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Summaries of Papers in this Issue

The Simultaneous Measurement of pH, Chloride and Electrolytic Conductivity in Soil Suspensions

The measurements of pH, chloride and electrolytic conductivity have been simplified and made simultaneous for suspensions of soil in water through the use of a triple electrode system mounted in a single unit. A glass electrode and a silver - silver chloride electrode with a common reference electrode and two pH meters are used for the determination of pH and chloride, respectively. Electrolytic conductivity is measured by an ohm-meter principle using silver electrodes. The outputs of the three meters are recorded on a three-pen recorder with electrically independent channels. The pH, over the range 0 to 10, is read from the chart while the values for chloride and electrolytic conductivity are obtained from graphs or tables. Once the instruments are set up they need little adjustment during the day.

S. McLEOD, H. C. T. STACE, B. M. TUCKER and P. BAKKER

Commonwealth Scientific and Industrial Research Organization, Division of Soils, Adelaide, Australia.

Analyst, 1974, **99**, 193–202.

A Potentiometric Method for the Determination of Chloride in Boiler Waters in the Range 0·1 to 10 μg ml^{-1} of Chloride

A potentiometric method for determining chloride in boiler water has been developed, which is based on the potential of a silver - silver chloride wire electrode *versus* a mercury(I) sulphate reference electrode immersed in a buffered solution of the sample. The method was tested in the range 0·1 to 10·0 μg ml^{-1} of chloride and the standard deviations of the results at 0·1, 1·76 and 10·0 μg ml^{-1} of chloride were approximately 0·04, 0·06 and 0·2 μg ml^{-1} of chloride, respectively. Substances normally present in boiler water do not interfere appreciably but the method is not suitable if octadecylamine is present.

K. TORRANCE

Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey.

Analyst, 1974, **99**, 203–210.

Filter-papers as a Source of Error in Ammonia Determinations

A selection of filter-papers was tested for the presence of ammonia. The degree of contamination of filtrates was also determined. In both instances, significant levels of ammonia were found. Hardened filter-papers contained significantly higher levels of ammonia.

LIAM Ó hALMHAIN and DONAL Ó DANACHAIR

Analytical Chemistry Section, Institute for Industrial Research and Standards, Ballymun Road, Dublin 9.

Analyst, 1974, **99**, 211–213.

THE ANALYST

The Simultaneous Measurement of pH, Chloride and Electrolytic Conductivity in Soil Suspensions

BY S. McLEOD, H. C. T. STACE, B. M. TUCKER AND P. BAKKER

(Commonwealth Scientific and Industrial Research Organization, Division of Soils, Adelaide, Australia)

The measurements of pH, chloride and electrolytic conductivity have been simplified and made simultaneous for suspensions of soil in water through the use of a triple electrode system mounted in a single unit. A glass electrode and a silver - silver chloride electrode with a common reference electrode and two pH meters are used for the determination of pH and chloride, respectively. Electrolytic conductivity is measured by an ohm-meter principle using silver electrodes. The outputs of the three meters are recorded on a three-pen recorder with electrically independent channels. The pH, over the range 0 to 10, is read from the chart while the values for chloride and electrolytic conductivity are obtained from graphs or tables. Once the instruments are set up they need little adjustment during the day.

WE have developed an apparatus for the simultaneous measurement of pH, chloride and electrolytic conductivity in aqueous soil suspensions. This apparatus has greatly reduced the time taken for these analyses because previously these determinations were carried out separately on aliquots of the same suspension in a manner similar to that described by Piper.[1] Soil suspensions are preferred to pastes, which are not adaptable to rapid standardised procedures and which have uncertainties in liquid junction potentials.

The measuring electrodes are mounted together in a plastic holder. The glass electrode (for measurement of pH) and the silver - silver chloride electrode (for measurement of chloride) with a common calomel reference electrode are connected to two pH meters and then through suitable attenuator circuits to two pens of a three-pen potentiometric recorder. The pens of the recorder must be electrically independent. The electrolytic conductivities are measured by the current between two silver electrodes from a solid-state oscillator using the ohm-meter principle. This technique is preferred to the use of a conductivity bridge because it requires no change in the ranges used. The oscillator current is recorded by the third pen of the

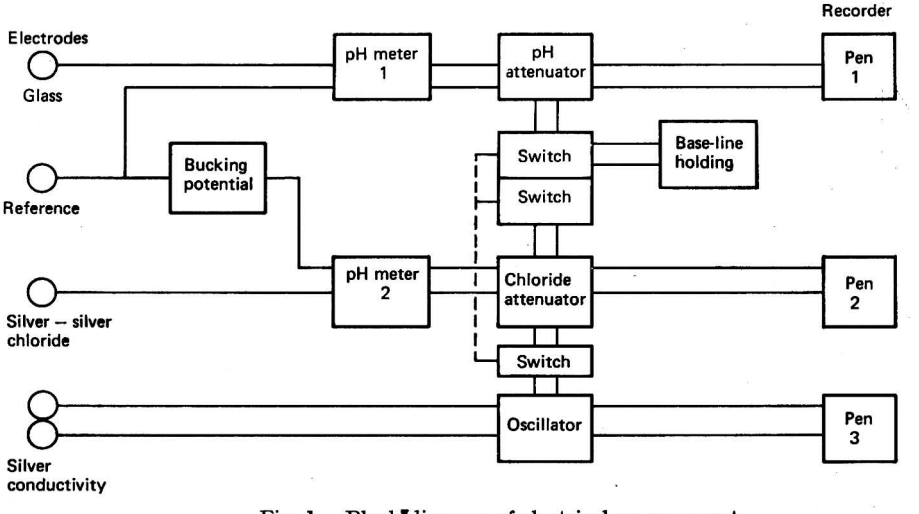


Fig. 1. Block diagram of electrical arrangement

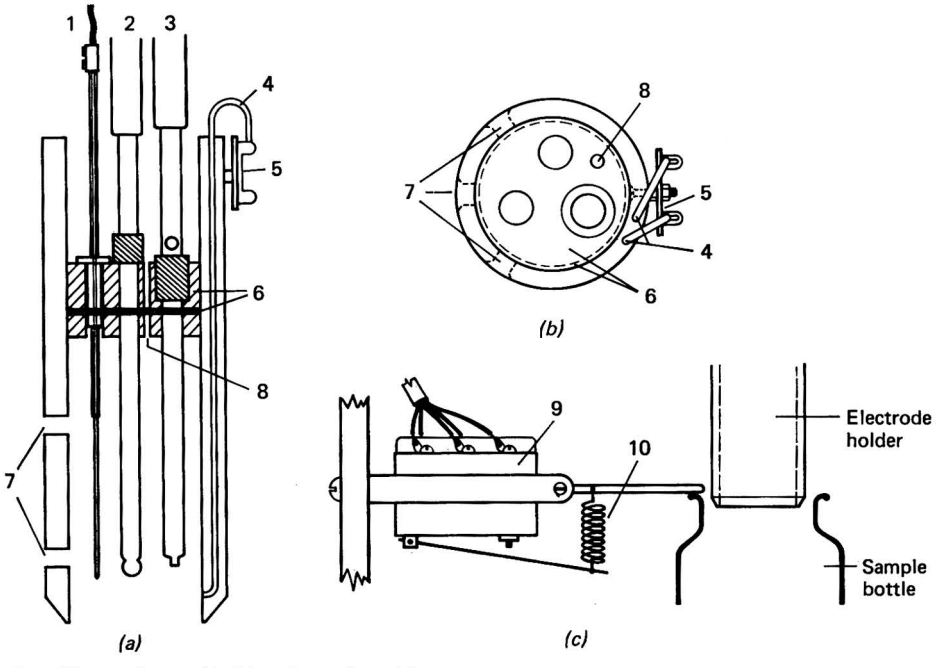
recorder. In order to prevent damage to the pens, the electrode system is automatically isolated from the recorder while samples are being changed. A block diagram of these arrangements is shown in Fig. 1. The pH and chloride attenuator circuits and the oscillator circuit, together with the bucking and base-line holding circuits, are incorporated in one unit, the attenuator box. The functions of the bucking and base-line holding circuits and the isolating switch are explained below.

As some soil suspensions contaminate the silver - silver chloride electrode, this electrode is washed between determinations with a dilute solution of sodium chloride. The holder and electrodes are then washed with distilled water and the electrodes dried. The whole operation can be carried out manually or semi-automatically. The precision of each measurement with this equipment has been found to be satisfactory.

ELECTRODE SYSTEM

ELECTRODE HOLDER—

Two types of electrode holder have been developed, the first of which (type A) is of simple design in which the washings between determinations are effected by jets from wash-bottles and the system is dried with soft tissue-paper, while the second (type B) incorporates channels and small jets through which the wash liquids and air for drying the system are forced. These jets are positioned so that all surfaces of the electrodes and the holder are washed. The flow of wash liquids through the jets is controlled by non-return valves.



1 = Silver - silver chloride electrode with a Perspex holding collar
2 = Glass electrode with a rubber holding collar
3 = Calomel electrode with rubber holding collar inserted into block to prevent wash entering the filling hole
4 = Silver conductivity electrodes cemented into wall of holder
5 = Terminal block for conductivity electrodes
6 = Perspex block with O-ring to hold electrodes
7 = Wash holes
8 = Air vent
9 = Micro-switch with adjustable height
10 = Overload spring allowing for reasonable changes in bottle size

Fig. 2. Electrode holder, type A: (*a*) side elevation; (*b*) plan; and (*c*) micro-switch

Type A—This holder is made in two parts from Perspex [Fig. 2 (*a*) and (*b*)]. The outer tube is 17 cm in length and 3 cm in diameter with a wall thickness of 3 mm, and has three

holes for washing the electrodes between measurements. The silver electrolytic conductivity electrodes, made from 1·6 mm diameter silver wire, are set with Araldite in slots milled in the outer surface of this tube and tested for electrical leakage between them. They are about 2 mm apart and are bent so that their exposed faces are at the inner surface of the tube, a distance of about 5 mm from the bottom. They are connected to a terminal block at the top of the tube. Although platinum is the usual metal for use in conductivity electrodes, it could not be used in this work because it gave drifting readings.

The glass, the silver - silver chloride and the calomel electrodes are mounted in holes in a block 2·3 cm in length. The glass and the calomel electrodes are held in place by rubber collars, and with the calomel electrode this collar also acts as a seal to prevent water from the washing procedure entering the filling hole of the electrode. The silver - silver chloride electrode is held in place with a Perspex collar. The block is supported in the outer tube by an O-ring seal and is provided with a small air vent. Except for the electrolytic conductivity electrodes, these dimensions are not critical and can be altered to suit the suspension bottles provided that sufficient space is allowed to accommodate the electrodes. The glass and calomel electrodes are placed as far as possible from the electrolytic conductivity electrodes. This arrangement minimises the effect on the cell constant if the electrodes have to be replaced and reduces the electrical interference by the conductivity current on the electrode potentials.

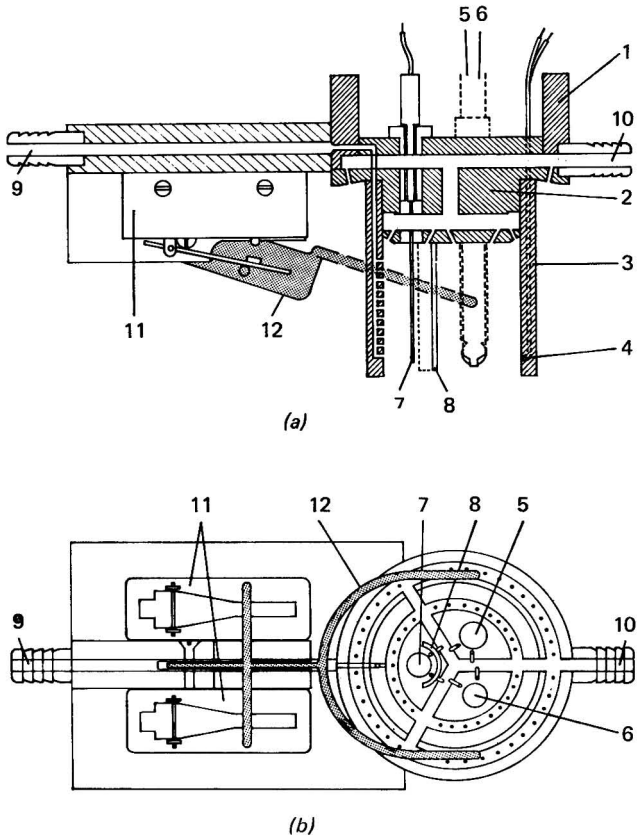
Type B—This holder is made of Perspex in five parts [Fig. 3 (*a*) and (*b*)], which are glued together with a suitable Perspex cement so as to form a complete unit. In our unit, the external diameter of the upper cylinder is 6·5 cm with a wall thickness of 6 mm and the length is 3·5 cm. The lower cylinder has an external diameter of 5·5 cm with a wall thickness of 3 mm and is 6·5 cm in length. This lower cylinder supports the electrolytic conductivity electrodes, which are made of silver wire as described for the type A holder, but in this unit these electrodes are soldered with silver to flexible insulated copper leads that pass through the various parts of the unit to one of the micro-switches (see Electrical circuits). A small air-vent is drilled near the top of the lower cylinder.

The wall of the lower cylinder contains a channel of small diameter, which is connected through the various parts of the unit to a channel in the side-piece that supports the micro-switches. This channel is for the sodium chloride wash and it is also connected to the water and air supplies. The lower part of the channel has several small outlet jets, which are directed towards the silver - silver chloride electrode.

The block that supports the glass, the silver - silver chloride and calomel electrodes in the same manner as described for the type A holder is held between these cylinders and is 2·5 cm in depth. This block contains an interconnected upper and lower set of channels. Each set has three channels, which radiate from the centre of the block as shown in Fig. 3 (*b*). These channels are connected to the water and air supplies by the side inlet 9. A channel 0·5 cm deep is machined into the bottom of the upper cylinder, and when the electrode block is fitted to the upper cylinder an annular space is formed to which the upper channels supply either water or air, as required.

The lower set of channels are also connected to a similar annular space between the block and the lower cylinder. Small jets, which enter the annular spaces, are placed at 0·5-cm intervals around the upper and lower rims of the electrode block and these jets are directed towards the outer and inner surfaces of the lower cylinder. The angle at which these holes are drilled is critical and should be $22 \pm \frac{1}{2}°$. This angle has been found the most satisfactory to ensure an even flow of water down the walls of the cylinder. Small jets that are directed towards the electrodes are also drilled in the lower set of channels. A small shield is placed at the back of the silver - silver chloride electrode so as to protect the other electrodes during the washing with sodium chloride solution.

In the simplest arrangement that we have used for the washing procedure with this unit, the sodium chloride solution is dispensed from a bottle equipped with a squeeze-bulb, the distilled water is pumped from a large container by a small centrifugal pump and the flow is controlled by a solenoid valve. The pump and valve operate together when the pump is switched on for the water wash. Non-return valves are also placed in the lines as close as possible to the holder and ensure that any liquid that remains in the lines does not flow into the unit during the measurements. The air for drying the whole system must be free from oil and is supplied at low pressure by a simple on - off valve.



1 = Upper cylinder
2 = Block
3 = Lower cylinder
4 = Silver electrolytic conductivity electrodes
5 = Calomel electrode
6 = Glass electrode
7 = Silver - silver chloride electrode
8 = Chloride electrode wash shield
9 = NaCl and water inlet
10 = Water and air inlet
11 = Ganged micro-switches
12 = Lever arm

Fig. 3. Electrode holder, type B: (*a*) side elevation; (*b*) plan

GLASS ELECTRODE—

The glass electrode is 15 cm long and has a 6 mm diameter stem with a bulb no larger than the stem diameter. The glass of the electrode is chosen so as to give a rapid response (105AR, Titron Instruments, Australia). An electrode of small dimensions is used in order to facilitate washing and to minimise liquid hold-up.

SILVER - SILVER CHLORIDE ELECTRODE—

This electrode is made from a length of about 15 cm of silver wire of 1·6 mm diameter. A length of about 2 cm at one end of this wire is cleaned in dilute nitric acid until effervescence just begins, and is then washed thoroughly with distilled water. A length of about 1 cm of this cleaned portion is immersed in 0·1 N hydrochloric acid and a current of 3 to 4 mA is passed for 1 minute between this wire and a platinum cathode, which results in a thin brown coating of silver chloride that is less susceptible to contamination than a thick coating. After washing with distilled water, the electrode is ready for use. We have recently found that the stability of this electrode can be improved by standing it for a few hours (overnight) in distilled water.

The silver chloride coating is gradually dissolved and eroded by continued use, but experience has shown that the performance of the electrode is satisfactory for up to 200 determinations. The used electrode is stripped by wiping it with a piece of soft material and re-coated.

REFERENCE ELECTRODE—

The reference electrode is common to the pH and chloride cells and is operated at earth potential. A calomel electrode (K401, Radiometer, Denmark) is used and it must have only a low flow-rate of potassium chloride solution through the liquid junction so as to prevent errors in the chloride and electrolytic conductivity measurements.

A mercury - mercury(I) sulphate electrode (K601, Radiometer) was used originally as it avoids the risk of chloride contamination, which is possible from a calomel electrode. However, comparative measurements gave soil pH values from 0·3 to 0·6 unit lower than a conventional arrangement in which a calomel reference electrode was used. This error was found to be due to the use of potassium sulphate to form the liquid junction of the mercury electrode. The potassium and sulphate ions have unequal transference numbers, so that the liquid junction potential is not almost zero, as assumed with potassium chloride, but is dependent on the ionic content of the external solution. The error is made up of two components: a suspension effect, due to the presence of charged particles in the test solution and which is generally small, giving values about 0·1 pH unit too low, and a standardisation error due to differences in ionic content between the standardising buffer and the test solution, which gives pH values that are too low by about 0·2 to 0·5 unit.

A similar effect could also occur in the measurement of chloride content with the silver - silver chloride electrode, but this would be partly compensated for by the empirical calibration of the electrode potentials against standard chloride solutions to the extent that the soluble salts may contain a large and fairly constant proportion of chloride. It is possible to minimise these liquid junction effects by making the soil suspensions in dilute salt solutions, but this expedient inhibits the use of conductivity measurements for soluble salts. The use of mercury - mercury(I) sulphate electrodes was therefore discontinued and the customary calomel electrodes were used. Tests on these calomel electrodes showed that detectable errors in the chloride concentration appeared only after standing for about 20 minutes and then only in the standard with the lowest chloride content. This result agreed with the earlier experience that diffusion of salts from the liquid junction did not affect the conductivity measurements in unstirred suspensions over much longer periods than the 1 to 2 minutes required to complete the set of analyses. New reference electrodes must nevertheless be tested for excessive leakage before being put into regular service.

MOUNTING THE HOLDERS—

The electrode holder is mounted vertically, in an earthed aluminium box (open at the front), so that the electrodes are immersed in the top 1 cm of soil suspension when the bottle is in place on the stand. On no account should the electrodes enter the sediment, as the glass electrode may be damaged and the conductivity electrodes will give completely false readings. Preferably, the calomel reference electrode should just enter the liquid surface. The lever-arm of the micro-switch is adjusted so that the top of the bottle operates it when it is placed in position or removed. It is desirable that the micro-switch operates after the electrodes are immersed and before they emerge from the suspension. The box has a drain to take the wash liquids to waste.

