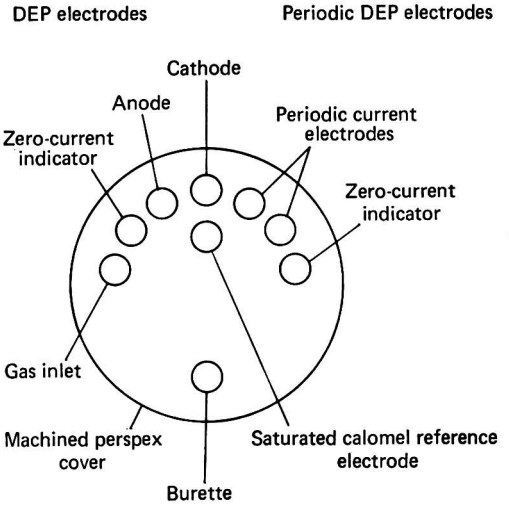

Fig. 3. Position of the electrodes in the titration cell. The machined, 1-cm thick, Perspex lid is drilled as shown

THE EXPERIMENTAL ASSEMBLY—

The general layout is shown in Fig. 4, in which only the periodic differential electrolytic potentiometric electrodes are shown. The d.c. differential electrolytic potentiometric circuitry has been described,[42] and is not included in the figure. Alternative methods of measuring periodic potentials, depending on the frequency, are used. The d.c. differential electrolytic potentiometric and other d.c. potentials are measured on a Vibron 39A pH meter. The electrical circuitry and instrumentation are described, and the performance discussed, under the headings Waveform generators, Periodic and Direct current potential measurement,

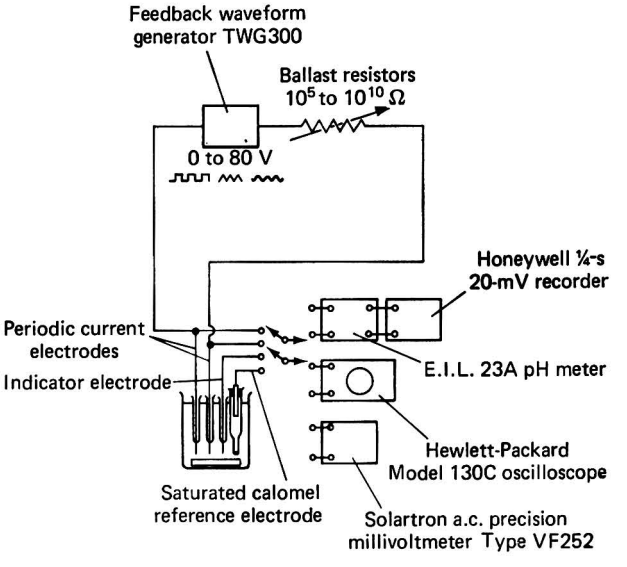

Fig. 4. The general titration assembly

Waveform monitoring and The working assembly. When this phase of the work was completed,[3] polarisation current stabilisation depended on high source voltages and high-value ballast resistors, a situation that has been examined.[43] A better solution consists in the use of constant-current sources,[44] which are now viable for use in d.c. polarisation, but stabilisation is more readily attained for high frequencies than for the low or very low frequencies used in this work.

WAVEFORM GENERATORS—

The requirements are for as high an output voltage as possible, in order to allow adequate resistance stabilisation, a variable frequency extending well below 1 Hz, and, if possible, a selection of waveforms that can be carefully shaped. It soon became obvious that waveform symmetry was of vital importance, and that any d.c. bias in the output was severely deleterious to the periodic signal from the electrodes. The instruments used are described below together with their performance, disadvantages and limitations.

Heathkit AG-9U Audio Generator—This instrument is based on a Clapp-Colpitts valve oscillator, and produces a sine wave output of 0 to 10 V r.m.s. and 2 Hz to 100 kHz. It lacked a very low frequency range, had a small maximum output, thus limiting current stabilisation, showed a drifting voltage output at any given attenuator setting and had a d.c. output component of up to 15 per cent. of the a.c. r.m.s. output. The last defect, which is of little importance in the higher frequency a.c. mode, is due to leakage via the capacitors in the output stage. An attempt was made to back-off the d.c. component by applying an equal and opposite signal from a 1·4-V Mallory cell and a 1-MΩ potentiometer, but it failed because of the signal drift in the generator. Insertion of a 1·0-μF polyester-foil capacitor in series with the output attenuated the d.c. component, but did not eliminate it; integration (see below) of the maximum signal output produced a ramp voltage slope of about 1·0 mV s^{-1}, whereas without the capacitor the slope was 100 mV s^{-1}. Insertion of the capacitor did not prevent the build-up of a high d.c. potential, and also presented an impendance of 30 kΩ at 5 Hz.

Advance H-1 sine wave - square wave generator—Although this instrument produced an output of up to 80 V peak-to-peak on the square wave, and 40 V peak-to-peak on the sine wave, its lowest frequency setting was 15 Hz and both waveforms became excessively distorted at low frequencies.

Feedback waveform generator TWG 300—This is a sophisticated valve circuit that operates in the fashion of an operational amplifier wave shaper, although no operational amplifiers are used in the circuit. The basic waveform is triangular, produced by an integrator ramp generator from which other waveforms are synthesised and shaped. It provides an output of 0 to 80 V peak-to-peak over a frequency range from 0·0008 to 1200 Hz, of square, sine, triangular and clipped triangular (of adjustable slope) waveforms. Although not high by d.c. differential electrolytic potentiometric standards, the output voltage is sufficient, with the aid of ballast resistors, to restrict variations in the differentiating current, I_Δ to $\pm$0·125 per cent. on a differential peak potential, E_Δ, of 100 mV peak-to-peak.

The circuit is shown in Fig. 5, and it is possible to adjust the shape of individual half-cycles of the output. The pre-set potentiometers P3, P4 and P5 can be used to balance or vary the mark to space ratio of the square waveform (rise time 10 to 90 per cent. less than 5 μs, overshoot less than 1 per cent.). The pre-set potentiometers P6 and P7 are used to optimise the shape of the sine wave output, one affecting the positive-going half-cycle and the other the negative-going half-cycle; there is some interaction between the two, necessitating repeated adjustment until the wave form is perfect. The amplitude can be balanced with respect to the 0 V reference level by means of the pre-set potentiometer P9, which can also be used to introduce a known d.c. offset (amplitude) into the output. Initially, the output frequency was calibrated against the 50-Hz mains supply by using Lissajou figures, but later the crystal clock was substituted. The scale accuracy was $\pm$3 per cent., the setting discrimination 0·2 per cent. and the hour-to-hour stability of the frequency was typically better than 0·1 per cent. The output amplitude stability was checked on the precision a.c. millivoltmeter and did not drift or wander by more than $\pm$0·3 per cent. over a 10-hour period. Internal or external relays could also be operated by the instrument.

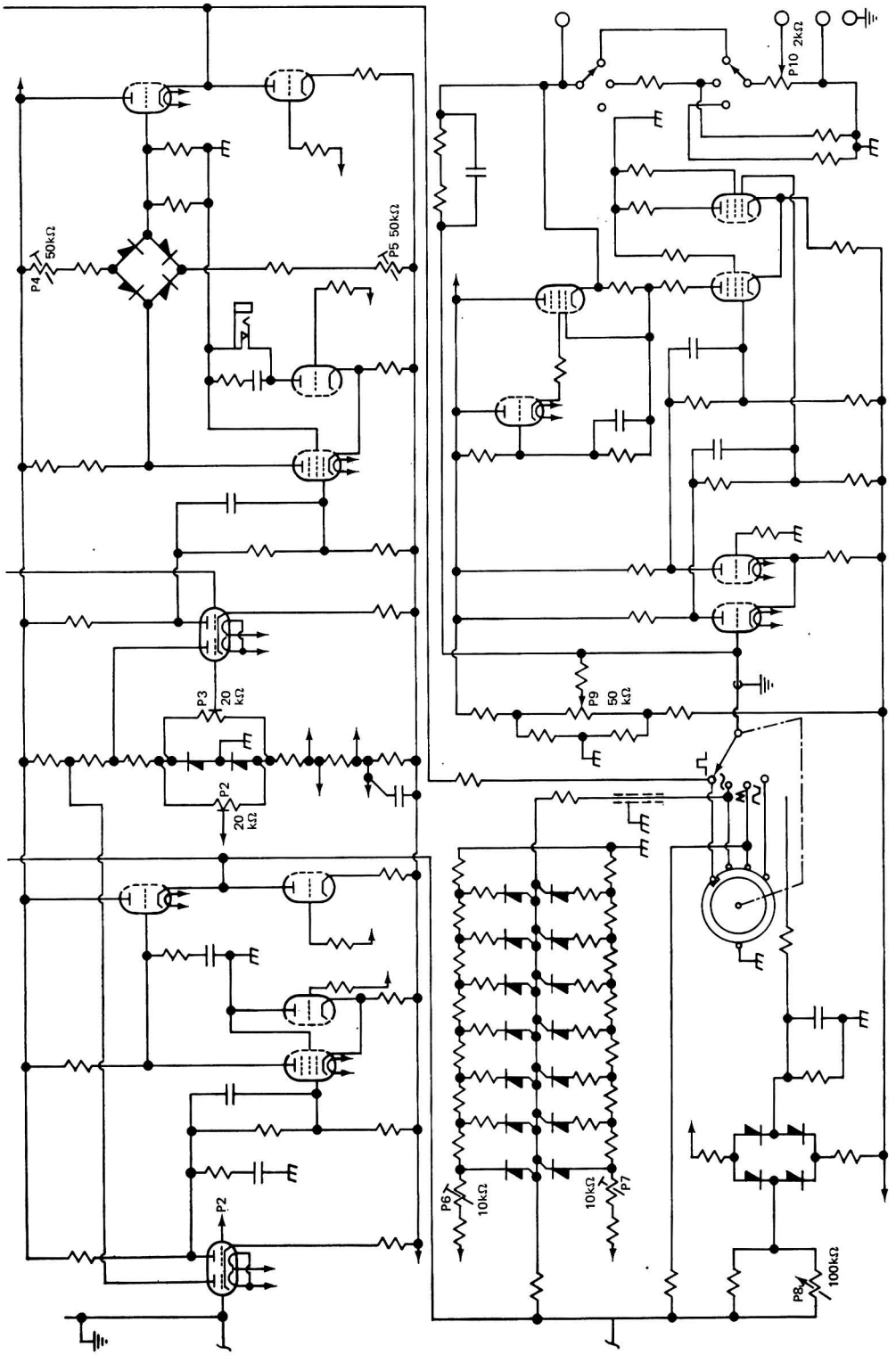

Fig. 5. Relevant part of circuit diagram of the Feedback waveform generator, showing pre-set potentiometers P_2 to P_{10}

Measuring instruments

Periodic current measurement—

Three main methods were employed, depending on waveform and frequency, for measuring or monitoring periodic signals.

*Solartron a.c. precision millivoltmeter, VF*522—This is a direct-reading meter with nine ranges from 1·5 mV to 15 V full scale on a 6-inch meter, and a nominal frequency range of 10 Hz to 100 kHz. Calibration showed the indicated values to be within 1 per cent. of full-scale deflection from 20 Hz to 100 kHz for pure sine waves. Below 20 Hz, an increasing negative error appeared, but it proved possible to use the instrument down to 3 Hz by calibration with the 130C oscilloscope (see below). No useful readings could be made below 3 Hz. The instrument is calibrated for pure sine wave signals; it was re-calibrated for square and triangular waveforms.

Hewlett-Packard 130*C measuring oscilloscope*—A wide range of frequencies can be used with an accuracy of ±3 per cent. peak-to-peak, 0·1 Hz to 1 MHz, with a useful response extending to 60 MHz. Modes of X - Y or Y - time could be used, both axes covering 200 μV cm^{-1} to 20 V cm^{-1} in sixteen ranges. The cathode-ray tube has an internal graticule so that parallax errors are eliminated. With a short persistence tube, the accuracy at low frequencies is limited by the spot size. Fig. 2 shows the distortion of a sine wave near the equivalence point. Square wave inputs to the electrodes also produce distorted output waveforms, depending on frequency, electrode kinetics, poising and concentration of the solution; the magnitude of such signals was read from the peak-to-peak oscilloscope display, or as an empirical r.m.s. value on the a.c. millivoltmeter.

E.I.L. 23*A pH meter and Honeywell* 0·25-*s*, 20-*mV recorder*—This combination was used to measure periodic signals up to about 4 Hz. Higher frequencies produced negative errors, but could be dealt with on the a.c. millivoltmeter, so that the whole spectrum could be measured with good accuracy, with the oscilloscope as a general-purpose monitor. The 23A pH meter is a direct-reading instrument of range 0 to 800 mV and accuracy ±2 mV (0 to 1600 mV accuracy ±4 mV with a doubler plug) and an input impedance of 10^{12} Ω. A coarse zero control allowed the zero point of the meter to be set in the centre of the scale so that the positive and negative half-cycles of a signal could be monitored. The recorder output of the meter was connected via a 1·5-kΩ helipot to a Honeywell ¼-s high-speed recorder. The recorder should show no loss of response at applied frequencies up to at least 2 Hz. The time constant of the meter, τ, is equal to RC, where R is the input resistance and C the input capacitance of the meter and τ is the time required for the response to reach $1/\sqrt{2}$ of the applied signal. The response of the system to periodic signals is shown in Fig. 6.

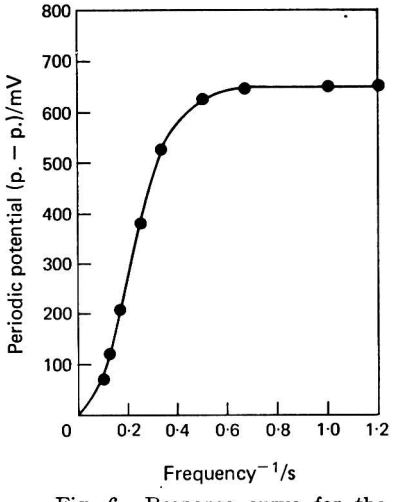

Fig. 6. Response curve for the combination of an E.I.L. 23A pH meter, centre-zeroed, and a Honeywell ¼-s high-speed recorder

DIRECT CURRENT POTENTIAL MEASUREMENT—

All d.c. potentials were measured on an E.I.L. 39A Vibron pH meter with an input impedance of 10^{15} Ω and a precision of 0·1 mV. As it has a relatively long time constant, this instrument could be used to measure the d.c. component in a periodic signal greater than about 0·2 Hz in frequency. A general-purpose converter has been designed for interfacing such devices as pH meters with potentiometric-type recorders; this is shown in Fig. 7. The total resistance between the input terminals should be selected so as to match the manufacturer's specified output impedance, in this instance 5 kΩ. The converter can be used for scale expansion, thus a 10-mV range anywhere between −1400 and +1400 mV can be expanded to full scale on the recorder, which can be centre zeroed so that noise and drift can be measured with great sensitivity. Such applications have been described earlier.[36,43,44] By use of a Honeywell 2·5-s, 10-mV recorder, ±10, 140, 500 or 1400 mV full-scale deflections can be displayed, and can be backed off by ±0 to 1400 mV by means of the band spread and backing-off controls on the 39A pH meter.

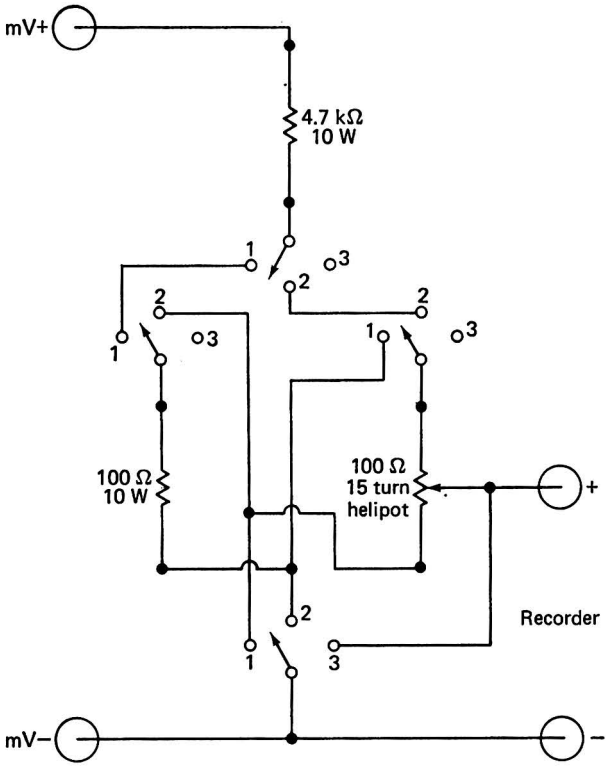

Fig. 7. Circuit diagram for converter unit for interfacing outputs of measuring instruments to recorders. The total resistance should equal the output impedance of the source, here 5 kΩ, for matching a 39A pH meter to a Honeywell 10-mV strip-chart recorder (1·5 kΩ for a 23A). Switch position 1: meter reading of ±1400, 500 or 140 mV gives full-scale deflection on the recorder. Switch position 2: meter displacement of 10 mV gives full-scale deflection on the recorder. Switch position 3: isolates the recorder

WAVEFORM MONITORING—

The presence of a d.c. component in the applied periodic signal, whether as an external or internal d.c. offset, or as any inequality of amplitude, shape or duration of the two half-cycles of the periodic signal, exerts a considerable effect on the results, as will be shown.

It is therefore essential to be able to monitor the waveform, and to produce a perfectly symmetrical and amplitude and time balanced signal, or to introduce a known amount of offset, amplitude or mark to space (time) bias. The 130C oscilloscope is not sufficiently accurate adequately to shape and balance amplitudes and cycle times, but is useful for initial settings.

Venner millisecond crystal clock, TSA 3314—In addition to its use for interval measurement,[36] this instrument has a versatile triggering system and can be used to measure pulse width, pulse interval and relay contact timing. It was used to measure the interval between the leading edges of two consecutive pulses, either positive-going or negative-going, and so to measure full-cycle frequencies from 100 μs to 10 000 s. It was also used to measure the interval between the leading edge of a positive-going pulse and the leading edge of a negative-going pulse, and *vice versa*, thus measuring the duration of individual half-cycles. The trigger level is 500 mV, and gating presented no problems with square waveforms, but the gating rise time is such that sine waves would trigger only at frequencies above 14 Hz at maximum generator output. At lower frequencies the clock would either not trigger at all, or else it registered impossibly small intervals. This effect arose from slight differences in trigger levels and slopes at the two inputs, so that triggering could occur on leading or trailing edges of a single excursion of the waveform when the slope of the trailing edge was low. The calibration of this clock has been described,[36] and the accuracy is about 1 p.p.m. It was used to calibrate both the waveform generator and the time base of the oscilloscope.

The clock could be used for precise frequency measurement, and for accurate balancing of the duration of the two half-cycles for square waves of any frequency and sine waves above 14 Hz, but would not give any help in amplitude balancing or offset detection. The oscilloscope afforded preliminary assistance, but a much more accurate and sensitive balancing, or bias detection, is afforded by integrating the signal over a period of time. Pure periodic waveforms have a zero integral, but any bias will show a positive or negative integral proportional to its magnitude. Earlier work has already been faulted because no precautions were taken to ensure that pure, bias-free, symmetrical waveforms were obtained, and this will be shown to be a crucial factor in periodic wave titrimetry. Electronic integration and d.c. differential electrolytic potentiometry have been used for balancing.

Electronic integration—The simple integrator circuit is shown in Fig. 8, and is of conventional design.[45] The output is re-set to zero by discharging the capacitor, and when the input is open-circuited the output remains constant. The relation with the inverting amplifier is

$$E_{out} = -\frac{1}{RC}\int_0^t E_{in}\,dt \qquad (1)$$

The input is taken directly from the waveform generator output terminals and the integrator output is taken to a 39A pH meter connected to a recorder. The presence of any d.c. offset, amplitude bias or mark-to-space bias in the input signal produced a rising or falling ramp on the pH meter. A cheap utility differential amplifier is used; the input leads are short-circuited and the offset pre-set potentiometer of the amplifier is adjusted to give minimum

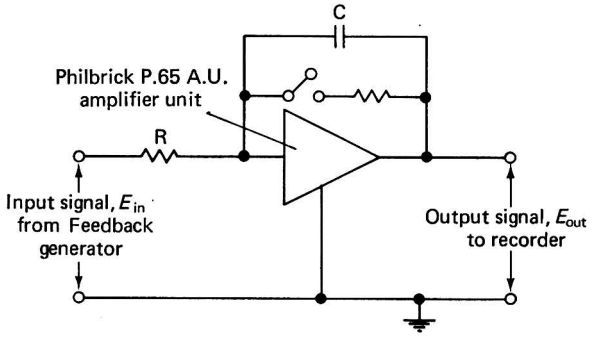

Fig. 8. Simple integrator used for waveform balancing: R = 10 MΩ, C = 10 μF, re-set resistor = 100 kΩ

drift, which was typically less than 5 mV h^{-1}. The presence of a 0·1-V d.c. component in an 80-V peak-to-peak periodic signal produced a rising or falling ramp of slope 500 mV min^{-1}, in good agreement with the 600 mV min^{-1} predicted by use of equation (1), remembering that the capacitor has a ± 10 per cent. tolerance. This therefore provided a precise and accurate method of determining and eliminating any d.c. component in the output of the waveform generator. The limiting factor was the coarseness of the winding of the 50-kΩ offset potentiometer, P9 in Fig. 5 (precision limited to $\pm 0{\cdot}02$ V). Later, this component was advantageously replaced with a miniature 15-turn helipot.

Chemical d.c. differential electrolytic potentiometry—With a reaction of high Q value in which titrant and titrand systems participate in fast electrode processes, the d.c. differential electrolytic potentiometric curve is a sharp peak, with the value of E_Δ falling steeply to zero on both sides of the end-point. Bromate (or bromine) - copper(I) is such a system. When a pair of small platinum electrodes, as closely similar as possible in area, response, surface condition, etc., are immersed in an end-point solution of such a titration system, and fed with the periodic signal, a small d.c. component will produce a large d.c. potential between the two electrodes. Adjustment of the waveform to give zero E_Δ indicates the removal of the d.c. component. It should have been possible to avoid preparation of the solution by titration of copper(I) with bromate by using a stoicheiometric mixture of copper(II) chloride and potassium bromide in acidic solution, but this method was not satisfactory, presumably on account of the presence of impurities. Consequently, 0·1 M copper(I) in 1 M hydrochloric acid was titrated with 0·016 67 M potassium bromate in a fully oxygen-free environment to a d.c. differential electrolytic potentiometric end-point; the zero-current potential of a platinum indicator electrode - S.C.E. reference electrode cell in this solution was perfectly stable at 520 mV.

The sensitivity is indicated by the results in that the presence of 1·0 V of d.c. in an 80-V peak-to-peak periodic signal of r.m.s. current density 20 μA cm^{-2} (d.c. density, 0·25 μA cm^{-2}) produced an E_Δ of 220 mV, while a normal titration at 1 μA cm^{-2} d.c. gives a peak E_Δ of 600 mV and a peak width at half-height of 0·02 ml. E_Δ is logarithmically related to I_Δ, but the peak breadth is directly proportional to I_Δ, and the peak breadth at half-height at 0·25 μA cm^{-2} is only 0·005 ml, so the source of the loss of sensitivity is apparent. Better results were obtained by using more dilute, unstirred solutions.

Relay-driven high source voltage circuit—With d.c. differential electrolytic potentiometry, values of ballast load below 10^{10} V Ω were found to be undesirable. Differentiation can be quenched at lower values, and E_Δ becomes erratic and dependent on the ballast load. In the event, the 80-V output of the Feedback generator proved to be adequate, but a square waveform of up to ± 480 V was examined. This waveform was first produced by using the internal relays of the waveform generator, triggered by a 2-Hz square wave, but the output showed excessive and destructive contact bounce. The circuit illustrated in Fig. 9 was therefore used, with an Electrothermal Flat-Pak Reed Relay, GR831/FO3. The equality of the individual half-cycles was checked on the crystal clock, and the oscilloscope was used to examine contact bounce. The circuit functioned well up to about 4 Hz, although contact bounce was becoming marked at this frequency. The limitation on higher frequencies was that the duration of the half-cycles became unequal, although this inequality was probably due to contact bounce triggering the clock at the wrong point in the half-cycle.

THE WORKING ASSEMBLY—

The basic circuit is that shown in Fig. 4. Because it became apparent that higher periodic current densities were required in periodic than in d.c. differential electrolytic potentiometry, the ballast resistance (and ballast load) could be reduced considerably and finally four decades of (1 to 10) × 10^4, 10^5, 10^6 and 10^7 Ω, built from 1 per cent. 2-W high-stability resistors, were used.

The signal was applied to a pair of indicator electrodes of the appropriate type, properly activated, in the titration solution. A third, zero-current indicator electrode and a reference electrode, S.C.E., S.M.S.E. or glass as appropriate, were included so that the d.c. and periodic potentials generated at each of the electrodes could be followed as well as the differential periodic and d.c. potentials. For comparison purposes, a conventional d.c. differential electrolytic potentiometric assembly of three indicator electrodes and a reference electrode

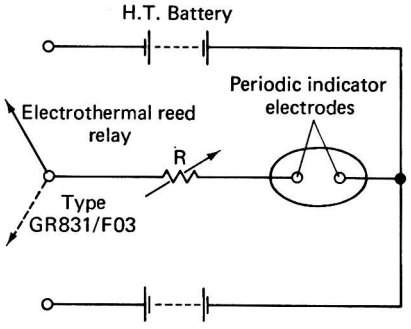

Fig. 9. Relay circuit used to provide a large source voltage for low frequency square wave differential electrolytic potentiometry

was included, but it is not shown in the figure. The principal initial difficulty encountered was with a.c. pick-up and noise, mainly 50-Hz ripple, but extending up to 60 MHz, and this interference sometimes amounted to tens of millivolts, which is more than 50 per cent. of the output signal. Various measures were taken to combat these interferences, as follows. (*a*) All mains supply cables at the back of the bench were enclosed with earthed aluminium sheet. (*b*) Co-axial wiring was used throughout; decade resistance boxes were earthed and carried co-axial sockets. (*c*) One of the pair of differential indicator electrodes was earthed. (*d*) At first, co-axial cable was fitted to the electrodes (instead of plain tinned copper connectors) when they were made so that the braiding extended down inside the glass sheath to the glass - metal seal, but this was inconvenient. Instead, the titration cell was surrounded on all sides, including top and bottom, but excluding the front, by earthed aluminium sheet that acted as a Faraday cage; co-axial cables were taken to the tops of the electrode sheaths and connections made by means of miniature crocodile clips. (*e*) When the d.c. differential electrolytic potentiometric electrodes were included, 50-Hz ripple appeared in the output signal from the periodic differential electrolytic potentiometric electrodes. This ripple was traced to the high-tension batteries that supplied current to the d.c. electrodes, and was removed by placing the batteries in an earthed metal box provided with co-axial sockets. (*f*) Great care was taken to ensure that there were no earth loops that could give rise to induced loop currents and erroneous voltages.

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NOTE—References 1, 2, 4, 6, 7, 39 and 42 are to Parts I, XVII, XVIII, XX, XIX, II and VI, respectively, of this series.

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Differential Electrolytic Potentiometry with Periodic Polarisation

Part XXII.* Symmetrical Periodic Current Differential Electrolytic Potentiometry in Oxidation - Reduction Titrimetry†

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The application of pure, symmetrical, bias-free square, sine and triangular wave periodic polarisation to all types of oxidation - reduction titrations is reported. Electrode configuration and earthing and the destructive effect of bias are examined. Titration curve shapes are the same as those of classical d.c. differential electrolytic potentiometry, but the periodic current densities required are much higher than for d.c. The electrode response speed is greatly accelerated, unpoised potentials are steady, warning is given of the approach of the end-point in type II (*b*) titrations, electrodes retain full activity for very long periods and errors in titrations of iron(II) with dichromate or cerium(IV) are eliminated in the periodic method as against the d.c. method. Discrimination in type II reactions is slightly attenuated in the periodic method, and in titrations at low concentrations it is considerably attenuated. The nature and the conditions of the titration, the speed of the electrode charge-transfer process, the ballast load, the current density, the electrode area, the shape of the applied waveform, the applied frequency, and the deactivation of electrodes, are examined in detail. The benefit of the constant-current mode over the constant-potential mode is demonstrated.

IN d.c. differential electrolytic potentiometry, two types of differential titration curve can occur,[1] depending on the speed of electrode processes concerned. For fast or moderately fast reactions[2] of comparable speed, a symmetrical peak of type I results, the differential potential falling near to zero on either side of the equivalence point. Slowness of the charge-transfer process produces an asymmetrical curve, although the peak remains sharp, and the differential potential fails to reach zero (by the amount of charge-transfer overpotential, η_a) on one or both sides of the peak. When the charge-transfer process becomes very slow for one reactant, type II curves result; if the slowness is associated with the titrant species, a rising-Z curve of type II (*a*) occurs, while if the titrand species have a very slow charge-transfer rate, a falling-Z curve of type II (*b*) is obtained. The same shape of curves for type I reactions was expected for periodic polarisation,[3] and there was little reason to expect any difference in type II reactions. The investigation of polarisation with perfectly symmetrical periodic waveforms of two platinum indicator electrodes in oxidation - reduction titrimetry is reported in this paper, together with an examination of the variables concentration, equilibrium constant, ballast load, current density, and the shape and repetition frequency of the applied waveform. It must be strongly emphasised that the investigation and results appertain only to perfectly symmetrical inputs. Previous work has been reviewed,[4] and has been faulted on this matter of symmetry.

EXPERIMENTAL

The apparatus and instrumentation have been described and evaluated in the previous paper.[4]

GLASSWARE—

Graduated glassware was of N.P.L. A grade, and was calibrated before use.[5] All of the glassware was initially cleaned with a mixture of 25 ml of saturated aqueous AnalaR

* For Part XXI of this series, see p. 697.

† Presented at the Second SAC Conference, Nottingham, 1968.

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grade chromium trioxide and 2·5 litres of AnalaR grade sulphuric acid. By keeping the apparatus continually wet, such cleaning was required only at long intervals; care was taken to remove all traces of adsorbed chromium species by thorough washing. The water used in this work has been defined in the previous paper[4] and titrimetric manipulation has been described earlier.[5]

SOLUTIONS—

AnalaR or Aristar reagents were used without further purification unless otherwise specified, and weighings were made on a Stanton CL3 balance of 10-μg sensitivity.

Potassium bromate solution, 0·016 67 M—This solution in water was prepared by direct weighing of the solid after drying for 3 hours at 160 °C, and it was used as a primary standard.

Hydrazinium sulphate solution, 0·025 M—This solution in water was prepared by direct weighing of the solid as received.

Arsenic(III) solution, 0·05 M—Arsenic(III) oxide was dried at 120 °C for 3 hours and about 9·981 g were weighed accurately and dissolved in 80 ml of a warm, 2·5 M solution of sodium hydroxide in water. The solution was acidified with 80 ml of 2·5 M hydrochloric acid, cooled and made up to 2 litres with water.

Antimony(III) solution, 0·05 M—This solution in water was made by direct weighing of potassium oxotartratoantimonate(III) that had been dried at 110 °C for 2 hours.

Iron(II), 0·1 M, *in* 0·5 M *sulphuric acid*—This was prepared from ammonium iron(II) sulphate hexahydrate by direct weighing and dissolution in water that contained sufficient sulphuric acid to yield the required concentration on dilution to volume with water.

Cerium(IV), 0·1 M, *in* 0·5 M *sulphuric acid*—An amount of 110 g of ammonium hexanitratocerate(IV) was macerated with 56 ml of sulphuric acid, and 100 ml of water were added slowly to the mixture. After heating to remove nitric acid, the solution was further diluted, cooled and finally made up to 2 litres with water.

Manganese(VII) solution, 0·02 M—This solution in water was prepared by direct weighing of potassium permanganate.

Chromium(VI) solution, 0·016 67 M—This solution in water was prepared by direct weighing of potassium dichromate that had been dried at 170 °C for 2 hours, and it was used as a primary standard.

Vanadium(V) solution, 0·1 M—This solution was prepared by dissolution of 23·4 g of ammonium metavanadate(V) in a solution of 12 g of potassium hydroxide in water. The ammonia was boiled out, and the solution cooled and made up to 2 litres with water.

Copper(I) solution, 0·1 M—Volumes of hydrochloric acid and water were fully de-oxygenated. About 2·48 g of copper(I) chloride was accurately weighed and dissolved in 150 ml of 10 M hydrochloric acid under nitrogen. The solution was transferred to a calibrated 250-ml flask, flushed with nitrogen and made up to the mark with water. It was stored, and subsequently handled, under nitrogen.

STANDARDISATION OF SOLUTIONS—

The two primary standards, potassium bromate and potassium dichromate, were used to standardise the other solutions by potentiometric titrimetry. The standard deviation on a 25-ml titration was 0·01 ml.

Antimony(III) and arsenic(III) solutions in a medium of 1·0 M hydrochloric acid, and 0·1 M potassium bromide solution, were all standardised by direct titration with standard potassium bromate solution. Hydrazine solution in a medium of 2·5 M hydrochloric acid and 0·1 M potassium bromide solution was similarly standardised. Arsenic(III) and hydrazine solutions remained stable for long periods, but the antimony(III) solution slowly deteriorated because of atmospheric oxidation. Copper(I) in a medium of de-oxygenated 1 M hydrochloric acid and 0·1 M potassium bromide solution blanketed with nitrogen was standardised by direct titration with potassium bromate solution. The iron(II) solution deteriorated owing to atmospheric oxidation at a rate of about 0·2 per cent. per day. It was standardised by direct titration in 0·5 M sulphuric acid with standard dichromate, or by double excess back-titration with bromate. An aliquot of iron(II) in 2·0 M hydrochloric acid, 0·1 M potassium bromide solution and 0·78 M phosphoric acid was treated with an excess of bromate. After allowing 20 minutes for complete oxidation of the iron(II), an excess and known amount of arsenic(III) solution was added, and the resulting excess of arsenic(III) titrated with bromate

either potentiometrically or with the aid of rosaniline as a visual indicator. Cerium(IV) was standardised against standardised arsenic(III) in 1·0 M sulphuric acid with the aid of osmium(VIII) oxide as a catalyst, so referring it to bromate. It was also, together with manganese(VII) and vanadium(V), titrated against iron(II) in 0·5 M sulphuric acid, so referring these oxidants to dichromate.

GENERAL PROCEDURE—

The titration cell[4] is set up in its Faraday cage. Six activated platinum electrodes and the extension salt bridge of the reference half-cell are fitted in the machined Perspex lid. The selected signal from the Feedback generator is balanced, first by using the gated crystal counter to ensure periodic equality of each half-cycle, and then by integration (most conveniently by the operational amplifier integrator) to ensure the absence of any d.c. component. The d.c. differential electrolytic potentiometric assembly follows normal practice. An aliquot of the standardised titrand (usually the reductant) is transferred by pipette into the cell together with any other necessary reagents and water in such amount that, allowing for washings, the volume at the end-point will be 200 ml. The titrand is then de-oxygenated if required; alternatively, the water and all the reagents are de-oxygenated before use and the cell kept under continuous nitrogen purge. For ordinary titrations, the titrant, de-oxygenated if necessary, is delivered from a burette, through the hole provided, up to within 2 ml of the expected end-point after which incremental addition is used, the size of the increment decreasing so as to traverse the end-point in 0·005-ml increments, and thereafter increasing. After each increment the various periodic and d.c. potentials are monitored until the drift becomes less than 1 mV min^{-1}, and the relaxation times for the three methods (zero-current potentiometry and d.c. and periodic differential electrolytic potentiometry) noted. The noise level in the circuit before applying the signal was about 0·2 mV r.m.s., as measured on the a.c. millivoltmeter, and it was halved by switching off the magnetic stirrer. The pipette dilution method[5] was used more frequently than the ordinary titration method in order to study minutely the form of the titration curve in the end-point region, and to compare the discrimination of the various methods. In this method, a pipette is used to deliver titrant into the cell in order to bring the titration to within 0·5 or 0·05 ml of the expected end-point, and the titration is completed with a 10 or 100-fold dilution of the original titrant solution.

PRELIMINARY WORK—

Electrode arrangement—Franck[6] used a three-electrode arrangement as in Fig. 1 (*a*), electrode C being a spinning ring. Other arrangements have been used, such as in Fig. 1 (*b*) and (*c*).[7] In Laitinen and Hall's work,[7] configuration 1 (*c*) is transposed to $E_{applied}$ and I_{out}. In the present work, stationary electrodes in vigorously stirred solutions were used, and

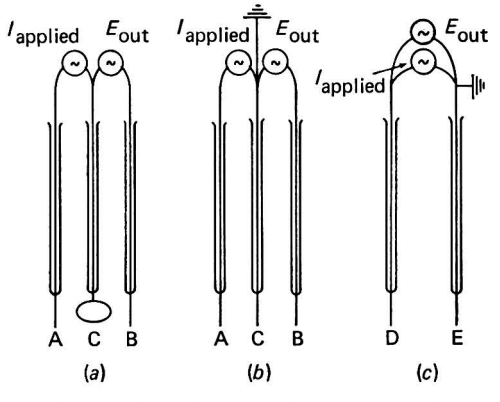

Fig. 1. Periodic electrode arrangements: (*a*), Franck's arrangement[6]; (*b*), three-electrode arrangement in stirred solutions; and (*c*), two-electrode arrangement used in this work. Input and output are reversed by Laitinen and Hall[7]

two and three-electrode modes [Fig. 1 (*c*) and (*b*)] were tested, by immersion of activated electrodes in 200 ml of a solution containing 25 ml of 5 M sulphuric acid, 25 ml of iron(II) and sufficient cerium(IV) to adjust the zero-current electrode potential to 690 mV *versus* S.C.E.; this is within 0·002 ml of cerium(IV) of the equivalence point. A symmetrical periodic signal of various waveforms and frequencies was applied to the electrodes, and the potentials were read as peak-to-peak values on the oscilloscope, with the results shown in Table I.

TABLE I

EXAMINATION OF ELECTRODE CONFIGURATIONS SHOWN IN FIG. 1

Applied signal 80 V peak-to-peak, ballast resistance 2 MΩ

	Potential/mV, measured between			
Frequency/Hz	AC	AB	CB	DE
Sine wave—				
10	112	57	55	115
5	200	104	96	206
3	300	156	144	308
Square wave—				
10	186	96	90	189
5	320	165	155	327
3	450	233	217	460

The output potential measured between B and C (or B and A) (Fig. 1) was offset and distorted about the zero axis of the oscilloscope in all instances. Franck[6] must have been observing a distorted signal, offset from zero reference, except for reactions so fast that η_a is negligible and all the mass-transfer rates are equal.

The contrast between the two modes, constant applied voltage and constant applied current, is important. In the constant applied current method, the output is the sum of the mass-transfer concentration term, η_c, and the charge-transfer overpotential, η_a, and for very fast reactions is η_c alone. In the constant applied potential method,[7] the potential is distributed between mass transfer, η_c, and charge transfer, η_a, and so the maximum output is obtained for very fast reactions, but for slow reactions the output will decrease as η_a increases. The advantage clearly lies with the constant applied current method.

The potential between A and C (or D and E) is the sum of the potentials AB and CB, and, apart from the benefit of doubling the amplitude of the signal, as previously adumbrated,[8] this mode of measurement eliminated offset and distortion and gave a balanced, symmetrical output. The slight differences between AB and CB could arise from many factors, such as slight differences in electrode area or activity, in mass and charge transfer rate parameters, or in electrode position. Such measurements in configuration 1 (*b*) could therefore be used as a test of functional identity of a pair of platinum electrodes of the same physical dimensions. Configuration 1 (*c*), which is the same as in d.c. differential electrolytic potentiometry, was chosen. Removal of the earth connections in 1 (*b*) or 1 (*c*) did not affect the magnitude of the potentials measured, but resulted in a large increase in noise, mainly 50-Hz pickup, present in the output signal.

Signal source—Initial work was performed with the Heathkit AG-9U generator,[4] and the various sources of interference and noise were eliminated as described.[4] The first titrations of iron(II) with cerium(IV) gave disappointing results; the curves were not sharp, the potentials were very low, the end-points did not coincide with the zero-current potentiometric end-points and the platinum surfaces quickly became dull and deactivated. A typical curve is shown in Fig. 2. These effects were traced to the presence of a d.c. component (about 15 per cent.) in the signal generator output, and emphasise the insistence on the use of pure, symmetrical, bias-free signals. An attempt to back off the d.c. component with a potentiometer comprising a Mallory cell and a 1-MΩ radio-potentiometer failed because of signal drift. Insertion of a 1-μF polystyrene capacitor in series with the circuit mitigated the effect, but did not eliminate it, as integration of the resulting signal showed. The Feedback TWG 300 generator was therefore used in the remainder of the work.

TITRATIONS AT 0·1 M OR EQUIVALENT CONCENTRATIONS—

The precision, accuracy and discrimination of periodic differential electrolytic potentiometry were examined and compared with those of classical d.c. differential electrolytic

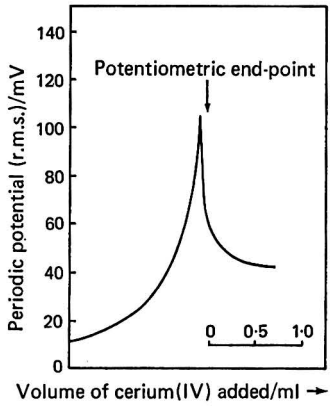

Fig. 2. Titration of iron(II) with cerium(IV) by using the AG-9U signal generator: sine wave, 3 Hz; current density, 20 μA cm^{-2} r.m.s.; 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid titrated with 0·1 M cerium(IV) solution

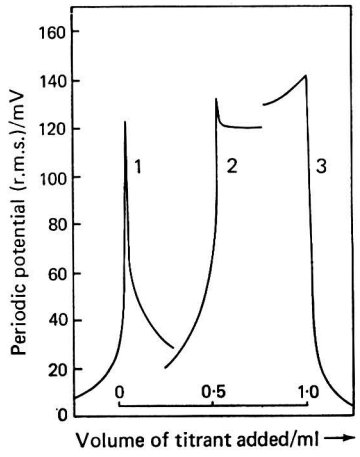

Fig. 3. Expanded end-point regions of titration curves of the three types, produced at 3 Hz sine wave: 1, 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid, titrated with 0·1 M cerium(IV), r.m.s. current density 20 μA cm^{-2}; 2, 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid, titrated with 0·016 67 M chromium(VI), r.m.s. current density 25 μA cm^{-2}; and 3, 200 ml of 0·003 M hydrazine in 1·0 M hydrochloric acid - 0·1 M potassium bromide, titrated with 0·016 67 M potassium bromate, r.m.s. current density 10 μA cm^{-2}

potentiometry and zero-current potentiometry. The titration curve shapes fall into the same groups, I, II (*a*) and II (*b*), as before. The results, which are representative of a large number of titrations involving many different reactions, are summarised in Table II; the mean figures were obtained under nearly optimum electrical conditions. The standard deviations of all results recorded are 0·01 ml or less. One titration curve of each type is given in Fig. 3. The discrimination (precision of location of the end-point) is about the same for a.c. and d.c. differential electrolytic potentiometry in type I titrations, where slopes in excess of 20000 mV ml^{-1} are obtained, but a.c. discrimination is slightly less than d.c. discrimination for type II curves.

Response or relaxation times for the three methods varied with the system being studied, the proximity of the end-point and the particular potential being measured. The relaxation time of a.c. differential electrolytic potentiometry was found to be very much less than that of d.c. differential electrolytic potentiometry or zero-current potentiometry after a perturbation by addition of an increment of titrant, and potential drift was much less than for d.c. differential electrolytic potentiometry. Moreover, in type II (*b*) titrations, zero-current and d.c. differential electrolytic potentiometric potentials drift haphazardly before the equivalence point, because there is no reaction of adequate exchange current to stabilise the cathode or zero-current electrode. This undesirable behaviour is eliminated by a.c. differential electrolytic potentiometry with which the periodic potentials stabilise within a few seconds and remain steady, then, just before the equivalence point, a small increase occurs, warning of the approach of the end-point, and finally the usual abrupt decrease follows. Presumably, the periodic current stabilises around the oxygen reduction process in the cathodic half-cycle; the anodic half-cycle is stabilised by, for example, oxidation of bromide in the titration medium.

Further interesting phenomena were observed. In the titration of iron(II) with cerium(IV) or dichromate, there was a marked discrepancy between the various end-points of the order of 0·01 to 0·02 ml, which is easily examined by use of the pipette dilution method. This

positive error had been detected at a very early stage,[1] but at that time it was ascribed to experimental error, and the fact that the errors were always of the same sign had been overlooked. In these titrations, the a.c. differential electrolytic potentiometric end-point was error-free and agreed with the zero-current inflection point, whereas the positive error in the d.c. differential electrolytic potentiometric results was confirmed. The a.c. (the same results were obtained with other waveforms also) and d.c. differential electrolytic potentiometric electrode processes must therefore differ, and many explanations have been canvassed. Theory predicts that the only explicit factors that can shift the end-point away from the equivalence point are differences in current density (*i.e.*, electrode area) and stirring speed at the two electrodes; other factors give rise to asymmetrical curves, but do not displace the end-point. The care taken in matching the electrodes and positioning them in the titration vessel invalidates such an explanation. Moreover, changing the function of the various electrodes from a.c. to d.c. to zero current produced the same phenomenon, independently of the position of the electrode.

TABLE II

TITRATION RESULTS

	Current density/μA cm^{-2}		Consumption of titrant/ml, at—		
Reaction*	r.m.s. a.c.	d.c.	periodic peak	d.c. D.E.P. peak	potentiometric inflection point
Type I—					
(*i*)	20	0·5	19·47	19·48	19·47
(*ii*)	20	0·5	23·88	23·88	23·88
(*iii*)	25	0·5	24·21	24·21	24·21
Type II (a)—					
(*iv*)	25	0·5	24·73	24·73	24·71
(*v*)	25	0·5	19·91	19·92$_5$	19·91
Type II (b)—					
(*vi*)	25	0·5	24·93	24·93	24·93
(*vii*)	25	0·5	24·61	24·61	24·61
(*viii*)	25	0·5	24·78	24·78	24·78

* REACTION—

Type I—

(*i*) 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid titrated with 0·1 M cerium(IV).

(*ii*) 200 ml of 0·0125 M copper(I) in 3·5 M oxygen-free hydrochloric acid, titrated under nitrogen with 0·016 67 M bromate.

(*iii*) 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid, titrated with 0·02 M manganese(VII).

Type II (a)—

(*iv*) 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid, titrated with 0·1 M vanadium(V).

(*v*) 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid, titrated with 0·016 67 M chromium(VI).

Type II (b)—

(*vi*) 200 ml of 0·003 125 M hydrazine in 1·0 M hydrochloric acid - 0·1 M bromide, titrated with 0·016 67 M bromate.

(*vii*) 200 ml of 0·006 25 M arsenic(III) in 1·0 M hydrochloric acid - 0·1 M bromide, titrated with 0·016 67 M bromate.

(*viii*) 200 ml of 0·006 25 M antimony(III) in 1·0 M hydrochloric acid - 0·1 M bromide, titrated with 0·016 67 M bromate.

Standard deviation of all titrations = 0·01 ml.

Theory also predicts that the intervention of a different, extraneous, reaction, such as electrolysis of the solvent or its ions, could result in the d.c. differential electrolytic potentiometric end-point being early if the anodic process is affected, or late if the cathodic reaction changes; however, this effect should act upon the a.c. electrodes in a similar manner. Ross and Shain[9] observed hysteresis in zero-current potentiometry of these two reactions in the forward and reverse directions, amounting to 0·1 ml of 0·1 M iron(II) solution, and attributed this behaviour to oxidation of the platinum surface, but, without denying that oxide is formed *after* the end-point with oxidant titrant, and reduced *after* the end-point with reductant titrant, their argument is based on the converse of these facts and is not valid. In d.c. differential electrolytic potentiometry, with an oxidant titrant, a layer of oxide can form

on the anode before the equivalence point, which could cause a potential halt, so displacing the anode curve in such a manner as to produce a late differential electrolytic potentiometric end-point. Theory indicates that an anomalous effect occurs, because if the anode reaction changed from oxidation of iron(II) to oxidation of water, the differential potential would be inverted. This effect could take place, but has not been observed with these particular reactions. In order to simulate the experimental curves, it is necessary at *this* stage to change the charge-transfer rate constant for the oxidation of water by fourteen orders of magnitude. In the titration of iron(II) with cerium(IV) the d.c. differential electrolytic potentiometric electrodes become deactivated after repeated use (*cf.*, Fig. 9, below), and the error of +0·005 ml with fresh electrodes increases with repeated use to as much as +0·05 ml.

To the above examples must be added that of the iron(II) - vanadium(V) reaction. In this instance, both a.c. and d.c. differential electrolytic potentiometric end-points are late in comparison with the zero-current inflection point, and the a.c. and d.c. curves have different shapes, as shown in Fig. 4. The Q of this reaction is rather low for the precision demanded, and would be improved by depressing the conditional potential of the reductant and increasing the conditional potential for the oxidant by increasing the sulphuric acid concentration to 5·0 M.

The three reactions that produce anomalies involve oxidant species with unusual electrochemical behaviour that has been the subject of other investigations in these laboratories. Cerium(III) has been oxidised to cerium(IV) in perchloric acid, in which the conditional potential is 1·65 V, giving a distinct voltammetric wave indicative of a fairly fast reaction and well separated from the oxidation wave of water. Pattern theory[10] analysis of the latter showed that k_0 had been decreased to about 10^{-23}. The oxide layer on platinum, in the presence of cerium(IV), seems to have unique charge-transfer properties, supporting the late end-point generation by the anode, which must go through a transition state close to equivalence. Chromium(VI) and chromium(III) have been shown to be very strongly and specifically adsorbed on platinum,[11] and to interfere greatly with charge-transfer processes. Vanadium(V) or vanadium(IV) species, or both, have also been shown to enter into disturbing adsorption processes.[11,12] The other species encountered in the reactions in Table II show no such behaviour, and although bromide is specifically adsorbed on platinum it does not interfere with the charge-transfer processes. It is not surprising that deactivation should be cumulative with d.c. differential electrolytic potentiometry, but prevented by the short relaxation time of the periodic method. The very low exchange current of the vanadium(V) - vanadium(IV) system,[12] and the extremely slow equilibration of the zero-current potential at gold or platinum electrodes,[12] suggest that the zero-current potentiometric inflection could be expected to be early, so that the d.c. and a.c. methods do give the correct result.

Titrations at lower concentrations—

Titrations of all the reactions in Table II were performed at lower concentrations with satisfactory results, but it will suffice to illustrate the point with the titration of iron(II) with cerium(IV). Curves obtained under optimum conditions with 10^{-2} and 10^{-3} M titrant are shown in Fig. 5, from which it is apparent that there is a marked deterioration compared with curve 1 in Fig. 3. There is much less deterioration with d.c. differential electrolytic potentiometry, but the potential equilibration time is considerably lengthened, whereas the permitted relaxation time in the periodic method remains constant at a given frequency. A marked decrease in the charge-transfer rate is evident in Fig. 5, and this, added to the abbreviated relaxation time for a reaction further slowed down by the decrease in reactant concentration, adequately explains the deterioration. Many titrations were performed under widely varying electrical conditions, but none was productive of results comparable with those from d.c. differential electrolytic potentiometry. Both Franck[13] and Stock[14] state that periodic titrimetry permits location of the equivalence point in dilute solution, but, while this statement is true, it must be concluded that the technique is markedly inferior to d.c. differential electrolytic potentiometry in dilute solutions.

Ballast load—

Ballast load is the product of the source voltage and the ballast resistance, and a high value is required to stabilise the differentiating current.[1] Values below 10^9 VΩ lead to unstable and erratic potentials, and the value has been found to affect the magnitude of

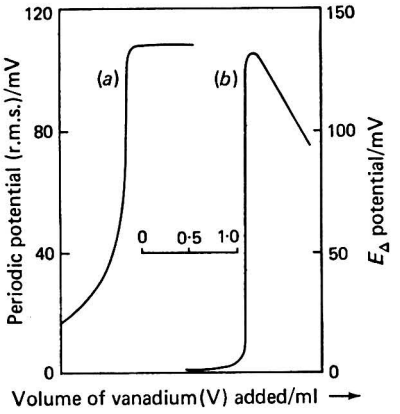

Fig. 4. Variation in curve form in vanadium(V) titrations. Two hundred millilitres of 0·0125 M iron(II) in 0·5 M sulphuric acid titrated with 0·1 M vanadium(V): (*a*), sine wave, 3 Hz, 25 μA cm^{-2} r.m.s. current density; and (*b*), d.c. 0·5 μA cm^{-2} current density

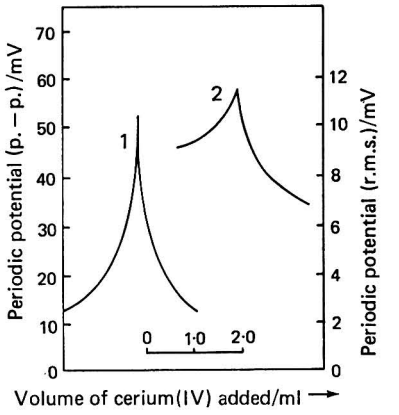

Fig. 5. Titrations at lower concentrations: 1, 200 ml of 1·25 × 10^{-3} M iron(II) in 0·5 M sulphuric acid, titrated with 10^{-2} M cerium(IV), sine wave, 3 Hz, 5 μA cm^{-2} r.m.s. current density; and 2, 200 ml of 1·25 × 10^{-4} M iron(II) in 0·5 M sulphuric acid, titrated with 10^{-3} M cerium(IV), sine wave, 3 Hz, 2 μA cm^{-2} r.m.s. current density

$E_{\Delta max.}$.[15] The rationale of this form of stabilisation has been examined,[16] and the technique applied was simple and was found to be necessary in the absence of constant-current sources of very low stable outputs. The peak periodic potential was therefore determined under fixed electrode conditions, *viz.*, on the copper(I) - bromate system within 0·005 ml of the end-point at a zero-current potential of 510 mV, by using an applied square-wave signal at 5 Hz and of peak-to-peak voltage from $\pm$1 to $\pm$240 V with first the Feedback generator as the source, then changing to the external reed-relay - battery source.[4] Frequencies above 5 Hz could not be examined because contact bounce in the relay produced unequal half-cycles. The results shown in Table III indicate that no significant difference existed over the ballast load range of 10^{10} to 10^6 V Ω. Although small compared with the values used in d.c. differential electrolytic potentiometry, erratic and drifting signals, symptomatic of inadequate stabilisation, did not occur so that the Feedback generator, with its limited output of $\pm$40 V, is adequate, possibly because the periodic current densities are much higher than the d.c. current densities.