ELECTRICAL CIRCUITS

The complete circuit is shown in Fig. 4; two Radiometer pH meters, Model PHM28, are used. The use of other meters will require appropriate changes in the values of the attenuator components. Meters with high impedance recorder outputs (above 30 kΩ) are not satisfactory with the present recorders. It is possible that such meters could be used with high input-impedance recorders, but we have not tried this arrangement. The potentiometric recorder has three electrically independent channels, each with a minimum span of 10 mV and a full-scale zero adjustment (B34, Rika Denki). Chart speeds of 5, 10 and 20 mm min^{-1} are suitable.

The pH meter 1 is direct-reading with a recorder output. The meter scale is not folded in the soil pH range so as to avoid off-scale readings that might damage the meter. An

adjustable attenuator is used to reduce the output to 2 mV per pH unit, giving a working range of 20 mV for 10 pH units. The resistor values in the attenuator are matched to the pH meter output and the recorder input. When the soil suspension bottle is removed from the electrodes, the micro-switch [Figs. 2 (*c*), 3 (*a*) and 3 (*b*)] substitutes a 20-mV output from the base-line holding circuit (supplied from a 1·5-V dry cell) for the pH meter output to the recorder. This output drives the recorder pen to full scale in order to avoid irregular fluctuations during the washing of the electrodes and to separate the pen traces from each other. The pH channel normally works on the 20-mV range but its sensitivity can be doubled by switching to the 10-mV range.

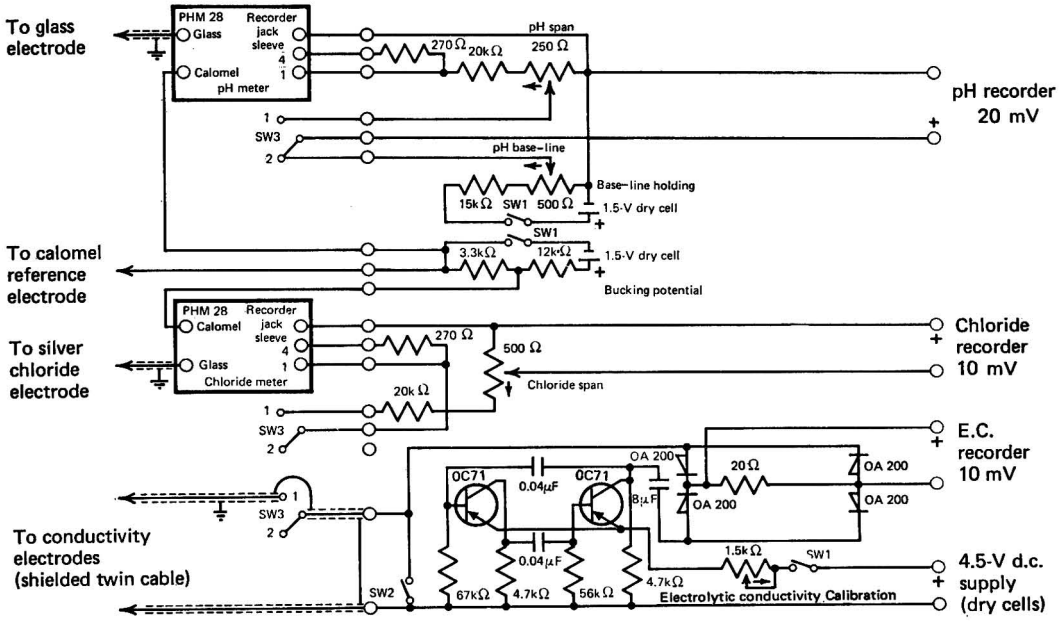


Fig. 4. Circuit and connections for attenuator box used with two Radiometer pH meters, Model PHM28. Switches: SW1 = 3-pole, 3-position and SW2 = 1-pole, 3-position (combined rotary); SW1 and SW2 off = off; SW1 and SW2 on = electrolytic conductivity calibrate; SW1 on and SW2 off = read-out. SW3 = 3-pole, 2-position micro-switch; 1, recorder reads; 2, recorder standby

The pH meter 2 is also direct-reading with a recorder output. The pH scale is used for log [Cl^-] measurements so that the buffer adjustment can be used for standardisation. Like the glass electrode, the silver - silver chloride electrode is connected to the meter with a lead that is shielded to earth so as to protect it from electrical interference. A constant bucking potential of about 400 mV is supplied from a dry cell through a high-resistance network in order to bring the electrode potential to a suitable value. An adjustable attenuator reduces the meter output to 4 mV per log [Cl^-] unit, giving a working range of 2·5 units. On removal of the soil suspension bottle, the same micro-switch disconnects the meter from the attenuator so that the chloride recorder pen remains at its electrical zero during the washing process.

The electrolytic conductivity oscillator circuit has been described by Tucker and Raupach.[2] The meter in this original circuit is replaced with a 20-Ω resistor connected in parallel to the third recorder pen. Adjustment of the input voltage to the oscillator to give a full-scale (10 mV) reading on the recorder calibrates the maximum current to 0·5 mA. The oscillator is driven by three 1·5-V dry cells. A mains-operated rectifier supply was originally used but fluctuations in the mains voltage gave considerable instability, which could not be overcome by using a constant-voltage transformer. The micro-switch disconnects the electrodes during the washing cycle so that the recorder pen returns to zero between readings.

CALIBRATION

pH—

The pH recorder is standardised against two buffers so that the pH can be read directly from the chart. The pH meter has a value for which the recorder output is zero, and the meter is standardised with a buffer whose pH is close to this zero by means of its buffer adjustment. For the Model PHM28 pH meter, the zero output is at pH 8 and a buffer of pH 7·78 is used. The recorder is adjusted to read the same value by means of its zero adjustment. After washing the electrodes with distilled water, a second buffer, with a pH as far from the first as possible (pH 4·00), is used to check the correctness of the pH meter reading. Small errors can be corrected by adjusting the sensitivity control or temperature setting; large errors indicate that the glass electrode is faulty. The recorder is then set to read the buffer value by means of its pH span control. When the recorder is first set up, it may be necessary to repeat this calibration procedure so as to obtain complete correspondence between the buffer pH values, pH meter reading and chart reading, but subsequently it may only be necessary to make day-to-day calibrations with the buffer adjustment. A third buffer is used occasionally in order to check the settings, the other two buffers and the linearity of the glass electrode potentials.

CHLORIDE—

The chloride meter cannot be matched against the recorder because of the scale expansion used. The recorder is set to a fixed value for the lowest chloride standard using the recorder zero adjustment and the buffer adjustment of the meter. The pen is set to a second fixed value for a second chloride standard using the chloride span control. A third standard is used as a check. For soils, convenient values for these standards are 10, 1000 and 100 mg l^{-1} of chloride made from sodium chloride; these values are equivalent to 25, 2500 and 250 mg kg^{-1} of chloride in the soil when a ratio of soil to water in the suspension of 1:2·5 is used. A plot of chart reading against the logarithm of the chloride concentration in the soil is used to calculate the results. Provided that the chart is always standardised at the same points and that the silver - silver chloride electrode is kept in good condition, this plot is a straight line. It is then more convenient to use a table that relates chart reading to chloride content.

ELECTROLYTIC CONDUCTIVITY—

The electrolytic conductivity recorder pen is set at zero with the oscillator switched off and it should remain at zero when the oscillator is switched on with the electrodes in distilled water. In the calibration setting (electrodes short-circuited), the recorder pen is set to full scale (10 mV) by its calibration control. A plot of chart reading against electrolytic conductivity of standard salt solutions is used for the calculation of the results. The electrolytic conductivities of standard sodium chloride solutions at 20 and 25 °C are given in Table I.

TABLE I

ELECTROLYTIC CONDUCTIVITY OF SODIUM CHLORIDE SOLUTIONS CALCULATED FROM DATA IN THE INTERNATIONAL CRITICAL TABLES

NaCl concentration/M	Electrolytic conductivity/mS cm^{-1}	
	20 °C	25 °C
0·0005	0·0562	0·0625
0·001	0·1114	0·1241
0·002	0·2208	0·2460
0·005	0·542	0·604
0·01	1·065	1·186
0·02	2·082	2·316
0·05	4·995	5·550
0·07	6·86	7·63
0·10	9·60	10·66
0·20	18·30	20·30
0·50	42·2	46·66

Regular checks on standard solutions are required so as to ensure that the true values are being recorded. For these calculations also, a table can be used instead of the chart.

The state of the supply batteries is indicated by the position of the calibration control and they must be replaced as soon as they begin to fail, as the shape of the calibration curve may change when faulty batteries are used. The instrument is less precise at high conductivities (above about 3 mS cm^{-1}) and dilution of the suspension gives better values in such instances.

Sample measurement

Weigh the sample of soil into a suspension bottle that contains the required amount of aerated distilled water and shake the bottle for 1 hour. During this period, the apparatus is set up and calibrated ready for use. After shaking, allow the suspension to settle for about 0·5 hour. Place the first bottle under the electrodes and raise it to the correct height by using a stand; the rim of the bottle should then operate the micro-switch. Either when the readings are steady, or after 2 minutes if some drift occurs, the suspension bottle is lowered, thus releasing the micro-switch. The silver - silver chloride electrode is washed with a 2 g l^{-1} solution of sodium chloride before washing the whole electrode system thoroughly with distilled water. Periodic checks on one pH buffer, one chloride standard and the electrolytic conductivity calibration should be made and, in addition, it is good practice to include a standard soil each day.

Comparison with usual methods

Comparisons of values for electrolytic conductivity and chloride content for twenty soils obtained with this simultaneous system and those obtained by the methods used previously in these laboratories are shown in Fig. 5 (*a*) and (*b*). The results, together with many others that are not shown, were considered to be acceptable and the simultaneous method has now been in use for 4 years.

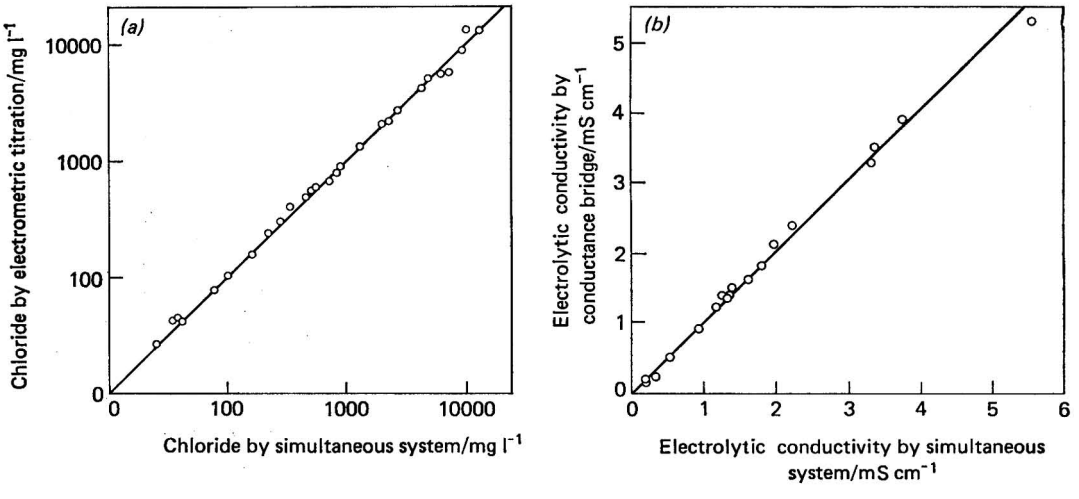


Fig. 5. (*a*) Comparison of chloride determinations by electrometric titration and simultaneous system. (*b*) Comparison of electrolytic conductivity determinations by conductance bridge and simultaneous system

Precision of the methods

In order to estimate the precision of the values for pH, chloride content and electrolytic conductivity obtained by this simultaneous method, twenty soil samples were analysed by two operators on each of two occasions (about 1 month apart). Each occasion was divided into a morning and an afternoon run and the operators changed runs between the occasions. Each soil was analysed in duplicate during each run and a different random order for the observations was used for each run, that is, eight separate estimations for each variable were obtained for each soil sample.

The results are presented in Table II in the form of analyses of variance showing the observed and expected mean squares assuming a random model for operators, occasions and repeat determinations made on the same day.[3] The results showed no effect due to

operators, occasions or any interaction for pH and electrolytic conductivity. For pH, there was a significant effect due to repeat runs on the same day. For chloride, there was a significant effect due to repeat analyses on the same day and a significant interaction between operators and occasions, although neither effect alone was significant.

TABLE II

ANALYSIS OF VARIANCE AND DEFINITION OF VARIANCE COMPONENTS

Variation due to	Degrees of freedom	Mean square: pH	Mean square: Chloride content	Mean square: Electrolytic conductivity	Expected value of mean square
Operators (OP)	1	0·0073	44	0·0038	$\sigma^2 + 20\sigma_R^2 + 40\sigma_{OP \times OC}^2 + 4\sigma_{S \times OP}^2 + 80\sigma_{OP}^2$
Occasions (OC)	1	0·0034	10 660	0·0016	$\sigma^2 + 20\sigma_R^2 + 40\sigma_{OP \times OC}^2 + 4\sigma_{S \times OC}^2 + 80\sigma_{OC}^2$
OP × OC	1	0·0013	64 080*	0·0004	$\sigma^2 + 20\sigma_R^2 + 40\sigma_{OP \times OC}^2$
Between runs within OP × OC	4	0·0166†	5227*	0·0018	$\sigma^2 + 20\sigma_R^2$
Soils (S)	19	14·0054	850 009	1·2296	
S × OP	19	0·0009	1494	0·0010	$\sigma^2 + 2\sigma_{S \times OP \times OC}^2 + 4\sigma_{S \times OP}^2$
S × OC	19	0·0013	2017	0·0023	$\sigma^2 + 2\sigma_{S \times OP \times OC}^2 + 4\sigma_{S \times OC}^2$
S × OP × OC	19	0·0017	1812	0·0006	$\sigma^2 + 2\sigma_{S \times OP \times OC}^2$
Error	76	0·0028	1351	0·0013	σ^2
Total ..	159				

Significance of the observed mean squares: *, $P < 0{\cdot}05$; †, $P < 0{\cdot}01$.

Definitions of variance components:

σ^2 = technical + sampling variance.

$\sigma^2_{S \times OP \times OC}$ = variance due to interaction of soils × operators × occasions.

$\sigma^2_{S \times OP}$ = variance due to interaction of soils × operators.

$\sigma^2_{S \times OC}$ = variance due to interaction of soils × occasions.

σ^2_R = variance associated with analyses repeated on same day.

$\sigma^2_{OP \times OC}$ = variance due to the interaction of operators and occasions.

σ^2_{OC} = variance associated with analyses carried out on different days.

σ^2_{OP} = variance associated with analyses carried out by different operators.

Estimates of the variance components can be calculated by equating the observed and expected mean squares, and the precision of estimation for varying numbers of samples, operators and occasions can be derived. For example, for calculating the variance associated with analyses repeated on the same day (σ_R^2) for the variable pH:

$$\begin{aligned} \sigma^2 + 20\,\sigma_R^2 &= 0{\cdot}0166 \\ \sigma^2 &= 0{\cdot}0028 \\ \hline 20\,\sigma_R^2 &= 0{\cdot}0138 \end{aligned}$$

Therefore, $\sigma_R^2 = 0{\cdot}00069$

Similarly, estimates of all other variance components can be calculated. The usual practice is to assume that a component is not different from zero unless a significant F-test (= effect mean square/error mean square) is obtained. Hence, for the results in Table II, the only variance components taken as greater than zero are those for σ_R^2 for pH and chloride content and $\sigma^2_{OP \times OC}$ for chloride content. The estimates of the variance components and standard deviations and coefficients of variation of single determinations are given in Table III. The precision obtained for the determination of each variable was satisfactory.

TABLE III

SUMMARY OF ESTIMATES OF ERROR

Variance component	pH	Chloride content	Electrolytic conductivity
σ^2 (technical + sampling)	0·0028	1351	0·0013
σ^2 (OP × OC)	—	1421	—
σ_R^2	0·0007	194	—
Total variance for a single sample	0·0035	2966	0·0013
Mean	8·42	748	1·034
Standard deviation (technical + sampling)	0·053	36·7	0·036
Standard deviation (total)	0·059	54·4	0·036
Coefficient of variation (technical + sampling), per cent.	0·63	4·91	3·48
Coefficient of variation (total), per cent.	0·70	7·27	3·48

Variances associated with determinations made with different numbers of samples, operators and days can be derived. For example, if r samples are analysed by s operators on each of t days, then:

$$\text{Variance (mean)} = \frac{\sigma^2 + \sigma_R^2}{r \times s \times t} \quad \text{for pH}$$

$$\text{Variance (mean)} = \frac{\sigma^2 + \sigma_R^2}{r \times s \times t} + \frac{\sigma^2_{OP \times OC}}{s \times t} \quad \text{for chloride content}$$

$$\text{Variance (mean)} = \frac{\sigma^2}{r \times s \times t} \quad \text{for electrolytic conductivity}$$

CONCLUSIONS

This method of simultaneous measurement has greatly reduced the time required for analyses, largely by eliminating the need for recording results at the bench and by avoiding the manipulation required for silver nitrate titrations and conductivity bridge measurements. The system has proved reliable over a period of 4 years and gives adequate precision. The instruments have required only slight day-to-day adjustments in order to maintain their standardisation. Throughout the development of the apparatus, the possibility of its incorporation into an automatic system has been kept in mind, but this would not be justified unless a much greater output of results is required.

We thank Mr. K. M. Cellier of the Division of Mathematical Statistics, who designed the precision tests and analysed the data for this study. We also thank Mr. L. Smith and Mr. B. Zarcinas, who were the operators in this study, and Mr. R. Sands, who made the electrode holders.

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A Potentiometric Method for the Determination of Chloride in Boiler Waters in the Range 0·1 to 10 μg ml^{-1} of Chloride

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A potentiometric method for determining chloride in boiler water has been developed, which is based on the potential of a silver - silver chloride wire electrode *versus* a mercury(I) sulphate reference electrode immersed in a buffered solution of the sample. The method was tested in the range 0·1 to 10·0 μg ml^{-1} of chloride and the standard deviations of the results at 0·1, 1·76 and 10·0 μg ml^{-1} of chloride were approximately 0·04, 0·06 and 0·2 μg ml^{-1} of chloride, respectively. Substances normally present in boiler water do not interfere appreciably but the method is not suitable if octadecylamine is present.

The presence of chloride ion in the steam - water circuit of power stations can give rise to corrosive acidic conditions, and therefore its concentration in boiler waters has to be kept below a specified limit. The controlled chloride levels in many of the boilers used by the Central Electricity Generating Board lie in the concentration range 0·1 to 5·0 μg ml^{-1}, a range in which, because of the solubility of the silver chloride, the normal Nernstian plot of the e.m.f. *versus* the logarithm of the chloride ion activity in the sample is curved. For most analytical purposes it is an advantage to have a linear calibration graph, and calibration functions that would provide this facility were investigated. The most convenient function of concentration to be plotted against e.m.f. for this concentration range was found to be log $(c + 1)$, where c μg ml^{-1} is the concentration of chloride in the sample.

Theoretical basis of the function log $(c + 1)$—

At chloride concentrations in the region of the solubility of silver chloride, $\sqrt{K_s}$ (where K_s is the solubility product of silver chloride), the contribution to the total chloride present from dissolution of the electrode surface becomes significant. Let the concentration of chloride present before dissolution be m and that dissolved from the electrode surface be s. The total chloride concentration in solution, T_{Cl}, is then $(m + s)$. K_s can be written

$$K_s = (m + s)\,(s)\,\gamma_{\pm}^2 \qquad (1)$$

where $\gamma_{\pm}$ is the mean univalent activity coefficient of the silver and chloride ions. By solving equation (1) for s, the real root, an expression for the total chloride present is

$$T_{Cl} = \frac{m}{2} + \left(\frac{\gamma_{\pm}^2 m^2 + 4K_s}{4\gamma_{\pm}^2}\right)^{\frac{1}{2}} \qquad (2)$$

The relationship between the e.m.f. of a silver - silver chloride electrode and the activity of chloride ions in solution, a_{Cl}, is

$$E = E^{\circ}_{Ag/Ag^+} + \frac{RT}{F}\ln K_s - \frac{RT}{F}\ln\left[\frac{\gamma_{\pm} m}{2} + \left(\frac{\gamma_{\pm}^2 m^2 + 4K_s}{4}\right)^{\frac{1}{2}}\right] \qquad (3)$$

Equations of this form have been derived by other workers.[1,2]

Equation (3) can be rearranged such that

$$E = E^{\circ}_{Ag/Ag^+} + \frac{RT}{2F}\ln K_s - \frac{RT}{F}\ln\left[\frac{\gamma_{\pm} m}{2\sqrt{K_s}} + \left(\frac{\gamma_{\pm}^2 m^2}{4K_s} + 1\right)^{\frac{1}{2}}\right] \qquad (4)$$

On binomial expansion of the term raised to the power of one half, and considering the first two members only, the expression within the square brackets becomes

$$\left[1 + \frac{\gamma_{\pm} m}{2\sqrt{K_s}} \quad \frac{\gamma_{\pm}{}^2 m^2}{8K_s}\right]$$

At values of m of less than $\sqrt{K_s}$, this expression tends to

$$\left[1 + \frac{\gamma_{\pm} m}{2\sqrt{K_s}}\right]$$

which becomes

$$\left[\frac{\gamma_{\pm}\ \mu g\ ml^{-1}\ \text{of chloride}}{0{\cdot}89} + 1\right]$$

when expressed in concentration terms of micrograms per millilitre. At the ionic strength of the preferred buffer concentration, the mean univalent activity coefficient was calculated to be 0·8, thus the simple expression ln [μg ml^{-1} of chloride + 1] was considered to be a practical calibration function. In order to confirm the application of this approximate but convenient expression the following procedure was adopted.

Six standard chloride solutions were prepared, the log (c + 1) values of which were evenly spaced between 0·1 and 18·67 μg ml^{-1} of chloride. The e.m.f. values of two portions of each solution were measured in a random order, as described under Method, and the resulting graph was tested statistically for linearity. As no significant difference was observed at the 95 per cent. probability level the calibration graph was assumed to be essentially linear over the concentration range tested.

Experimental

Effect of sodium hydroxide—

Alkaline conditions are maintained in boiler water in order to minimise corrosion and it was noted that the response of a silver - silver chloride electrode in solutions that had a constant chloride concentration varied with the pH. It was thought that this effect, probably caused by the association of silver with hydroxyl ions, could be eliminated by adding an acidic buffer to the sample solutions before measuring their e.m.f. values. The choice of buffer solutions is discussed below.

Choice of buffer solution—

An ammonium acetate - acetic acid buffer system was investigated as it has been used successfully in argentimetric titrations of chloride in boiler water. Two buffer concentrations were investigated: one solution contained 60 g l^{-1} and 78 g l^{-1} of acid and salt, respectively, and the other was prepared with one tenth of these concentrations. The experimental procedure was that described under Method, in which 4 ml of buffer were added to 100 ml of sample, giving a final pH of approximately 4·7.

The effect of adding 40 μg of sodium hydroxide per millilitre to 10 μg ml^{-1} standard chloride solutions was studied by using both buffer ionic strengths; this concentration of alkali is in excess of that normally present in boiler waters, *viz.*, 2 to 5 μg ml^{-1} of sodium hydroxide. The e.m.f. values, for both buffer ionic strengths, of the 10 μg ml^{-1} chloride solutions with and without the sodium hydroxide were measured alternately. A comparison of the two means indicated no statistically significant difference (for the 95 per cent. probability level and 10 degrees of freedom) between the standards when using the more concentrated buffer but a significant difference when the less concentrated buffer was used. The results are summarised in Table I.

Effects of other substances—

The effects of other substances were tested at two concentrations of chloride.

The electrode potentials were measured alternately in standard chloride solutions and in the same concentration of standard with other substances added at concentrations greater than those normally found in boiler water. The experimental procedure was as described

TABLE I

CHOICE OF CONCENTRATION OF ACETATE BUFFER

Mean e.m.f. of 10 μg ml^{-1} standard chloride solution/mV	Mean e.m.f. of 10 μg ml^{-1} chloride solution + 40 μg ml^{-1} of sodium hydroxide/mV		Standard deviation of the difference of the two means/mV	Calculated value of t (for 10 degrees of freedom)
	Buffer A*	Buffer B†		
191·4	192·8	—	0·34	4·12‡
197·7	—	198·0	0·39	0·77 (N.S.)

* Buffer A: 6·00 g l^{-1} of acetic acid and 7·78 g l^{-1} of ammonium acetate.
† Buffer B: 60·0 g l^{-1} of acetic acid and 77·8 g l^{-1} of ammonium acetate.
‡ Significant at 1 per cent. but not significant at 0·1 per cent. probability level.
N.S. denotes not significant.

under Method and the results are shown in Table II. None of the substances tested caused any effect that would interfere significantly with the analytical technique when present in the concentrations normally found in boiler water.

TABLE II

EFFECT OF OTHER SUBSTANCES

Substance	Concentration of substance/μg ml^{-1}	Apparent* chloride concentration, μg ml^{-1}, at concentrations of	
		1 μg ml^{-1}	10 μg ml^{-1}
Ammonia	1	1·04	10·00
Cyclohexylamine	1		
Morpholine	1		
Hydrazine	0·1		
Na_3PO_4	10	1·04	10·00
SiO_2	10	1·02	9·70
NaOH	20	1·03	10·00
Fe^{3+}	10	0·95	9·80
Na_2SO_4	10	0·96	9·70
Ca^{2+}	10		
Mg^{2+}	10		

Braces denote substances that were tested together.

*If other substances had no effect the result would be expected to fall within the following ranges: 1·00 $\pm$ 0·04 and 10·00 $\pm$ 0·30 μg ml^{-1}, for 95 per cent. confidence limits.

The effect of iron(III) ion on the electrode was significant at the 1 μg ml^{-1} level but its concentration was considered to be greatly in excess of that expected under the alkaline reducing conditions of boiler water.

EFFECT OF TEMPERATURE ON ELECTRODE ASSEMBLAGE—

The effects of temperature on the electrode assemblage are complex. The potential of both the reference and the silver chloride electrode will vary according to the thermodynamic factor, $\frac{RT}{F}$. In addition, both the mercury(I) sulphate and the chloride electrode will have a temperature dependence related to the solubility of their respective sparingly soluble salts. No detailed study of temperature effects was undertaken as the problem is best solved by ensuring that all solutions are equilibrated in a water-bath thermostatically controlled to within $\pm$0·1 °C. Experiments indicated that a decrease in temperature of 1 °C resulted in an increase in potential of approximately 1 mV at 25 °C, which would correspond to changes in concentration of 0·40 and 0·04 μg ml^{-1} at the 10 and 1 μg ml^{-1} of chloride levels, respectively.

EFFECT OF LIGHT—

No effect of light was noted during the course of an analysis, but it is important to stress that the apparatus was screened from strong natural light.

METHOD

REAGENTS—

Except where otherwise stated, analytical-reagent grade chemicals should be used.

Water—Use water that has a low chloride content. Water of suitable quality (conductivity less than 10 μS m^{-1}) can be obtained by passing distilled water through a mixed-bed de-ionisation unit.

Acetic acid - ammonium acetate buffer solution—Dissolve 77·8 g of ammonium acetate in approximately 250 ml of water and add 57 ml of glacial acetic acid (sp. gr. 1·05). Dilute the mixture to 1 litre with water and store in a polythene bottle. This solution has been found to be stable for at least 9 weeks.

Standard chloride solution A—Dry sodium chloride in an oven at 250 °C for 2 to 3 hours. Weigh 1·649 g of the dry salt, and wash it with water into a 1-litre calibrated flask, make the solution up to the mark with water and store it in a borosilicate glass bottle. This solution has been found to be stable for at least 1 year.

1 ml of solution A $\equiv$ 1000 μg of chloride.

Standard chloride solution B—With a pipette, transfer 1·0 ml of solution A into a 1-litre calibrated flask and dilute to the mark with water. Transfer the contents of this flask into a polythene bottle and add 40 ml of acetate buffer solution. This buffered standard has been found to be stable for at least 1 month.

1 ml of solution B (excluding buffer solution) $\equiv$ 1 μg of chloride.

Standard chloride solution C—With a pipette, transfer 10·0 ml of solution A into a 1-litre calibrated flask and dilute to the mark with water. Add 40 ml of buffer solution and store as for solution B. This buffered solution has been found to be stable for at least 1 month.

1 ml of solution C (excluding buffer solution) $\equiv$ 10 μg of chloride.

Hydrochloric acid (1 + 99)—This solution should be freshly prepared each time a batch of electrodes is to be chloridised.

APPARATUS—

Measuring cylinders—Borosilicate glass measuring cylinders (100 ml), fitted with glass stoppers, should be used to prepare samples for analysis.

Water-bath—A water-bath of sufficient depth to cover approximately three quarters of the height of a 100-ml measuring cylinder should be used. It should be fitted with a circulating pump and a heating coil such that the temperature can be thermostatically controlled to within $\pm$0·1 °C.

Magnetic stirrer—A variable-speed magnetic stirring unit should be used to stir the solution being analysed. A PTFE or polythene-covered magnet, 20 mm long, is suitable for use in a 50-ml borosilicate glass beaker and a constant stirring rate that is just sufficient to rotate the magnet should be chosen.

Silver - silver chloride electrodes—The electrodes can be prepared from 19 s.w.g. (1·016 mm diameter) silver wire and a 120 to 140-mm length of this wire should be sealed into a soda-glass tube that is 5 to 7 mm in diameter and 100 mm long. As the seal may not be perfect, it is advisable to apply a thin coating of Araldite epoxy resin at the glass - silver surface. The length of wire protruding from the seal should be 10 to 15 mm.

Prior to electrolysis, the silver wire should be cleaned by washing it first with several small amounts of benzene and then cautiously with dilute nitric acid. Finally, the electrodes should be washed thoroughly with water. The silver electrode should be chloridised by placing it in a solution of dilute hydrochloric acid (1 + 99) and electrolysing against a platinum cathode at a current of 0·4 mA for a period of 4 hours. After electrolysis, the silver chloride electrode should be washed thoroughly with distilled water and stored in water in the dark when not in use. Electrodes prepared in this manner were used daily over a period of 3 months.

Mercury(I) sulphate reference electrode—An Electronic Instruments Ltd., Model RH 23/3, electrode with a ground-glass sleeve junction was used. The internal electrolyte used should be 1 M sodium sulphate solution.

Millivoltmeter—A millivoltmeter capable of discriminating to $\pm$0·1 mV should be used. A Beckman Research pH meter is suitable.

PROCEDURE—

Sample collection—Collect samples that have been cooled to below 35 °C in stoppered

glass or polythene bottles, taking care to avoid contamination of the sample. Samples of boiler water have been found to be stable for at least 2 weeks.

Analysis of samples—Pour 100 ml of sample into a measuring cylinder and add 4·0 ml of buffer solution. Replace the stopper, shake and place the cylinder in the water-bath, allowing approximately 10 minutes for equilibration of a sample whose initial temperature was 5 °C above or below that of the water-bath. Standard solutions B and C (with buffer added) should previously be temperature equilibrated in the water-bath.

Clamp the reference and silver chloride electrodes together; if they are mounted on a retort stand, it is convenient to mark the height at which the electrodes are clear of the stirrer. This level should be kept constant throughout the analysis. Rinse a 50-ml beaker three times with small portions of standard solution C, before adding sufficient solution for the potentiometric measurement, then immerse the electrodes in the solution and note the e.m.f. reading after 2 minutes. Take the electrodes out of the solution and remove the surplus solution from the reference electrode with a soft tissue. The silver chloride wire electrode will have very little solution on its surface and it is advisable to avoid damaging its surface by any mechanical contact. This measuring procedure is repeated for the following sequence of solutions: standard B, the sample solutions, standard C, standard B.

Calculation of results—Calculate the mean e.m.f. values of the two standard solutions and plot these values against their respective log $(c + 1)$ values, *i.e.*, 2 and 11. It is convenient to use semi-logarithmic paper for this purpose. The values of log $(c + 1)$ corresponding to the measured e.m.f. of the samples are read from the calibration graph. When the chloride concentration in the samples is known to be less than 1 μg ml^{-1}, greater precision will be obtained by using 0·1 and 1 μg ml^{-1} chloride standards to define the calibration graph.

Results

Precision—

Six solutions containing known amounts of chloride in the range 0·1 to 10 μg ml^{-1} were prepared. Two independent 1 and 10 μg ml^{-1} solutions were prepared as calibration standards, and to these were added ammonium acetate buffer solution in the recommended proportions. The e.m.f. values of these two solutions provided the within-batch calibration points throughout the precision tests.

The e.m.f. values of two portions of each of the six solutions were measured. In addition, the e.m.f. value of each of the two standard solutions was measured before and after each batch. Readings were taken after 2 and 4 minutes and the within-batch, between-batch and total standard deviations were calculated for each electrode for both the 2 and 4-minute readings. The results are summarised in Table III.

Table III

Precision of determination of chloride

Concentration of chloride added /μg ml^{-1}	Concentration of chloride found*/μg ml^{-1}	Within-batch standard deviation/μg ml^{-1}	Between-batch standard deviation/μg ml^{-1}	Total standard deviation/μg ml^{-1}
Results after 2 minutes—				
0·10	0·08	0·01	0·04	0·04
0·74	0·72	0·03	N.S.	0·04
1·76	1·77	0·04	N.S.	0·06
3·38	3·49	0·09	N.S.	0·09
5·94	6·12	0·04	0·14	0·15
10·00	9·80	0·22	N.S.	0·23
Results after 4 minutes—				
0·10	0·09	0·01	0·05	0·05
0·74	0·73	0·02	N.S.	0·03
1·76	1·78	0·04	N.S.	0·06
3·38	3·51	0·10	0	0·10
5·94	6·15	0·04	N.S.	0·06
10·00	9·82	0·23	0	0·23

* Mean of ten results.

Although the internal calibration procedure described under Method was chosen because changes in the electrode potential were feared, the effect of using a fixed calibration graph on the precisions given in Table III was investigated. A mean calibration graph was con-

structed from the 10 and 1 μg ml^{-1} of chloride calibration points used in five batches. The values of the other six solutions were calculated from this calibration graph and the results, from the 2-minute readings, are presented in Table IV.

A comparison of the results presented in Tables III and IV shows that both the between-batch and the total standard deviations are increased by using a mean calibration graph.

TABLE IV

PRECISION OF DETERMINATION OF CHLORIDE FROM A MEAN CALIBRATION GRAPH

Concentration of chloride added/μg ml^{-1}	Concentration of chloride found*/μg ml^{-1}	Within-batch standard deviation/μg ml^{-1}	Between-batch standard deviation/μg ml^{-1}	Total standard deviation/μg ml^{-1}
0·10	0·09	0·01	0·06	0·06
0·74	0·74	0·03	0·13	0·14
1·76	1·78	0·03	0·15	0·15
3·38	3·49	0·09	0·19	0·30
5·94	6·10	0·04	0·25	0·35
10·00	9·82	0·11	0·93	0·93

* Mean of ten results.

Three boiler waters from two power stations were also analysed by the described method. In each instance a batch consisted of five replicate analyses of the boiler water, together with five replicates of the same water *plus* a standard addition of chloride, e.m.f. readings being taken after 2 minutes. The results (shown in Table V) indicate satisfactory recovery of the added chloride and within-batch standard deviations similar to those obtained with standard solutions.

TABLE V

ANALYSIS OF BOILER WATER SAMPLES (AT CERL)

Sample	Chloride found/μg ml^{-1}	Recovery of added chloride, per cent.	Within-batch standard deviation/μg ml^{-1}
Boiler water A	3·08	—	0·08
+ 2·37 μg ml^{-1} of chloride ..	5·46	100·4	0·06
Boiler water A′	3·05	—	0·06
+ 2·50 μg ml^{-1} of chloride ..	5·60	101·2	0·04
Boiler water B	3·00	—	0·09
+ 2·50 μg ml^{-1} of chloride ..	5·51	100·4	0·03

TABLE VI

PRECISION OBTAINED WITH SYNTHETIC SOLUTIONS AT POWER STATIONS

Station	Chloride concentration/μg ml^{-1} Added	Found*	Total standard deviation
A	0·1	—	—
	0·5	0·51	0·09
	2·0	2·08	0·11
	6·0	6·14	0·15
B	0·1	0·10	0·03
	0·5	0·49	0·04
	2·0	2·04	0·07
	6·0	—	—
C	0·1	—	—
	0·5	0·47	0·06
	2·0	2·22	0·09
	6·0	—	—
D	0·1	0·09	0·03
	0·5	0·48	0·05
	2·0	2·05	0·13
	6·0	—	—

* Mean of ten results.

The analytical method was used at a number of power stations for the determination of chloride in synthetic and boiler water samples. The precisions reported for synthetic solutions (Table VI) are similar to those reported in this laboratory.

Table VII contains the results of the analyses of boiler waters and spiked boiler water samples and it can be seen that the recovery is satisfactory in all instances.

TABLE VII

ANALYSIS OF BOILER WATER SAMPLES AT POWER STATIONS

Sample	Chloride found/μg ml^{-1}	Recovery of chloride added, per cent.	Within-batch standard deviation/μg ml^{-1}
Station A	6·83	—	Nil
+ 6·1 μg ml^{-1} of chloride	12·62	95	0·32
Station B	0·07	—	0·03
+ 0·05 μg ml^{-1} of chloride	0·12	100	0·02
Station C	0·47	—	0·04
+ 0·5 μg ml^{-1} of chloride	0·97	100	0·06
Station D	0·22	—	0·02
+ 0·25 μg ml^{-1} of chloride	0·45	92	0·02

DISCUSSION

The precisions reported in Table III were considered adequate for daily analysis of boiler water. In view of the similarities of the precisions found after 2 and 4 minutes, it was decided that the recommended procedure would be to measure the e.m.f. after 2 minutes. The time required for analysis depends principally on the batch size and the precision required by the analyst. One obvious method of improving the time taken for analysis would be to use a predetermined calibration graph. Estimates of the precisions that could be obtained by this procedure are reported in Table IV. A mean calibration graph was obtained from the ten standard solutions in five series of analyses.