TABLE III

EFFECT OF BALLAST LOAD ON PERIODIC POTENTIALS

Ballast load/V Ω	Source voltage/V	Ballast resistance/Ω	Peak potential/mV
1·4 × 10^{10}	$\pm$240	3 × 10^7	290
3·6 × 10^9	$\pm$120	1·5 × 10^7	285
4 × 10^8	$\pm$40	5 × 10^6	284
10^8	$\pm$20	2·5 × 10^6	286
1·6 × 10^7	$\pm$8	10^6	288
4 × 10^6	$\pm$4	5 × 10^5	286
10^6	$\pm$2	2·5 × 10^5	288

CURRENT DENSITY—

The effect of varying the current density in order to determine the optimum value (defined as that current at which the breadth of the peak at half-height is twice the smallest volume increment that it is desired to distinguish in locating the end-point) was investigated under identical conditions of applied voltage, frequency and waveform. A family of curves on an expanded volume scale is illustrated in Fig. 6. It is evident from the curve shapes that charge-transfer overpotential is present, and that, as with d.c. differential electrolytic

potentiometry, the peak height increases logarithmically with increasing current density while the peak width at half-height increases linearly with increasing current density. However, there is a strong contrast in the current densities required for periodic and d.c. differential electrolytic potentiometry. The current densities recorded in Table II approximate to the optimum values for the two methods, but the periodic current densities are the larger by as much as 50-fold r.m.s. or 18-fold peak-to-peak. Moreover, the periodic peak height is much lower than the d.c. peak height; a decrease of 80 per cent. is not uncommon. These differences arise from first, the charging of the double layer capacitance each half-cycle, secondly, clipping and loss of signal when the ratio of electrode relaxation time to repetition time becomes significant, as it will even for fast reactions near the equivalence point where concentrations are low, and thirdly, the fact that the lower the conditional charge-transfer rate constant, k, becomes, the longer the relaxation time becomes in relation to frequency and the more current is used in double layer charging owing to the mounting value of η_a, again leading to clipping and attenuation of signal.

Variation of the electrode area at constant current density has little or no effect in either periodic or d.c. differential electrolytic potentiometry, unless the electrode area becomes so small that a change, usually a decrease, in the electrode slope factor occurs. The extremely small electrodes used by Morisaka and Harada[4] were chosen on the false premise that current and not current density was the relevant factor.

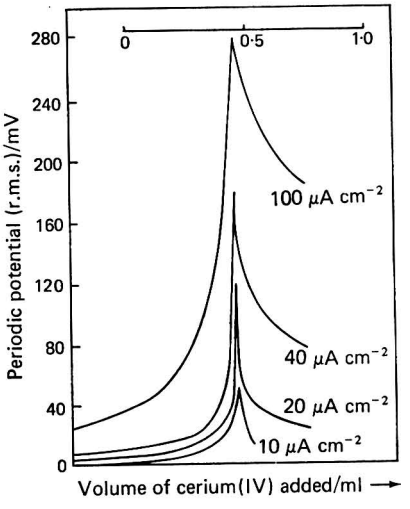

Fig. 6. Variation of applied current density, expanded end-point region. Titration of 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid with 0·1 M cerium(IV); sine wave, 3 Hz, r.m.s. current densities of 10, 20, 40, and 100 μA cm^{-2}

VARIATION OF APPLIED WAVEFORM—

Variation of the applied waveform from square, through sine, to triangular was investigated. A square waveform allows the electrode the longest relaxation time to approach its equilibrium potential in each half-cycle. For a given peak-to-peak signal a square wave has a larger effective amplitude; the r.m.s. values are $\frac{1}{2}$ peak-to-peak for a square wave, $1/(2\sqrt{2})$ peak-to-peak for a sine wave and $\frac{1}{4}$ peak-to-peak for a triangular wave. In terms of coulombs passed at the same peak-to-peak amplitude, the square waveform produces 41 per cent. more charge than the sine and 100 per cent. more than triangular waveforms. It is probable that only in the instance of fast reactions in which more electrolysis can occur, will the square waveform offer material advantage.

Titrations of iron(II) with cerium(IV) and of copper(I) with bromate, under the conditions given in Table II, were conducted with applied square, sine and triangular wave-

forms under otherwise identical conditions. No detectable difference was found in the discrimination or accuracy of the various titration curves; with the fast electrode processes of the copper(I) titration an increase in the peak potential of about 50 per cent. was observed for the square waveform over the sine waveform over the frequency range 50 to 3 Hz, as shown in Table IV, but the difference was much smaller with the slower electrode processes of the iron(II) titration [*cf.*, Fig. 8 (*a*)]. Laitinen and Hall[7] assert that the relatively large double-layer capacitance charging current limits the sine wave response, but not the square wave response. After using the fast electrode process iodine - iodide, they concluded that as the frequency increases, the charging current occupies a greater fraction of the square

TABLE IV

RESPONSE FOR FAST ELECTRODE PROCESSES; TITRATION OF COPPER(I) WITH BROMATE

Current density 10 μA cm^{-2} r.m.s.

	Applied waveform frequency/Hz						
Peak height/mV peak-to-peak	100	50	20	10	5	3	1
Square wave	22	42	97	176	285	400	600
Sine wave	14	27	62	112	186	265	510

wave, and the output corresponds increasingly to capacitance charging, until at 1000 Hz the response is purely capacitance. However, their method is amperometric, in which the concentration of an electroactive species is linearly related to the current resulting from its reaction at the electrode. Consequently, the output current reflects the charging current as well as the electrolysis current and the method will rapidly lose sensitivity at low frequencies, apart from the loss occasioned by η_a. This complication does not arise with a constant-current bipotentiometric method, and a marked difference between sine wave and square wave signals would be expected only with fast electrode processes, such as that in Table IV. For a square wave input, a perfect square waveform output would be obtained only if the electrode processes were infinitely fast, the double-layer capacitance were infinitesimal and the titration curve were a straight line. Roll-off on the leading edges therefore occurs, related to the electrode process relaxation time, the amount of roll-off increasing as the applied frequency increases and as the electrode response time increases. This last condition occurs when concentrations are low in the equivalence point region and when the charge-transfer rate is slow. The output waveform changes during the course of a titration,[4] and even with so fast a system as copper(I) - bromate, an output signal that is almost perfectly triangular in shape is obtained in the end-point region, the electrode system thus behaving as an almost perfect integrator.

VARIATION IN APPLIED FREQUENCY—

The effect of varying the applied frequency under otherwise identical conditions is shown in the family of expanded end-point region curves in Fig. 7. The peak height decreases as the frequency increases, as is to be expected because the time allowed for the electrodes to reach the equilibrium potential decreases with increasing frequency. The lower the requency, the longer is the relaxation time and the closer will be the approach to the equilibrium potential corresponding to the limiting condition of d.c. differential electrolytic potentiometry. Graphs of titration peak height against (applied frequency)$^{-0.5}$ are shown in Fig. 8 (*a*) for a fast electrode reaction system, and in Fig. 8 (*b*) for a slower ($k \approx 10^{-6}$ l cm^{-2} s^{-1}, $\alpha \approx 0.5$) but not very slow, electrode reaction system.

For the fast electrode processes [Fig. 8 (*a*)] there is a considerable frequency range (50 to 2 Hz) over which the peak periodic titration potential is proportional to the (applied frequency)$^{-0.5}$, the inverse square root of frequency law, for both square and sine waveforms. Over this frequency range, the square waveform has the advantage of an increase of about 50 per cent. in the titration peak potential over the sine waveform. The maximum peak periodic potential of 650 mV peak-to-peak is attained at 0·5 Hz for square, and 0·1 Hz for sine, waveforms; this is indicative of the high charge-transfer rates of this system. In comparison, the d.c. differential electrolytic potentiometric peak height (which obeys the law $E_\Delta = a + b \log I_\Delta$) is 600 mV at a current density of 1 μA cm^{-2}.

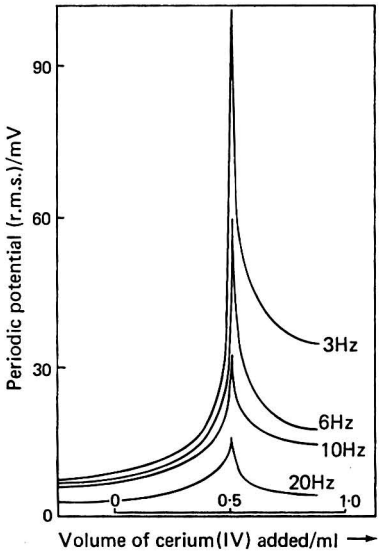

Fig. 7. Variation of applied frequency, expanded end-point region. Titration of 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid with 0·1 M cerium(IV); sine wave, r.m.s. current density 20 μA cm^{-2}; frequency 3, 6, 10 and 20 Hz

For the slower, but still reasonably fast, electrode processes [Fig. 8 (*b*)] the rectilinear part of the graph embraces a smaller frequency range, and in this range, the square waveform shows, at most, a 20 per cent. advantage over the sine waveform, as theory predicts

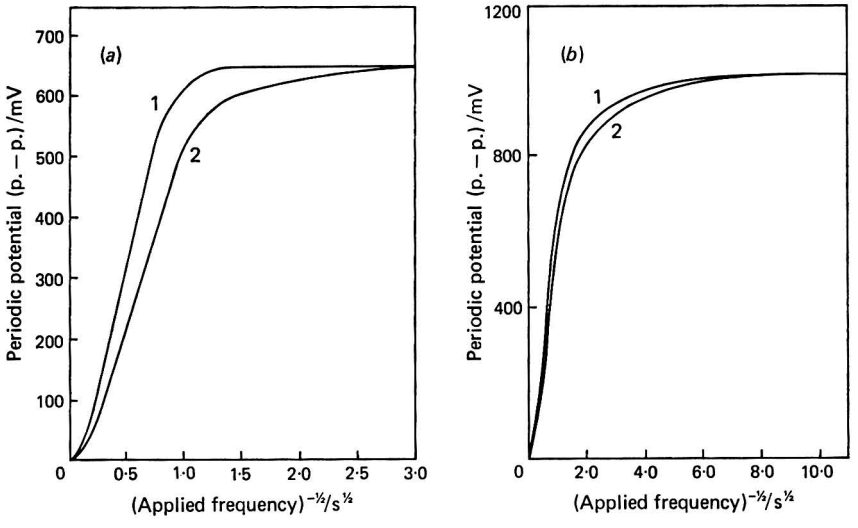

Fig. 8. (*a*) Frequency - peak height relationship for fast reactions. Titration of 200 ml of oxygen-free 0·0125 M copper(I) in 3·5 M hydrochloric acid with 0·016 67 M potassium bromate: (1), square wave; and (2), sine wave, r.m.s. current density 10 μA cm^{-2}. (*b*) Frequency - peak height relationship for moderately fast reactions. Titration of 200 ml of 0·0125 M iron(II) in 0·5 M sulphuric acid with 0·1 M cerium(IV): 1, square wave; and 2, sine wave, r.m.s. current density 10 μA cm^{-2}

for slower reactions. The maximum peak periodic potential of about 1000 mV is considerably greater than the 400 mV attained by d.c. differential electrolytic potentiometry at a current density of 1·0 μA cm^{-2}, and derives from potential enhancement by the addition of charge-transfer overpotential, as can be seen in Fig. 7. At low frequencies, below about 1 Hz, charge-transfer overpotential becomes progressively more marked. At the current densities used, 20 μA cm^{-2} r.m.s., the electrode surface becomes more deactivated during the anodic half-cycle, by adsorption and oxide formation, than reactivated during the cathodic half-cycle, and so η_a is enhanced.

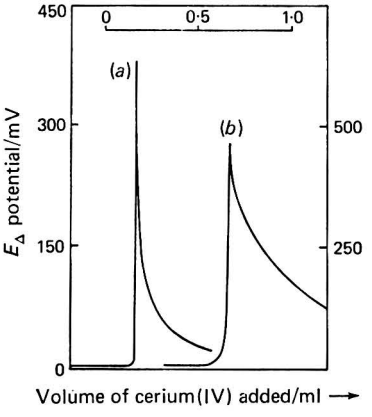

Fig. 9. Effect of electrode deactivation in d.c. differential electrolytic potentiometry. Two hundred millilitres of 0·0125 M iron(II) in 0·5 M sulphuric acid titrated with 0·1 M cerium(IV), d.c. density 0·5 μA cm^{-2}: curve (*a*), (left-hand scale), freshly activated electrode[4]; and curve (*b*), (right-hand scale), fourth titration in a series without intervening reactivation

ELECTRODE ACTIVATION AND DEACTIVATION—

In d.c. differential electrolytic potentiometry, deactivation of the electrodes does not necessarily result in a decrease in response, which would cause a reduction in the height and sharpness of the titration peak. Deactivation can, as predicted by theory, cause an increase in peak height and sharpness with a change in the charge-transfer rate parameters. However, the slower the charge-transfer process becomes, the longer the electrode response time will be, and, while this merely exacts a penalty of increased tedium in d.c. differential electrolytic potentiometry, it assumes major significance in periodic current titrimetry because of the small and finite time allowed in each half-cycle for the electrode to respond. The period of time for which electrodes could be used without reactivation was investigated by carrying out series of repetitive d.c. and periodic differential electrolytic potentiometric titrations without intervening reactivation of the electrodes. The titration of iron(II) with cerium(IV) was chosen because electrodes are fairly, but not very quickly deactivated by the formation of a unique anodic film. Exposure to cerium(IV) will oxidise the electrodes, but immersion in the iron(II) solution of the following titration should reduce this film and reactivate the electrode although the reactivation is much less efficient than the stripping and electro-reduction used in this work.[4] The two expanded end-point region d.c. differential electrolytic potentiometric curves shown in Fig. 9 are (*a*), the first, and (*b*), the fourth in a series of titrations without intervening electrode treatment. Charge-transfer overpotential is evident after the end-point in the first curve but is clearly very much enhanced in curve (*b*); the difference is exactly in accord with theory for a decrease in k and α for both reactions. Indeed, values of the over-all conditional charge-transfer rate constants and charge-transfer coefficients can be calculated from such curves. When η_a is greater than 100 mV, as it is in Fig. 9 (*b*), the parameters for cerium and iron reactions can be calculated by pattern theory,[2,10] taking

the co-ordinates of four points because two systems are involved. Computer curve fitting at lower charge-transfer overpotentials is equally suitable.

Electrodes used for periodic differential electrolytic potentiometry, however, retained their activity for a large number of titrations, as could be judged by the exact reproducibility of the titration curves on an expanded volume scale, provided that they were stored under water when not in use, were not exposed to cerium(IV) solutions for too long, and were not subjected to an applied signal containing any d.c. bias component. It is evident from their titration curves for the same reaction that Laitinen and Hall[7] were using severely deactivated electrodes; indeed, the titration curves corresponded to type II (*a*)[1] for a totally inactive oxidant and they probably used an asymmetrical or offset waveform. Comparison of Figs. 3 and 9 shows that in this titration d.c. differential electrolytic potentiometric electrodes suffer considerable fouling, with deterioration of the titration curve, whereas the periodic differential electrolytic potentiometric curves remain reproducible. Similar behaviour was observed with some other titrations, but not with any of those involving bromide or iodide, in which specific adsorption of halide ions keeps the electrodes clean.

Conclusions

The application of pure, symmetrical, bias-free periodic waveforms to all types of oxidation - reduction titrations has been examined, and the titration conditions and electrical parameters have been optimised. The titration curves are of the same shape as with d.c. differential electrolytic potentiometry, with the possible exception of the vanadium(V) titration of iron(II). The precision and discrimination of periodic current titrimetry at customary concentrations are comparable with those of d.c. differential electrolytic potentiometry for type I reactions, and slightly inferior for type II reactions. The periodic mode has the advantage over the d.c. mode in giving error-free end-points in cerium(IV) and chromium(VI) titrations, where the d.c. method gives slight but definite positive errors.

However, the periodic method has several advantages. Electrode response speed is greatly accelerated, and electrode fouling and deactivation are very substantially minimised. The latter factor is of importance in the context of industrial process control. Further, with the falling-Z type II (*b*) titrations the potentials stabilised in seconds rather than hours in the unpoised condition before the end-point, and gave warning of the approach of the end-point by a small rise. With the d.c. method the pre-end-point potentials are meaningless and little or no notice of the end-point is given.

At lower concentrations, however, the discrimination deteriorated markedly below that of the d.c. method. This result is in accord with theoretical prediction; the decrease in exchange current density limits the electrode response speed, so that the periodic signal deteriorates because of the limited relaxation time, whereas in the d.c. method a longer time can be allowed for relaxation after a concentration perturbation and the achievement of good results merely requires more patience.

The use of pure, symmetrical, bias-free waveforms has been found to be of great importance in achieving good results, a necessity that previous workers have overlooked.

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Note—References 1, 4, 8 and 15 are to Parts II, XXI, XVIII and III, respectively, of this series.

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The Detection of Light Elements by X-ray Emission Spectroscopy with Use of Low-energy Satellite Peaks

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A method for the indirect detection of light elements, L (L = C, N, O or F), by using X-ray emission spectroscopy is described. The technique relies upon the formation of certain low-energy satellite peaks to those X-ray emission peaks which originate from electronic transitions involving the valence shell of an element, A, when A–L bonds are made. The energy difference between the main peak and the satellite peak is characteristic of the ligand (F, 20 ± 1 eV; O, 14 ± 2 eV; N, 9 ± 2 eV; and C, about 5 eV). Applications to compounds that contain more than one type of ligand are described and experimental limitations are discussed.

X-RAY emission spectroscopy is widely used for both qualitative and quantitative analyses for the elements present in a wide variety of minerals, glasses, ceramics and other materials.[1–5] Characteristic X-ray emission can be brought about either by irradiation with electrons (*e.g.*, microprobe and betaprobe) or with X-rays (X-ray fluorescence), but in either instance it is found that the efficiency of X-ray emission diminishes with decrease in atomic number.

Coupled with the inherent difficulties in the production of characteristic X-ray emission from light elements there are concomitant difficulties in detection. The wavelengths of the Kα lines of the light elements are boron 6·76 nm, carbon 4·47 nm, nitrogen 3·16 nm, oxygen 2·36 nm and fluorine 1·83 nm. Only the last two elements can be detected easily by the use of acid phthalate crystals with 2d spacings of about 2·6 nm. Other crystals and "soap films" have been developed[6] with larger 2d spacings but these are not in universal use. A second difficulty in detecting radiations from light elements is again associated with their low energies. Not only will the sample itself readily absorb them but so also will any gas present in the spectrometer. This difficulty can be overcome by carrying out the operation in a vacuum. Even so, it remains true to say that the light elements cannot readily be detected by X-ray emission spectroscopy. The purpose of this paper is to demonstrate that the presence of such light elements in a sample can, however, easily be established by a detailed investigation of specific low-energy satellite peaks associated with characteristic X-ray emissions of other elements in the sample.

THEORETICAL

When atoms form chemical bonds, atomic orbitals are perturbed, especially those of the valence shell.[7–9] The nature and extent of these perturbations will depend upon the energies and spatial characteristics of the orbitals themselves. As a result of this orbital interaction, molecular orbitals (ψ) are formed, which can be described, to a first approximation, as a linear combination of atomic orbitals (ϕ)—

$$\psi_l = \Sigma a_{rl} \phi_r \qquad (1)$$

The coefficients a_{rl} describe the contribution of the atomic orbital ϕ_r to the molecular orbital ψ_l. Wide variations in the values of a_{rl} are possible, subject only to the normalisation requirement, $\Sigma(a_{rl})^2 = +1$. Similar values of a_{rl} for different values of r would be characteristic of covalent bonding and disparate values of a_{rl} indicative of ionic bonding.

X-ray emissions that result from electronic transitions from valence orbitals to inner vacancies will reflect the perturbations brought about by bond formation.[10] Main peaks (diagram lines) may be altered in their characteristic wavelength and new satellite peaks formed. High-energy satellite peaks accompany most X-ray emission lines and are caused by transitions in doubly ionised species.[11,12] Low-energy satellites, however, seem to be restricted to transitions from valence shell orbitals and to be intimately associated with bond formation. The origin of these low-energy satellites can be understood by means of a very simple molecular orbital model.[10]

Consider the p valence shell orbitals on the central atom (A) in a complex, or in a crystal environment, where it is surrounded by x ligand atoms (L). The K transition (p → 1s) will now exhibit structure that reflects the various molecular orbitals in AL_x. Light elements such as carbon and nitrogen will use both their 2s and 2p orbitals in bond formation. There will therefore be two types of molecular orbitals involving A,p electrons, (Ap,L2p) and (Ap,L2s). The actual energies of these orbitals can be estimated by using the molecular orbital method. It can be shown that the energy difference between these two groups of orbitals is closely related to the ionisation energy difference between the 2s and 2p orbitals of the ligand. The energy difference between the (Ap,2p) and (Ap,2s) orbitals can be directly observed experimentally as Δ, the difference in energy between the main K peak and the low-energy satellite (for second-row elements, $K\beta$ and $K\beta'$, respectively). This difference occurs because these two peaks arise from transitions to an A 1s vacancy involving the Ap character present in the two groups of molecular orbitals and, as the ionisation energy difference between 2s and 2p orbitals varies considerably (C 5·4 eV, N 6·0 eV, O 14·8 eV and F 20·4 eV[13,14]), it is possible to identify unambiguously the nature of the ligand bound to A merely by observing Δ. It is true that Δ is also a function of the central atom, but these changes are only of the order of 2 to 4 eV and cause Δ to decrease from left to right in the periodic table.[10]

Experimental

A series of compounds of elements in the second row of the periodic table in association with light ligands was investigated in order to test the general applicability of the theoretical model discussed above. The compounds used were obtained from normal commercial sources (without further purification) or were prepared by standard techniques. Samples were presented to the spectrometer in the form of pressed discs backed with terephthalic acid. All of the spectra were recorded on a Philips PW 1410 spectrometer fitted with a chromium 2·7-kW X-ray tube and Harwell 2000 series counting equipment. The fine collimator of the spectrometer was used to obtain the best resolution and the X-ray tube was operated at 50 kV and 50 mA. The output of the head amplifier of the proportional counter (1-μm polypropylene window, argon - methane flow gas) was fed directly to a low-level amplifier (2024). The remainder of the counting equipment comprised a high-level amplifier (2025), a pulse-height analyser (2010) and a rate meter (2101). A high-voltage supply to the proportional counter was supplied by an Isotope Developments Limited 532 EHT unit. The results are given in Table I and typical spectra are presented in Figs. 1 to 6.

Table I

Energies of $K\beta_{1,3}$ X-ray emission peaks and associated low-energy satellites

Compound	See Fig.	Energy/eV $K\beta_{1,3}$	Energy/eV $K\beta'$	Energy/eV $\Delta = (K\beta - K\beta')$
MgO	1	1296	1281	15
MgF_2	1	1294	(1274)*	20
Al_2O_3	2	1553	1537	16
Na_3AlF_6	2	1552	1532	20
Topaz	3	1551	1532	20 (Fig. 3d)
		1553	1538	15 (Fig. 3c)
Aluminium 8-hydroxyquinolinate	3	1552	1538	14 (Fig. 3a)
		1555	1545	10 (Fig. 3b)
SiC	4	1835	1827	8
Si_3N_4	4	1835	1824	11
SiO_2	4	1834	1819	15
Na_2SiF_6	4	1833	1813	20
Na_2HPO_4	5	2135	2120	15
KPF_6	5	2134	2115	19
Na_2PO_3F	5	2135	2113	22
			2121	14
$MgSO_4.7H_2O$	6	2466	2452	14
KSO_3F	6	2466	2452	14
			shoulder ~ 2446	~20

* See text.