As only one reference electrode was used, the major difference in precision should be due to the small variations in the condition of the silver chloride electrode over a period of 2 weeks. Although the results obtained with a fixed calibration graph are much less precise than those given in Table III, operational conditions in power stations can be foreseen when a rapid result of moderate accuracy is required in the shortest possible time. If the highest precision is sought, then measurements will involve a period of temperature equilibration, the duration of which will depend on the difference between the sample temperature and the temperature at which the procedure is carried out.

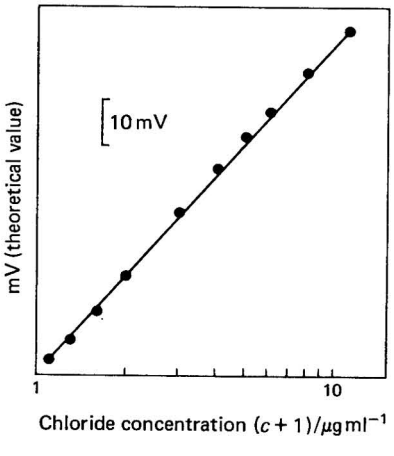

Fig. 1. Display of bias from calibration function

In the precision tests, a positive bias was observed in the region 1 to 10 μg ml^{-1}, which can be shown to be due to the choice of the function log $(c + 1)$ and the method of construction of the calibration graph. An estimate of this bias was obtained by plotting the theoretical e.m.f. values calculated from equation (3) *versus* the logarithm of the chloride concentration expressed as $(c + 1)$. These results, together with the calibration graph that would be obtained by joining the 10 and 1 μg ml^{-1} calibration points, are displayed in Fig. 1. The agreement between the theoretical and observed values cannot be checked unless accurate values of K_s and $\gamma_\pm$ are known. By using values of $1{\cdot}6 \times 10^{-10}$ for K_s[3] and 0·8 for $\gamma_\pm$, a theoretical bias of $+0{\cdot}20$ μg ml^{-1} is calculated for solutions containing 5·94 μg ml^{-1} of chloride, which agrees with the observed bias of $+0{\cdot}20$ μg ml^{-1}.

The method is free of interference from most substances normally found in boiler waters. In the presence of octadecylamine, the signal from the electrodes drifted in the direction of increasing chloride concentration, a similar effect being noted with solutions containing small amounts of commercial preparations used as corrosion inhibitors.

The proposed method is considered to be more convenient and precise than the spectrophotometric methods of analysis for chloride ions[4,5] in the range 0·1 to 10 μg ml^{-1} in boiler waters.

This work was carried out at the Central Electricity Research Laboratories and is published by permission of the Central Electricity Generating Board.

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Filter-papers as a Source of Error in Ammonia Determinations

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A selection of filter-papers was tested for the presence of ammonia. The degree of contamination of filtrates was also determined. In both instances, significant levels of ammonia were found. Hardened filter-papers contained significantly higher levels of ammonia.

THE determination of ammonia, or the determination of protein and nitrogen contents via ammonia, in samples is an important test in many analytical laboratories. Prior filtration of the sample through a filter-paper is often carried out and indeed recommended in some instances.[1,2] Actual digestion of the filter-paper with the sample is recommended in the determination of nitrogen in some types of sample.[3]

Our colleagues in our Water and Effluents Laboratory noted anomalously high ammonia contents in murky samples that had been filtered prior to carrying out the ammonia determinations, and it was suggested that ammonia leached from the filter-paper might be the source of the anomaly.

A search of the literature revealed two references[4,5] to such a source of error. As both references were rather old (18 and 27 years, respectively) and as no reference was apparently made to this problem in the more recent standard analytical texts and official methods of analysis, it was decided to investigate modern filter-papers. It is of interest to note that the authors of both of these previous papers claimed no originality for detecting the presence of ammonia in filter-papers, assuming it to be generally known, but remarked that they found no references to it elsewhere.

Ammonia has certainly been used in the past in the production of hardened ashless grades of filter-paper.[6]

EXPERIMENTAL

A range of Whatman filter-papers (from sealed cellophane-wrapped boxes), a sample of old stock of Green's filter-paper and some Schleicher and Schüll Selecta filter-papers were tested as follows.

TEST 1—

Quantitative tests on all samples—About 20 g of filter-paper were weighed into a round-bottomed flask, which was fitted to a conventional macro-Kjeldahl distillation apparatus (the ammonia evolved was collected by dipping the outlet into 50 ml of 2 per cent. boric acid solution), 400 ml of 25 per cent. sodium hydroxide solution were then added and the flask was heated until 50 ml of distillate were collected. The distillate solution was made up to 250 ml with ammonia-free water and a suitable aliquot (1 to 5 ml) was further diluted to 50 ml; 2 ml of Nessler's reagent were added to the latter solution and the resulting colour was read after 15 minutes against the relevant disc in a Lovibond Nessleriser.

By using phosphate buffer[7,8] of pH 7·4 instead of the above sodium hydroxide solution, approximately one tenth of the ammonia evolved by the former method was collected.

TEST 2—

The determination of total nitrogen (by the Kjeldahl method, which is exclusive of nitrate or nitrite, if present) was carried out on one sample (Whatman No. 541).

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TEST 3—

Filter-papers were folded and placed in glass funnels in the usual mode for filtration and four successive 25-ml portions of ammonia-free water were passed through each paper. The ammonia content of each of these 25-ml portions was measured by the Nessleriser method.

TEST 4—

A qualitative spot test with Nessler's reagent (BDH Chemicals Ltd.) applied directly on the paper gave positive results in all cases, with rather higher levels indicated for hardened ashless papers.

RESULTS AND DISCUSSION

All results are expressed as parts per million *m/m* of ammonia in the filter-paper except when otherwise indicated.

Test 1 showed that the ammonia content of analytical filter-papers ranged from 20 to 275 p.p.m. *m/m*. Lower values were obtained for qualitative filter-papers such as Whatman No. 1. The lowest result (20 p.p.m.) was obtained on the sample from old stock of Green's 401. The values increased with the degree of ashlessness and hardness of the filter-paper. Values of 210 p.p.m. were obtained for Whatman No. 542 and 275 p.p.m. for Schleicher and Schüll No. 589/3 (Blue band). While values were constant within a given box of filter-papers, considerable variation existed between different boxes of the same grade, manufacturer and even batch number, *e.g.*, two boxes of the same batch number of Whatman No. 542 filter-papers gave 210 and 110 p.p.m. of ammonia.

Test 2 showed that a Whatman No. 541 filter-paper, which had an ammonia content by Test 1 of 65 p.p.m., had a total nitrogen content (as NH_3) of 160 p.p.m.

The results of Test 3 on four different single papers (A to D) from the same box of Whatman No. 541 filter-papers are shown in Table I. Filter-papers from this box gave 50 p.p.m. of ammonia when subjected to Test 1.

TABLE I

RESULTS FOR AMMONIA DETERMINATION BY TEST 3

Portion of water (25 ml) passed through paper	Amount of ammonia in the filtrate/μg			
	Paper A	Paper B	Paper C	Paper D
First	14	20	12	12
Second	5	8	8	7
Third	4	4	3	5
Fourth	2	4	3	4

It is recommended[2] that when filtering murky water samples the first 25 ml of filtrate be discarded. The above results show that more than 100 ml need to be discarded if filter-paper is used.

When it is considered that the mass of a typical 12·5-cm filter-paper is slightly more than 1 g, the amount of ammonia, and indeed nitrogen, added in an analysis when the entire filter-paper is included with the sample can be of the order of 300 μg. Table I gives an indication of the amounts added when filtering solutions through filter-paper.

It is to be noted that Schleicher and Schüll in their publication "Selecta Filter Papers—Description of Types" do state that hard filters are "unsuitable for all work where the filter must be burnt together with the sediments, in accordance with Kjeldahl" because of the nitrogen content that results from the manufacturing process. No warning is given of the unsuitability of any filter-papers for ammonia determinations or of other than hardened papers for nitrogen determinations.

CONCLUSION

While the ammonia content of filter-paper might be known to some analysts, a survey of the literature, and of standard and reference methods, indicates a lack of awareness of the necessity of avoiding the use of filter-papers when determining low levels of ammonia and nitrogen.

The present work re-emphasises the necessity of carrying out blank determinations with all reagents including filter-paper and of taking the blank determination through all the stages of the analysis.

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Determination of Impurities in Oxygen by Mass Spectrometry

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The difficulties in analysing high-purity gaseous oxygen are overcome by using a copper-based catalyst to remove the oxygen completely, allowing impurities to be readily determined by mass spectrometry. The sensitivity is estimated to be 1 p.p.m. and the uncertainty is at most 5 per cent. of the concentration. The equipment is described, together with the results of experiments confirming the accuracy, linearity and reproducibility for the particular impurities nitrogen, argon and krypton.

THIS paper describes a method of determining a number of impurities in oxygen with an uncertainty of up to 5 per cent. of the concentration and a sensitivity in the region of 1 p.p.m. atomic. The method is based on the use of a mass spectrometer to determine impurities following the complete removal of oxygen by means of a copper-based catalyst.

The need for such measurements arose in the course of work to establish the low-temperature fixed points of the 1968 International Practical Temperature Scale. These require high-purity gases (less than 10 p.p.m. of total impurities) if the temperatures are to be realised with sufficient accuracy (better than 0·3 mK). In the case of oxygen, the significant impurities are nitrogen, argon and krypton. The more volatile gases, such as helium, hydrogen and neon, are improbable impurities, although important if present, while the less volatile gases, such as carbon dioxide and water vapour, are likely to be frozen out without influencing the fixed-point temperatures.

As is well known,[1,2] it is difficult to analyse oxygen mass spectrometrically owing to the reaction between oxygen and carbon on the filament. The life of the filament is drastically reduced as a result of this reaction, and the copious amount of carbon monoxide produced masks the main peak of nitrogen at *m/e* 28. However, the removal of oxygen by chemical means, in order to permit determination of the residual gases, is well established. Many absorbents have been used, including phosphorus,[3] chromium(III) sulphate,[4] hot copper turnings[5] and sodium - potassium alloy.[6] The present work utilises the copper-based catalyst R3-11, manufactured by BASF, which removes oxygen by the reaction

$$Cu + \tfrac{1}{2}O_2 \rightarrow CuO \ (H = -37{\cdot}1 \text{ kcal})$$

The advantages of such a catalyst are cheapness, ease of handling in the oxidised form, straightforward regeneration to the reduced form and facility for operation at room temperature. However, as the oxygen absorption capacity increases with temperature, the present system used a nominal catalyst temperature of 150 °C.

APPARATUS—

The apparatus, shown in Fig. 1, consists essentially of two 60-cm³ metering chambers, V and W, from either of which gas can be passed through the catalyst and thence into the mass spectrometer batch inlet system. Simple Bourdon gauges monitor the pressures in V and W. In normal use, V alone is used to define the volume of sample gas, which is admitted to it through the needle valve N_1. The second chamber is used, as described later, in experiments to test the linearity and accuracy of the system. All interconnections in the system are of 6 mm diameter tubing.

The catalyst is held in a chamber, 60 cm in length and 2 cm in diameter, supported vertically and filled to within 5 cm of the top with 140 g of catalyst, which is in the form of pellets of diameter 5 mm and length 3 mm. A plate, P, pierced with small holes, retains the catalyst in the chamber, while a 7-μm filter, F, prevents the transport of dust into the

inlet system of the mass spectrometer. A heating tape is wrapped around the outside of the catalyst chamber, and the temperature, indicated by two monitoring thermocouples, is easily maintained within 5 °C of the nominal 150 °C. Taps T_3 and T_4 provide connections to a diffusion pumping system with a liquid nitrogen trap and allow evacuation of all or part of the equipment as desired. With the exception of taps T_1, T_2, T_3 and T_4, and the Bourdon gauges, the entire system is constructed of stainless steel, the parts having been carefully cleaned before assembly.

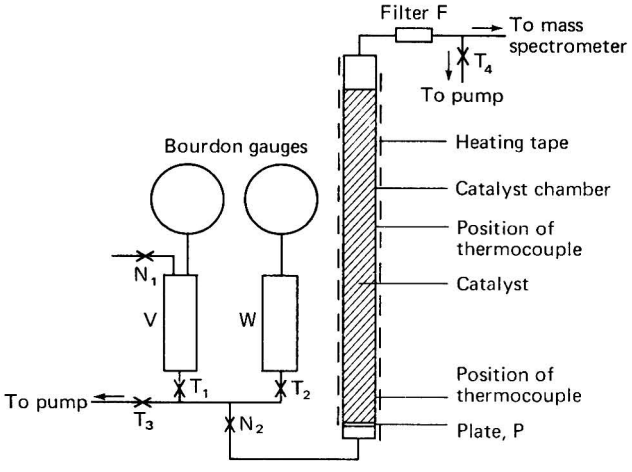

Fig. 1. Schematic representation of the analysis system

Before initial use, and infrequently thereafter, the catalyst must be reduced by passing hydrogen at 200 °C through it, setting up the reaction

$$CuO + H_2 \rightarrow Cu + H_2O \ (H = -20{\cdot}7 \text{ kcal})$$

The water is removed from the catalyst chamber by baking and pumping it which, at the same time, serves to remove other adsorbed contaminants. Although the manufacturers advise against exceeding a temperature of 250 °C, an overnight bake at 300 °C caused no discernible impairment of the catalyst. Regeneration of the catalyst was necessary after the analysis of approximately eighty oxygen samples (each of 60 cm^3 at atmospheric pressure).

EXPERIMENTAL METHOD—

The system is normally kept under a high vacuum, and an analysis is carried out in the following way. A sample of oxygen is admitted to the metering chamber V, filling it to a measured pressure (normally 1 atm). Under the control of the needle valve N_2, this sample is then bled into the catalyst chamber over a 3-minute period (the bleeding is carried out slowly so as to avoid possible local overheating of the catalyst, which is not made to withstand exposure to almost pure oxygen). The oxygen is now completely absorbed and, after an interval of 10 minutes in order to ensure equilibrium at the low pressure of the residual impurities, these remaining gases are admitted to the spectrometer batch inlet system. Peak heights are measured at *m/e* 28, 40 and 84 and are then converted, by means of calibration data, into the partial pressures of nitrogen, argon and krypton present in the original oxygen sample. At low impurity levels (less than 100 p.p.m.), a correction is applied for outgassing of nitrogen in the spectrometer inlet system, the correction being determined in a separate experiment. The system is readily calibrated by using pure samples of nitrogen, argon or krypton in order to determine the relationship between spectrometer peak height and the initial pressure in V.

Except when the catalyst requires regeneration, the oxygen present does not exceed its detection limit, estimated to be 0·5 p.p.m., while the estimated detection limits for the three impurities under consideration are of a similar magnitude.

VERIFICATION OF ACCURACY—

In order to check the linearity of the system for each of the three major contaminants, tests were carried out in the following manner. Chamber V was filled with one contaminant at a measured pressure, P_0, of approximately 0·1 atm. This gas was then expanded into the empty chamber, W, which, after closing tap T_1, was evacuated. The gas remaining in V was again expanded into W, which was once more isolated and evacuated. This expansion process was repeated as many times as desired until, after the final expansion, gas was allowed to remain in W, which thus contained contaminant at the same pressure P as did V. After again closing T_1, the gas from W was fed through the catalyst chamber to the spectrometer, thus yielding a measurement of P. This value for pressure could be compared with that calculated from the known volume expansion factor, D, because the measured pressure, P, is related to the number of expansions, n, by the relationship $P/P_0 = D^n$. D itself was determined by measurement of the pressure change upon performing an expansion from 1 atm. In Fig. 2, P (on a logarithmic scale) is plotted against n for the representative case of nitrogen. Each point represents a separate set of n expansions, while the line shows the theoretical relationship based upon the direct determination of D.

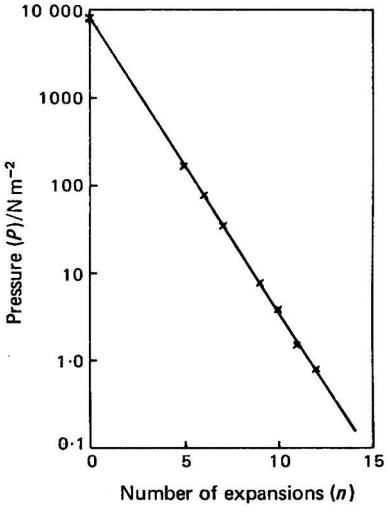

Fig. 2. Pressure as a function of the number of expansions of the sample gas. The crosses are experimental points, while the straight line represents the calculated pressure based upon the measured expansion factor

In the second stage of the test, the purest available oxygen was added to the contaminant still retained in V, making up the total pressure to 1 atm. This doped oxygen was then analysed, yielding the over-all partial pressure of the contaminant. Subtraction of the pressure previously found to be present in the identical volume W yielded the partial pressure of contaminant intrinsically present in the oxygen, which could be compared with that measured directly in similar, but undoped, oxygen. The results of these tests are shown in Table I. The agreement of the results to within 1 p.p.m. or 5 per cent., whichever is the greater, together with the satisfactory agreement shown in the expansion experiments, are taken to confirm the linearity of the system and the absence of perturbing absorption effects for the three gases nitrogen, argon and krypton.

It is possible that the exothermic absorption of oxygen promotes the desorption of small amounts of gas from the catalyst. This effect is unlikely in view of the thorough baking to which the catalyst has been subjected, and certainly cannot exceed the amounts measured in high-purity oxygen and given in Table I.

Carbon monoxide is another possible and potentially important contaminant of high-purity oxygen. It was hoped that carbon monoxide might be detected after oxidation to carbon dioxide, but it was found to be largely absorbed, yielding only an insignificant amount of carbon dioxide. The present equipment is therefore unsuitable for the determination of carbon monoxide in oxygen.

TABLE I

RESULTS OF MEASUREMENTS ON PURE AND DOPED OXYGEN

Test gas	Partial pressure of test gas/N m^{-2}			
	With 1 atm of added oxygen in V	Alone in W	Difference	As measured in oxygen alone
Argon	2·0	1·9	0·1	0·1
	1·0	0·9	0·1	
Krypton	4·9	4·4	0·5	0·6
	2·9	2·2	0·7	
	1·4	0·8	0·6	
Nitrogen	9·3	7·2	2·1	1·7
	5·6	3·6	2·0	
	3·3	1·4	1·9	

CONCLUSIONS

As a result of the absorption of oxygen by a cheap, convenient and re-usable catalyst, it is possible to use a mass spectrometer to measure impurity concentrations in oxygen with a sensitivity of 1 p.p.m. and an accuracy of 5 per cent. Self-consistency checks indicate that calibration results for nitrogen, argon and krypton can be reliably extrapolated over several orders of magnitude. The absence of perturbing sorption effects has also been demonstrated for these three gases.