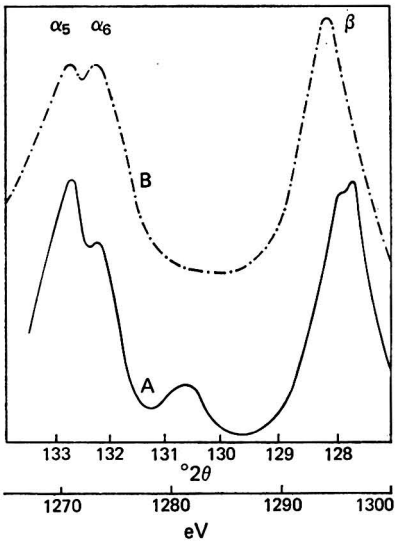

Fig. 1. Magnesium X-ray emission in the Kβ region, with use of an ammonium dihydrogen orthophosphate (101) crystal (first order): A, MgO; and B, MgF_2

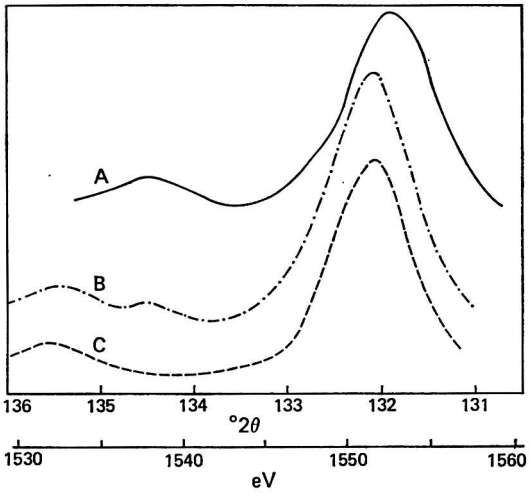

Fig. 2. Aluminium X-ray emission in the Kβ region, with use of a pentaerythritol crystal (first order): A, Al_2O_3; B, AlF_3; and C, Na_3AlF_6

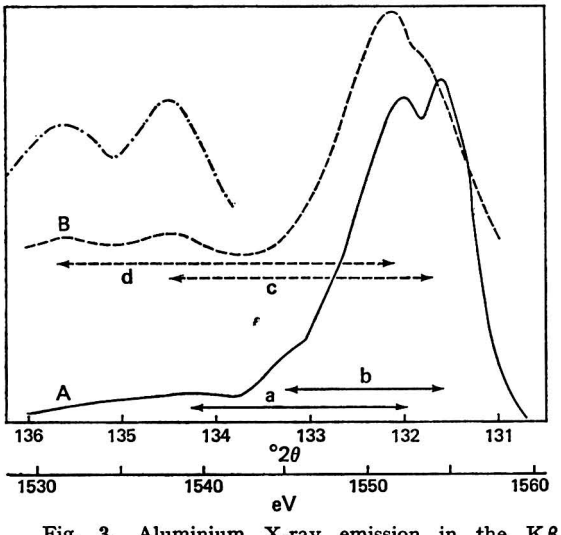

Fig. 3. Aluminium X-ray emission in the Kβ region, with use of pentaerythritol crystal (first order): A, aluminium 8-hydroxyquinolinate: a, Kβ—Kβ′ for oxygen; and b, Kβ—Kβ′ for nitrogen. B, topaz (— · — · —, section of topaz spectrum, intensity × 10): c, Kβ — Kβ′ for oxygen; and d, Kβ—Kβ′ for fluorine

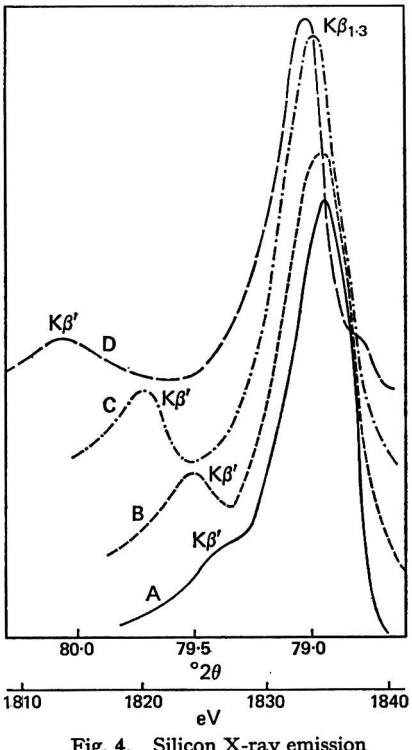

Fig. 4. Silicon X-ray emission in the Kβ region with use of an ammonium dihydrogen orthophosphate (101) crystal (first order): A, silicon carbide; B, silicon nitride; C, silica; and D, sodium hexafluorosilicate

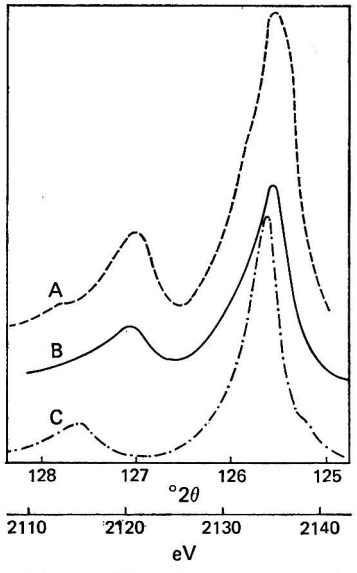

Fig. 5. Phosphorus X-ray emission in the Kβ region with use of a germanium (111) crystal (first order): A, disodium monofluorophosphate; B, disodium hydrogen orthophosphate; and C, potassium hexafluorophosphate

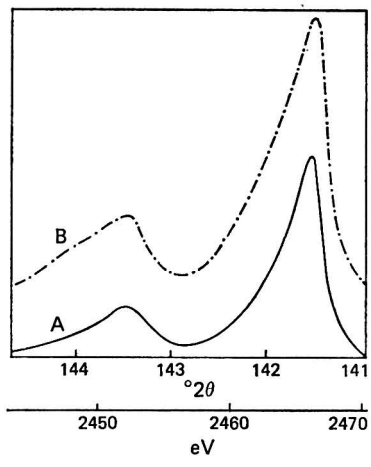

Fig. 6. Sulphur X-ray emission in the Kβ region with use of an ammonium dihydrogen orthophosphate (101) crystal (second order): A, magnesium sulphate ($MgSO_4.7H_2O$); and B, potassium fluorosulphonate (KSO_3F)

Discussion

Central atom surrounded by one type of ligand—

As can be seen from Figs. 1 to 6, when an atom is surrounded by fluorine, oxygen or nitrogen ligands a distinct Kβ′ satellite peak is readily observed, with an intensity between 10 and 30 per cent. of the main Kβ peak. The Kβ - Kβ′ energy separation, Δ, is different for nitrogen (9 ± 2 eV), oxygen (12 ± 2 eV) and fluorine (20 ± 2 eV) ligands, thus enabling them to be readily distinguished. An interesting problem exists with magnesium fluoride, for which Mendel[15] first reported the presence of a Kβ′ satellite peak. It is apparent from the magnesium oxide spectrum that some of the high-energy satellite peaks associated with magnesium Kα emission are of not much less energy than the Kβ emission itself, indeed, the $K\alpha_6$ - Kβ energy difference is only 20 eV. This proximity means that the low-energy satellites of magnesium Kβ emission might overlap with high-energy satellites from Kα emission. With the oxide, this overlap does not occur but with the fluoride it would seem as though it does. The Kβ′ emission for magnesium fluoride is anticipated at about 20 eV less than the Kβ emission and this is the energy of the $K\alpha_6$ satellite. Fig. 1 therefore provides no clear indication of a Kβ′ satellite for magnesium fluoride associated uniquely with fluorine, which association was claimed by Mendel. A comparison of the magnesium oxide and fluoride spectra reveals that the intensities of peaks at about 1271 eV ($K\alpha_5$) and 1274 eV ($K\alpha_6$) are in the ratio 1:0·8 for the oxide but about 1:1 for the fluoride. It therefore seems reasonable to suggest that the increased relative intensity at 1274 eV with the fluoride is due to the superposition of the $K\alpha_6$ peak and the Kβ′ satellite. This unfortunate example clearly shows that some care must always be exercised when low-energy satellites are used for light-element detection. Carbon ligands also give rise to satellite peaks, but because the 2s - 2p ionisation energy difference is not great the Kβ - Kβ′ separation is small (about 5 eV) and the Kβ′ emission is usually observed as a shoulder on the low-energy side of the Kβ peak.

It is interesting to notice that as well as the creation of a new satellite Kβ′ peak, chemical bond formation also has a profound effect upon the general shape of the Kβ peak itself.[16–18] It is much less asymmetrical than in the free element, presumably because of the formation of rather localised bonds as opposed to the extensively delocalised bond structure characteristic of a metal. However, under high resolution the Kβ peaks show some interesting

structures; in the oxide, the magnesium Kβ peak is split into two overlapping peaks of similar intensity,[17,18] in alumina, the aluminium Kβ peak has a distinct high-energy component,[17,18] and the Kβ peak of silica shows a high-energy shoulder of less intensity than the main peak.[19]

The complexities of Kβ peak shape do not usually intrude into a simple analysis for light elements. It is usually not possible to associate the particular components of the Kβ peak with particular ligands, but rather with particular features of the general structure about each atom. The Kβ peak of magnesium fluoride can be contrasted with that of the oxide. In the fluoride, the Kβ emission is not split, in keeping with the general idea that the localised pairs in the Mg–F bonds, which are strongly polarised towards the fluorine atom, will interact with each other less than corresponding electron pairs in the Mg–O bonds. In the latter example, bond–bond interactions cause the magnesium Kβ emission to be split.

CENTRAL ATOM SURROUNDED BY LIGANDS OF MORE THAN ONE TYPE—

The examples considered above really constitute special cases and it is more probable that a particular atom will be in a ligand environment of two or more different types of atom. In this event, the simple molecular orbital model of the bonding orbitals becomes more complex, but for the satellite peaks, which derive from orbitals that are mostly ligand 2s in character and with ionisation energies very near to ligand 2s ionisation energies, there should be very little interaction between molecular orbitals corresponding to Ap interaction with different ligand 2s orbitals. Distinct molecular orbitals should therefore result that will generate distinct Kβ' peaks, thus permitting the different elements, present as ligands, to be identified individually. That this is true can be seen from Fig. 3. Aluminium in topaz clearly shows both oxygen and fluorine satellites, while in the 8-hydroxyquinolinate complex an oxygen satellite peak and a nitrogen shoulder can both be observed. In the other examples, fluorophosphate (Fig. 5) and fluorosulphate (Fig. 6), the angular resolution at which the spectra were recorded was not so favourable and a clear distinction between the various Kβ' peaks was not possible. One would be justified in inferring the presence of fluorine as well as oxygen from the fluorophosphate spectrum but unfortunately fluorosulphate can hardly be distinguished from sulphate.

The spectrum of a sample of aluminium fluoride is given in Fig. 2. In addition to the anticipated fluorine Kβ' satellite there is also clearly an oxygen satellite, which shows that oxygen is present in the sample, probably as water, indicating a partially hydrated specimen.

The bonding involvement of the central atom p orbitals is much greater with 2p ligand orbitals than with 2s orbitals. This greater involvement means that molecular orbitals with mixed ligand character (as well as Ap orbitals) will be formed in structures of the type $AL'_x.L''$ (x atoms of one type of ligand L' and y atoms of another ligand type L''). It is therefore to be anticipated that the Kβ peak will exhibit some complex structure reflecting the degree of Ap orbital participation in the various molecular orbitals that involve ligand atoms. Even so, it seems possible to separate the structure of the main Kβ peak into oxygen Kβ', fluorine Kβ', etc., peaks. These names indicate a relationship between the energy of the particular molecular orbital and the ionisation energy of a ligand 2p orbital, rather than a molecular orbital exclusively between Ap orbitals and the 2p orbitals of a given type of ligand. In the aluminium Kβ spectra from both aluminium 8-hydroxyquinolinate and topaz it is possible to identify Kβ - Kβ' pairs that can be associated with nitrogen, oxygen or fluorine ligands (as indicated in Fig. 3). The values of Δ thus obtained are similar to those found when only one type of ligand surrounds the central atom.

STRUCTURAL IMPLICATIONS—

The occurrence of low-energy satellites in the X-ray emission spectrum of A, which can be identified as being due to ligand L, not only indicates that L is present in the sample but that L is bound to A. Indeed, if it were not bound to A it could not be detected by the method described in this paper. "Bound" is used in the broadest sense because, while covalent bonds will give the most intense satellite peaks, ionic bonds are never completely ionic, and even a fairly small amount of covalent character is sufficient to generate the appropriate emission peaks (*e.g.*, the Kβ peak in magnesium fluoride).

The presence of low-energy satellites can therefore be used not only to detect the presence of particular light elements but also to indicate the existence of bonds between particular pairs of atoms. However, the presence of more than one satellite does not indicate that

two different types of ligand are necessarily bound to the same atom; it is equally probable that the sample is a mixture in which central atoms are co-ordinated, either wholly by one, or wholly by the other, ligand type—or even by a variable mixture of co-ordination situations.

Quantitative analysis—

Having established that light elements can easily be detected by using the low-energy satellite technique, the next step is to see if the method can be used for quantitative work. The main difficulty in using satellite peaks for quantitative analysis is the large number of factors that control the intensities of these peaks. Peak intensities will be related to the coefficients in equation (1). These coefficients will vary not only from element to element and ligand to ligand, but also with structural features, such as bond length and co-ordination number. If, however, attention is focused on the compounds of a single element, then it should be possible to establish relative satellite intensities for particular ligands in particular bonding situations.

A further complication that will restrict the use of low-energy satellites in any form of quantitative analysis is the fact that only the immediate environment of the atom under investigation is studied. The number of co-ordinated ligands might be estimated but it will not be possible to establish whether or not the ligand atoms themselves are being shared between the atoms being studied. Thus, the intensity ratio of $K\beta$ to $K\beta'$ will be more or less the same for both AlF_6^{3-} and AlF_3, because in both instances the aluminium is six-co-ordinate. This example shows clearly that a simple determination of the $K\beta:K\beta'$ intensity ratio cannot be used to determine the ratio of the concentration of the ligand to that of the central atom in the bulk material. Nevertheless, the technique can be used to estimate approximately the sample contamination. Fig. 2 shows the $K\beta$ emission spectrum of a sample labelled aluminium fluoride. The presence of the oxygen satellite at about 1540 eV shows that some Al–O bonds exist as well as Al–F bonds. A direct comparison of the sizes of the $K\beta'$ peaks in AlF_3 with those of the Al_2O_3 and AlF_6^{3-} spectra indicates that the ratio (aluminium bonding to fluorine) to (aluminium bonding to oxygen) is about 2:1. The most probable chemical reason for the presence of oxygen is partial hydration due to exposure of the sample to the air. This example shows that the potential for quantitative analysis is extremely limited.

Conclusion

Low-energy satellites to X-ray emission peaks that involve transitions from valence bond orbitals can be used to establish the presence of light elements that cannot be readily detected directly by use of conventional X-ray emission apparatus, although the technique is not really suitable for quantitative analysis. All of the examples considered in this paper have involved light elements bound to a second-row element, but the arguments are general and comparable X-ray emission features can be found in the spectra of other, heavier, elements when they are chemically bound to light elements. It is interesting to note that low-energy satellites do not seem to be formed if the ligand atoms belong to rows of the periodic table other than the first.

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Stability of Dilute Standard Solutions of Antimony, Arsenic, Iron and Rhenium Used in Colorimetry

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A study has been made of the stability of dilute standard solutions of antimony (4 μg ml^{-1}), arsenic (20 μg ml^{-1}), iron (50 μg ml^{-1}) and rhenium (5 μg ml^{-1}) used in colorimetry. The standard elements in these solutions were determined over a period of 2 months by using colorimetric procedures developed in this laboratory and reported previously. Tests were carried out on standard solutions stored in soda-glass, in borosilicate glass and in rigid polyethylene containers.

The dilute standard antimony solutions, prepared either by dissolving antimony potassium tartrate in water, or by dissolving elemental antimony in sulphuric acid and diluting the solution with water, were found to be stable (*i.e.*, to deteriorate by less than 2 per cent.) over a period of 50 days. Similar dilute standard antimony solutions containing hydrochloric acid deteriorated rapidly, however.

The dilute standard arsenic solutions prepared either by dissolving arsenic(III) oxide in sodium hydroxide solution and then neutralising the solution with hydrochloric acid, or by dissolving disodium hydrogen arsenate heptahydrate in water, were found to be stable. Arsenic(III) in the former standard solution was oxidised slowly by dissolved oxygen, but the total arsenic present in the solution remained unchanged and could be determined by the molybdenum-blue method.

An iron(III) standard solution, 0·06 M in hydrochloric acid and prepared from ammonium iron(III) sulphate, was stable for at least 2 months, as was a standard potassium perrhenate solution in a buffer solution of pH 6.

Light in the laboratory and the material of the containers did not adversely affect the solutions reported to be stable. Light accelerated the deterioration of the antimony solutions that contained hydrochloric acid, and the material of the containers had a slight effect on the rate of deterioration.

The preparation and use of standard metal-ion solutions of known concentration is essential for the accurate application of many instrumental techniques. For the application of the more sensitive techniques, very dilute standard solutions are required and it becomes increasingly difficult to ensure that these solutions have the concentration intended. Metal ions may be lost from solution by adsorption on to the container walls and by hydrolysis. For example, DeMars and Shain[1] found that cadmium ions were adsorbed on to the container walls from standard cadmium solutions and that this adsorption affected the analytical results at concentrations of cadmium less than 0·1 μg ml^{-1}. They prepared each of their standard cadmium solutions five times in the same calibrated flask and rejected the first four solutions. Equilibrium between the container walls and the solutions was then ensured and the final solution had the intended concentration of cadmium ions.

Simple hydrated metal ions and other unstable metal complexes are hydrolysed if the standard solution contains insufficient acid. The amount of acid required in order to prevent hydrolysis depends on the particular metal ion and on the ligands present. The hexachloroantimonate(V) ion, for example, is reported to be hydrolysed rapidly even in 6 M hydrochloric acid to form singly charged mixed hydroxochloro complex anions,[2] which cannot be extracted with basic dyes.[3]

Despite the extensive studies that have been made on the hydrolysis of metal ions[4] and on adsorption, insufficient consideration is often given to the stability or instability of the standard solutions used in the development and application of analytical procedures. Often, users are instructed to prepare dilute standard solutions freshly each day from more concentrated standard solutions, but rarely are any quantitative data given on the stability of the dilute standard solution that is prepared. Usually, it is assumed that the freshly

prepared working standard solution does not deteriorate during the day, but this assumption is not always valid, as was shown recently[5] for dilute standard tin solutions prepared by an accepted procedure. These solutions were shown to deteriorate within a few hours of preparation owing to the addition of insufficient acid.[5]

This paper describes a preliminary study of the stability of dilute standard metal-ion solutions (4 to 50 μg ml^{-1}) used in colorimetry. It is intended that studies of solutions of lower concentrations that are used in more sensitive techniques will be made subsequently. The metals selected for study here are those for which colorimetric procedures of determination have been developed in this laboratory. These procedures are based on the complete chemical reaction of the species determined, and for this reason they are precise and reliable; precision and reliability are vital in assessing the stability of standard solutions. The studies on standard solutions of antimony and arsenic are of particular interest as many published colorimetric procedures for these elements give low recoveries and are unreliable, and assessment of these standards had previously been difficult.

EXPERIMENTAL

Storage tests were carried out in borosilicate glass, soda-glass and rigid polyethylene bottles. All of the containers were new and were pre-treated with concentrated hydrochloric acid and then washed thoroughly with water. The polyethylene bottles were obtained from Fisons Scientific Apparatus, and were reported to be prepared from first-grade natural high-density polythene. The effects of storing solutions in the dark as well as in the light in the laboratory were investigated.

Absorbance measurements were made with a Unicam SP600 spectrophotometer. The wavelength and absorbance scales were checked by using didymium and neutral filters.

Stability of standard antimony solutions (4 μg ml^{-1} of antimony)

Standard solutions—

Standard antimony solution A—Pure antimony metal (0·1000 g) was dissolved in 15 ml of concentrated sulphuric acid by heating the mixture strongly. The solution was allowed to cool and diluted carefully to 500 ml in a calibrated flask with 6 M hydrochloric acid. Standard antimony solution A was prepared by diluting 10 ml of this solution to 500 ml in a calibrated flask with 6 M hydrochloric acid.

Standard antimony solution B—This solution was prepared in the same way as standard antimony solution A, except that the dilutions were made with water, no hydrochloric acid being added.

Standard antimony solution C—Pure antimony potassium tartrate (0·267 g) was dissolved in 500 ml of 6 M hydrochloric acid in a calibrated flask. Standard antimony solution C was prepared by diluting 10 ml of this solution to 500 ml in a calibrated flask with 6 M hydrochloric acid.

Standard antimony solution D—This solution was prepared in the same way as standard antimony solution C, except that dilutions were made with water, no hydrochloric acid being added.

Procedure for determining antimony—

Precautions that have to be taken when using Brilliant green as a colorimetric reagent have been discussed previously.[6,7] A procedure for determining antimony, which has given good results, was used in this work exactly as described previously.[7]

Oxidation of antimony with cerium(IV) was carried out in 6 M hydrochloric acid, and extraction of antimony(V) into toluene was made from 1·7 M hydrochloric acid. When using standard antimony solutions B and D, which do not contain hydrochloric acid, an equal volume of concentrated hydrochloric acid was added before oxidising the antimony with cerium(IV).

Stanton and McDonald[8] indicated that toluene should be pre-treated with potassium dichromate in order to remove trace amounts of reducing agents. Previously in this laboratory it had not been found necessary to pre-treat the toluene, but in the present work pre-treatment was found to be necessary with one batch of toluene for which absorbance values that were 20 per cent. low were obtained at the 20 μg of antimony level when pre-treatment was not carried out.

RESULTS—

In tests lasting up to 57 days, standard antimony solutions B and D were found to be stable (*i.e.*, to deteriorate by less than 2 per cent.). This stability was independent of the material of the container and of whether the solutions were stored in the dark or in the light in the laboratory (see Tables I and II).

TABLE I

STABILITY TESTS ON THE STANDARD ANTIMONY SOLUTION CONTAINING ANTIMONY AND SULPHURIC ACID (STANDARD ANTIMONY SOLUTION B)

	Absorbance*					
	In borosilicate glass		In soda-glass		In polyethylene	
Time/days	In dark	In light	In dark	In light	In dark	In light
0	0·616	0·616	0·616	0·616	0·616	0·616
18	0·610	0·614	0·613	0·615	0·612	0·616
57	0·613	0·614	0·612	0·615	0·611	0·610

* 20 μg of antimony. Each value is the mean of three determinations ($\sigma < 2$ per cent.).
Measurements made after 1, 5, 11, 27 and 37 days gave the same mean absorbance (0·614 $\pm$ 2 per cent.).

Standard antimony solutions A and C, which contained hydrochloric acid, deteriorated rapidly (see Tables III and IV). The rate of deterioration was less when the solutions were stored in the dark, and was dependent to a moderate extent on the material of the container.

No detailed study was made of the oxidation state in which the antimony exists in these dilute standard solutions. Antimony(V) in 6 M hydrochloric acid is known to be hydrolysed to an unreactive form.[2,3] On the other hand, Maren[9] has indicated that effective loss of antimony can occur by the formation of an oxidation state intermediate between antimony(III) and antimony(V). The species in this state is not oxidised to antimony(V) by cerium(IV) unless the species is first reduced to antimony(III) with sulphite.

TABLE II

STABILITY TESTS ON THE AQUEOUS ANTIMONY POTASSIUM TARTRATE SOLUTION (STANDARD ANTIMONY SOLUTION D)

	Absorbance*					
	In borosilicate glass		In soda-glass		In polyethylene	
Time/days	In dark	In light	In dark	In light	In dark	In light
0	0·618	0·618	0·618	0·618	0·618	0·618
18	0·615	0·613	0·615	0·616	0·615	0·614
52	0·612	0·614	0·610	0·616	0·615	0·612

* Measurements made after 5 and 37 days gave the same mean absorbance (0·615 $\pm$ 2 per cent.).

In the present work, a study was made of a standard antimony solution, containing hydrochloric acid, which had lost 24 per cent. of its effective antimony content after 21 days. Treatment with sulphite prior to oxidation with cerium(IV) enabled 51 per cent. of the loss to be recovered. After storage for 39 days only 14 per cent. of the loss was recovered, and after storage for 50 days no increase in recovery was obtained. Furthermore, for a second solution, it was found that 50 per cent. of the loss after 20 days could be recovered by passing oxygen through the solution for 48 hours. A combination of the treatments with oxygen and sulphite gave 100 per cent. recovery of the loss after 20 days, but no recovery after 50 days. At the end of the storage period, an attempt was made to detect adsorbed antimony-containing species on the walls of the containers by treating the drained containers with concentrated hydrochloric acid at 100 °C; no antimony was detected.

This study of the hydrolysis of standard antimony solutions containing hydrochloric acid is incomplete, but the effective loss of antimony appears to be caused by the formation of one or more soluble hydrolysed species, possibly of a mixed or intermediate oxidation state. A rigorous study of the antimony species that exist under such highly acidic conditions would be difficult to carry out, but further studies are planned in order to establish whether

TABLE III

STABILITY TESTS ON STANDARD ANTIMONY SOLUTIONS CONTAINING ANTIMONY, SULPHURIC ACID AND HYDROCHLORIC ACID (STANDARD ANTIMONY SOLUTION A)

	Absorbance					
	In borosilicate glass		In soda-glass		In polyethylene	
Time/days	In light	In dark	In light	In dark	In light	In dark
0	0·615	0·615	0·615	0·615	0·615	0·615
1	0·589	0·610	0·565	—	0·590	—
5	0·542	0·597	0·526	0·570	0·557	0·580
18	0·445	0·560	0·424	0·538	0·498	0·533
37	0·386	0·535	0·361	0·525	0·427	0·520

the antimony that is not available for colorimetry is available for atomic-absorption and polarographic studies. If, as seems probable, the antimony is still present in a true or even colloidal solution, then it should be possible to determine it by atomic-absorption spectrophotometry.

TABLE IV

STABILITY TEST* ON STANDARD ANTIMONY SOLUTION CONTAINING ANTIMONY POTASSIUM TARTRATE AND HYDROCHLORIC ACID (STANDARD ANTIMONY SOLUTION C)

Time/days	0	1	5	11	18	27	37
Absorbance	0·610	0·581	0·570	0·475	0·342	0·303	0·240

* In light, stored in soda-glass.

STABILITY OF STANDARD ARSENIC SOLUTIONS (20 μg ml^{-1} OF ARSENIC)

STANDARD SOLUTIONS—

Standard arsenic solution A—Pure arsenic(III) oxide (0·660 g) was dissolved in a small volume of 1 M sodium hydroxide solution and the resulting solution was neutralised to litmus with 1 M hydrochloric acid. The solution was then diluted to 500 ml with water in a calibrated flask. Standard arsenic solution A was prepared by diluting 10 ml of this solution to 500 ml with water in a calibrated flask.

Standard arsenic solution B—Pure disodium hydrogen arsenate heptahydrate (4·17 g) was dissolved in 500 ml of water in a calibrated flask. Standard arsenic solution B was prepared by diluting 10 ml of this solution to 1 litre with water in a calibrated flask.

PROCEDURE FOR DETERMINING ARSENIC—

Arsenic can be determined conveniently by allowing arsenic(V) to react with molybdate and reducing the molybdoarsenate formed to molybdenum blue with hydrazinium sulphate; iodine is suitable for oxidising arsenic(III) to arsenic(V) prior to its reaction with molybdate. This procedure was recently used as the colorimetric finish in a method of determining arsenic in steel.[10] The apparent molar absorptivity of the molybdenum blue based on the arsenic concentration was found to be $2{\cdot}55 \times 10^4$ l mol^{-1} cm^{-1} and, as the reaction appeared to be complete under the conditions used, this value is probably the true molar absorptivity.

As the colorimetric finish was given previously only as part of a procedure for determining arsenic in steel samples,[10] the simplified procedure used in this work is described below. The acidity at which the molybdenum blue is formed in this procedure (0·57 M) is lower than that used in the previous procedure (0·8 to 0·9 M). At this lower acidity, the reduction of molybdoarsenate to molybdenum blue is more rapid.

Iodine solution, 1 per cent. m/V—Dissolve 0·5 g of iodine and 1 g of potassium iodide in 50 ml of water.

Ammonium molybdate solution, 1 per cent. m/V—Add 56 ml of concentrated sulphuric acid carefully to 400 ml of water. Dissolve 5 g of ammonium molybdate in the solution and dilute the resulting solution to 500 ml with water in a calibrated flask. This solution has been used for 40 days without signs of deterioration.