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Analysis of Steroids

Part XXIV.* A Specific Method for the Spectrophotometric Determination of 17-Ethynyl Steroids

BY G. SZEPESI AND S. GÖRÖG

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The investigation of the equilibrium between 17-keto steroids, acetylene and 17-ethynyl steroids has afforded two possibilities for its analytical application. On the one hand, the 17-ethynyl steroids can be quantitatively converted into 17-keto steroids and the latter determined spectrophotometrically as their 16-glyoxalyl derivatives ($\lambda_{max.}$ = 294 nm, ϵ = 10 700 and σ = 1·1 per cent.). On the other hand, at 0 °C the 17-keto steroid contaminants in 17-ethynyl steroids can be selectively determined on the same principle.

These methods cannot be applied in the investigation of 3-keto steroids.

THE introduction of the 17-ethynyl group into various steroid hormones greatly enhances their hormonal activity. The resulting "super gestogens" and "super oestrogens" can be used in very small doses, hence the micro-determination of these compounds is very important.

Most of the existing micro-scale methods for the determination of 17-ethynyl steroids involve ultraviolet[1,2] and infrared[3] spectrophotometric, colorimetric[4–6] and fluorimetric[6] procedures, which usually depend on other functional groups in the steroid molecule such as the α,β-unsaturated 3-keto group of the phenolic A-ring. These and many other methods of similar nature are very sensitive, but are not, however, specific for the ethynyl group. The specific determination of the 17-ethynyl group can be performed by argentimetric - acidimetric titration,[7,8] but obviously this method cannot be used on the micro-scale. Gas-chromatographic methods[9–12] have also proved to be suitable for the micro-determination of 17-ethynyl steroids.

In this paper, a selective spectrophotometric method is reported for the determination of 17-ethynyl steroids, which is based on the "de-ethynylation" reaction of the 17-ethynyl steroid leading to the formation of the 17-keto steroid, and determination of the 17-keto group by our previously described diethyl oxalate method.[13–16] Examination of the equilibrium between the 17-ethynyl and 17-keto steroids has also suggested the possibility of determining 17-keto steroid contamination in 17-ethynyl steroids.

EXPERIMENTAL

APPARATUS—

A Spektromom 202 spectrophotometer with 1-cm quartz cells was used.

REAGENTS AND MATERIALS—

Solvent mixture—Mix 900 ml of analytical-reagent grade t-butyl alcohol, distilled over sodium, with 100 ml of analytical-reagent grade cyclohexane. The water content of this mixture should not exceed 0·05 per cent., as determined by the Karl Fischer method.

Sodium t-butoxide reagent, 0·25 N—Dissolve 2·88 g of sodium in 400 ml of the above mixture of t-butyl alcohol and cyclohexane by boiling the mixture, cool and dilute the solution to 500 ml.

Diethyl oxalate reagent, 1 N—Dilute 75 g (67·3 ml) of redistilled diethyl oxalate to 500 ml with the above solvent mixture.

Ethanol, 96 *per cent.* V/V.

Hydrochloric acid, 0·5 N.

Cyclohexane.

The steroids used in this study were products of G. Richter Ltd., Budapest, and their purity was checked by titration of the ethynyl group[7] and thin-layer chromatography. The

* For details of Part XXIII of this series, see *J. Chromat.*, 1973, **76**, 502.

three ethynyl steroids used in the control experiments for the determination of 17-keto contamination (see Table II) were highly purified materials with 17-keto contents of less than 0·05 per cent.

PROCEDURE FOR THE DETERMINATION OF 17-ETHYNYL STEROIDS—

Dissolve an accurately weighed amount of the sample equivalent to about 0·03 g of 17-ethynyl steroid in the solvent mixture and dilute this solution to 50 ml in a calibrated flask. Transfer 2·0 ml of this stock solution into a carefully dried 50-ml flask. Add 5 ml of solvent mixture, 2·5 ml of sodium t-butoxide reagent and reflux the mixture for 2 to 3 minutes. Cool the mixture and add 0·5 ml of diethyl oxalate reagent. Allow the mixture to stand at room temperature for 15 minutes, then add 3 ml of 0·5 N hydrochloric acid, transfer the mixture into a 50-ml calibrated flask and dilute to volume with ethanol.

Prepare the reference solution in a similar manner, except that the 2·0-ml aliquot of the stock solution is added after the addition of the hydrochloric acid.

Determine the absorbance against the reference solution at 294 nm and calculate the 17-ethynyl steroid content on the basis of the absorbance of a similarly treated standard solution or from the molar absorptivities given in Table I.

TABLE I

SPECTRAL DATA FOR THE DETERMINATION OF 17-ETHYNYL STEROIDS AFTER DE-ETHYNYLATION AND FORMATION OF 16-GLYOXALYL DERIVATIVES

	Purity,[7] per cent.	Molar absorptivity (294 nm)	Relative standard deviation,* per cent.
Mestranol	99·9	10 700	±1·1
17-α-Ethynyl-androst-5-en-3β,17-diol	99·8	10 800	±1·0
17-α-Ethynyloestradiol	99·9	10 700	±1·0
Ethynodiol diacetate	99·5	10 600	—
Ethynodiol	98·9	10 700	—

* Six parallel runs.

PROCEDURE FOR DETERMINING THE 17-KETO STEROID CONTENT IN 17-ETHYNYL STEROIDS—

Accurately weigh about 0·03 to 0·05 g of the sample to be tested into a 50-ml calibrated flask. Dissolve the material in a mixture of 2·5 ml of cyclohexane, 2·5 ml of solvent mixture and 0·5 ml of diethyl oxalate reagent. Before the addition of sodium t-butoxide reagent, place the flask containing the mixture in an ice-bath and allow it to stand for 15 minutes. Then add 2·5 ml of sodium t-butoxide reagent and allow the mixture to stand for an additional period of 30 minutes in the ice-bath. Add 3 ml of 0·5 N hydrochloric acid and dilute to volume with ethanol.

Prepare the reference solution in a similar manner but in this case weigh the substance to be tested into the flask after the addition of the hydrochloric acid. The difference between the two weighings must not exceed 1 per cent. Calculate the 17-keto content on the basis of the extinction recorded at 294 nm by using the molar absorptivities given in Table I. In instances of very low 17-keto contents the use of 2-cm cells is advisable.

RESULTS AND DISCUSSION

The results for the determination of various 17-ethynyl steroids by the recommended method are summarised in Table I, and those for the determination of 17-keto steroid contaminants in the 17-ethynyl steroids are given in Table II.

The 17-ethynyl steroids are produced commercially from 17-keto steroids by treating the latter with sodium acetylide according to the following reaction.

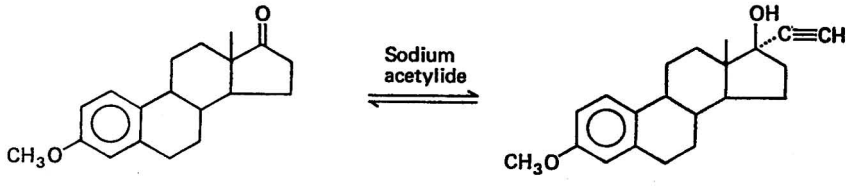


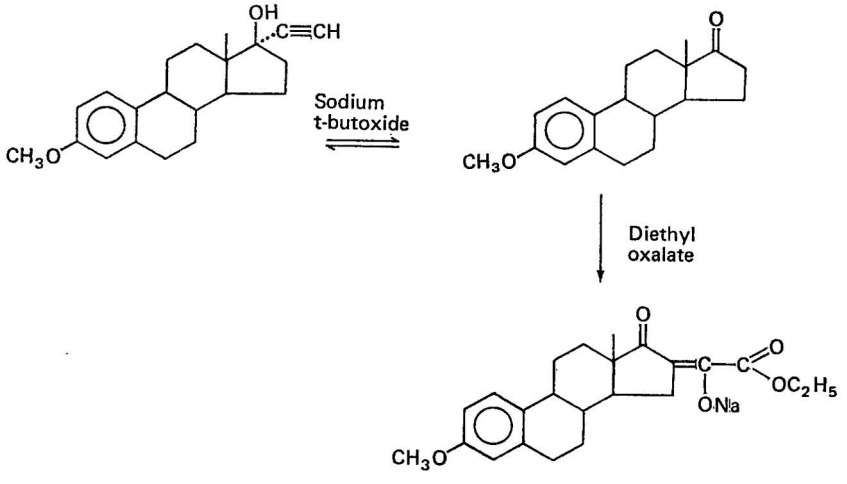
TABLE II

DETERMINATION OF 17-KETO STEROID CONTENT IN 17-ETHYNYL STEROIDS

Main component	Contaminant	Amount of contamination: Taken, per cent.	Found, per cent.	Standard deviation*
Mestranol	Oestrone-3-methyl ether	0·060	0·064	±0·005
		0·212	0·210	
		0·320	0·320	±0·05
		0·420	0·424	
17-α-Ethynyloestradiol..	Oestrone	0·180	0·182	
		0·480	0·482	±0·03
		0·620	0·622	
17-α-Ethynyl-androst-5-en-3β,17-diol	Dehydroepi-androsterone	0·200	0·205	±0·01

* Six parallel runs.

In the presence of a large excess of sodium acetylide at low temperatures, this equilibrium is shifted to the right. At high temperatures, however, in the presence of a large excess of sodium alcoholate and in the absence of acetylene, this equilibrium can be shifted in the opposite direction. In the procedure described above, the elimination of the ethynyl group followed by the determination of the 17-keto group formed offers the possibility of determining the 17-ethynyl steroids as shown by the reactions given below.



The effect of the temperature on the equilibrium in the presence of a 100-fold excess of sodium t-butoxide was investigated, and the results obtained are shown in Table III.

At 0 °C, this equilibrium shifts towards the formation of the ethynyl group, but with increasing temperature the extent of the conversion by the de-ethynylation reaction also increases. At 81 °C, which is the boiling-point of the solvent mixture used, the latter reaction becomes quantitative. The results given in Table III were obtained by the spectrophotometric determination of the 17-keto group after its conversion into the corresponding 16-glyoxalyl derivative as described under Experimental. At high temperatures, the equilibrium is established almost instantaneously.

TABLE III

EFFECT OF TEMPERATURE ON THE EQUILIBRIUM

Temperature/°C	0	5	25	50	81
Conversion of the de-ethynylation reaction, per cent.	0	1·1	4·9	53·0	100·0

The quantitative nature of the de-ethynylation reaction at the boiling-point temperature was proved by subjecting the reaction mixture to thin-layer chromatography, which showed that the 17-ethynyl steroids had completely disappeared and that the 17-keto steroids were formed exclusively. It was therefore concluded that the problem of the quantitative conversion of 17-ethynyl into 17-keto steroids could easily be solved.

As both the de-ethynylation reaction and the formation thereafter of the glyoxalyl derivative occur in the presence of sodium t-butoxide, the favourable situation exists that after the completion of the elimination reaction, the conversion of the 17-keto steroids into their 16-glyoxalyl derivatives with diethyl oxalate can be carried out in the same reaction mixture without the need for any separation step. The latter reaction reaches completion within 15 minutes at room temperature. The 16-glyoxalyl derivatives of 17-keto steroids are very stable and possess a strong absorption band at 294 nm, at about pH 2.[13]

In Table I, the spectral data of some 17-ethynyl steroids that were investigated after the above treatment with sodium t-butoxide and diethyl oxalate are summarised.

With oestrogens the ring "A" is aromatic and it has an absorption band near the peak of the 16-glyoxalyl-17-keto group, the effect of which can be nullified by our recently described differential spectrophotometric method.[14] For the purpose of uniformity, the latter procedure was used for all materials investigated. It is to be noted, however, that the method described is not suitable for the determination of 17-ethynyl steroids that contain an unsaturated 3-keto group in the A-ring, *e.g.*, ethisterone and norethisterone, as these compounds undergo oxidation in boiling alkali solution giving 6-keto derivatives.[17,18]

As mentioned in the introduction, examination of the equilibrium enabled us to determine the 17-ketone content of 17-ethynyl steroids, which determination cannot be carried out by the standard diethyl oxalate method[13] because the de-ethynylation reaction discussed above occurs at room temperature.

As can be seen from the results given in Table III, this interference can be eliminated by carrying out the reaction at 0 °C. By using this method, trace amounts of 17-ketone impurity in 17-ethynyl steroids can be determined, which procedure is of value for analytical control in the manufacture of some 17-ethynyl steroids. Some results obtained are collected in Table II. Obviously, the limitation described in the determination of 17-ethynyl steroids applies also to this determination.

The relative standard deviations given in Tables I and II are characteristic of the precision of the method.

The authors thank Mrs. S. V. Ramakrishnan for her technical assistance.

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NOTE—References 7 and 14 are to Parts II and XVII of this series, respectively.

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A Stable and Sensitive Colorimetric Method for the Determination of Ergocalciferol (Vitamin D_2) by Using Trifluoroacetic Acid

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A sensitive and fairly stable colour for the spectrophotometric determination of ergocalciferol (vitamin D_2) following reaction with trifluoroacetic acid has been developed. In the method hydroquinone was used to stabilise the yellow colour, which reached maximum absorbance in not less than 80 s and remained stable for at least 20 s, thus giving the analyst convenient time to carry out the determination. Maximum absorbance was reached at a wavelength of 490 nm. The colour could be destroyed within 2 minutes by the addition of hydrogen peroxide solution, which property was used to advantage in differential spectroscopy. The absorbance is linear for ergocalciferol solutions containing from 0 to 32 μg, optimum results being obtained in the 5 to 15-μg range. The relative standard deviation of the method is $\pm 1{\cdot}76$ per cent.

ERGOCALCIFEROL (vitamin D_2) is isomerised to isotachysterol by reaction with antimony trichloride,[1] acetyl chloride[2] and trifluoroacetic acid.[3] This reaction has provided the basis for several chemical methods of assay for ergocalciferol. The method that is widely used is the colorimetric method involving the use of antimony trichloride,[4,5] but it suffers from several disadvantages.[6] Clements, Forbes, Olliff and Rogers[7] used the fairly stable yellow colour produced with trifluoroacetic acid at 403 nm for the determination; they also reported that a maximum appeared at 496 nm during the first 90 s and then rapidly disappeared. Recently, Sklan and Budowski[3] reported that trifluoroacetic acid produced a yellow colour, which reached maximum absorption at 500 nm after 25 s and faded rapidly, but which could be analytically useful. They used the ultraviolet absorbance of the esterified isotachysterol at 290 nm for their determinations after first extracting the ester.

During the investigation of chemical methods for the determination of ergocalciferol in pharmaceutical preparations it was noted that the stability of the absorbance at 496 and 403 nm when using trifluoroacetic acid was affected by oxidation in air. It was found that low concentrations of a solution of hydroquinone in chloroform, which acts as an antioxidant, increased the sensitivity of the yellow colour and stabilised the absorbance at 490 nm. This absorbance could be abolished rapidly by the addition of a small volume of hydrogen peroxide solution. This paper briefly describes the optimum conditions for utilising the reaction with trifluoroacetic acid for the determination of ergocalciferol.

EXPERIMENTAL

REAGENTS—

Trifluoroacetic acid, "OR" grade.

CAUTION—Trifluoroacetic acid is an extremely corrosive acid and should be handled with care.

Chloroform, analytical-reagent grade.

Hydroquinone solution, 0·1 per cent.—Dissolve 50 mg of crystalline hydroquinone in 1 ml of diethyl ether and dilute to 50 ml with chloroform. Prepare a fresh solution daily.

Hydrogen peroxide, 30 per cent. solution, reagent grade.

Ergocalciferol, U.S.P. Reference Standard.

INSTRUMENTATION—

Perkin-Elmer, Coleman Model 124, spectrophotometer.

Rotary evaporator.

PROCEDURE—

Mix the sample under test, containing 5 to 15 μg of ergocalciferol, with 2 ml of the 0·1 per cent. hydroquinone solution in an organic solvent contained in a 25-ml Erlenmeyer flask. Completely evaporate the mixture under vacuum at 35 to 40 °C. To the residue add 0·5 ml of chloroform, mix, and add 2·0 ml of trifluoroacetic acid, mixing them well and then transferring the mixture to a 1-cm spectrophotometer cuvette within 50 s. Measure the highest absorbance reached at 490 nm within 1 to 3 minutes from mixing, zeroing the instrument with a solvent blank of chloroform - trifluoroacetic acid (1 + 4). After measurement add 2 drops of hydrogen peroxide solution to the cuvette, mix, and measure the absorbance 2 minutes $\pm$10 s after the addition of hydrogen peroxide. Subtract the latter measurement from the former and calculate the amount of ergocalciferol by comparison with a similarly treated standard solution.

RESULTS AND DISCUSSION

Various parameters were studied in an attempt to increase the intensity and stability of the yellow colour developed with trifluoroacetic acid. The presence of chloroform was necessary for the maximum development of the sensitive colour, while alcohol and, to a lesser extent, ether, hindered its development. The amount of chloroform could be increased from 0·5 to 2·0 ml with no significant effect on the stability of the colour but with a corresponding decrease in sensitivity due to the larger volume of solution. The 4:1 ratio of chloroform to trifluoroacetic acid recommended by Clements *et al.*[7] resulted in an appreciable decrease in both sensitivity and stability. Hydroquinone in higher concentrations than 0·1 per cent. tended to crystallise out from the chloroform on standing; however, 2 ml of the 0·1 per cent. solution were sufficient to stabilise the colour. The residue obtained by evaporating this solution in chloroform to dryness on a rotary evaporator resulted in only a 4 per cent. decrease in the yield of colour if the residue was maintained under vacuum at 40 °C for 45 minutes.

The addition of 30 per cent. hydrogen peroxide solution was sufficient to cause the colour at 490 nm to fade rapidly in the first minute and then to reach a stable intensity after 90 s. The slight change in volume on addition of hydrogen peroxide solution had a negligible effect on the blank value. Nitric acid and potassium permanganate were found to be unsuitable for destroying the colour; one drop of concentrated nitric acid gave an initial incomplete oxidation, followed by a slight increase in absorbance at 490 nm while potassium permanganate dissolved in trifluoroacetic acid, had a slow, decreasing effect on the absorbance at 490 nm with the appearance of a new maximum at 448 nm.

Various amounts and dilutions of U.S.P. Reference Standard ergocalciferol in chloroform were used to construct a linear calibration graph. The maximum absorbance attained at

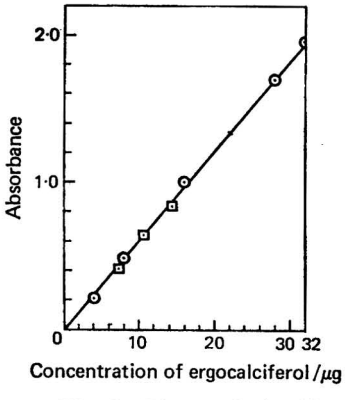

Fig. 1. Linear relationship of two different samples and their dilutions of reference standard ergocalciferol to the colour development in the trifluoroacetic acid procedure

490 nm was rectilinear with the amount of ergocalciferol over the range from 0 to 32 μg (Fig. 1). The relative standard deviation of the absorbance for six identical aliquots containing 9·1 μg of ergocalciferol was $\pm$1·76 per cent. The visible spectrum obtained approximately 4 minutes after the addition of trifluoroacetic acid (Fig. 2) illustrates the absorbance band at 490 nm with only a slight contribution at 403 nm. The maximum intensity of absorbance at 490 nm was attained in not less than 80 s, and was stable for at least 20 s before decreasing slowly, thus leaving ample time for the determinations. Ergocalciferol similarly treated with trifluoroacetic acid but in the absence of hydroquinone, attained its maximum absorbance in less than 1 minute, the value decreasing rapidly (Fig. 3).

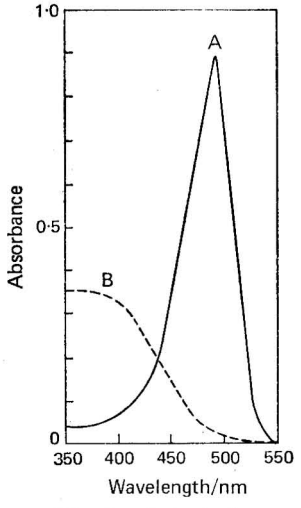

Fig. 2. A typical spectrum of the colour developed in the reaction of ergocalciferol with trifluoroacetic acid in the presence of hydroquinone before (A) and after (B) the addition of hydrogen peroxide

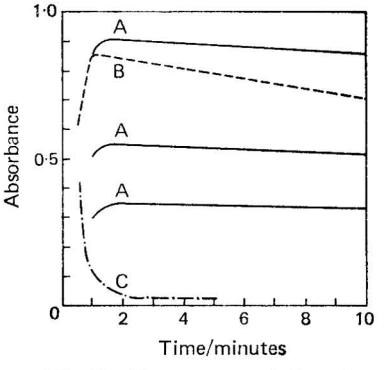

Fig. 3. Time course of the colour formation of ergocalciferol with trifluoroacetic acid: A, with hydroquinone (at three concentrations of ergocalciferol); B, without hydroquinone; and C, with hydrogen peroxide

The method for the determination of ergocalciferol described in this paper holds several important advantages over the colorimetric methods reported in the literature. The colour produced, in addition to being proportional to the concentration, is more sensitive and more stable than those produced by similar procedures. The addition of hydroquinone in order to stabilise the colour overcomes the technical difficulty of obtaining an accurate absorbance value from an unstable colour. The procedure is simple and rapid and requires no special skills; moreover, in the presence of spectral interferences of a general background nature, the use of hydrogen peroxide for differential spectroscopy provides a distinct advantage. No significant variation in results was observed by different analysts using similar samples.

The use of this method for the determination of ergocalciferol in pharmaceutical formulations containing other vitamins after preliminary purification is currently under investigation.

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Determination of the Substituted Pyrimidine Pesticides Ethirimol, Dimethirimol, Pirimiphos-methyl, Pirimiphos-ethyl and Pirimicarb in Technical and Formulated Materials by Gas Chromatography*

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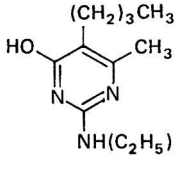
Gas chromatography can be used to determine the members of a group of substituted pyrimidines that comprises two systemic fungicides, ethirimol and dimethirimol; two insecticides, pirimiphos-methyl and pirimiphos-ethyl; and an aphicide, pirimicarb.

The two fungicides, ethirimol and dimethirimol, are hydroxy compounds, which are determined by gas chromatography with flame-ionisation detection after conversion into their volatile trimethylsilyl ethers. The remaining three compounds are determined directly without derivative formation. All separations are carried out on a column packed with 100 to 120-mesh Celite coated with 10 per cent. silicone elastomer. Depending upon the retention time of the compound to be determined, either n-octadecane or n-nonadecane is added as an internal standard. The procedures are applicable to the technical pesticides and to a range of formulated products.

The methods are sufficiently specific and accurate to be recommended for referee purposes, and their precision has been shown to be satisfactory for routine control.

THE applications in agriculture of five new substituted pyrimidines that have insecticidal or fungicidal activity have previously been reported.[1–4] The technical pyrimidines are manufactured by ICI Organics Division and are formulated and marketed by ICI Plant Protection Limited.

The structural formulae of the five compounds are as follows—



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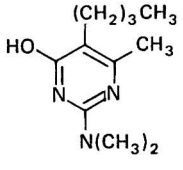

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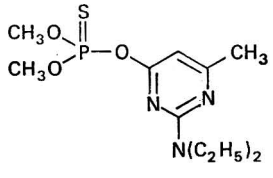

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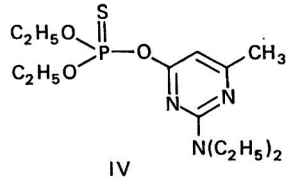

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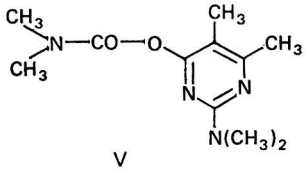

V

* Based on a paper presented at the CIPAC Symposium, Stockholm, June, 1972.

Ethirimol† ('Milstem'‡) (5-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine, **I**) and dimethirimol† ('Milcurb'‡) (5-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine, **II**) are systemic fungicides that are effective in controlling mildew in cereals and cucurbits, respectively. Both compounds when pure are white crystalline solids (ethirimol, m.p. 159 to 160 °C, and dimethirimol, m.p. 102 °C). Pirimiphos-methyl† ('Actellic'‡) [*O*-(2-diethylamino-6-methylpyrimidin-4-yl)-*OO*-dimethyl phosphorothioate, **III**] and pirimiphos-ethyl† ('Primicid'‡) [*O*-(2-diethylamino-6-methylpyrimidin-4-yl)-*OO*-diethyl phosphorothioate, **IV**] are substituted pyrimidine phosphates that possess insecticidal activity in a wide variety of applications. Both compounds are pale, straw-coloured liquids. The fifth compound, pirimicarb† ('Pirimor'‡) (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, **V**), which is a white crystalline solid (m.p. 91·5 °C), is a fast-acting, selective aphicide.

Gas - liquid chromatographic methods for determining each of the five pesticides were first developed in ICI Organics Division for process control during the manufacture of the technical materials. It was found that with minor variations in technique, all five compounds could be determined on the same chromatographic column, using either n-octadecane or n-nonadecane as an internal standard according to the retention times for the individual compounds. Subsequently, this method was successfully applied to the analysis of formulated products, embracing aqueous solutions and suspensions, emulsifiable concentrates, dispersible powders and impregnated granules. For certain analyses, methods based upon ultraviolet absorption measurements in methanol have been used. Ethirimol and dimethirimol can be determined by ultraviolet spectrophotometry in methanol based on absorbance measurements at 297 nm ($\epsilon = 7820$) and 303 nm ($\epsilon = 7149$), respectively. Pirimiphos-methyl and pirimiphos-ethyl can also be determined by ultraviolet spectrophotometry.

For technical and formulated materials, clean-up on Florisil columns is followed by hydrolysis in boiling 3 N hydrochloric acid to the parent pyrimidine, 2-diethylamino-4-hydroxy-6-methylpyrimidine. The latter is then determined absorptiometrically in methanolic solution at 298 nm ($\epsilon = 8010$). Pirimicarb, on the other hand, has been determined by routine alkaline hydrolysis, distillation of the liberated dimethylamine into 2 per cent. boric acid solution and titration with standard acid.

Quantitative thin-layer chromatographic procedures have also been developed for all five compounds, in which, after development on Kieselgel GF_{254} plates by the use of a selected solvent system, eluted bands corresponding to the active pesticide are removed by the "vacuum cleaner" technique[5] and the determination is completed by measuring the ultraviolet absorbance of a methanolic extract. With the ethirimol system a 75 + 10 + 15 chloroform - acetone - glacial acetic acid mixture is used for development and the absorbance of a methanolic extract is measured at 297 nm.

For dimethirimol a 1 + 9 methanol - dichloromethane mixture is used and measurement made at 303 nm. In the determination of pirimiphos-methyl and pirimiphos-ethyl, 3 + 1 and 9 + 1 n-hexane - acetone mixtures, respectively, are used for development. Both of the latter compounds are measured at 248 nm [ϵ(pirimiphos-methyl) = 20 761; ϵ(pirimiphos-ethyl) = 21 345]. A 9 + 1 chloroform - dioxan developing solvent is used for pirimicarb and ultraviolet measurement made at 245 nm ($\epsilon = 22\,291$).

All of these alternative procedures have been applied in our laboratories. Because of the specificity achieved and its ease of application we believe that the gas - liquid chromatographic method has most to recommend it and the procedure involved is described in detail in this paper. Results obtained by using the various methods are included for comparison.

Gas-chromatographic methods

Reagents and materials—

Chloroform—Analytical-reagent grade.
Concentrated hydrochloric acid—Analytical-reagent grade.
Sodium hydroxide solution, 0·5 M.
Bromothymol blue indicator.
*Silicone elastomer E*301—As supplied by Phase Separations Ltd.

† Approved common names.
‡ Registered trade names.

Silylating agent, BSA [NO-*Bis*(*trimethylsilyl*)*acetamide*]—As supplied by Phase Separations Ltd.

Silanising agent, Silyl 8—As supplied by Phase Separations Ltd.

Internal standards, n-octadecane and n-nonadecane—As supplied by BDH Chemicals Ltd.

Analytical standards—Pure samples of ethirimol, dimethirimol, pirimicarb, pirimiphos-methyl and pirimiphos-ethyl can be obtained on request from ICI Plant Protection Ltd., Yalding, Kent. Pirimiphos-methyl and pirimiphos-ethyl standards must be stored at 0 °C for maximum long-term stability. Dimethirimol and pirimicarb standards are also available from the National Physical Laboratory, Division of Chemical Standards.

CAUTION—Pirimicarb, pirimiphos-methyl and pirimiphos-ethyl are cholinesterase inhibitors. Care must be taken when using samples containing these compounds to avoid inhalation of dust or vapour or allowing them to make contact with skin.

Internal standard solutions—Prepare solutions containing either n-octadecane or n-nonadecane in chloroform as follows:

Solution No.	Compound to be determined	Internal standard	Concentration of solution/g l^{-1}
I	Ethirimol, pirimicarb	n-Nonadecane	10·00
II	Dimethirimol	n-Octadecane	10·00
III	Pirimiphos-ethyl	n-Nonadecane	2·00
IV	Pirimiphos-methyl	n-Octadecane	1·80

Calibration solutions—Prepare calibration solutions by dissolving accurately weighed amounts of pure pesticides in exact volumes of the appropriate internal standard solutions as follows:

Compound to be determined	Amount of pure pesticide/g	Internal standard solution	
		No.	Volume/ml
Ethirimol	0·15	I	20·0
Dimethirimol	0·20	II	20·0
Pirimicarb	0·15	I	10·0
Pirimiphos-ethyl	0·30	III	50·0
Pirimiphos-methyl	0·35	IV	50·0

Convert ethirimol and dimethirimol into their corresponding trimethylsilyl ethers by mixing a 1·0-ml aliquot of either calibration solution with 0·10 ml of silylating agent in a stoppered test-tube. Allow the mixture to react for 10 minutes before use.

Celite 545, 100 *to* 120 *mesh, acid-treated*—Commercially available grades of acid-treated Celite may be suitable but should be checked for the absence of residual acidity. By using column packing prepared as described below, chromatograms should be reproducible and give peaks with negligible tailing. Distorted peaks and excessive retention times may indicate that the Celite was insufficiently washed to become neutral following treatment with acid. Unwashed grades can be treated as follows.

Weigh 250 g of 100 to 120-mesh Celite 545 into a 2-litre beaker and add concentrated hydrochloric acid until the Celite is covered with a 2-inch layer of acid. Heat the mixture on a boiling water bath in a fume cupboard for 2 to 3 hours, stirring occasionally. Decant the acid and repeat the treatment with fresh acid. Filter off the Celite into a sintered-glass funnel, wash it several times with distilled water, transfer it into a clean 2-litre beaker and stir it for 15 minutes with about 1 litre of distilled water. Filter off the Celite and repeat the procedure with fresh water until the filtrate remains neutral after stirring the mixture for 15 minutes. Filter off the Celite and dry it overnight in an oven at 130 °C. Sieve the dried Celite through 100 and 120-mesh sieves and use the portion retained on the 120-mesh sieve to prepare the column packing.

Column packing—Dissolve 5 g of silicone elastomer E301 in 290 ml of chloroform, which is most conveniently achieved by allowing the mixture to stand overnight in a stoppered flask. Add 45 g of acid-treated Celite to the solution of stationary phase and distil off the chloroform under vacuum on a water-bath by using a rotary evaporator. Remove the last traces of solvent by drying the packing in an open dish at 100 °C. Sieve the dried material, retaining the 100 to 120-mesh fraction.

APPARATUS—

The work described in this paper was carried out with with a Pye Series 104, Model 4, gas chromatograph equipped for flame-ionisation detection and single-column operation with on-column injection. A Hewlett-Packard, Model 3370A, integrator was used for peak area evaluation and chromatograms were monitored with a potentiometric recorder. Equivalent equipment of other manufacture may be suitable.

Column—A 5 foot by 4 mm i.d. glass column was packed with 10 per cent. silicone elastomer E301 coated on 100 to 120-mesh acid-treated Celite and conditioned by heating for 8 hours at 250 °C with carrier gas flowing. The column was then silanised, with the temperature maintained at 250 °C, by injecting three successive 10-μl portions of Silyl 8. Re-silanisation was carried out from time to time in order to prevent peak tailing.

Operating conditions—Gas flow-rates measured at the detector outlets were: carrier gas (oxygen-free nitrogen), 50 ml min^{-1}; hydrogen, 50 ml min^{-1}; and air, 750 ml min^{-1}. Satisfactory chromatograms were produced by using column oven temperatures within the range 200 to 220 °C, the following settings being regarded as optimal for the Pye 104 gas chromatograph: ethirimol and pirimiphos-ethyl, 220 °C; pirimiphos-methyl, 215 °C; pirimicarb, 210 °C and dimethirimol, 200 °C. The most suitable oven temperature settings for gas chromatographs of other designs may differ from those recommended and should be ascertained by the user. Amplifier attenuation settings were selected to give about 80 per cent. full-scale deflection on a 10-mV recorder for peak area evaluation or to give an integrator count of about 2000×10^2 μA s for each major peak. Settings for the Hewlett-Packard 3370A integrator were: slope sensitivity (up), 0·3 mV min^{-1}, slope sensitivity (down), 0·1 mV min^{-1}; and base-line re-set delay, zero.

PROCEDURE FOR PREPARATION OF SAMPLE SOLUTIONS—

Technical samples—Prepare solutions in chloroform containing amounts of substituted pyrimidine and internal standard similar to those present in the corresponding calibration solutions, using internal standard solutions I, II, III or IV as appropriate.

Ethirimol aqueous suspensions—Weigh accurately sufficient sample to contain about 0·15 g of ethirimol, add 20·0 ml of internal standard solution I and mix by shaking them for 1 minute in a stoppered test-tube. Allow sufficient time for the aqueous and chloroform layers to separate and for any solid matter to settle. Remove 1·0 ml of the chloroform layer and treat with silylating agent as directed for the preparation of the ethirimol calibration solution.

NOTE—The above procedure has been used satisfactorily for samples containing up to 60 per cent. *m/m* of water.

Dimethirimol hydrochloride solutions—Weigh accurately sufficient sample to contain about 0·20 g of dimethirimol, dilute it with 50 ml of water, add three to four drops of bromothymol blue indicator and titrate the mixture with approximately 0·5 N sodium hydroxide solution to the green end-point. Extract it four times with 25-ml portions of chloroform and filter the combined extracts through phase-separating paper. Wash the paper with 20 ml of chloroform, then evaporate the filtrate and washings to dryness in a rotary evaporator. Dissolve the residue in 20 ml of internal standard solution II, then proceed as directed for the preparation of the dimethirimol calibration solution.

Pirimiphos-methyl and pirimiphos-ethyl emulsifiable concentrates—Weigh accurately sufficient sample to contain about 0·35 g of pirimiphos-methyl or 0·30 g of pirimiphos-ethyl and dissolve it in 50·0 ml of the appropriate internal standard solution.

Pirimiphos-ethyl granular products—Weigh accurately sufficient sample to contain about 0·30 g of pirimiphos-ethyl. Shake it with 50·0 ml of internal standard II in order to extract the pesticide and allow the solids to settle. Chromatograph aliquots of the supernatant liquid.

Dispersible powders containing pirimicarb—Proceed as directed for the technical material and calibration solution, using an equivalent amount of sample. Allow the undissolved solids to settle and chromatograph aliquots of the supernatant liquid directly.

ANALYSIS OF SOLUTIONS—

Aliquots (1 μl) of calibration and sample solutions are chromatographed in turn and integrator counts for pesticide and internal standard peaks are recorded. Alternatively,

peak areas from chart-recorder traces can be calculated from the product of peak height × peak width at half-height.

Typical chromatograms given by samples of the technical pesticides are shown in Fig. 1 (*a* to *e*). Approximate relative retentions (internal standard = 100) are as follows: ethirimol trimethylsilyl ether, 53; dimethirimol trimethylsilyl ether, 47; pirimiphos-methyl, 149; pirimiphos-ethyl, 142; and pirimicarb, 73. Other significant peaks due to impurities are not usually observed for materials that are typical of normal manufacture.

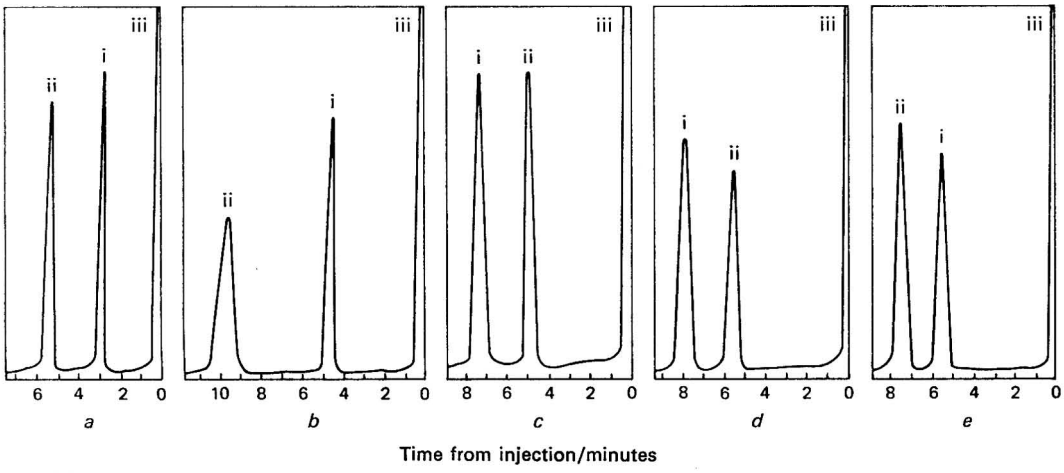

Fig. 1. Gas - liquid chromatograms obtained for chloroform solutions of technical pyrimidines with internal standards present, using a 5 foot × 4 mm i.d. column packed with 10 per cent. silicone elastomer E301 on 100 to 120-mesh acid-treated Celite.

a: (*i*), Ethirimol trimethylsilyl ether; (*ii*), n-nonadecane; and (*iii*), solvent. Temperature 220 °C
b: (*i*), Dimethirimol trimethylsilyl ether; (*ii*), n-octadecane; and (*iii*), solvent. Temperature 200 °C
c: (*i*), Pirimiphos-methyl; (*ii*), n-octadecane; and (*iii*), solvent. Temperature 215 °C
d: (*i*), Pirimiphos-ethyl; (*ii*), n-nonadecane; and (*iii*), solvent. Temperature 220 °C
e: (*i*), Pirimicarb; (*ii*), n-nonadecane; and (*iii*), solvent. Temperature 210 °C

CALCULATION—

$$\text{Calibration factor } (F) = \frac{a}{b} \times \frac{d}{c}$$

$$\text{Percentage } (m/m) \text{ of pesticide in the sample} = \frac{f}{e} \times \frac{g}{h} \times F \times 100$$

where *a* g is the amount of standard pesticide in the calibration solution; *b* g, the amount of internal standard in the calibration solution; *c*, the integration or peak area for the pesticide in the calibration solution; *d*, the integration or peak area for the internal standard in the calibration solution; *e* g, the amount of sample taken; *f* g, the amount of internal standard in the sample solution; *g*, the integration or peak area for the pesticide in the sample solution; and *h*, the integration or peak area for the internal standard in the sample solution.