Hydrazinium sulphate solution, 0·15 per cent. m/V—Dissolve 0·15 g of hydrazinium sulphate in 100 ml of water. Satisfactory results were obtained when this solution was used within 40 days of preparation.

Method—Transfer 5 ml of standard arsenic solution with a pipette into a 100-ml conical flask and add, in turn, 4 drops of iodine solution, 1 ml of ammonium molybdate solution and 1 ml of hydrazinium sulphate solution, swirling the mixture thoroughly after each addition. Cover the flask with a watch-glass and place the mixture on a boiling water bath for 12 minutes. Allow the solution to cool, transfer it into a 50-ml calibrated flask and dilute it to 50 ml with water. Measure the absorbance of the solution at 840 nm in 1-cm cells against water. Subtract the absorbance of a blank determination.

RESULTS—

The total arsenic concentration of both standard solutions was found to be constant for at least 50 days, regardless of the material of the container used and of whether the solutions were stored in the light or in the dark, as shown by the results in Tables V and VI; Table V also includes some results obtained with standard solutions containing 4 μg ml^{-1} of arsenic. By omitting the oxidation step with iodine, it was possible to determine arsenic(V) alone and thus follow the oxidation by air of arsenic(III) in standard solution A. As an example, 50 per cent. of the arsenic(III) was found to be oxidised to arsenic(V) after storage for 33 days in a glass container. Nevertheless, the total arsenic in the solution, as measured by the complete molybdenum-blue method, remained unchanged.

TABLE V

STABILITY TESTS ON THE AQUEOUS SOLUTION OF DISODIUM HYDROGEN ARSENATE (STANDARD ARSENIC SOLUTION A)

	Absorbance*						
	In borosilicate glass		In soda-glass			In polyethylene	
Time/ days	In light, 20 μg ml^{-1}	In dark, 20 μg ml^{-1}	In light, 20 μg ml^{-1}	In dark, 20 μg ml^{-1}	In light, 4 μg ml^{-1}	In light, 20 μg ml^{-1}	In dark, 20 μg ml^{-1}
0	0·682	0·682	0·682	0·682	0·682	0·682	0·682
22	0·680	0·678	0·681	0·682	0·681	0·682	0·681
56	0·680	0·681	0·679	0·680	0·678	0·681	0·682

* 100 μg of arsenic. Each value is the mean of three determinations ($\sigma < 2$ per cent.).
Measurements made after 1, 6, 15 and 41 days gave the same mean absorbance (0·681 $\pm$ 2 per cent.).

STABILITY OF STANDARD IRON(III) SOLUTIONS [50 μg ml^{-1} OF IRON(III)]

STANDARD SOLUTION—

Standard iron(III) solution, 50 μg ml^{-1}—Pure ammonium iron(III) sulphate dodecahydrate (0·432 g) was dissolved in water, 5 ml of concentrated hydrochloric acid was added and the resulting solution was diluted to 1 litre with water in a calibrated flask.

TABLE VI

STABILITY TESTS ON THE STANDARD ARSENIC SOLUTION PREPARED FROM ARSENIC(III) OXIDE (SOLUTION B)

	Absorbance*			
	In soda-glass		In polyethylene	
Time/ days	In dark	In light	In dark	In light
0	0·680	0·680	0·680	0·680
39	0·677	0·677	0·678	0·675
100	0·675	0·677	0·679	0·677

* Measurements made after 2, 6, 12, 24, 32, 47, 66 and 85 days gave the same mean absorbance (0·678 $\pm$ 2 per cent.).

PROCEDURE FOR DETERMINING IRON(III)—

Iron(III) was determined with bis(2-hydroxy-3,5-dichlorophenyl) sulphide (bithionol) by

using a procedure developed in this laboratory.[11] The coefficient of variation of this procedure had previously been shown to be less than 1 per cent.

RESULTS—

The results of the stability tests are shown in Table VII. Loss of iron from the solutions after storage for 2 months is less than 2 per cent.

TABLE VII

STABILITY TESTS ON STANDARD IRON(III) SOLUTION

	Absorbance*			
	In soda-glass		In polyethylene	
Time/ days	In dark	In light	In dark	In light
0	0·510	0·510	0·510	0·510
30	0·500	0·506	0·500	0·506
69	0·503	0·501	0·504	0·504

* 250 μg of iron. Each value is the mean of three determinations ($\sigma < 2$ per cent.). Measurements made after 1, 10, 20, 38 and 46 days gave the same mean absorbance (0·505 $\pm$ 2 per cent.).

STABILITY OF STANDARD RHENIUM(VII) SOLUTIONS [5 μg ml^{-1} OF RHENIUM(VII)]

STANDARD SOLUTION—

Standard rhenium(VII) solution, 5 μg ml^{-1}—A buffer solution (pH 6) was prepared by diluting 500 ml of 0·1 M potassium dihydrogen orthophosphate solution *plus* 56 ml of 0·1 M sodium hydroxide solution to 1 litre with water in a calibrated flask.

Pure potassium perrhenate (0·1553 g) was dissolved in water and diluted to 1 litre with water in a calibrated flask. The standard rhenium solution was prepared by diluting 50 ml of this solution to 1 litre with buffer of pH 6 in a calibrated flask.

PROCEDURE FOR DETERMINING RHENIUM(VII)—

Rhenium(VII) was determined with Brilliant green by using a procedure developed in this laboratory.[6]

RESULTS—

The results of the stability tests are shown in Table VIII. No evidence of loss of rhenium(VII) from the solution was apparent after 2 months.

TABLE VIII

STABILITY TESTS ON STANDARD RHENIUM(VII) SOLUTION

	Absorbance*			
	In soda-glass		In polyethylene	
Time/ days	In dark	In light	In dark	In light
0	0·431	0·431	0·431	0·431
34	0·431	0·425	0·433	0·428
84	0·430	0·428	0·426	0·430

* 25 μg of rhenium. Each value is the mean of three determinations ($\sigma < 2$ per cent.). Measurements made after 1, 5, 10 and 54 days gave the same mean absorbance (0·429 $\pm$ 2 per cent.).

DISCUSSION

In colorimetric procedures for the determination of metals, instructions are commonly given that the dilute working standard be prepared freshly each day from a more concentrated stock standard solution. The present work has shown that the working standards of certain elements (antimony, arsenic, iron and rhenium) can themselves be stored and used satisfactorily for at least 2 months.

Standard solutions are used initially in order to prepare calibration graphs, but they are also used subsequently in order to check whether a procedure is working satisfactorily. This latter use applies particularly to those colorimetric methods which are known to be erratic; procedures for determining antimony and arsenic might be considered to fall within this category.

When constructing initial calibration graphs, it is advisable to prepare the standard solutions freshly from the solid standard substance. Even if the solutions are to be used immediately, however, it is more satisfactory to prepare solutions that are known to be stable. The use of hydrochloric acid in preparing standard antimony solutions, for example, is an unnecessary risk when other, more stable, solutions can be prepared and used just as readily.

When possible, the apparent molar absorptivity obtained with the procedure should be calculated and compared with the known true molar absorptivity of the coloured compound formed. Failure to reach the full true molar absorptivity indicates either that the standard solution has deteriorated or that the procedure is not being applied satisfactorily for some reason, provided that the wavelength control of the spectrophotometer does not require adjustment and the absorbance scale has been checked with a neutral filter. For the purpose of making an occasional check on the procedure, there appears to be no reason why stable stored dilute working standard solutions should not be used. If a low recovery is obtained, then a fresh working standard should be prepared from the solid standard rather than from a concentrated stock solution.

The full tests reported in this study were restricted to the use of only one container of each type, but preliminary investigations with other similar containers gave similar results. One cannot be absolutely certain, however, that any particular standard solution has not deteriorated during storage. This possibility cannot be overlooked if the apparent molar absorptivity is calculated.

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Spectrophotometric Determination of Phosphorus(V) Oxide in Cements and Clinkers with Molybdovanadate Reagent

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The current method of determining phosphorus(V) oxide in cements and clinkers has been investigated in an attempt to improve its reliability. The method in use at the Building Research Station is that for U.K. internal trade, as described in B.S. 4550 : Part 2 : 1970, in which phosphate is complexed as the molybdovanadophosphate and the intensity of the yellow colour produced is measured. Investigations have shown that silica in solution interferes with the development of the colour and a modified determination procedure has been developed in order to minimise this interference and to reduce the relative error for phosphorus(V) oxide determination in cements.

The Building Research Station has for many years investigated the problems associated with the presence of certain minor constituents, *e.g.*, phosphorus(V) oxide, in cements. These minor constituents can affect the cementitious properties of the end product disproportionately to the amount present. High-temperature phase studies have led to an understanding of the mechanisms by which these minor constituents act and this knowledge has been applied to the production of Portland cements from phosphatic limestones[1] and phosphogypsum[2] on a successful, commercial basis. These phase investigations, and the commercial production of cement, require a reliable and accurate method of determining the phosphorus(V) oxide content of a variety of materials.

In the usual complete analysis of a cement, the opening up procedure, as specified in the relevant British Standard,[3] involves the use of hydrochloric acid in the presence of ammonium chloride. The latter is used to dehydrate the silica gel and convert it into a more readily filterable form. The bulk of the silica is filtered off and determined gravimetrically. The filtrate is then made up to 500 ml and aliquots are taken for the determination of various components, including the silica remaining in solution and phosphorus(V) oxide but excluding the alkalis, which are determined on a separate solution of the cement in nitric acid. The silica remaining in solution is usually less than 0·2 per cent. of the sample and in ordinary British Portland cement the phosphorus(V) oxide content is generally less than 0·5 per cent. However, when phosphatic limestones or phosphogypsum are used as raw materials, the phosphorus(V) oxide content of clinkers and cements can be as high as 2·5 per cent.

In some recent work on a batch of cement clinkers manufactured from phosphatic limestone, only the sodium, potassium, fluorine and phosphorus(V) oxide contents were required, and it was thought that an aliquot from the solution in nitric acid used for alkali determination would suffice for the phosphorus determination. However, high and erratic results were obtained. Observation indicated that an inverse relationship existed between the amount of residue (silica) filtered off after nitric acid attack and the apparent phosphorus(V) oxide content. The present investigation was therefore undertaken to establish the influence of soluble silica and other conditions on the spectrophotometric determination of phosphorus(V) oxide with molybdovanadate reagent, particularly with regard to cement analysis.

The use of the molybdovanadophosphate complex in the determination of phosphorus has been described.[4–13] Barton[4] was the first to use it in the analysis of phosphate rock and to combine the vanadate, molybdate and acid into one reagent. Misson[5] formulated the complex as $(NH_4)_3PO_4.NH_4VO_3.16MoO_3$, whereas Donald, Schwehr and Wilson[6] suggested the formula $H_3PO_4.VO_3.11MoO_3.nH_2O$. The first formulation would require an ammonium vanadate, NH_4VO_3, to ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, mass ratio in the reagent

mix of 1 : 24·2, while the corresponding ratio for the second formulation would be 1 : 16·6. In practice, ammonium molybdovanadate reagents of varying composition have been used by different investigators, as follows—

Investigators	Reference	Mass ratio, vanadate to molybdate	Combined (C) or separate (S)
B.S. 4550 : Part 2	3	1:40	C
Barton	4	1:40	S
		1:40	C
Misson	5	1:41	S
Donald, Schwehr and Wilson	6	1:20	C
Gericke and Kurmies	7	1:20	C
Baadsgaard and Sandell	8	1:20	S
Hanson	9	1:20	C
Kitson and Mellon	10	1:20	S
Murray and Ashley	11	1:43	S
Gee and Deitz	12	1:20	C
Hill	13	1:80	S

Whichever of the complex compositions is assumed to be correct, the vanadate to molybdate ratio of 1 : 20 is close to the ratio calculated for both formulations. This ratio also has a bearing on the stability of the combined reagent solution, as with a 1 : 40 ratio solids separate out within a few weeks, but with a 1:20 ratio the solution is still clear after several months.

No reason could be found why the B.S. method[3] should specify exactly 426 nm for the wavelength at which the absorbance of the complex is measured. Donald, Schwehr and Wilson[6] indicate that the wavelength is not critical, any wavelength between 420 and 480 nm being satisfactory. Barton[4] suggests that 400 nm is the optimum as he found maximum separation between the spectra of the complex and the reagent blank at this wavelength. Gericke and Kurmies[7] give a wavelength of 436 nm, this being the maximum transmission wavelength of the filter used in their colorimeter. Those papers[5,7,10,11,13] which deal with the determination of phosphorus in iron and steel recommend the use of the longer wavelengths in order to reduce the interference from the iron colour.

Interference from silica, present during the analysis, has been observed by various investigators. Kitson and Mellon[10] state that the error introduced in the determination of 10 mg ml^{-1} of phosphorus by up to 1 mg ml^{-1} of silicate ions was less than 2 per cent. Gee and Deitz[12] found that the interference from silica was serious at 390 nm, with 1 part of silica equivalent to 0·8 part of phosphorus(V) oxide, but at 450 nm this interference decreased to about half of that at 390 nm. The most comprehensive assessment of silica interference was carried out by Gericke and Kurmies.[7] They pointed out the slowness of development of the silica complex relative to the phosphorus complex, and found that if the silica content was below 1 mg per per 100 ml of solution, no interference was found when measuring after a 5-minute development time. Separation of the silica was found to be necessary with concentrations above 1 mg per 100 ml.

Experimental

All absorbance measurements were made by using a Hilger and Watts H700 Uvispek spectrophotometer and absorption spectra were obtained on a Unicam SP800 recording spectrophotometer.

Reagents—

Two ammonium molybdovanadate reagent solutions were prepared as follows.

Solution 1 (1 : 40 *ratio*)—Dissolve 1 g of ammonium metavanadate, NH_4VO_3, in 300 ml of distilled water *plus* 140 ml of nitric acid (sp. gr. 1·42). Then add 400 ml of a 10 per cent. *m/V* solution of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, and dilute the mixture to exactly 1 litre with distilled water.

Solution 2 (1 : 20 *ratio*)—This solution was prepared as for solution 1, but with 200 ml of 10 per cent. *m/V* ammonium molybdate solution.

Standard phosphate solution—Weigh 0·186 g of dry diammonium hydrogen orthophosphate, $(NH_4)_2HPO_4$ (analytical-reagent grade), dissolve it in distilled water and dilute the solution to exactly 500 ml.

1 ml of solution ≡ 0·2 mg of P_2O_5.

Standard silica solution—Mix 0·500 g of freshly ignited high-purity silica with 2·5 g of anhydrous sodium carbonate and fuse the mixture in a platinum crucible. Dissolve the cooled melt in distilled water and dilute the solution to exactly 500 ml. Store the solution in a polythene bottle.

1 ml of solution ≡ 1 mg of SiO_2.

CALIBRATION—

Calibration graphs were prepared by using aliquots of the standard phosphate solution to which were added 15 ml of ammonium molybdovanadate solution 1 or 2, diluting to 100 ml in every instance. After 30 minutes the absorbance of each solution was measured at 426 nm. Solutions were examined in 10-mm cells and the appropriate diluted ammonium molybdovanadate solution was used as reference. The results are given in Table I.

TABLE I

RESULTS FOR CALIBRATION FOR PHOSPHORUS

	Absorbance† at 426 nm with ammonium molybdovanadate solution	
P_2O_5/mg per 100 ml*	Solution 1	Solution 2
1	0·199	0·200
2	0·403	0·405
3	0·605	0·597
4	0·794	0·797

* At dilutions used in B.S. 4550: Part 2, 1 mg of P_2O_5 per 100 ml ≡ 1 per cent. of sample.

† Net absorbance, corrected for cell blank, at 30 minutes.

Table II shows the net absorbance values obtained after development for 30 minutes when the ammonium molybdovanadate solutions were added to aliquots of the standard silica solution.

TABLE II

RESULTS FOR CALIBRATION FOR SILICA

	Absorbance at 426 nm with ammonium molybdovanadate solution	
SiO_2/mg per 100 ml	Solution 1	Solution 2
1	0·093	0·029
2	0·180	0·059
3	0·242	0·086
4	0·306	0·114

EFFECT OF TIME—

The rate of development of the coloured complexes was studied by taking aliquots containing the equivalent of 2 mg of either standard phosphate or standard silica solution, adding 15 ml of the appropriate ammonium molybdovanadate solution and diluting the mixture to 100 ml. The absorbance of each solution was measured at 426 nm in 10-mm cells at times ranging from 2 to 45 minutes. The results are shown in Fig. 1. The two complexes with solution 2 were also measured at 2·5 hours (silica complex absorbance, 0·135; phosphorus complex absorbance, 0·396) and at 24 hours (silica complex absorbance, 0·216; phosphorus complex absorbance, 0·396).

ABSORPTION SPECTRA—

Fig. 2 shows the absorption spectra obtained for phosphorus and silica when solution 2 was used for sample and reference solutions. The base-line was set to zero with reference solution in both beams. Inspection of the spectra indicated that measurement of absorbance at 450 nm might minimise the interference from silica. Therefore, calibrations for both silica and phosphorus(V) oxide, with ammonium molybdovanadate solution 2, were obtained at 450 nm and are shown in Tables III and IV. For comparison purposes, results obtained at 426 nm are also included.

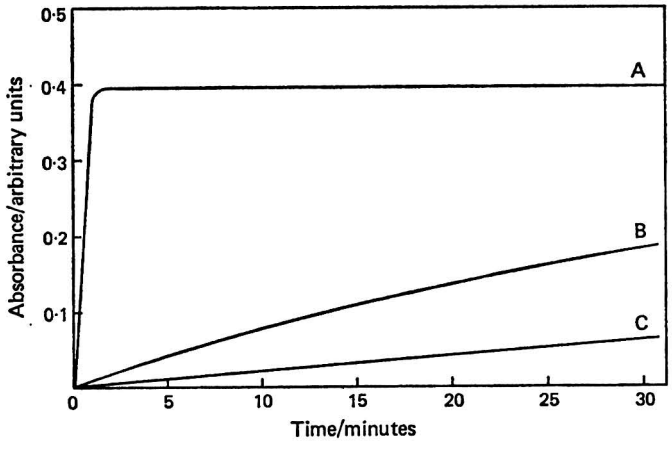

Fig. 1. Development of molybdovanadate complexes: A, 2 mg of phosphorus(V) oxide per 100 ml with ammonium molybdovanadate solutions 1 and 2; B, 2 mg of silica per 100 ml with ammonium molybdovanadate solution 1; and C, 2 mg of silica per 100 ml with ammonium molybdovanadate solution 2. A wavelength of 426 nm was used, with 10-mm cells

Table III

Results for calibration for phosphorus(V) oxide with solution 2

Development time = 2 minutes to 24 hours

P_2O_5/mg per 100 ml	1	2	3	4
Absorbance* at 450 nm	0·124	0·240	0·362	0·483
Absorbance* at 426 nm	0·200	0·405	0·597	0·797

* Corrected for cell blank, 10-mm cells.

Table IV

Effect of wavelength and time on silica calibration when using solution 2

SiO_2/mg per 100 ml	1		2		3		4	
Wavelength/nm	426	450	426	450	426	450	426	450
Absorbance at 10 minutes ..	0·011	—	0·023	0·002	0·034	0·003	0·046	0·004
20 minutes ..	0·021	0·001	0·044	0·005	0·065	0·007	0·087	0·011
30 minutes ..	0·029	0·002	0·059	0·010	0·086	0·013	0·114	0·017

Discussion

The results in Tables I and II and Fig. 1 show that while both ammonium molybdovanadate solutions behave identically towards standard phosphate, solution 1 reacts faster, and to a greater extent, with silica than does solution 2. This effect is probably caused by the excess of molybdate. Fig. 1 also indicates that the effect of silica can be reduced by shortening the development time from 30 minutes to, say, 5 or 10 minutes. The phospho-complex is formed quantitatively within 2 minutes and is stable for at least 24 hours, whereas the silico-complex develops much more slowly and is still developing after 24 hours. The absorption spectra in Fig. 2 indicate that peak absorption occurs at 330 to 340 nm, which is considerably lower than the 400 nm indicated by Barton.[4]

Ordinary British Portland cements generally contain less than 0·5 per cent. of phosphorus(V) oxide, and from experience in the use of the methods of B.S. 4550 : Part 2, it is usual to find approximately 0·2 per cent. of silica remaining in solution. At the dilutions used in these methods, 0·5 per cent. of phosphorus(V) oxide is equivalent to 0·5 mg of phosphorus(V) oxide in 100 ml of solution for spectrophotometry, and 0·2 per cent. of silica to

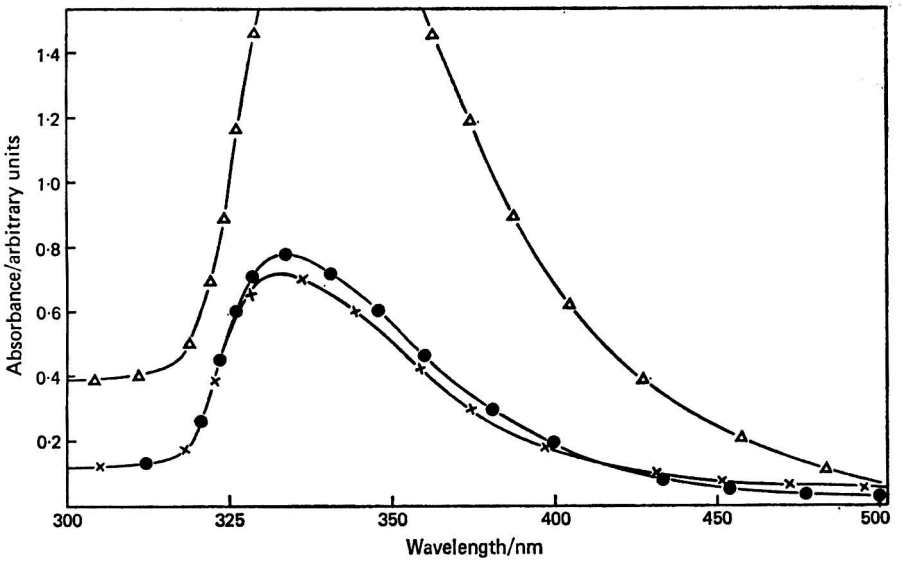

Fig. 2. Absorption spectra of molybdovanadate complexes: △, 2 mg of phosphorus(V) oxide per 100 ml; ×, 0·4 mg of phosphorus(V) oxide per 100 ml; and ●, 2 mg of silica per 100 ml. The reference solution was ammonium molybdovanadate solution 2

0·2 mg of silica in 100 ml. From the results shown in Tables I to IV and Fig. 1, some calculations of the error introduced by 0·2 per cent. of silica when determining 0·5 per cent. of phosphorus(V) oxide have been made and are shown in Table V. If the actual phosphorus(V) oxide content is less than 0·5 per cent., then the relative errors shown in Table V will be increased.

TABLE V

EFFECT OF SOLUBLE SILICA (0·2 PER CENT.) ON DETERMINATION OF 0·5 PER CENT. OF PHOSPHORUS(V) OXIDE

Conditions			Absorbance due to 0·2 per cent. of SiO_2	Equivalent P_2O_5, per cent.	Error, per cent., relative to 0·5 per cent. of P_2O_5
Ammonium molybdovanadate solution	Wavelength/nm	Time/minutes			
1	426	30	0·018	0·09	18
1	426	10	0·008	0·04	8
2	426	30	0·006	0·03	6
2	426	10	0·002	0·01	2
2	450	30	0·001	0·008	1·6
2	450	10	0·0002	0·0017	0·3

CONCLUSIONS

With British cements, phosphorus is a minor constituent that is not normally considered to be of great importance, and for works purposes is not usually determined. When such a determination is required, with the use of the ammonium molybdovanadate reagent and the method recommended for U.K. internal purposes in B.S. 4550 : Part 2 : 1970, a relative error of 20 per cent. can arise. A ten-fold decrease in the error can be achieved by halving the amount of molybdate in the reagent, to give an ammonium vanadate to ammonium molybdate mass ratio of 1 : 20, and decreasing the development time to 10 minutes while still retaining the wavelength of 426 nm. Reduction of the concentration of molybdate in the reagent also provides the additional advantage that the combined reagent solution remains stable for several months.

When the error must be even smaller, as for example in studies concerned with the manufacture of cements from phosphatic raw materials or with high-temperature phase relationships in which phosphate is a very important minor constituent[1,2] requiring accurate control, this requirement can be achieved by changing the wavelength to 450 nm in addition to implementing the recommended reductions in molybdate concentration and development time.

Mrs. F. Halliwell assisted with the experimental work. This work forms part of the programme of the Building Research Establishment of the Department of the Environment and this paper is published by permission of the Director.

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Determination of Cyclamate in Soft Drinks by Reaction with Nitrous Acid

Part II.* Manual and Semi-automated Methods: Determination of Cyclohexyl Nitrite by Diazotisation and Coupling with Bratton - Marshall Reagent

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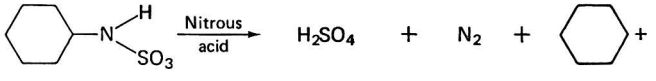
The determination of cyclamate at a concentration of approximately 1 mg ml^{-1} in soft drinks is described. Cyclohexyl nitrite, derived from cyclamate by reaction of the latter with nitrous acid, is determined in a non-aqueous system by initial diazotisation with sulphanilamide and subsequent coupling with 2-aminoethyl-1-naphthylamine. Manual and semi-automated procedures have been evaluated and interference has been studied.

THE initial products of the reaction of nitrous acid with cyclamate are sulphuric acid, nitrogen and carbonium ions—



Subsequently, the unstable carbonium ions are rapidly converted into cyclohexene (with a 24 per cent. yield) and cyclohexyl nitrite (with a 76 per cent. yield, under strong nitrosating conditions).[1] Published methods for the determination of cyclamate depend on the determination of sulphate, nitrite or cyclohexene. These include gas - liquid chromatographic,[2–4] thin-layer chromatographic,[5,6] titrimetric,[7] gravimetric[8] and infrared spectrophotometric[9] procedures. Determinations based on cyclohexyl nitrite arouse particular interest because of the specificity of this compound and its formation in high yield. Previously, organonitrites have been determined by diazotisation and coupling in a two-phase system,[10] but the one-phase system, as described in this paper, affords increased simplicity and precision of technique.

EXPERIMENTAL

REAGENTS FOR MANUAL METHOD—

Standard sodium cyclamate solution, aqueous, 2·5 mg ml^{-1}.

Standard sodium nitrite solution, aqueous, 0·5 M.

Sulphuric acid, 5 N.

Tetrachloroethylene, redistilled, boiling range 120 *to* 121 °C.

Ethanol, 95 *per cent.*

Bratton - Marshall mixed reagent[11]—A 0·5 per cent. *m/V* solution of sulphanilamide in 1 + 1 hydrochloric acid - 95 per cent. ethanol (1 + 1) (this solution is stable for 4 weeks) is mixed with an equal volume of 0·1 per cent. *m/V* solution of 2-aminoethyl-1-naphthylamine dihydrochloride in 95 per cent. ethanol (this solution is stable for 4 weeks at 5 °C in the dark).