RESULTS AND DISCUSSION

LINEARITY AND REPRODUCIBILITY OF RESPONSE—

The relationship between relative response and composition of sample solutions is demonstrated by the results given in Table I for the dimethirimol trimethylsilyl ether - n-octadecane and pirimiphos-ethyl - n-nonadecane calibration systems.

In each system the amount of internal standard was fixed and that of the pure pesticide varied, and *vice versa*. The mean calibration factors were calculated from single chromatograms on groups of five solutions in chloroform containing different ratios of internal standard to pesticide. Within each group the coefficient of variation was of the order of 2 per cent.,

thus confirming that the relative responses for dimethirimol and pirimiphos-methyl are rectilinear over a reasonably wide range of calibration ratios. These results confirm that a single calibration point is sufficient for determining each pesticide. Similar rectilinear relationships can be demonstrated for ethirimol, pirimiphos-methyl and pirimicarb.

TABLE I

LINEARITY OF RESPONSE FOR CALIBRATION SOLUTIONS OF DIMETHIRIMOL - n-OCTADECANE AND PIRIMIPHOS-ETHYL - n-NONADECANE MIXTURES

Range of amounts/g		Mean calibration factor for five calibration points	Coefficient of variation, per cent.
n-Octadecane	Dimethirimol		
0·20	0·05 to 0·40	1·224	2·1
0·05 to 0·40	0·20	1·214	1·7
n-Nonadecane	Pirimiphos-ethyl		
0·10	0·10 to 0·50	2·413	2·3
0·06 to 0·16	0·30	2·410	2·4

For routine analysis of numbers of samples by using the same packed column, it is only necessary to check calibration factors on a daily basis. The factors are also consistent from day to day, a typical variation observed over a period of several weeks being within ± 1 per cent. of the mean.

PRECISION AND ACCURACY—

The precision was determined by replicate analysis of samples of unformulated pesticides that were typical of material produced in normal manufacture. Each sample was analysed six times, by using a freshly prepared calibration and sample solution for each determination. Each solution was injected once only into the chromatograph. With two of the compounds, dimethirimol and pirimicarb, the exercise was repeated, using duplicate injections of each solution. The mean and standard deviations obtained for each group of six determinations are shown in Table II.

TABLE II

PRECISION OF GAS - LIQUID CHROMATOGRAPHIC DETERMINATIONS OF TECHNICAL SUBSTITUTED PYRIMIDINES

Technical compound	Active pesticide content, per cent. *m/m* (mean and standard deviation for six determinations)
Ethirimol	96·7 $\pm$ 1·6
Dimethirimol	92·7 $\pm$ 0·9 (92·1 $\pm$ 1·3)
Pirimiphos-methyl	91·1 $\pm$ 1·8
Pirimiphos-ethyl	96·4 $\pm$ 1·5
Pirimicarb	100·3 $\pm$ 1·5 (99·5 $\pm$ 0·6)

Values in parentheses are based on duplicate injections of each solution used.

The results show that the standard deviations of all five procedures are less than ± 2 per cent. The results in parentheses for duplicate injections do not indicate any significant improvement in precision over the use of single injections and confirm experience found from numerous routine analyses that the methods are sufficiently reliable to permit economy in the numbers of chromatograms needed for an individual determination.

The applicability of the gas - liquid chromatographic methods to the analysis of formulated products is illustrated by the results given in Table III. These results were obtained by analysing a range of standardised formulations, which had been accurately prepared on the laboratory scale, and the standard deviations and mean recoveries were calculated from groups of six replicate analyses on each sample. The results confirm that for formulations with contents of nominal active ingredients ranging from 4·5 to 50 per cent., the analytical precision and accuracy are satisfactory for purposes of routine quality control. The lower recovery of 97·9 per cent. obtained with the granular preparation of pirimiphos-ethyl is attributed to the difficulty in accurately preparing a homogeneous sample of this type of formulation on a small scale.

TABLE III

DETERMINATION OF SUBSTITUTED PYRIMIDINES IN STANDARDISED FORMULATIONS

Formulation	Pesticide content, per cent. *m/m*		
	Added	Found (mean and standard deviation for six determinations)	Mean recovery, per cent.
Ethirimol, aqueous suspension	40·6	40·3 ± 0·25	99·2
Dimethirimol hydrochloride, aqueous solution ..	10·35	10·25 ± 0·09	99·1
Pirimiphos-ethyl on pumice granules	4·64	4·53 ± 0·06	97·9
Pirimiphos-ethyl, emulsifiable concentrate ..	27·21	27·40 ± 0·30	100·7
Pirimiphos-methyl, emulsifiable concentrate ..	49·31	49·70 ± 0·20	100·8
Pirimicarb, dispersible powder	25·76	25·43 ± 0·15	99·0

SPECIFICITY—

Quantitative gas - liquid chromatographic procedures depend upon measurement of the area of a peak corresponding to the compound of interest and should be less prone to interference from impurities than the methods based on ultraviolet absorption or acid - base titration. In quantitative thin-layer chromatographic methods ultraviolet absorption is used as the final method of determination, but as this step is performed after separation and extraction of the band corresponding to the active pesticide the specificity should be comparable with that of gas - liquid chromatography.

TABLE IV

COMPARISON OF RESULTS BY GAS - LIQUID CHROMATOGRAPHY WITH THOSE BY QUANTITATIVE THIN-LAYER CHROMATOGRAPHY, ULTRAVIOLET ABSORPTION MEASUREMENT AND TITRIMETRY

Material	Substituted pyrimidine content, per cent. *m/m*, by			
	Gas - liquid chromatography	Thin-layer chromatography	Ultraviolet absorption	Titration
Ethirimol, technical sample 1	97·6	96·8	97·3	—
2	95·1	93·1	97·8	—
Ethirimol, aqueous suspension	40·1	41·2	41·4	—
Dimethirimol, technical sample 1	85·8	83·4	92·0	—
2	92·7	87·4	96·5	—
Dimethirimol hydrochloride, aqueous solution ..	10·2	10·3	11·6	—
Pirimiphos-methyl, technical	91·1	88·3	91·6	—
Pirimiphos-ethyl, technical	96·5	94·2	96·2	—
Pirimiphos-methyl, emulsifiable concentrate ..	49·5	48·8	49·4	—
Pirimiphos-ethyl, emulsifiable concentrate ..	27·2	28·1	26·9	—
Pirimiphos-ethyl on pumice granules	4·5	4·5	4·3	—
Pirimicarb, technical sample 1	100·3	100·1	—	98·2
2	100·8	100·5	—	97·4
Pirimicarb, dispersible powder sample 1	25·2	25·5	—	25·1
2	50·2	51·2	—	49·6

The gas - liquid chromatographic procedures carried out as described in this paper were compared with the thin-layer chromatographic, ultraviolet and titrimetric methods developed in these laboratories. Table IV gives results of single determinations by each method on a range of technical and formulated materials.

It was found that for samples of technical ethirimol, which usually have purities higher than about 95 per cent., results by gas - liquid and thin-layer chromatography and ultraviolet absorption show fairly good agreement. With material with lower concentrations of active ingredient, as illustrated by dimethirimol, ultraviolet measurement may overestimate the true concentration as a result of a greater degree of interference by impurities, which can usually be seen to be present on thin-layer chromatograms. Similarly, dimethirimol hydrochloride in solutions prepared with low concentrations of technical materials tends to be overestimated by ultraviolet measurement because of interfering impurities. On the other hand, satisfactory agreement is found between all three methods for ethirimol suspensions prepared from technical material that has a purity in excess of 95 per cent.

For samples of technical and formulated pirimiphos-methyl and pirimiphos-ethyl, agreement between gas - liquid and thin-layer and ultraviolet methods is generally found to be acceptable, the differences being well within the limits of precision for each of these procedures.

The purity of technical pirimicarb is usually close to 100 per cent., as illustrated by the gas - liquid and thin-layer chromatographic results, although titrimetry has been found to give slightly lower values. On the other hand, for dispersible powders of 25 and 50 per cent. nominal pirimicarb contents, results by all three procedures show satisfactory agreement.

Conclusions

In this paper, examples have been given of the application of gas - liquid chromatography to the analysis of five pyrimidine pesticides. The methods have been shown to be of acceptable precision for routine quality control of batches of technical materials and formulated products. The methods are believed to be accurate and specific, as shown by satisfactory recovery experiments with standardised formulations and by good agreement of results with those given by other methods. The relative response factors are rectilinear over a wide concentration range and are highly reproducible from day to day. Calibration checking during routine analysis can therefore be kept to a minimum and single chromatograms run for each sample to be analysed.

The specificity of the gas - liquid chromatographic procedures indicates that they can be used satisfactorily for the analysis of low-purity samples or materials that have been degraded, for example, as a result of storage at elevated temperatures. Extensive routine use of these methods has not so far given evidence of interference by breakdown products, or by wetting or dispersing agents incorporated in formulations. Freedom from such interference must nevertheless be confirmed when applying the methods to new formulations.

Experience with the gas - liquid chromatographic methods for the substituted pyrimidine group of pesticides indicates that they would serve as referee procedures, taking precedence over quantitative thin-layer chromatography, ultraviolet absorption or titrimetry in cases of doubt or dispute.

The analytical procedures described are based upon the work of a number of colleagues within ICI Organics Division and Plant Protection Ltd. In particular, the contributions made by S. Kaminsky, D. Myles, S. H. Yuen, D. W. Delo, R. J. Parker and K. Hulston are gratefully acknowledged. Thanks are also due to M. G. Ashley for his valuable comments during the preparation of this paper.

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Analytical Methods Committee

REPORT PREPARED BY THE PROPHYLACTICS IN ANIMAL FEEDS SUB-COMMITTEE

The Determination of Clopidol in Animal Feeds

THE Analytical Methods Committee has received the following Report from its Prophylactics in Animal Feeds Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. J. Markland (Chairman), Mr. B. J. Allen, Mr. R. J. Anderson, Dr. I. E. Burrows, Mr. A. G. Croft, Mr. G. Drewery, Mr. C. E. Dodd, Dr. K. Field, Mr. R. S. Hatfull, Mr. J. S. Leahy, Mr. D. H. Mitchell, Mr. R. C. Spalding, Mr. J. A. Stubbles and Dr. D. R. Williams, with Mr. P. W. Shallis as Secretary.

INTRODUCTION

Clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) is a coccidiostat used at a level of about 200 p.p.m. in poultry feeds, to which it is added as a pre-mix; Coyden 25 is an example of a typical commercial pre-mix.

When the Sub-Committee began its work, only one method was available for consideration. This method, details of which were made available by the Dow Chemical Company Ltd., is based on extraction of the drug from the feed with methanolic ammonia solution, clean-up of the extract on an alumina column, separation of the drug on an anion-exchange resin, elution from the resin with 40 per cent. acetic acid and, finally, spectrophotometric measurement in the ultraviolet region. Certain modifications to the method were made during the course of the work, but the basis of the method recommended in the Appendix is unaltered.

After the Sub-Committee had begun its work, details of a gas-chromatographic method were published.[1] Some members tried this method and found it to be reasonably satisfactory and more specific, although the apparatus required is not readily available. In consequence, it was decided to investigate fully only the spectrophotometric method and to suggest that the gas-chromatographic method could find application when interferences render the spectrophotometric method unusable.

EXPERIMENTAL

A preliminary investigation of the method as made available to the Sub-Committee was carried out by one laboratory. In this work clopidol was determined in four different feedingstuffs to which it had been added as the pre-mix Coyden 25; the mean of thirty recovery tests was 93·3 per cent. for results within the range 79·1 to 106·3 per cent. The main problem encountered in the method was the physical blocking of the columns with oil and fat from the feed. A preliminary extraction with light petroleum to remove oil and fat was tried, but there was evidence of loss of clopidol when this procedure was used.

The method was then tested collaboratively in five laboratories. A single sample of an unmedicated feedingstuff was circulated, to which pure clopidol was added in each laboratory at a level of 150 p.p.m., and three determinations were carried out. The range of the fifteen recoveries reported was 104·5 to 84·1 per cent. with a mean of 93·1 and a coefficient of variation of 4·51 per cent. Again, some difficulties were experienced owing to blockage of the columns with fat from the feedingstuff and one laboratory noted a comparatively large "apparent clopidol" content of the blank meal.

At this stage of its investigations, the Sub-Committee received details of an improved version of the method. The basis of the method was the same, but some improvements in technique had been effected. Consequently, a collaborative test of this improved method was arranged in which nine laboratories took part. Each collaborator received a single unmedicated feedingstuff, a supply of clopidol of about 98 per cent. purity and portions of a common supply of Dowex 1-X8 anion-exchange resin and alumina. Medication of the blank meal was effected in each laboratory at the level of 200 p.p.m. and three separate determinations of clopidol and of the reagent blank value were carried out. The results, and a statistical assessment of them, are shown in Tables I and II.

TABLE I

DETERMINATION OF CLOPIDOL BY THE RECOMMENDED METHOD

Laboratory	Clopidol added/mg	Reagent blank /mg	Clopidol found/mg	Clopidol recovered/mg	Recovery, per cent.	Mean, per cent.
A	10·0	0·6	10·5	9·9	99·0	97·0
	10·0	0·6	9·9	9·3	93·0	
	10·0	0·5	10·4	9·9	99·0	
B	10·5	0·4	10·6	10·2	97·1	96·0
	9·7	0·4	9·7	9·3	95·9	
	10·1	0·5	10·1	9·6	95·1	
C	10·0	0·1	9·2	9·1	91·0	91·7
	10·0	0·0	9·2	9·2	92·0	
	10·0	0·0	9·2	9·2	92·0	
D	10·0	0·5	11·7	11·2	112·0	111·0
	10·0	0·5	11·5	11·0	110·0	
	10·0	0·5	11·6	11·1	111·0	
E	10·0	1·3	10·0	8·7	87·0	85·0
	10·0	1·7	10·5	8·8	88·0	
	10·0	1·9	9·9	8·0	80·0	
F	10·1	0·8	10·0	9·2	91·1	94·0
	9·9	0·7	10·4	9·7	98·0	
	10·0	1·2	10·5	9·3	93·0	
G	10·0	1·6	12·5	10·9	109·0	109·3
	10·0	1·2	12·7	11·5	115·0	
	10·0	1·9	12·3	10·4	104·0	
H	10·5	3·2	14·0	10·8	102·9	97·3
	10·6	4·1	14·4	10·3	97·2	
	9·9	2·9	12·0	9·1	91·9	
I	10·3	2·8	13·0	10·2	99·0	100·3
	10·0	2·7	12·8	10·1	101·0	
	10·0	2·7	12·8	10·1	101·0	

Over-all performance—

Number of tests	27
Over-all mean, per cent.	98·0
Minimum	80·0
Maximum	115·0
Standard deviation, per cent.	8·4
Coefficient of variation, per cent.	8·5

It can be seen from Table II that the main contribution to the total variation is that between laboratories. The standard deviation of a single test would be that shown in Table I, *i.e.*, 8·4, and the 95 per cent. confidence limits would be the actual clopidol content of the sample $\pm$ 16·8 in terms of percentage recovery.

The Sub-Committee is of the opinion that the performance of the method is similar to the performances of other methods recommended for the determination of prophylactic levels of drugs in animal feedingstuffs. However, before making any recommendation, the Sub-Committee decided to investigate more fully the observation of some members that

some feeds gave interfering peaks at about the wavelength of maximum absorption of clopidol. In all, seventeen different feeds were tested, eight of which gave low or negligible apparent clopidol contents, three of which gave high (up to 30 p.p.m.) apparent values and the remainder gave low values in some laboratories and high values in others. In view of these conflicting findings, which proved little other than the fact that a constituent of some feeds produced irrelevant absorption that could interfere, it was decided not to pursue this investigation. For feeds that exhibit high levels of interference, the calculation criteria of the method are not met.

TABLE II

ANALYSIS OF VARIANCE

Source of variation	Sum of squares	Degrees of freedom	Variance	"F" ratio*
Between laboratories	1599·12	8	199·89	15·41
Within laboratories	215·98	18	12·00	
Overall	1815·1	26	69·81	

* Limiting values of "F" for 8 or 18 degrees of freedom: 5 per cent. $P = 2{\cdot}51$; 1 per cent. $P = 3{\cdot}71$; 0·1 per cent. $P = 5{\cdot}76$.

RECOMMENDATION

The Sub-Committee recommends that the method given in the Appendix to this report should be used for the determination of clopidol in animal feedingstuffs. If, however, interference from the feed renders the method unusable, it is suggested that the clopidol content be determined by a gas-chromatographic procedure, such as that proposed by Kutschinski.[1]

Appendix

RECOMMENDED METHOD FOR THE DETERMINATION OF CLOPIDOL IN ANIMAL FEEDS

SCOPE AND FIELD OF APPLICATION—

The method is applicable to the determination of clopidol in animal feeds at concentrations of 170 to 300 p.p.m. (By suitable adjustment of the size of the sample, the volume of extract taken through the procedure and the volume of the final solution, the method can be applied to lower and higher levels of clopidol in feeds and up to 25 per cent. of clopidol in pre-mixes.)

PRINCIPLE—

Clopidol is extracted from the feed with methanolic ammonia solution, and a portion of the extract is passed through a column of alumina on to a column of ion-exchange resin. The clopidol is retained on the resin and interfering substances are removed by washing the column with 80 per cent. methanol. The clopidol is eluted from the resin with 40 per cent. acetic acid and the absorbance is measured at a wavelength of 267 nm. The concentration of clopidol present in the sample is calculated by reference to a calibration graph.

REAGENTS*—

Alumina, 100 to 250 mesh, alkaline, Brockmann activity **1.**

Anion-exchange resin, AG 1-X8 or Dowex 1-X8, 100 to 200 mesh—In order to convert Dowex resin in the chloride form into the acetate form, add 1 litre of 6 N hydrochloric acid to 350 g of resin in a 3-litre beaker and heat the mixture on a steam-bath for 2 to 3 hours. Pour the slurry into a glass Büchner funnel and wash the resin with water until the washings are free from chloride (about 6 litres of water are required). Transfer the resin into a 5 cm diameter glass column that has a coarse sintered-glass disc at the bottom end, and wash it with 5 to 10 per cent. m/V sodium acetate solution until the column effluent gives only a cloudy solution on addition of silver nitrate solution. Return the resin to the glass Büchner funnel, and wash it with water. Next, transfer the resin into a 3-litre beaker, add 1 litre of 40 per cent. V/V acetic acid, and heat the mixture on a steam-bath for at least 3 hours.