PROCEDURE FOR MANUAL METHOD—

Weigh accurately about 10 g of the sample (containing approximately 1 mg ml^{-1} of cyclamate) and transfer it quantitatively into a 100-ml separating funnel. Adjust the volume to a total of 25 ml by the addition of distilled water and add, by pipette, 10 ml of 5 N sulphuric acid, 10 ml of tetrachloroethylene and 15 ml of 0·5 M sodium nitrite solution. Shake the

* For details of Part I of this series, see reference list on p. 748. For Part III, see p. 749.

mixture for 2 minutes, then separate the organic solvent layer and wash it with 10 ml of distilled water. Discard the washings, filter the organic solvent layer through a Whatman No. 5 filter-paper and dilute an aliquot of the filtrate exactly ten times with 95 per cent. ethanol. Transfer an aliquot (1 ml) of the resulting solution into a dry 25-ml calibrated flask; add, by pipette, 20 ml of 95 per cent. ethanol and 2 ml of Bratton - Marshall mixed reagent. Dilute to volume with 95 per cent. ethanol, mix the solution and, after 20 minutes, determine the optical density at $\lambda_{max.}$ (550 nm) by using 1-cm cells with 95 per cent. ethanol in the reference cell. Read off the cyclamate content by reference to the calibration graph prepared as described below.

CALIBRATION GRAPH FOR MANUAL METHOD—

Range 0 to 12·5 mg of cyclamate—To a series of 100-ml separating funnels add 0, 1, 2, 3, 4 and 5-ml portions of standard sodium cyclamate solution (2·5 mg ml^{-1}). Adjust the volume in each instance to 25 ml and continue as described under Procedure for manual method as far as ". . . by using 1-cm cells with 95 per cent. ethanol in the reference cell." Construct a calibration graph relating cyclamate concentration to optical density. (Under our conditions the calibration graph obeyed the equation $y = 0{\cdot}55x$, where x is the amount of cyclamate in milligrams and y is the optical density.) The calculated ϵ value for cyclamate based on this graph was $3{\cdot}07 \times 10^4$ at $\lambda_{max.}$ (550 nm).

PROCEDURE FOR AUTOMATED METHOD—

By using Technicon AutoAnalyzer equipment and the manifold shown in Fig. 1, set the base-line by continuously sampling air through the sample line A, aqueous sodium cyclamate

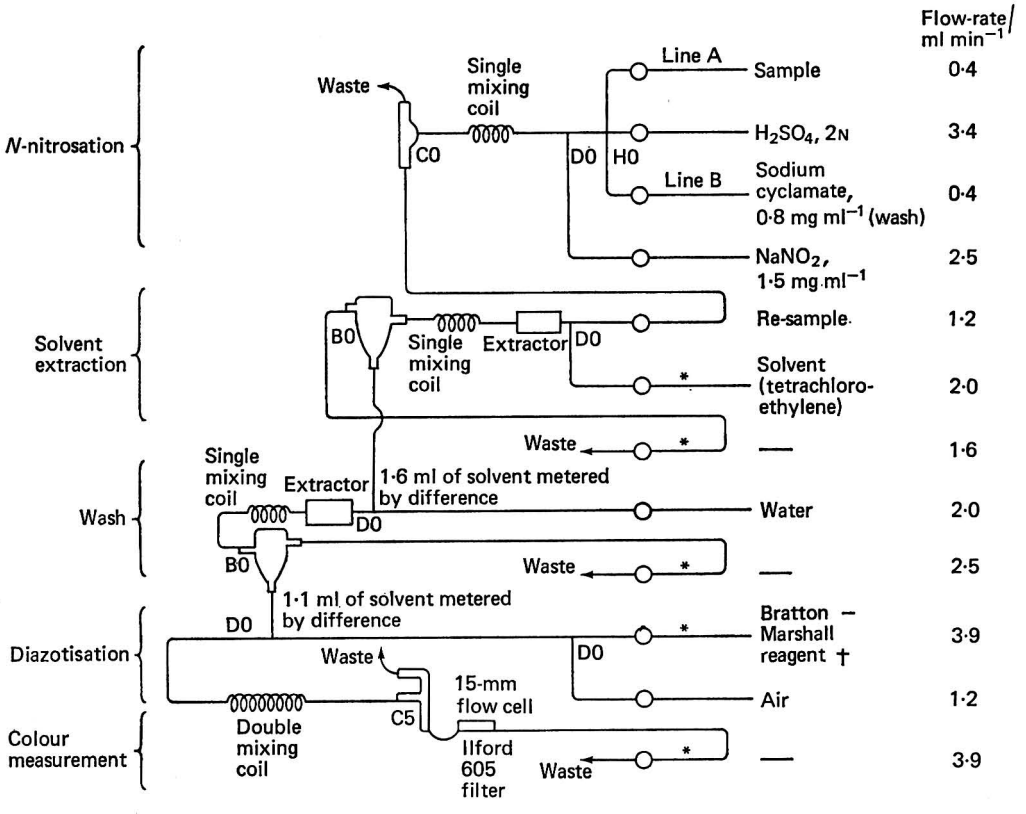


Fig. 1. Manifold for determination of cyclamate in the concentration range 0·80 to 1·30 mg ml^{-1} All Tygon tubing except where marked *, when yellow Tygon tubing was used.

† Reagent specified in manual method but diluted twenty times with water

solution (0·8 mg ml^{-1}) through the wash line B and other reagents as specified in Fig. 1. Construct a calibration graph by sampling standard sodium cyclamate solutions, covering the range 0·80 to 1·30 mg ml^{-1}, through the sample line A at the rate of 40 per hour. (In our work, a change of 0·10 mg ml^{-1} in the cyclamate concentration produced a change in optical density of about 0·1 unit.) Sample the test solutions and determine the cyclamate content of the samples by reference to the calibration graph.

Results and discussion

Results of analyses are given in Table I.

Table I

Determination of cyclamate in soft drinks with 1 mg ml^{-1} of added cyclamate

	Manual method		Automated method	
Type of soft drink	Orange	Lemon	Orange	Lemon
Number of samples analysed	10	10	20	20
Mean apparent recovery, per cent.	106·4	106·9	106·1	106·6
Apparent recovery range, per cent.	104 to 109	104 to 110	103 to 109	103 to 110
Mean deviation, (±) per cent.	1·6	2·0	1·5	2·1

The manual procedure is simple and fairly rapid. Maximum colour is developed within 20 minutes and the colour remains stable for at least 1 hour. The automated procedure has proved somewhat difficult to perform over long periods of time, because of the problem of maintaining equilibrium in each of the two-phase separators. This problem would probably be overcome by using the techniques described by Carter and Nickless.[12]

Assessment of the interference caused by various classes of substances has been made by applying the conditions of nitrosation, extraction and colorimetric determination, as specified above in the Procedure for manual method, to several test substances (see Table II).

Table II

Interference of functional groups

Class of substance	Test substance	Interference, per cent.*
Alcohol	Propan-2-ol	100
	D-Linalol	38
Terpene	D-Limonene	0·5
Aldehyde	Formaldehyde	0·5
Amine	Methylamine	<0·1
Amino-acid	DL-Serine	<0·1
	L-Cysteine	<0·1
Phenol	Phenol	<0·1
	Phloroglucinol	<0·1
Reductant	Ascorbic acid	<0·1
	Sulphur dioxide	<0·1

$$\text{* Interference, per cent.} = \frac{\text{Molar absorbance of test substance}}{\text{Molar absorbance of sodium cyclamate}} \times 100.$$

Alcoholic constituents are a major cause of interference (Table II) but attempts to remove these compounds from control samples that were free from cyclamate have not reduced sample blank values by more than 10 per cent. Preliminary treatments examined include removal of steam volatiles, oxidation with permanganate, bromination and extraction with diethyl ether of the sample saturated with sodium chloride.

Interference associated with the basic constituents of soft drinks has been evaluated by determining the apparent cyclamate content of control samples that were free from added cyclamate (Table III).

TABLE III

INTERFERENCE DUE TO BASIC CONSTITUENTS OF DRINKS*

Twenty replicate analyses

Type of drink	Orange	Lemon
Mean	5·9	6·2
Range	4 to 8	4 to 10
Mean deviation (±)	0·9	1·4

* Expressed as a percentage for a nominal cyclamate content of 1 mg ml^{-1}.

The results show that the interference is reasonably constant between samples. By deducting the appropriate mean values (listed in Table III) from the apparent recovery values (listed in Table I), recovery values of added cyclamate of approximately 100 to 101 per cent. are obtained.

High reagent blanks can arise from impurities present in tetrachloroethylene, which readily undergo nitrosation, but, by limiting the volume of solvent used at the nitrosation - extraction stage, the solvent blank has been effectively reduced. Only trace amounts of inorganic nitrite remain entrained in the solvent phase and they contribute negligibly to the solvent blank.

Investigations have shown that quantitative extraction of cyclohexyl nitrite is effected by the single-extraction stage specified in the manual method.

Use of a pre-mixed diazotisation and coupling reagent results in increased precision and only a small reduction in sensitivity, compared with procedures that involve the separate addition of diazotisation and coupling reagents.

The one-phase non-aqueous system for colour development described in this paper gives a quantitative reaction, rapid colour development and good reproducibility, and is preferred to a two-phase system described by earlier workers.[10]

The contribution of cyclohexyl nitrite and cyclohexene to the final colour measured has been determined; ϵ values are $4{\cdot}16 \times 10^4$ for cyclohexyl nitrite and $0{\cdot}09 \times 10^4$ for cyclohexene (compared with $3{\cdot}07 \times 10^4$ for cyclamate). Under the conditions defined for the *N*-nitrosation of cyclamate, the yield of cyclohexyl nitrite is 76 per cent. and that of cyclohexene 23 per cent. The calculated ϵ value for cyclamate based on the contribution of these products is $3{\cdot}17 \times 10^4$; this result is in good agreement with the determined value of $3{\cdot}07 \times 10^4$.

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NOTE—Reference 2 is to Part I of this series.

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Determination of Cyclamate in Soft Drinks by Reaction with Nitrous Acid

Part III.* Manual and Semi-Automated Methods: Determination of Excess of Nitrous Acid with Safranine

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The determination of cyclamate in soft drinks at a concentration of approximately 1 mg ml^{-1} is described. Excess of nitrous acid is added to the sample and unconsumed nitrous acid is determined colorimetrically with safranine. Factors that affect the sensitivity of the latter reaction and interference due to other components of soft drinks have been investigated. Preliminary precipitation with zinc hexacyanoferrate(II) effectively reduces this interference.

IN the automated determination of cyclamate, via the determination of cyclohexyl nitrite by diazotisation and coupling with Bratton - Marshall reagent, some difficulty was encountered at the extraction stage.[1] A more simple process, based on the addition of excess of nitrite to cyclamate followed by the determination of unconsumed nitrite with safranine, has now been investigated. This determination depends on the change in colour of safranine from red to blue in the presence of nitrite ions. The mechanism of this reaction is not fully understood but Carboni[2] has suggested that nitrous acid reacts with the two amino groups present in the safranine molecule to form a hexatomic ring.

EXPERIMENTAL

REAGENTS FOR MANUAL METHOD—

Safranine solution, 0·50 mg ml^{-1}—Dissolve 0·50 g of safranine O [Gossypimine; C.I. 50240 (841)] in water. Filter the solution through a Whatman GF/C glass filter-paper into a 1-litre calibrated flask, dilute to volume with water and mix.

Standard sodium nitrite solution, aqueous, 0·100 mg ml^{-1}.

Standard sodium cyclamate solution, aqueous, 0·500 mg ml^{-1}.

Hydrochloric acid, 1·0 N.

Modified Carrez reagent.—Mix, *in situ*, equal volumes of zinc sulphate reagent (14·3 per cent. solution of $ZnSO_4.7H_2O$ in 0·5 N sulphuric acid) and potassium hexacyanoferrate(II) reagent (10·6 per cent. solution of $K_4Fe(CN)_6.3H_2O$ in water).

PRELIMINARY CLARIFICATION OF SAMPLE—

Transfer an accurately weighed amount of sample containing 3·5 to 6·5 mg of sodium cyclamate into a 200-ml calibrated flask. Dilute it to approximately 100 ml with distilled water, add 20 ml of zinc sulphate reagent and mix. Add 20 ml of potassium hexacyanoferrate(II) reagent, dilute to volume with distilled water, mix and filter the mixture on a Whatman No. 4 filter-paper. Discard the precipitate.

ANALYSIS OF CLARIFIED SAMPLE BY MANUAL METHOD—

Transfer by pipette 20 ml of the clarified filtrate into a 200-ml calibrated flask and dilute to nearly 130 ml with water. With a pipette, add 25 ml of 0·100 mg ml^{-1} sodium nitrite solution and 20 ml of 1 N hydrochloric acid. Shake the solution occasionally during the next 30 minutes, then add by pipette 20 ml of 0·500 mg ml^{-1} safranine solution, dilute to volume with distilled water and mix. After 20 minutes, read the optical density at wavelength 610 nm by using a 1-cm cell with water in the reference cell. Read off from the calibration

* For details of Parts I and II of this series, see reference list on p. 754.

graph (see below) the corresponding cyclamate content and hence calculate the cyclamate content of the sample.

CALIBRATION GRAPH FOR MANUAL METHOD—

Range 3·5 to 6·5 mg—To a series of 200-ml calibrated flasks add volumes of standard sodium cyclamate solution containing 3·5, 4·5, 5·0, 5·5 and 6·5 mg of sodium cyclamate. Dilute to almost 130 ml with water and complete the analysis by using the general procedure described above under Analysis of clarified sample by manual method. Construct a calibration graph relating optical density to amount of cyclamate in milligrams. (Under our conditions this graph obeyed the equation $y = -0{\cdot}028x$, where y is the optical density and x μg ml^{-1} is the equivalent concentration of cyclamate in the final solution.)

APPARATUS AND REAGENTS FOR SEMI-AUTOMATED METHOD—

Technicon AutoAnalyzer equipment is used with the manifold shown in Fig. 1 and a sampling rate of 40 per hour. The dialyser is fitted with a cuprophane membrane and the colorimeter with Ilford 607 filters.

Safranine solution, 0·20 mg ml^{-1}—Dissolve 0·20 g of safranine O [Gossypimine; C.I. 50240 (841)] in water. Filter the solution through a Whatman GF/C glass filter-paper into a 1-litre calibrated flask. Add by pipette 10 ml of concentrated hydrochloric acid followed by 300 ml of 95 per cent. ethanol. Dilute to volume with water and mix.

Standard sodium nitrite solution, aqueous, 0·42 mg ml^{-1}.

Standard sodium cyclamate solution, aqueous, 0·7, 0·8, 0·9, 1·0, 1·1 and 1·2 mg ml^{-1}.

Hydrochloric acid, 0·6 N.

Modified Carrez reagent.—Prepare as described under Reagents for manual method.

PROCEDURE FOR SEMI-AUTOMATED METHOD—

By using the manifold shown in Fig. 1, set the base-line with water flowing continuously through both the sample and the sodium nitrite lines and with other reagents flowing according

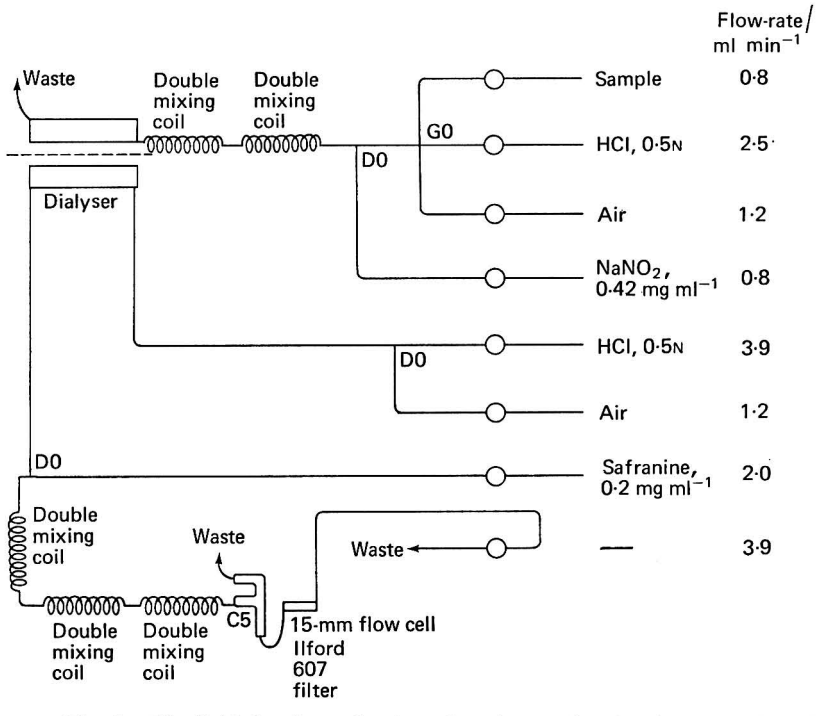

Fig. 1. Manifold for determination of cyclamate by the determination of excess of nitrite with safranine. All Tygon tubing

to the manifold diagram. After recording the base-line, transfer the sodium nitrite line from water to the 0·42 mg ml^{-1} sodium nitrite reagent. Adjust the concentration of the sodium nitrite reagent as necessary (so as to allow for day-to-day variation in the metering characteristics of the manifold) in order to give an upper setting of about 0·8 optical density unit. Check the base-line and upper setting, then sample a series of sodium cyclamate solutions (0·7 to 1·2 mg ml^{-1}), followed by the prepared samples (clarified as described above). From a calibration graph, relating optical density values to cyclamate contents of the aqueous standards, determine the cyclamate contents of the samples.

INTERFERENCE—

Investigations have shown that omission of the clarification stage in the analysis of control samples of soft drinks gives sample blank values corresponding to 7 per cent. interference, calculated for a nominal cyclamate level of 1 mg ml^{-1}. This interference is mainly caused by fruit components. Assessments of interferents that represent different classes of compounds are listed in Table I.

TABLE I

INTERFERENCE OF FUNCTIONAL GROUPS

Class of substance	Substance	Level of substance/mg ml^{-1}	Interference, per cent.
Reductants	Ascorbic acid	0·5	10
	Sulphur dioxide	0·5	10
	Hydroquinone	0·5	10
–SH group	Glutathione	0·05	10
	L-Cysteine	0·05	10
Phenol	Phloroglucinol	0·003	10
Carbonyls	Acetaldehyde	0·1	1
	Acetone	0·1	N.D.
Alcohols	Methanol	200	10
	Ethanol	13	10
	Propan-2-ol	10	10
	Citronellol	2·5	10
	Glycerol	40	N.D.
	Propane-1,2-diol	20	N.D.
Esters	Geranyl acetate	7	10
	Ethyl acetate	2	N.D.
Amino-acids	L-Glutamic acid	1	N.D.
	Arginine	1	N.D.
	Aspartic	1	N.D.
Amine	Methylamine	1	N.D.

N.D. = not detectable (limit of detection corresponds to 0·5 per cent. interference).

Various methods of reducing the 7 per cent. interference due to the basic constituents of cyclamate-free drinks have been investigated. Results in Table II show that precipitation with zinc hexacyanoferrate(II) decreases the interference in control samples to zero.

TABLE II

REMOVAL OF INTERFERENCE

Type of reaction	Method of treatment	Effect of interference
Removal of volatiles	Preliminary boil	Nil
Masking of amino-acids	Addition of 1 N NaOH - 0·1 per cent. HCHO	Nil
Precipitation	Addition of zinc hexacyanoferrate(II)	Decreased to zero
	Addition of neutral lead acetate	Decreased to 1 per cent.
	Addition of tungstophosphoric acid	Decreased to 1 per cent.

In further investigations, specific interferents have been added to solutions containing concentrations of sugar and acid similar to those used in soft drinks formulations and the percentage interference remaining after clarification has been determined, for a nominal cyclamate content of 1 mg ml^{-1} (Table III).

TABLE III

INTERFERENCE DUE TO SPECIFIC ADDITIVES (AFTER CLARIFICATION)

Additive	Concentration of additive/mg ml^{-1}	Interference, per cent.
Sulphur dioxide	0·5	1
Ascorbic acid	1·0	1
Phloroglucinol	0·003	2
Propan-2-ol	10·0	4
Glutathione	10·0	N.D.

N.D. = not detectable (limit of detection corresponds to 0·5 per cent. interference)

EFFECT OF ACIDITY ON THE RATE OF REACTION OF NITRITE WITH SAFRANINE—

The results obtained (Table IV) are in general agreement with those reported by Santacana.[3]

TABLE IV

EFFECT OF ACIDITY ON RATE OF REACTION OF NITRITE WITH SAFRANINE

Acidity (as N HCl)	0·03	0·07	0·10	0·20
90 per cent. reaction time/minutes ..	20	8	6	3
Optical density* (610 nm, 1-cm cell)	0·540	0·600	0·635	0·680

* Uncorrected for background absorption (see below).
Reaction mixtures: safranine, 50 μg ml^{-1}; sodium nitrite, 5 μg ml^{-1}; hydrochloric acid as specified.

OVERLAP OF THE ABSORPTION CURVES FOR SAFRANINE AND THE NITRITE - SAFRANINE COMPLEX—

In considering the optimum wavelength to choose for the colorimetric determination of nitrite with safranine, it is important to assess background interference due to unconsumed safranine remaining in the reaction mixture. In Fig. 2, the absorption spectra of safranine in 0·1 N hydrochloric acid medium, before and after reaction with nitrite, are shown. Similar curves have also been obtained by using hydrochloric acid concentrations of 0·07 and 0·20 N. The effect of acidity on the sensitivity of the reaction (ϵ) and on the percentage interference due to unconsumed safranine at various wavelengths is given in Table V. These values have been calculated on the basis that 6·65 μg of sodium nitrite reacts with 50 μg of safranine (Fig. 3); thus 5 μg of sodium nitrite is equivalent to 37·5 μg of safranine and the unconsumed

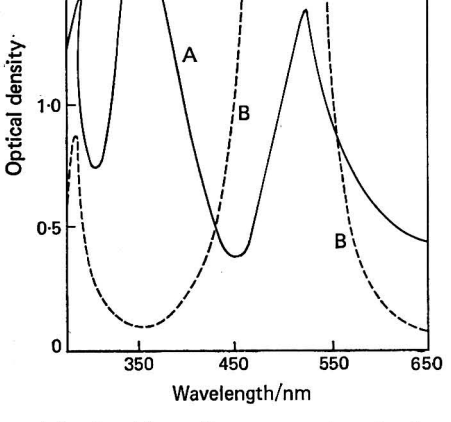

Fig. 2. Absorption curves: A, safranine (50 μg ml^{-1}) and sodium nitrite (5 μg ml^{-1}) in 0·1N HCl; and B, safranine (50 μg ml^{-1}) in 0·1N HCl

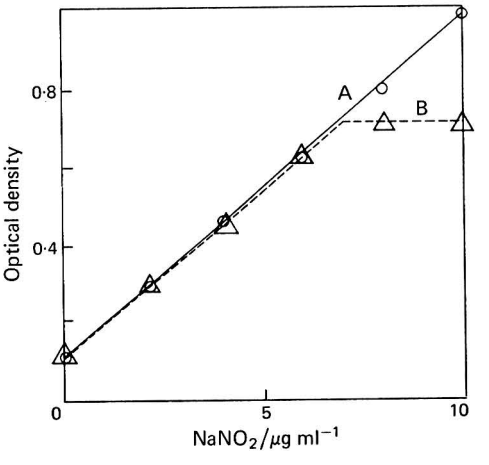

Fig. 3. Calibration graphs for the reaction of nitrite with safranine: A, safranine (100 μg ml^{-1}) and nitrite, in 0·1N HCl, at wavelength 610 nm; and B, safranine (50 μg ml^{-1}) and nitrite in 0·1N HCl, at wavelength 610 nm

safranine amounts to 25 per cent. of the safranine originally present; ϵ values in Table V have been calculated after correcting for the absorption due to unconsumed safranine. The percentage interference has been evaluated as the optical density due to 25 per cent. of the safranine originally present divided by the optical density determined after reaction and multiplied by 100. Although the results in Table V clearly indicate that at $\lambda_{max.}$ (352 nm) the sensitivity is at a maximum and interference at a minimum, the presence of ultraviolet-absorbing substances in soft drinks invalidates the value of measurements at 352 nm and a detailed investigation at the 600-nm region has therefore been made.

Table V

Calculated values* for the sensitivity and interference in the reaction of nitrite with safranine

	0·07 N hydrochloric acid		0·10 N hydrochloric acid		0·20 N hydrochloric acid	
Wavelength/nm	Sensitivity ($\epsilon \times 10^3$)	Interference, per cent.	Sensitivity ($\epsilon \times 10^3$)	Interference, per cent.	Sensitivity ($\epsilon \times 10^3$)	Interference, per cent.
352 ($\lambda_{max.}$)	—	—	23·5	3	—	—
568	8·2	12	8·7	13	8·6	19
575	8·1	11	8·5	11	8·3	18
588	8·1	6	8·2	7	7·8	14
606	6·7	3	7·1	3	6·7	12
625	5·6	2	6·0	2	5·4	7
645	4·1	3	4·8	2	5·4	4
667	2·0	4	2·4	4	5·4	2

* Corrected for background absorption due to unconsumed nitrite.
Reaction mixtures: safranine, 50 μg ml^{-1}; sodium nitrite, 5 μg ml^{-1}; hydrochloric acid as specified.

Calibration graphs for the nitrite - safranine reaction—

The results obtained are shown graphically in Fig. 3. Optical densities were determined at wavelength 610 nm in a 1-cm cell, after allowing colour development to proceed for 20 minutes. For the nitrite - safranine complex at 610 nm, $\epsilon = 6{\cdot}9 \times 10^4$.

Results and discussion

Results of analyses obtained by the manual and semi-automated procedures are given in Table VI.

Table VI

Determination of cyclamate in soft drinks with 1 mg ml^{-1} of added cyclamate

	Manual method		Semi-automated method	
Type of soft drink*	Orange	Lemon	Orange	Lemon
Number of samples analysed ..	10	10	50	50
Mean recovery, per cent.	100·4	101·2	100·6	101·6
Recovery range, per cent.	98 to 102	98 to 105	99 to 103	99 to 104
Mean deviation, (±) per cent. ..	1·0	2·2	0·9	1·8

*Drinks did not contain added sulphur dioxide.

Both the manual and semi-automated procedures are simple, relatively rapid and give good reproducibility. The semi-automated procedure with safranine, in contrast to the procedure based on the Bratton - Marshall reaction,[1] is free from technical difficulties and is the more reliable automated method to perform.