* The suitability of the batch of alumina and of the other reagents should be tested before use by analysing a sample to which a known amount of clopidol has been added.

Filter the mixture and then wash the resin again with water until the washings are free from chloride. Store the resin in water.

Clopidol—Purified for use as a standard.

Acetic acid, 40 per cent. V/V.

Methanol—Analytical-reagent grade.

Methanol, aqueous, 80 per cent. V/V.

Methanol - ammonia (sp. gr. 0·88) solution, 95 + 5 V/V.

Clopidol standard solution—Weigh. to the nearest 0·1 mg, 125 mg of pure clopidol into a beaker, add 25 ml of 2 per cent. *m/V* sodium hydroxide solution in order to dissolve the clopidol, transfer the solution into a 500-ml calibrated flask, and dilute to the mark with water.

APPARATUS—

Chromatographic column.

Ion-exchange column.

These two columns are illustrated in Fig. 1.

Recording spectrophotometer—With 10-mm silica cells.

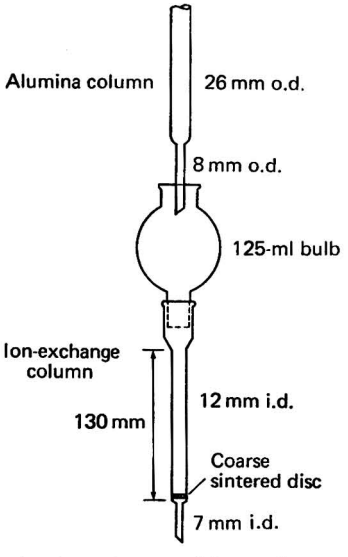

Fig. 1. The alumina column and ion-exchange apparatus

PREPARATION OF TEST SAMPLE—

The feed sample should, if necessary, be finely ground before being weighed out for analysis.

PROCEDURE

TEST PORTION—

Weigh, to the nearest 0·001 g, approximately 50 g of the test sample into a 500-ml calibrated flask and add 400 ml of methanolic ammonia solution. Place a magnetic stirring bar in the flask and stir the mixture on a magnetic stirrer for 20 minutes. Remove the stirring bar from the flask, dilute to the mark with methanolic ammonia solution, mix the contents well and set aside for 20 to 30 minutes.

CHROMATOGRAPHY—

Alumina column—For each column required, weigh approximately 25 g of alumina into an aluminium-foil dish and place it in an oven at 105 ± 5 °C for 1 hour. Remove the dish from the oven and cool it to room temperature in a desiccator. Make a slurry of the alumina with 25 ml of methanolic ammonia solution and filter on a Büchner funnel, then wash the

alumina with methanol until the washings are neutral. Form a slurry of the alumina with methanol (50 ml) and pour it into the column. Allow the methanol to drain through the column. Place a plug of glass-wool lightly on top of the alumina and then wash with 25 ml of methanol, not allowing the liquid in the column to fall below the top of the alumina. Discard the eluate.

Anion-exchange column—Form a slurry in 40 per cent. *V/V* acetic acid of sufficient resin to fill the columns required. Filter on a Büchner funnel, wash the resin with twice its own volume of the acetic acid, and then with aqueous methanol until the washings are neutral. Form a slurry of the resin with aqueous methanol and add sufficient to a column to give a resin bed 2 to 3 cm deep after settling. Place a small plug of glass-wool on top of the resin and wash the column with two 13-ml portions of aqueous methanol without allowing the liquid level in the column to fall below the top of the resin. Discard the effluent.

Chromatographic procedure—By pipette, transfer 10·0 ml of the extract of the feed sample directly on to an alumina column and also transfer a similar volume of methanolic ammonia solution directly on to another alumina column. Allow the solutions to drain to the top of the alumina and then wash each column with three 12-ml portions of aqueous methanol, allowing the liquid to drain to the top of the alumina on each occasion. Allow all of the eluate from each column to drain directly into a separate ion-exchange column, then remove the alumina columns.

Allow the liquid to drain to the top of the ion-exchange resin, then wash each column with four 13-ml portions of aqueous methanol. Discard all of the effluent in each instance.

Elute each column with two 10-ml portions and then one 4-ml portion of 40 per cent. *V/V* acetic acid. Collect the eluate from each column in a separate 25-ml calibrated flask and dilute the contents of each flask to the mark with 40 per cent. *V/V* acetic acid.

Determination—Record the absorption spectrum of the sample extract between 350 and 245 nm in a 10-mm silica cell against the reagent blank solution.

Calibration graph—By pipette, transfer 1, 5, 7·5, 10, 12·5 and 15-ml portions of clopidol standard solution into separate 250-ml calibrated flasks. Dilute the contents of each flask to the mark with 40 per cent. *V/V* acetic acid and then record the absorption spectra of these solutions in 10-mm silica cells between 350 and 245 nm against 40 per cent. *V/V* acetic acid. Construct a calibration graph by plotting the absorbance at 267 nm against the concentration of clopidol in micrograms per millilitre.

Expression of results

Determine the absorbance of the sample extract at 267 nm above a base-line determined by drawing a line through the absorbance at 327 and 297 nm and extending it through 267 nm (see Note).

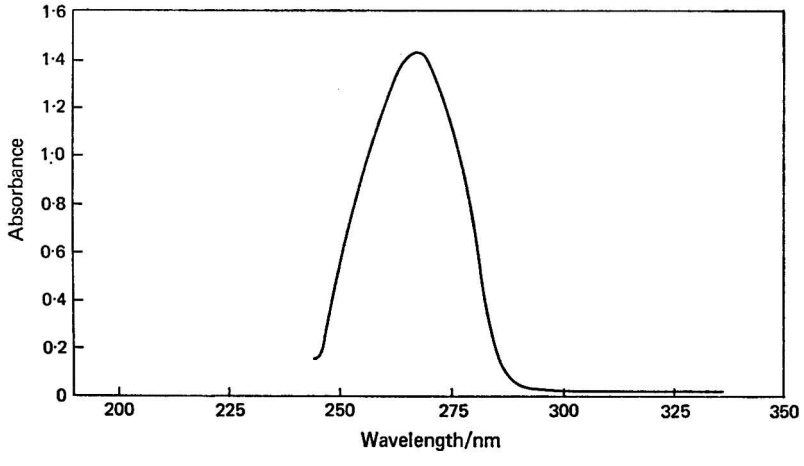

Fig. 2. Absorption spectrum for 200 p.p.m. of clopidol in an animal feed

Calculate the concentration of clopidol in the test sample from the expression—

$$\text{Clopidol, p.p.m.} = 23{\cdot}23\ C$$

where C is the concentration of clopidol (μg ml^{-1})read from the calibration graph, equivalent to the absorbance of the test solution and 23·23 is a factor that makes allowance for the volume of the feed sample in the flask.

NOTE—

Background absorption due to the feed normally approaches a linear function that can be described by the points on the curve at 296 and 327 nm. Occasionally this is not so, as can be detected by the presence of absorption peaks in the region between 350 and 297 nm. The absorbance at 327 and 297 nm should not differ by more than 0·05 unit and at both points it should be below 0·2. Results will be satisfactory provided that these criteria are kept in mind, together with any obvious distortion in the appearance of the curve. No maximum other than that of clopidol should be present.

An absorption spectrum for the determination of about 200 p.p.m. of clopidol in an animal feed is shown in Fig. 2.

REFERENCE

1. Kutschinski, A. H., *J. Agric. Fd. Chem.*, 1968, **16**, 913.

Book Reviews

STANDARD METHODS FOR THE ANALYSIS OF OILS, FATS AND SOAPS. INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY. Second Supplement. Pp. 60 (loose-leaf). London: Butterworths. 1973. Price £2·50.

There can be no more appropriate time than the present for the publication of standard analytical methods that have been tested by international collaboration. Such methods provide an important cornerstone for international trade, and assume particular importance in relation to the current efforts at harmonisation in the European Community.

The second supplement to the Fifth Edition of the IUPAC "Standard Methods for the Analysis of Oils, Fats and Soaps" is to be welcomed. The distinguished members of the Oils and Fats Section of IUPAC are continuing an international effort started in 1930.

The supplement contains six methods—

(1) An amplified version of the procedure for the iodine value already described in the Fifth Edition.

(2) The determination of di- and tri-unsaturated fatty acids in fats.

(3) The determination of epoxy-group oxygen in fat.

(4) The determination of arsenic in glycerol.

(5) The determination of unsaponified and unsaponifiable matter in soaps.

(6) The determination of minor amounts of glycerol in soaps.

As would be expected, the methods studied are established procedures, already fully described in journals, text-books and in various national collections of methods. The reviewer must therefore examine the supplement with regard to the special features that justify the reissue of the methods under IUPAC aegis. These features are as follows—

(*a*) International collaborative study.

(*b*) Bilingual issue in French and English.

(*c*) Suitability for the widest international usage in practice.

The over-all impression formed is that the methods comprise a substantial and useful publication. Nevertheless, a number of shortcomings require attention in future editions if the highest standards of usefulness are to be met.

The interesting introduction to the Fifth Edition states that the methods are submitted to a searching examination based on analyses of identical samples by the members of the section. It is a pity that each method does not therefore contain the statistical information on reproducibility which must be available from the study, and which would be especially pertinent in an international context.

The bilingual aspect of the publication is excellent and frequently causes one to admire the ability of the French language to be precise and at the same time almost poetically elegant. Is it not, however, an excessive luxury to reproduce twice a dimensioned diagram of a reaction tube containing no descriptive matter other than the section heading and the page number in each language?

Surprisingly, the reissued section II D7 on iodine value is somewhat unsatisfactory as regards presentation and content. Several opportunities for improvement have been missed.

The first question that may be asked is why the method of Hübl is still included. Its status is ambivalently characterised as "still official in some countries except for commercial transactions." Surely the main, if not only, purpose of IUPAC methods is for commercial applications in the widest sense. It may be noted that both Bolton (1928) and Hilditch rejected the Hübl method in favour of the Wijs method.

Next, it may be noted that pipettes are not listed under the apparatus required, nor is starch solution among the reagents although it is the indicator used in all the three methods described. As in the Fifth Edition, the preparation of Wijs reagent is described in detail from both iodine trichloride and iodine monochloride and the latter description contains the only new material, which consists of a quantitative test for the presence of excess of iodine, including a complicated equation. However, no target figure is given and instead the paragraph finishes with the anti-climactic statement that a qualitative indication of the excess is all that is required.

Two omissions of real practical significance in the performance of the analysis concern the need for a dry sample, and the desirability to shake vigorously near the end-point, so that the last trace of iodine is extracted from the carbon tetrachloride into the aqueous reaction phase.

A practical point is also omitted in the method for arsenic in glycerol. It would be worth stating that the silver diethyldithiocarbamate reagent, if stored in a well stoppered bottle in the dark, is stable for 2 months. On the other hand, the apparatus described includes an excessively elaborate absorption tube consisting of a glass U-tube with fifteen bubbles of various specified dimensions. In practice, a very simple tube is satisfactory, and this is implied by a footnote at the end of the method.

In summary, it may be said that the supplement more than adequately meets the first two criteria listed above. The instructions given are certainly good enough to enable the experienced analyst to carry out the analyses. Nevertheless, some careful editorial work is required before the third criterion is met at the desired standard.

The price of the supplement of thirty-two sheets (of which two are entirely blank) is £2·50, identical with that of the whole issue, including the hard cover, of the original Fifth Edition in 1964.

K. G. BERGER

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF CYCLOPENTADIENYL COMPOUNDS. By N. M. SERGEYEV. *Progress in Nuclear Magnetic Resonance Spectroscopy, Volume 9, Part 2.* Pp. iv + 71–146. Oxford, New York, Toronto, Sydney and Braunschweig: Pergamon Press. 1973. Price £2.

In general, a whole Part on the nuclear magnetic resonance spectra of a single type of compound would be expected to be fairly dull, but with the particular choice of cyclopentadienyl compounds this is far from true. When attached to metal atoms, the cyclopentadienyl groups, especially the substituted rings, have numerous isomeric possibilities concerning the ring-carbon to which the metal is attached; there are also the π-complexes in which the metal is partially bound to each of the five ring-carbon atoms. Each of these possibilities gives rise to identifiable nuclear magnetic resonance spectra, some of which require sophisticated nuclear magnetic resonance analysis, including the relative sign determination of coupling constants using double-resonance techniques. Furthermore, such clear determinations are often possible only at low temperatures, because kinetically controlled isomerisations occur at higher temperatures with appropriate spectral modification. There are pitfalls, as some high-temperature spectra resemble low-temperature spectra for other isomers, but a full interpretation of the nuclear magnetic resonance spectrum gives a great deal of information.

Cyclopentadienyl compounds therefore provide an ideal example of the power of nuclear magnetic resonance methods, and this account by N. M. Sergeyev of Moscow brings out this aspect strongly. While remaining fairly comprehensive in his tables, most of which collate material from a range of original publications, he has managed to write a clear and lively account in good English. As a result, this work can be recommended to all interested in cyclopentadiene complexes and to those who want an extensive example of the use of nuclear magnetic resonance spectroscopy.

D. H. WHIFFEN

APPOINTMENTS VACANT

Determination of Impurities in Oxygen by Mass Spectrometry

The difficulties in analysing high-purity gaseous oxygen are overcome by using a copper-based catalyst to remove the oxygen completely, allowing impurities to be readily determined by mass spectrometry. The sensitivity is estimated to be 1 p.p.m. and the uncertainty is at most 5 per cent. of the concentration. The equipment is described, together with the results of experiments confirming the accuracy, linearity and reproducibility for the particular impurities nitrogen, argon and krypton.

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Analyst, 1974, **99**, 214–217.

Analysis of Steroids
Part XXIV. A Specific Method for the Spectrophotometric Determination of 17-Ethynyl Steroids

The investigation of the equilibrium between 17-keto steroids, acetylene and 17-ethynyl steroids has afforded two possibilities for its analytical application. On the one hand, the 17-ethynyl steroids can be quantitatively converted into 17-keto steroids and the latter determined spectrophotometrically as their 16-glyoxalyl derivatives ($\lambda_{max.} = 294$ nm, $\epsilon = 10\,700$ and $\sigma = 1{\cdot}1$ per cent.). On the other hand, at 0 °C the 17-keto steroid contaminants in 17-ethynyl steroids can be selectively determined on the same principle.

These methods cannot be applied in the investigation of 3-keto steroids.

G. SZEPESI and S. GÖRÖG

Chemical Works, G. Richter Ltd., Budapest X, Hungary.

Analyst, 1974, **99**, 218–221.

A Stable and Sensitive Colorimetric Method for the Determination of Ergocalciferol (Vitamin D_2) by Using Trifluoroacetic Acid

A sensitive and fairly stable colour for the spectrophotometric determination of ergocalciferol (vitamin D_2) following reaction with trifluoroacetic acid has been developed. In the method hydroquinone was used to stabilise the yellow colour, which reached maximum absorbance in not less than 80 s and remained stable for at least 20 s, thus giving the analyst convenient time to carry out the determination. Maximum absorbance was reached at a wavelength of 490 nm. The colour could be destroyed within 2 minutes by the addition of hydrogen peroxide solution, which property was used to advantage in differential spectroscopy. The absorbance is linear for ergocalciferol solutions containing from 0 to 32 μg, optimum results being obtained in the 5 to 15-μg range. The relative standard deviation of the method is $\pm 1{\cdot}76$ per cent.

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Analyst, 1974, **99**, 222–224.

Determination of the Substituted Pyrimidine Pesticides Ethirimol, Dimethirimol, Pirimiphos-methyl, Pirimiphos-ethyl and Pirimicarb in Technical and Formulated Materials by Gas Chromatography

Gas chromatography can be used to determine the members of a group of substituted pyrimidines that comprises two systemic fungicides, ethirimol and dimethirimol; two insecticides, pirimiphos-methyl and pirimiphos-ethyl; and an aphicide, pirimicarb.

The two fungicides, ethirimol and dimethirimol, are hydroxy compounds, which are determined by gas chromatography with flame-ionisation detection after conversion into their volatile trimethylsilyl ethers. The remaining three compounds are determined directly without derivative formation. All separations are carried out on a column packed with 100 to 120-mesh Celite coated with 10 per cent. silicone elastomer. Depending upon the retention time of the compound to be determined, either n-octadecane or n-nonadecane is added as an internal standard. The procedures are applicable to the technical pesticides and to a range of formulated products.

The methods are sufficiently specific and accurate to be recommended for referee purposes, and their precision has been shown to be satisfactory for routine control.

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ICI Organics Division, Blackley, Manchester.

Analyst, 1974, **99**, 225–232.

The Determination of Clopidol in Animal Feeds

Report prepared by the Prophylactics in Animal Feeds Sub-Committee.

ANALYTICAL METHODS COMMITTEE
9/10 Savile Row, London, W1X 1AF.

Analyst, 1974, **99**, 233–238.

RS for the analysis of pesticides residue

On the recommendation of international organisations such as FAO and WHO, many nations throughout the world have felt the necessity to regulate the use and the control of pesticides in foodstuffs.
The analysis of pesticide residues presents unusual problems because of the small amounts of substances to be determined and the large number of possible interfering substances which must be first eliminated. In order to determine extremely small quantities, very sensitive analytical methods are required, which however cannot be applied directly to the substances under examination. The general procedure is:

- ☐ extraction of the pesticide from the alimentary product;
- ☐ concentration of the extract by evaporation of the solvent;
- ☐ removal from the extract of naturally-occurring substances which would interfere with the pesticides;
- ☐ determination of the extracted pesticide.

In these operations large quantities of polar and non polar solvents are used.
The usual Analytical Grade solvents, when subjected to a more detailed investigation (GLC e.c.d. and Na d.), show peaks due to impurities, whose positions coincide with those of the peaks of pesticides. The use of solvents from which these impurities have been eliminated is therefore indispensable.
Carlo Erba RS solvents for pesticides have been studied and developed in order to satisfy these requirements. Their main characteristic is that of having a greatly reduced quantity of any residue which may interfere with the analytical method. This has been attained by working under special conditions, with small batches which are controlled individually, and by special choice of packing materials, cleaning methods and bottle closure procedures. For chlorinated compounds, a maximum limit of 10^{-9} % as aldrin (GLC e.c.d.) is guaranteed, and for phosphorylated compounds a maximum limit of 10^{-8} % as parathion (GLC Na d.).
All these products are available in bottles of 1000 ml.

Acetone RS	**n-Hexane RS**
Acetonitrile RS	**Isooctane RS**
Benzene RS	**Isopropyl alcohol RS**
Chloroform RS	**Methanol RS**
Cyclohexane RS	**Methylene chloride RS**
Dioxane RS	**Petroleum ether 40°-60° RS**
Ethyl acetate RS	**n-Pentane RS**
Ethyl ether RS	**Toluene RS**

CARLO ERBA **Divisione Chimica/Via C. Imbonati 24/20159 Milano**

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