Interference associated with nitrous acid reactive substances in soft drinks has been largely eliminated by preliminary clarification with zinc hexacyanoferrate(II). In cyclamate determinations at the 1 mg ml^{-1} level, the presence of sulphur dioxide as preservative at the maximum permitted concentration, *viz.*, 350 p.p.m.,[4] causes less than 1 per cent. interference. Propan-2-ol, which is occasionally used as a solvent for minor ingredients of soft drinks, results in about 4 per cent. interference at the 1 per cent. concentration level.

Weak nitrosating conditions have been used for the reaction of nitrite with cyclamate as they preclude the formation of cyclohexyl nitrite, which is also reactive towards safranine.[5]

Investigations have also shown that, for the nitrite - safranine reaction, an acidity of 0·1 N hydrochloric acid can be considered to be the optimum acidity (Tables IV and V). When the concentration of safranine is limited to 50 μg ml^{-1}, a maximum of 6·65 μg ml^{-1} of sodium nitrite reacts (Fig. 3), which suggests that the molar ratio of safranine to nitrite is approximately 3:2, assuming a relative molecular mass of 360 for safranine. On the basis of this result, the safranine concentration used in the semi-automated procedure has been selected so as to limit the maximum colour development, thus enabling an adequate check to be maintained on the upper setting.

In conclusion, the safranine method of determining cyclamate described in this paper has been compared with other published procedures (Table VII). For small-scale routine control purposes, the manual safranine method is considered to be the most suitable. For large-scale application, when rapidity and simplicity of analysis are of importance, the semi-automated method is more appropriate. The procedure will, however, be of greater value when the clarification stage has also been automated, *e.g.*, by using a continuous filter-strip technique. For reference purposes, the gas - liquid chromatographic procedure,[6] which is highly selective, reliable and accurate, remains the method of choice.

TABLE VII

COMPARISON OF METHODS FOR DETERMINING CYCLAMATE AT THE 1 mg ml^{-1} LEVEL IN SOFT DRINKS

	Determination of cyclohexene by gas - liquid chromatography		Safranine method				Bratton - Marshall method			
			Manual		Semi-automated		Manual		Automated	
Samples*	O	L	O	L	O	L	O	L	O	L
Number of samples analysed	10	10	10	10	50	50	10	10	20	20
Mean recovery, per cent.	98·9	99·7	100·4	101·2	100·6	101·6	106·4	106·9	106·1	106·6
Recovery range, per cent.	98 to 100	99 to 101	98 to 102	98 to 105	99 to 103	99 to 104	104 to 109	104 to 110	103 to 109	103 to 110
Mean deviation, (±) per cent.†	0·54	0·56	1·0	2·2	(0·9) S.D.	(1·8) S.D.	1·6	2·0	1·5	2·1
Sample blank, per cent.	0		1	1	1	1	6	6	6	6
Interference	Nil		Sulphur dioxide, ascorbic acid, alcohols and phenols				Alcohols, aldehydes and terpenes			

*O = orange; L = lemon.
†S.D. = standard deviation.

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NOTE—References 1 and 6 are to Parts II and I of this series, respectively.

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The Determination of Non-fat Milk Solids in Milk Bread from the Orotic Acid Content

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A method is described for the determination of the content of non-fat milk solids in milk bread. Orotic acid (2,6-dihydroxypyrimidine-4-carboxylic acid) present in the milk solids is extracted from the bread with water and determined by a colorimetric procedure, and the content of non-fat milk solids is calculated from the orotic acid content of the milk bread. The mean orotic acid content of the non-fat milk solids examined was 62·5 mg per 100 g (range 48·0 to 74·5 mg per 100 g).

METHODS for the determination of milk solids in bread depend on the determination of a component of the milk that is not normally present in bread, such as lactose,[1] or of a component that, if present in bread, can be allowed for and a suitable correction applied, *e.g.*, calcium. The possible addition of permitted food additives that contain calcium, such as calcium hydrogen orthophosphate, calcium propionate or calcium carbonate, generally renders the latter method impracticable. Lactose in milk bread can be determined by a lengthy titrimetric method[2] or by paper chromatography,[3] but varying amounts of lactose may be lost during the production of the milk bread,[4] thus affecting the calculated milk solids content. Recently, following a suggestion made by Brieskorn and Wallrauch,[5] the orotic acid (2,6-dihydroxypyrimidine-4-carboxylic acid) content of milk has been used as a basis for calculating the buttermilk content of milk-containing baked foods.[6] The orotic acid content was determined by the method of Adachi, Tanimura and Asahina[7] as modified by Brieskorn and Wallrauch.[5] This procedure has been applied to the determination of non-fat milk solids in milk bread.

METHOD

REAGENTS—

Carrez solution I—A 23 per cent. *m/V* solution of zinc sulphate.
Carrez solution II—A 15 per cent. *m/V* solution of potassium hexacyanoferrate(II).
Saturated bromine water—An excess of liquid bromine should be present.
Ascorbic acid solution, 10 *per cent.* m/V.
p-*Dimethylaminobenzaldehyde solution*, 3 *per cent.* m/V *in propan*-1-*ol.*
n-Butyl acetate.
Standard orotic acid solutions—Dissolve 50 mg of orotic acid in a mixture of 10 ml of water and 1 ml of concentrated ammonia solution and dilute to 500 ml. Dilute 10 ml of this solution to 100 ml with water and dilute 2·5, 5, 10 and 15 ml of this solution to 50 ml with water to give solutions containing 2·5, 5, 10 and 15 μg of orotic acid per 5 ml.

PROCEDURE—

Determination of total solids content of milk bread samples—Dry the sliced loaf overnight at 40° C and mill the product to pass through a 1·5 mm diameter mesh screen; determine the total solids content by drying 2 g at 130 °C for 1 hour.

Extraction of orotic acid from skim milk powder—Add about 0·9 g of sample, accurately weighed, to 75 ml of warm water contained in a flask and mix gently so as to disperse the solid. Add 1 ml of Carrez solution I, mix and heat the mixture in order to produce a flocculent white precipitate. Add 1 ml of Carrez solution II, mix and heat to boiling. Filter the mixture through a fluted 15-cm Whatman No. 541 filter-paper (or equivalent) into a second flask. Wash down the sides of the first flask with about 50 ml of water, heat to boiling and pour the wash water through the same filter. Repeat the washing procedure with a further 20 ml of water. Cool the combined filtrates to room temperature, transfer the solution to a 250-ml

calibrated flask and dilute to volume with water. Take 5 ml of this solution for the determination of orotic acid.

Extraction of orotic acid from milk bread—Weigh 5 g of the sample (dried at 40 °C) into a 100-ml macerator flask (*e.g.*, M.S.E. Homogeniser, Catalogue No. 7700, or equivalent), add 100 ml of water and mix at as high a speed as possible for 1 minute. Allow the coarser particles to settle briefly and filter the supernatant liquid through a fluted 15-cm Whatman No. 541 filter-paper (or equivalent), rejecting the first 10 to 15 ml of filtrate. Take 5 ml of this solution for the determination of orotic acid.

Determination of orotic acid—With a pipette, transfer into a series of glass-stoppered test-tubes 5 ml of water (to act as a blank), 5 ml of each of the standard orotic acid solutions and 5 ml of the test solutions containing 2 to 15 μg of orotic acid per 5 ml of solution. Add to each tube 1·5 ml of saturated bromine water and allow the mixture to stand at room temperature for 1 minute (Note 1). Add 2 ml of the ascorbic acid solution to each tube and place the tubes in a water-bath at 40 °C for 5 minutes. Add 3 ml of the *p*-dimethylaminobenzaldehyde solution to each tube and return the tubes to the water-bath at 40 °C for a further 10 minutes. Remove the tubes from the water-bath, cool to room temperature and add to each tube 4·0 ml of n-butyl acetate (Note 2); shake the mixtures vigorously for 15 s and allow the layers to separate. Transfer the upper layer to a dry test-tube containing about 1 g of anhydrous sodium sulphate, mix gently, add approximately 1 g of anhydrous sodium sulphate, mix gently and allow the solid to separate out. Transfer the clear n-butyl acetate layer into a 1-cm cell and measure the absorbance at its maximum (at about 461 to 462 nm) against the colourless n-butyl acetate blank obtained from the 5 ml of water.

Calculate the mean value of the absorption for 10 μg of orotic acid per 5 ml of solution from the sum of the absorbances of the standard orotic acid solutions (or plot absorbance against concentration) and calculate the orotic acid content of the 5 ml of aqueous extracts and hence the orotic acid content of the samples. Calculate the orotic acid content of the milk bread on a dry basis.

The calcium was determined by atomic-absorption spectroscopy[8] and the lactose was determined by an enzymatic procedure.[9]

The results for the bread samples were calculated on a dry basis.

NOTES—

1. By the time that the bromine has been added to the last tube in a series, the first tube will have stood long enough. A bromination time of from 20 s to 5 minutes is satisfactory[7] and it is not necessary to measure the time needed for this stage. The ascorbic acid solution can then be added immediately to the tubes, and in the same order as the addition of the bromine water.

2. The n-butyl acetate can be conveniently added from a 50-ml burette.

DISCUSSION AND RESULTS

The method used for the extraction of orotic acid from skim milk powder was that of Brieskorn and Wallrauch[5] but their lengthy extraction and evaporation procedure with milk bread has been simplified. The period of bromination was found not to be critical, which is in agreement with the results obtained by Adachi *et al.*,[7] although Motz,[10] who used a similar procedure, specified a bromination time of 30 ± 2 s. The recovery of known amounts of orotic acid was unaffected by the presence of potassium bromide, potassium bromate, ascorbic acid, glyceryl monostearate or sodium stearyl fumarate at the levels normally present in bread.

The mean orotic acid content of thirty-nine samples of skim milk powder from various manufacturers was 62·5 mg per 100 g (range 48·0 to 74·5 mg per 100 g); eight samples of full cream milk powder gave a mean orotic acid content of 62·9 mg per 100 g (range 52·0 to 69·6 mg per 100 g) on a fat-free basis. These values are similar to those reported by Brieskorn and Wallrauch[5] for skim milk powders (60·0 to 71·0 mg per 100 g) and to those reported for full cream milk powders by Motz[10] (53·4 to 81·7 mg per 100 g; calculated on a fat-free basis, assuming a content of 26 per cent. of fat), but are approximately half those reported by Okonkwo and Kinsella[11] (112 to 134·6 mg per 100 g) for food milk powders. The mean orotic acid content of twenty bulk samples of cows milk was 65·3 mg l^{-1} (range 53·3 to 70·9 mg l^{-1}), which is similar to values found for cows milk in Germany,[5] Austria[12] and France,[13] but slightly less than that reported from North America.[11]

Two samples of skim milk powder were used to prepare milk loaves of known milk solids content and these loaves were analysed for their orotic acid and lactose contents. The results obtained were compared with the values calculated from the known composition of the skim milk powders used in preparing the milk loaves; these results are shown in Table I.

TABLE I

RECOVERY OF LACTOSE AND OROTIC ACID FROM STANDARD MILK LOAVES

	Non-fat milk solids added, per cent. on dry basis	Lactose			Orotic acid		
		Added/g per 100 g	Found/g per 100 g	Recovery, per cent.	Added/mg per 100 g	Found/mg per 100 g	Recovery, per cent.
A	0	0	0		0	0·09	
	1·1	0·46	0·30	65·2	0·54	0·52	96·3
	2·1	0·89	0·69	77·5	1·03	0·98	95·1
	3·2	1·35	1·07	79·2	1·56	1·44	92·3
	4·2	1·77	1·46	82·5	2·05	2·04	99·5
	6·2	2·62	2·44	93·5	3·03	3·02	99·6
B	0	0	0		0	0·15	
	2·1	0·91	0·72	79·0	1·23	1·25	101·5
	4·1	1·78	1·36	76·4	2·40	2·23	92·9
	6·1	2·64	2·08	78·9	3·57	3·39	95·0
			Mean ..	79·0		Mean ..	96·5

A. Non-fat milk solids contained 42·2 per cent. of lactose and 48·9 mg of orotic acid per 100 g.
B. Non-fat milk solids contained 43·4 per cent. of lactose and 58·6 mg of orotic acid per 100 g.

The recovery of lactose is lower and more variable than the recovery of orotic acid. This loss of lactose is in agreement with the results of Lee and Ronalds.[4] Samples of milk loaves available in New South Wales were also analysed for orotic acid, lactose and calcium; these results are shown in Table II. A value of 50 mg per 100 g was taken as a minimum value for the orotic acid content of skim milk powders available in New South Wales and this figure was used to calculate the non-fat milk solids contents shown in Table II. Most of the samples gave values close to the required standard of 4 per cent. of non-fat milk solids, calculated on a dry basis. The lactose content of the milk loaves shows an approximate correlation with the orotic acid content of the loaves, but in each instance the lactose content is less than that expected from the orotic acid content. The mean value for the ratio $\frac{\text{orotic acid (milligrams per 100 g)}}{\text{lactose (grams per 100 g)}}$ found in the skim milk powders was 1·16 (range 0·97 to 1·39), whereas in the milk loaves (Table II) the mean value for the ratio was 1·77 (range

TABLE II

OROTIC ACID, LACTOSE, CALCIUM AND CALCULATED CONTENTS OF NON-FAT MILK SOLIDS IN MILK LOAVES

Sample No.	Orotic acid/ mg per 100 g	Lactose/ g per 100 g	Calcium/ mg per 100 g	Calculated non-fat milk solids, per cent., from— orotic acid content	Calculated non-fat milk solids, per cent., from— calcium content
1	2·30	1·85	124	4·6	—
2	2·08	1·70	97	4·2	—
3	2·08	1·37	72	4·2	—
4	2·06	1·17	82	4·1	—
5	2·05	1·30	68	4·1	—
6	2·04	1·22	120	4·1	—
7	1·76	0·98	61	3·5	—
8	1·75	0·87	68	3·5	—
9	1·71	1·45	71	3·4	—
10	1·56	1·07	92	3·1	—
11	1·38	0·62	49	2·8	2·6
12	0·98	0·28	53	2·0	3·0
13	0·88	0·39	37	1·8	1·6
14	0·77	0·53	42	1·5	2·0

1·18 to 3·50). An approximate maximum content of non-fat milk solids for samples 11 to 14 can be calculated from the calcium content, assuming that a plain loaf contains 20 mg of calcium per 100 g and skim milk powder 1·1 per cent. of calcium. The results are shown in Table II and confirm the low values for the added non-fat milk solids calculated from the orotic acid content. This procedure will obviously give values that are too high when applied to bread containing added calcium compounds.

Conclusion

A procedure is described for the determination of added non-fat milk solids in milk bread, which is based on the orotic acid content of milk. Orotic acid appears to be unaffected by bread-making processes, in contrast to lactose, and the procedure is applicable in the presence of added calcium compounds.

The author is grateful to the Bread Research Institute of Australia, North Ryde, for the provision of the standard milk loaves and skim milk powder samples, and acknowledgement is made to the Director and Government Analyst and the New South Wales Department of Health for permission to publish this paper.

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Detection of Thioureas, Thiosemicarbazides and Monothiosemicarbazones with 2,3-Dichloro-1,4-naphthoquinone

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Thiosemicarbazones, thiosemicarbazides, thioureas and also isothiocyanates are made to react with 2,3-dichloro-1,4-naphthoquinone in an ethanolic medium and on rendering the mixtures alkaline with ethanolic ammonia solution, a red to violet colour is developed with absorption maxima between 500 and 570 nm. The reaction forms the basis of a spot test for these compounds.

Thioureas, thiosemicarbazides and monothiosemicarbazones can be detected with Grote's reagent,[1] 7,7,8,8-tetracyanoquinodimethan,[2] sodium pentacyanonitrosylferrate(III),[3,4] pentacyano complex iron salts[5] and molybdophosphoric acid.[6] 2,3-Dichloro-1,4-naphthoquinone is known to react in aqueous solution with compounds that contain –SH groups, such as cysteine, glutathione and thiamine, in the presence of potassium carbonate to give a coloured product.[7] As thio compounds are known to exist as a thio - thiol equilibrium mixture (reaction 1), it was thought to be of interest to extend the use of this reagent to the detection of thiourea and its derivatives.

$$\mathrm{RNH{-}\overset{\overset{S}{\|}}{C}{-}NH_2 \rightleftharpoons RN{=}\overset{\overset{SH}{|}}{C}{-}NH_2 \rightleftharpoons RNH{-}\overset{\overset{SH}{|}}{C}{=}NH} \quad \ldots \quad \ldots \quad (1)$$

However, under experimental conditions similar to those previously reported,[7] this reagent failed to produce any measurable colour change with the thiourea derivatives under investigation. Reyes and Silverstein[8] have noted that in non-polar solvents thioketones exist predominantly in the enolic form. Therefore, it was thought to be of interest to investigate the reaction in absolute ethanol, a less polar solvent than water. Because of its limited solubility in ethanol, potassium carbonate is unsuitable for making the solution alkaline and a number of bases such as pyridine, quinoline, ethylamine, aniline and ethanolic ammonia solution were therefore tried for this purpose. All of these compounds except ethanolic ammonia solution react with the reagent itself to give a red colour. Therefore, ethanolic ammonia solution, which caused only a slight change in colour, was used to make the reaction medium alkaline.

In the present work, experimental conditions were sought in order to develop a specific spot test for detecting micro-scale amounts of thioureas, thiosemicarbazides, monothiosemicarbazones and isothiocyanates (henceforth referred to as "thio compounds").

Experimental

Apparatus—

All spectral measurements were carried out on a Spectronic 20 spectrophotometer (Bausch & Lomb) with four matched 10-ml cells that had a 1-cm light path.

Reagents and materials—

Thiosemicarbazones of benzaldehyde, 4-methoxybenzaldehyde, 2-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3-methoxy-4-hydroxybenzaldehyde and acetone were prepared by an established method.[9] Substituted thiosemicarbazides and phenylthiourea were synthesised by a previously reported procedure,[10] and 2,3-dichloro-1,4-naphthoquinone was prepared by the method described earlier.[11] 4-Acetylaminobenzaldehyde thiosemicarbazone (B.P.C.), allyl isothiocyanate (E. Merck), phenyl isothiocyanate (Koch-Light Laboratories Ltd.), 1,4-phenylene diisothiocyanate (Hoechst), thiourea and absolute ethanol (Indian Pharmacopoeia) were also used. All other reagents were of analytical-reagent grade.

Preparation of 2·5 per cent. m/V ethanolic ammonia solution—Dry ammonia vapour was passed into absolute ethanol at −5 °C until the mass had increased by 20 per cent. The solution was then appropriately diluted with absolute ethanol.

2,3-Dichloro-1,4-naphthoquinone reagent solution—The reagent (65·0 mg) was dissolved in absolute ethanol and diluted to 250 ml. The solution was stored in a refrigerator.

PROCEDURE FOR DETECTION OF THIO COMPOUNDS—

Plate method—About three drops of a 0·1 mg ml^{-1} ethanolic solution of the thio compound were spotted on to a white porcelain spot plate and 0·5 ml of the reagent solution and 0·3 ml of ethanolic ammonia solution were added successively to the spot. After 5 minutes, the coloured spot was compared with the yellow-coloured reference spot given by the reagents alone.

Spot test on paper—About three drops of the test solution (containing thio compounds equivalent to 0·1 mg ml^{-1}) were transferred on to a 1 inch diameter Whatman No. 1 filter-paper and allowed to dry; 0·3 ml of the reagent solution was then spotted on to the spot and allowed to dry. The yellowish spot thus obtained changes to red and then violet when exposed to ammonia vapour for about 2 minutes. After 5 minutes, the coloured spot was compared with the yellow reference spot produced by the reagents alone.

DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORBANCE—

The ethanolic solution of the various thio compounds (1·0 ml; 1 μmol ml^{-1}) was mixed with 3·0 ml of ethanolic ammonia solution and 15·0 ml of the reagent solution in a 25-ml calibrated flask. The volume was adjusted to the mark with ethanol and the solution was allowed to stand for 1 hour at room temperature. The absorbance was measured in the range 420 to 600 nm against a reagent blank. The blank solution consisted of a mixture of 15·0 ml of the reagent solution and 3·0 ml of ethanolic ammonia solution diluted to 25 ml with absolute ethanol.

TABLE I

ABSORBANCE OF PRODUCTS OF THIO COMPOUNDS WITH 2,3-DICHLORO-1,4-NAPHTHOQUINONE

Thio compounds	Concentration/μmol per 25 ml	λ_{max}	Absorbance at λ_{max}.*
Thiosemicarbazones—			
4-Acetylaminobenzaldehyde thiosemicarbazone	1·00	560	0·680
Benzaldehyde thiosemicarbazone	1·00	540	0·780
2-Hydroxybenzaldehyde thiosemicarbazone	1·00	530	1·000
4-Hydroxybenzaldehyde thiosemicarbazone	1·00	560	0·335
4-Methoxybenzaldehyde thiosemicarbazone	1·00	560	0·690
3-Methoxy-4-hydroxybenzaldehyde thiosemicarbazone	1·00	560	0·310
Acetone thiosemicarbazone	1·00	520	0·200
Thiosemicarbazides—			
Thiosemicarbazide	1·00	500	0·045
4-Methyl-1-(3,4-methylenedioxybenzoyl)-3-thiosemicarbazide	1·00	530	0·025
4-Phenyl-1-(3,4-methylenedioxybenzoyl)-3-thiosemicarbazide	1·00	520	0·230
4-Methyl-1-benzoyl-3-thiosemicarbazide	1·00	520	0·025
4-Phenyl-1-benzoyl-3-thiosemicarbazide	1·00	520	0·200
Thioureas—			
Phenylthiourea	100·00	520	0·750
Thiourea	100·00	540	0·102
Isothiocyanates—			
Allyl isothiocyanate	100·00	520	0·025
Phenyl isothiocyanate	100·00	530	0·610
1,4-Phenylene diisothiocyanate	1·00	530	0·060

* Each value is the average of five determinations.

Discussion

Ethanolic solutions of all the thio compounds tested give a red to violet colour when allowed to react with a solution of 2,3-dichloro-1,4-naphthoquinone in the presence of ammonia. The colour develops within 5 minutes. The absorbance maxima of the coloured products formed are in the range 500 to 570 nm (Table I). Maximum colour intensity is obtained within 1 hour and remains constant for about 2 to 3 hours. Under the experimental conditions used, ureas, semicarbazides, semicarbazones and inorganic thiocyanates do not give detectable coloured products with the reagent.

As it is reported in the literature[12] that alkyl and aryl isothiocyanates give corresponding thioureas in the presence of ammonia, the investigation was extended to include isothiocyanates (Table I). It appears that in the presence of ammonia, isothiocyanates are first converted into corresponding thioureas, which then react with the reagent to yield coloured products, as indicated above.

It is evident from the results given in Table I that the sensitivity of the test decreases in the following order: thiosemicarbazones > thiosemicarbazides > thioureas > isothiocyanates.

Further, it is noted that substituents in thio compounds play an important rôle in the reaction. The presence of a phenyl group increases the resonance energy of the enolic tautomer to a greater extent than that of the ketonic form, which results in an equilibrium that favours the formation of the thioenolic tautomer.[13] This effect explains the higher sensitivity obtained when phenyl-substituted thiosemicarbazides, thioureas and isothiocyanates are tested with this reagent.

Further work on the detection and determination of thio - thiol forms in thio compounds and the nature of products formed in the reaction with 2,3-dichloro-1,4-naphthoquinone is in progress.

Conclusion

Thioureas, thiosemicarbazides, monothiosemicarbazones and isothiocyanates can be selectively detected in micro-scale amounts by a simple spot test with 2,3-dichloro-1,4-naphthoquinone in ethanolic medium.

The authors express their sincere thanks to Dr. C. S. Shah, Principal, L.M. College of Pharmacy, Ahmedabad-9, India, for the facilities provided for carrying out this work. They are also grateful to Hoechst Pharmaceuticals Ltd., Bombay, for their generous gift of 1,4-phenylene diisothiocyanate (Jonit).

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Spectrophotometric Determination of 4-Acetylaminobenzaldehyde Thiosemicarbazone (Thiacetazone) with 2,3-Dichloro-1,4-naphthoquinone

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4-Acetylaminobenzaldehyde thiosemicarbazone (thiacetazone) is made to react with 2,3-dichloro-1,4-naphthoquinone in an ethanolic medium. When the mixture is rendered alkaline with ammonia a purple product with an absorption maximum at 560 nm is obtained. A method based on this reaction is described for the quantitative determination of micro-scale amounts of thiacetazone. The procedure is applied to its determination in tablets. The results are compared with those obtained by the official method.

MONOTHIOSEMICARBAZONES are used as drugs in the therapy of tuberculosis,[1] viral and protozoal diseases,[2,3] and of certain types of tumours.[4] 4-Acetylaminobenzaldehyde thiosemicarbazone (thiacetazone) alone and in combination with isoniazid is used as an antitubercular agent. Various methods for its determination include titrimetric,[5–9] gravimetric,[10–12] colorimetric and spectrophotometric[13–16] procedures. The official method[17] involves the precipitation of thiacetazone with silver nitrate in methanolic solution. The complex formed between silver and thiacetazone is then determined gravimetrically. In a recent variation of this method, Campbell, Grzeskowiak and Turner[18] have titrated potentiometrically the acid liberated during the complexation of aromatic monothiosemicarbazones with silver ion.

The reaction of monothiosemicarbazones with 2,3-dichloro-1,4-naphthoquinone to form a coloured product has been reported in the preceding paper.[19] This reaction forms the basis of a specific quantitative method for the spectrophotometric determination of thiacetazone.

In the present work, reaction conditions were established for the determination of thiacetazone in pure samples and in tablets containing it. The results compare favourably with those obtained by the official procedure.[17]

EXPERIMENTAL

APPARATUS—

All the spectral measurements were carried out as described previously.[19]

REAGENTS AND MATERIALS—

4-Acetylaminobenzaldehyde thiosemicarbazone was repeatedly recrystallised from ethanol (melting-point of final product, 227 °C). 2,3-Dichloro-1,4-naphthoquinone was prepared by the method described earlier.[20] All other reagents were of analytical-reagent grade.

The reagents 2·5 per cent. ethanolic ammonia solution and 2,3-dichloro-1,4-naphthoquinone reagent solution were prepared as described previously.[19]

Standard solution of thiacetazone—An accurately weighed amount of thiacetazone (12·0 mg) was dissolved in sufficient warm absolute ethanol to give 100 ml of final solution; a 25-ml aliquot of this solution was further diluted to 100 ml with the same solvent.

PROCEDURE—

Determination of wavelength of maximum absorbance—A 5·0-ml volume of standard thiacetazone solution was mixed with 3·0 ml of ethanolic ammonia solution in a 25-ml calibrated flask; 15·0 ml of the reagent solution were added and the final volume was adjusted to the mark with absolute ethanol. The reaction mixture was then allowed to stand for 1 hour at room temperature. The absorbance was measured in the range 420 to 600 nm against the blank (Fig. 1). For the blank experiment, 15·0 ml of reagent solution and 3·0 ml of ethanolic ammonia solution were mixed and the mixture was diluted to 25 ml with absolute ethanol.

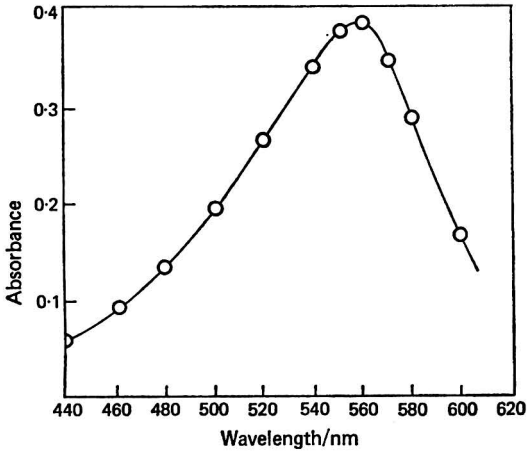

Fig. 1. Absorption spectrum of the reaction product of thiacetazone (0·625 μmol) with the reagent solution (15·0 ml) in the presence of 3·0 ml of ammonia solution in 25 ml of the reaction mixture

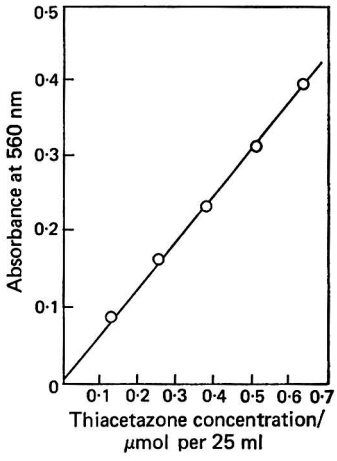

Fig. 2. Relationship between the amount of thiacetazone with the reagent solution (15·0 ml) when using 3·0 ml of ammonia solution in 25·0 ml of reaction mixture, and the absorption at 560 nm of the purple product formed

The amount of thiacetazone contained in the sample was determined by reference to a calibration graph. The absorbance at 560 nm was proportional to the amount of thiacetazone in the range 0·125 to 0·625 μmol per 25 ml of the reaction mixture (Fig. 2).

Analysis of thiacetazone tablets—Twenty tablets were weighed and powdered. An aliquot of the powder equivalent to 12·0 mg of thiacetazone was accurately weighed. Four portions of 20 ml of warm absolute ethanol were used to extract the thiacetazone from the powder and each extract was filtered through a Whatman No. 41 filter-paper. The residue on the filter-paper was then washed with 10 ml of warm ethanol. The filtrate and the washings were combined in a 100-ml calibrated flask and, after cooling, the volume was adjusted to the

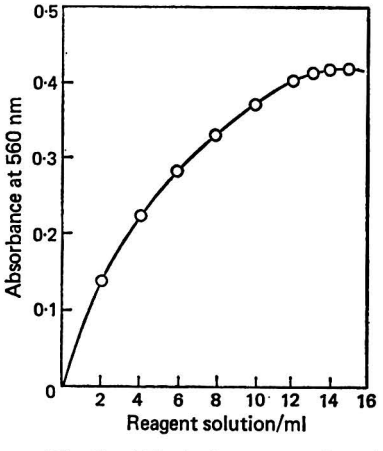

Fig. 3. Effect of concentration of the reagent solution on the absorption at 560 nm of the product formed on reaction with thiacetazone (0·625 μmol) when using 3·0 ml of ammonia solution in 25·0 ml of reaction mixture

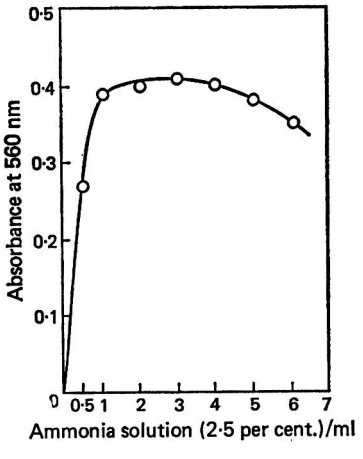

Fig. 4. Effect of concentration of added ammonia solution on the absorption at 560 nm of the product formed on reaction of thiacetazone (0·625 μmol) with the reagent solution (15·0 ml) in 25·0 ml of reaction mixture

mark with the same solvent; 25 ml of this solution was further diluted to 100 ml and analysed as described above.

FACTORS THAT AFFECT THE REACTION OF THIACETAZONE WITH 2,3-DICHLORO-1,4-NAPHTHOQUINONE—

Concentration of 2,3-dichloro-1,4-naphthoquinone—The absorbance at 560 nm of the coloured product formed by the reaction of thiacetazone (0·625 μmol) with this reagent increased with increasing concentration of the reagent. The maximum absorbance is obtained in the presence of 13·0 ml of reagent solution and remains constant on further increasing the volume of reagent solution added (Fig. 3).

Concentration of ammonia—The typical purple colour developed after the reaction of the ethanolic ammonia solution with the thiacetazone and the reagent. Maximum colour intensity was obtained in the presence of 3·0 ml of the ammonia solution (Fig. 4).

Temperature of reaction—The effect of temperature was studied at 0, 6, 22·5 and 37 °C. No appreciable change in absorbance was observed with these variations in temperature.

Time of reaction—The colour intensity was found to reach a maximum on maintaining the reaction mixture at room temperature for 50 minutes and remained constant for a further 3 hours (Fig. 5).

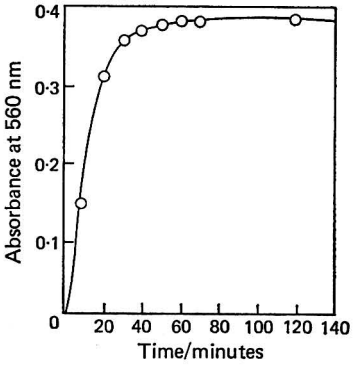

Fig. 5. Rate of development of purple colour after reaction of thiacetazone (0·625 μmol) with the reagent solution (15·0 ml) when using 3·0 ml of ammonia solution in 25·0 ml of reaction mixture

RESULTS

Thiacetazone samples obtained from market sources were analysed by the proposed procedure. The percentage recovery and the standard deviation calculated from a series of experiments are given in Table I.

TABLE I

DETERMINATION OF THIACETAZONE IN SAMPLES

Sample No.	Recovery, per cent., obtained by— Proposed method	Official method*
1	99·5 ± 1·1†	99·3 ± 2·2†
2	99·0 ± 1·1	99·2 ± 2·2

* Gravimetric procedure.[17]
† Standard deviation calculated from ten experiments.

Thiacetazone tablets were also analysed by the proposed procedure. The results obtained are shown in Table II and compare favourably with those obtained by the official method. The usual tablet diluents and lubricants do not interfere in the analysis by this method, which is simple, rapid and accurate.

TABLE II

DETERMINATION OF THIACETAZONE IN TABLETS

Sample	Labelled amount per tablet/mg	Recovery* per tablet by—	
		proposed method/mg	official method†/mg
A	25·00	24·94	25·39
B	50·00	49·34	49·03
C	50·00	47·79	49·50
D	75·00	76·56	76·35
E	125·00	127·40	128·60

* Each value is the average of five determinations.
† Spectrophotometric procedure.[17]

CONCLUSION

A spectrophotometric method has been established for the determination of thiacetazone in the concentration range 0·005 to 0·025 μmol ml^{-1}. The method is rapid and has a reproducibility of $\pm$1·1 per cent. and has been successfully applied to the analysis of thiacetazone tablets.

The authors express their sincere thanks to Dr. C. S. Shah, Principal, L.M. College of Pharmacy, Ahmedabad-9, India, for the facilities to carry out this work.

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Book Reviews

HIGH-PRECISION TITRIMETRY. By C. WOODWARD and H. N. REDMAN. *Analytical Sciences Monograph, No.* 1. Pp. viii + 63. London: Society for Analytical Chemistry. 1973. Price £2·50.

In the first part of this small book, which is the first in a series of Analytical Sciences Monographs, the authors describe the procedures required for the visual titrimetric determination of many substances with an accuracy and precision of within ±0·01 per cent. They describe the apparatus needed in titrimetric work, the preparation and assay of standard substances, namely hydrochloric acid, sodium carbonate and sulphamic acid, and the preparation of standard solutions for acid - base, redox, argentimetric and complexometric titrations. With inexpensive apparatus and careful attention to detail, they show that excellent accuracy and precision can be obtained by any competent analytical chemist.

The second part of the book covers instrumental methods and in 17 pages deals with photometric, potentiometric, amperometric, conductimetric and coulometric titrations. Information is presented to show that coulometric titration is probably the most accurate and precise titration procedure at present available. There is a useful compilation of 83 references.

This book is well written and should be read by all analytical chemists who carry out titrations, even if results that are accurate and precise to 1 part in 10 000 are not required. It contains many useful hints and snippets of information such as how to prepare stable solutions of sodium thiosulphate.

My only criticism of the contents of this monograph is that the authors have used normalities very extensively and it can be argued that molarities should preferably be used in expressing the concentrations of solutions, particularly for complexometric titrations. The price is on the high side for a soft-back book of only 70 pages. J. B. HEADRIDGE

SPECTROSCOPY AND ITS INSTRUMENTATION. By P. BOUSQUET. Translated by K. M. GREENLAND. Pp. xii + 239. London: Adam Hilger. 1971. Price £7.

This book provides a clear exposition of the theory and function of the various optical components of prism and grating spectrographs and spectrometers, including absorption instruments. Much of the treatment is necessarily mathematical, but this detracts little from the readability of the book. A comprehensive description of gratings and spectrographic mountings is included, and there is a reasonably large section (80 pages) on instrumentation for interference spectroscopy, *i.e.*, Fabry - Perot and Fourier spectroscopy.

The text appears to be reasonably up to date; current practice is emphasised, although historical perspectives frequently appear. However, appreciation of signal to noise ratios for optimising detectability (p. 26) appears to be a recent innovation in emission spectrography. The main value of the book lies in its authoritative description of the principles that govern the construction and operation of the optical components of spectrometers. It is not intended as, and does not provide, a guide to the use of the instruments or to their applications. The book is excellently produced, clear to read and well illustrated. The price, however, even for today, seems certain to ensure that sales will be only to libraries and very dedicated spectroscopists. A. TOWNSHEND

ANALYSIS OF RAW, POTABLE AND WASTE WATERS. By THE DEPARTMENT OF THE ENVIRONMENT. Pp. x + 305. London: H.M. Stationery Office. 1972. Price £2·15.

This is the official Government publication that gives approved procedures for the analysis of all types of water samples except high-purity water. It supersedes "Chemical Analysis as Applied to Sewage and Sewage Effluents" (H.M. Stationery Office, 1956), "Recommended Methods for the Analysis of Trade Effluents" (Society for Analytical Chemistry, 1958) and "Approved Methods for the Physical and Chemical Examination of Water" (Institute of Water Engineers, 1949, revised in 1953 and 1960), and as such will be an essential reference work for all laboratories concerned with the routine analysis of water samples. Apart from specialised tests, such as those for B.O.D., conductivity and suspended solids, most procedures involve titrimetric or spectrophotometric measurements and will therefore be suitable for even the most poorly equipped water laboratories. "Specialised techniques," including atomic-absorption spectrophotometry, flame photometry, ion-selective electrodes, gas - liquid chromatography and polarography, are detailed only very briefly and are rarely applied in any of the procedures adopted. This omission is a pity, because these techniques are now widely used in many water laboratories as the greater degree

of selectivity in many instances permits more accurate results to be obtained more rapidly with very little extra capital outlay. The committee responsible for this publication were well aware of these advantages and have recommended the setting up of a Standing Committee of Analysts to test and introduce new methods. It is to be hoped that this will soon result in the recommendation of a number of new methods as alternatives to those described in this book.

J. M. OTTAWAY

GAMMA-RAY SPECTROSCOPY WITH PARTICULAR REFERENCE TO DETECTOR AND COMPUTER EVALUATION TECHNIQUES. By P. QUITTNER. Pp. 111. London: Adam Hilger Ltd. 1972. Price £4.

A monograph that reviews the applications of computers to the evaluation of data in gamma-ray spectroscopy is a welcome addition to more general books on the subject of gamma-ray spectroscopy. While the topic is not exhaustively covered, virtually all aspects of importance and interest have at least some mention. A short chapter on statistics and spectrum smoothing follows the brief introduction. The next chapter, on the determination of detector response functions, is of little more than academic interest because the application of theoretical functions to experimental spectra is fraught with difficulty. However, the following three chapters, on peak-location methods, peak-area determination techniques and the quantitative measurement of gamma-ray spectra by least-squares resolution, are of enormous practical importance in present-day gamma-ray spectroscopy and are given good coverage. Three general peak location methods are detailed: those which simply seek maxima, those which depend on the examination of the smoothed first derivative of the spectral data and those which rely on the behaviour of the second difference of the spectral data. Peak-area determination is coupled with the absolute determination of gamma-ray intensities, which involves the subject of detector efficiency calibration. The two main techniques for peak-area calculation are dealt with, namely, the fitting of functions to peak data and base-line subtraction methods. The chapter on the least-squares resolution method includes useful discussions of the principles of the method, the selection of weighting factors, gain and threshold compensation, the effect of omitting to fit all of the components to an actual spectrum and lastly the method's value relative to a number of peak-area calculation techniques.

The final four chapters are a miscellany of related topics: experimental errors, decay curve analysis, spectrum stripping, applications in activation analysis, on-line computer methods, detection limits, optimisation programs and specialised counting methods.

The monograph will serve as a useful summary for those experienced in the application of computers to the evaluation of gamma-ray spectra, and as an excellent introduction to those unfamiliar with the subject.

J. W. MCMILLAN

DIFFERENTIAL THERMAL ANALYSIS. Volume 2. APPLICATIONS. Edited by R. C. MACKENZIE. Pp. xvi + 607. London and New York: Academic Press. 1972. Price £12·50.

When the first volume of a two-volume work reaches an enviably high standard, there is always some doubt whether the second volume will live up to similar expectations. Happily, the contents of Volume 2, "Applications," largely dispel these doubts, although, perhaps because of the continuing high standard, a reviewer is apt to be more critical of the second volume.

Dr. Mackenzie is, again, to be congratulated on his expert editing of this volume, which is divided into two sections. The first, entitled "Physical Chemistry," contains chapters on Determination of Thermal Constants, Calorimetric Measurements, Reaction Kinetics, Phase Studies and Low Temperature Studies. The second section, on "Applications in Industry," includes chapters on Ceramics, Building Materials, Cements, Glass, Mineral Industries, Soils, Catalysts, Atomic Energy, Explosives, Plastics and Rubbers, Textiles, Pharmaceuticals, Oils, Fats, Soaps and Waxes, Food Industries, Forest Products and General Applications in Industry with Special Reference to Dusts.

Dr. Mackenzie's skill as an editor is demonstrated by the complete complementary nature of the chapters on "Determination of Thermal Constants" and "Calorimetric Measurements," the easy pitfall of repetition being completely avoided. However, in "Calorimetric Measurements" it is disappointing to see such brief treatment of differential scanning calorimetry. While conceding that, in the Preface, Dr. Mackenzie specifically mentions limiting the subject matter to differential thermal analysis, the many similarities between differential thermal analysis and differential scanning calorimetry, should, perhaps, have merited fuller discussion of the latter technique.

One slight editorial inconsistency is noted regarding standard materials for differential thermal analysis temperature calibration: a footnote on p. 88 mentions that materials are commercially available for temperature calibration over the range 100 to 1000 °C, whereas on p. 141 the temperature range for these materials is given as 100 to 950 °C. The relevant literature references to McAdie are also inconsistent, p. 88 quoting "McAdie 1971, 1972" and p. 141 quoting "McAdie 1969, 1971."

It is a pity to find no mention of slate in Chapter 32 (Building Materials), particularly as its use as a cladding material is finding increasing application in the building and civil engineering industries.

Chapter 34, on Glass, was found to be disappointingly short, even though the authors have done such outstanding work in putting the mystique of glass-making on to a sound, scientific footing.

However, the above criticisms must, inevitably, pale before the immense wealth of sound information contained in this excellent volume. To give but a few examples: Dr. Sharp's chapter on Reaction Kinetics is a masterpiece, which must, surely, be essential reading for all workers and potential workers in the field. Chapter 42 (Pharmaceuticals) is also particularly useful in presenting a survey of a subject that is often difficult to discuss without contravening the plethora of patents and commercial secrets with which this field seems to abound. Finally, mention must be made of the fortunate choice of the contributor of Chapter 44 (Food Industries), who has been persuaded to render a valuable service by collating information, in English, in a field where the majority of the work has appeared in foreign language journals.

That a text-book has, so to speak, "arrived" is evidenced by reference to it by author rather than title, *e.g.*, "Partington," "Vogel," etc., and I would venture to predict that "Differential Thermal Analysis" will soon be referred to as "Mackenzie"—a fitting tribute to him.

C. J. KEATTCH

HANDBUCH DER ANALYTISCHEN CHEMIE. Edited by W. FRESENIUS and G. JANDER. Dritter Teil. QUANTITATIVE BESTIMMUNGS- UND TRENNUNGSMETHODEN. Band IIIaα2. ELEMENTE DER DRITTEN HAUPTGRUPPE. ALUMINIUM. Second Edition. By H. BENSCH. Pp. xx + 716. Berlin, Heidelberg and New York: Springer-Verlag. 1972. Price DM198; $61.60.

Fresenius and Jander's "Handbuch der analytischen Chemie" is the leading comprehensive work in German on analytical chemistry. It consists of nine volumes on qualitative and eight volumes (in twenty-five parts) on quantitative analysis. The present part-volume is concerned with the separation and determination of aluminium; it is more than half as long again as the first edition, which was published, in what must have been very difficult circumstances, in 1942.

After an introduction on the basic chemistry of the aluminium ion in aqueous systems, the longest section of the work (over 500 pages) deals with methods of determination. This section is divided into chapters on gravimetric, photometric, fluorimetric, nephelometric, polarographic, spectrographic (including atomic-absorption and X-ray fluorescence) and radiochemical methods, each with many sub-divisions and its own literature references (up to 1967). There follows a section on methods of separation, partly in the form of tables and cross-references to other sections of the volume as well as detailed procedures. The final section on "special methods" deals with the determination of metallic as distinct from total aluminium in various materials, and with the determination of aluminium oxide in aluminium, its alloys and iron and steel. The very detailed list of contents is probably of more use in a volume of this kind than an index, which is not provided.

This is a massive work of chemical scholarship containing virtually everything known on the subject, well arranged and presented. But so much can be done nowadays with a few powerful instrumental methods such as spectrography, X-ray fluorescence and atomic absorption that many of the methods in this book will be of interest to very few analysts. Nevertheless, it is a work to which anyone concerned with the determination of aluminium should have access, and as such should find a place in the larger technical libraries.

G. M. HOLMES

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Spectrophotometric Determination of Phosphorus(V) Oxide in Cements and Clinkers with Molybdovanadate Reagent

The current method of determining phosphorus(V) oxide in cements and clinkers has been investigated in an attempt to improve its reliability. The method in use at the Building Research Station is that for U.K. internal trade, as described in B.S. 4550 : Part 2 : 1970, in which phosphate is complexed as the molybdovanadophosphate and the intensity of the yellow colour produced is measured. Investigations have shown that silica in solution interferes with the development of the colour and a modified determination procedure has been developed in order to minimise this interference and to reduce the relative error for phosphorus(V) oxide determination in cements.

M. J. BOWLEY

Department of the Environment, Building Research Establishment, Building Research Station, Garston, Watford, Hertfordshire, WD2 7JR.

Analyst, 1973, **98**, 739–744.

Determination of Cyclamate in Soft Drinks by Reaction with Nitrous Acid

Part II. Manual and Semi-automated Methods: Determination of Cyclohexyl Nitrite by Diazotisation and Coupling with Bratton - Marshall Reagent

The determination of cyclamate at a concentration of approximately 1 mg ml^{-1} in soft drinks is described. Cyclohexyl nitrite, derived from cyclamate by reaction of the latter with nitrous acid, is determined in a non-aqueous system by initial diazotisation with sulphanilamide and subsequent coupling with 2-aminoethyl-1-naphthylamine. Manual and semi-automated procedures have been evaluated and interference has been studied.

A. JEAN SHENTON

Lyons Central Laboratories, London, W14 0QU.

and R. M. JOHNSON

Food, Drink and Tobacco Industry Training Board, Leon House, High Street, Croydon, CR9 3NT.

Analyst, 1973, **98**, 745–748.

Determination of Cyclamate in Soft Drinks by Reaction with Nitrous Acid

Part III. Manual and Semi-Automated Methods: Determination of Excess of Nitrous Acid with Safranine

The determination of cyclamate in soft drinks at a concentration of approximately 1 mg ml^{-1} is described. Excess of nitrous acid is added to the sample and unconsumed nitrous acid is determined colorimetrically with safranine. Factors that affect the sensitivity of the latter reaction and interference due to other components of soft drinks have been investigated. Preliminary precipitation with zinc hexacyanoferrate(II) effectively reduces this interference.

A. JEAN SHENTON

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and R. M. JOHNSON

Food, Drink and Tobacco Industry Training Board, Leon House, High Street, Croydon, CR9 3NT.

Analyst, 1973, **98**, 749–754.

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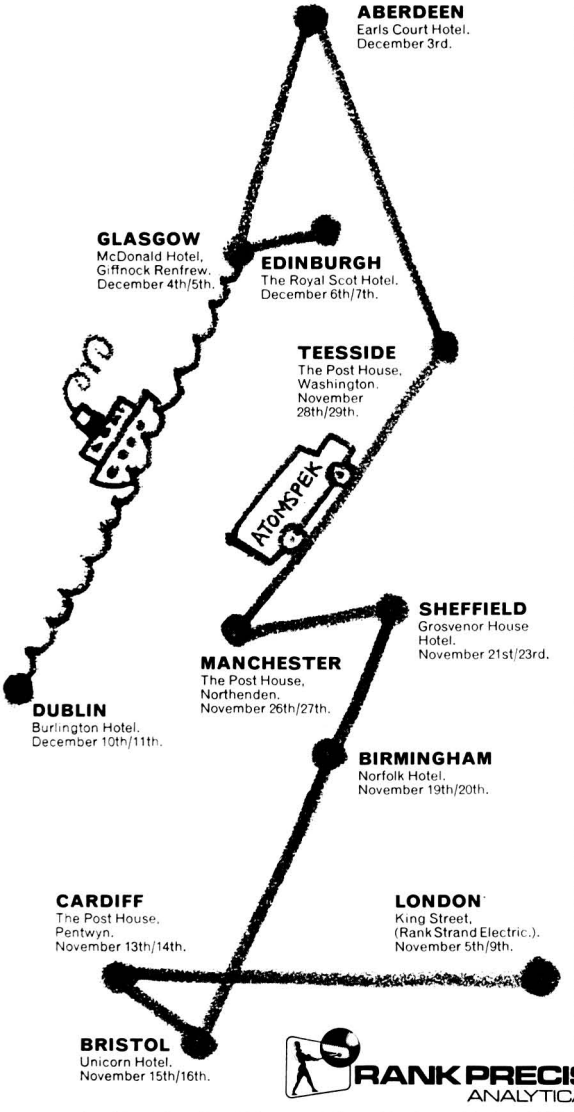

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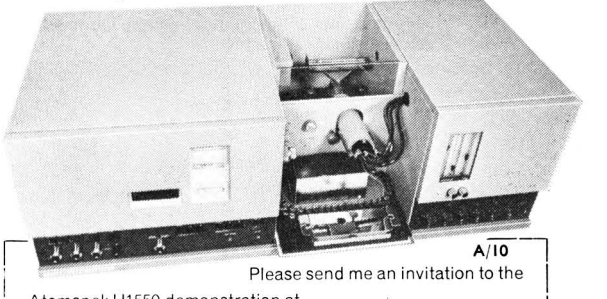

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ANALYTICAL DIVISION

Analytical Division, Hilger and Watts, Westwood Industrial Estate, Ramsgate Road, Margate, Kent. Telephone: Margate 24261.

The Determination of Non-fat Milk Solids in Milk Bread from the Orotic Acid Content

A method is described for the determination of the content of non-fat milk solids in milk bread. Orotic acid (2,6-dihydroxypyrimidine-4-carboxylic acid) present in the milk solids is extracted from the bread with water and determined by a colorimetric procedure, and the content of non-fat milk solids is calculated from the orotic acid content of the milk bread. The mean orotic acid content of the non-fat milk solids examined was 62·5 mg per 100 g (range 48·0 to 74·5 mg per 100 g).

A. W. ARCHER

Research Section, Division of Analytical Laboratories, Department of Health, P.O. Box 162, Lidcombe, New South Wales, Australia 2141.

Analyst, 1973, **98**, 755–758.

Detection of Thioureas, Thiosemicarbazides and Monothiosemicarbazones with 2,3-Dichloro-1,4-naphthoquinone

Thiosemicarbazones, thiosemicarbazides, thioureas and also isothiocyanates are made to react with 2,3-dichloro-1,4-naphthoquinone in an ethanolic medium and on rendering the mixtures alkaline with ethanolic ammonia solution, a red to violet colour is developed with absorption maxima between 500 and 570 nm. The reaction forms the basis of a spot test for these compounds.

M. B. DEVANI, C. J. SHISHOO and M. G. SHAH

Department of Pharmaceutical Chemistry, Lallubhai Motilal College of Pharmacy, Ahmedabad-9, India.

Analyst, 1973, **98**, 759–761.

Spectrophotometric Determination of 4-Acetylaminobenzaldehyde Thiosemicarbazone (Thiacetazone) with 2,3-Dichloro-1,4-naphthoquinone

4-Acetylaminobenzaldehyde thiosemicarbazone (thiacetazone) is made to react with 2,3-dichloro-1,4-naphthoquinone in an ethanolic medium. When the mixture is rendered alkaline with ammonia a purple product with an absorption maximum at 560 nm is obtained. A method based on this reaction is described for the quantitative determination of micro-scale amounts of thiacetazone. The procedure is applied to its determination in tablets. The results are compared with those obtained by the official method.

C. J. SHISHOO, M. B. DEVANI and M. G. SHAH

Department of Pharmaceutical Chemistry, Lallubhai Motilal College of Pharmacy, Ahmedabad-9, India.

Analyst, 1973, **98**, 762–765.

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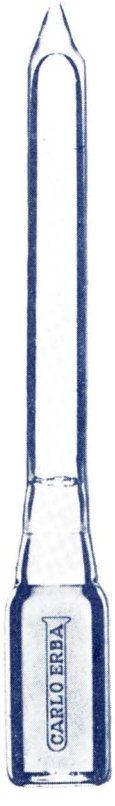

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This list includes the Methyl esters of the above mentioned Fatty acids, as well as the C3, C5, C7.

Alcohols

This list includes the normal C4 and C5 Alcohols, together with their secondary, tertiary and branched chain isomers; moreover the normal C6, C7, C8, C9, C10, C11, C12.

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