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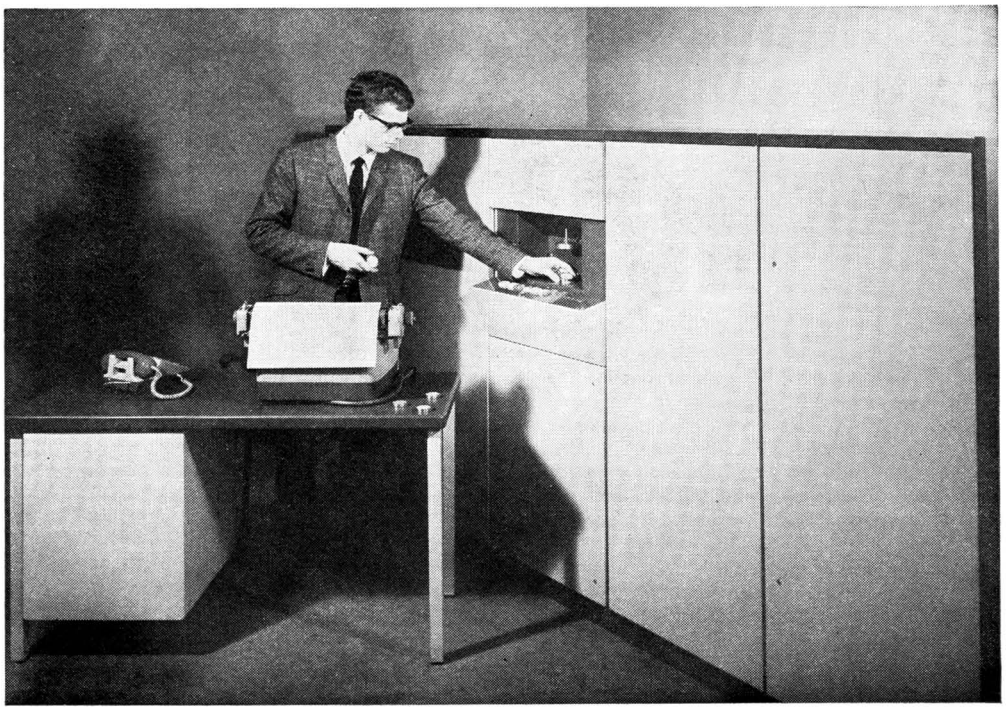

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Summaries of Papers in this Issue

The Application of Landolt Reactions in Quantitative Catalytic Analysis

The place of Landolt reactions among quantitative catalytic methods is reviewed. By measuring the time lapse of the incubation period of a Landolt reaction (the reaction time) and plotting its reciprocal values *versus* catalyst concentration, a calibration graph can be obtained. The advantages of the use of Landolt reactions are as follows: there is no need for instrumentation and temperature control is simple. Theoretical backgrounds of the method are presented on a kinetic basis. During the analyses the uncatalysed and the catalysed reactions proceed simultaneously. The uncatalysed reaction can be examined separately if reaction time measurements are taken in the absence of the catalyst. Having obtained the velocity constant of the former, results of measurements of the reaction times of the simultaneous reactions can be evaluated to obtain the velocity constant of the catalysed reaction alone. The experimentally obtained calibration graph shows a linear correlation between reciprocal reaction time *versus* catalyst concentration; the rigorous kinetic examination, however, yields to a partly exponential correlation. By expanding the exponential member into a Taylor series and examining the error that arises by neglecting the non-linear members the simple linear correlation can be explained. Sensitivity, selectivity and precision of the methods are briefly discussed.

G. SVEHLA

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Analyst, 1969, **94**, 513–521.

A Catalytic Method for the Determination of Ruthenium

A method is outlined for the determination of ruthenium based on its catalysis of the periodate oxidation of the tris(1,10-phenanthroline)iron(II) complex. This reaction has been used to detect 10^{-10} M ruthenium and to determine 10^{-9} to 2×10^{-8} M ruthenium, with a coefficient of variation of 4·8 per cent. The interference effects of various ions have been studied and the method is shown to have only three serious interferences, iridium, osmium and rhenium. The sensitivity attained compares favourably with other similar methods by which ruthenium can be determined.

J. M. OTTAWAY, C. W. FULLER and J. J. ALLAN

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Analyst, 1969, **94**, 522–526.

Separation of Gold from Platinum Metals on Cation Exchangers in Concentrated Hydrobromic Acid Solutions

Separation of gold from platinum metals in the Dowex 50W × 8 [H^+] - hydrobromic acid *plus* bromine system was investigated. Distribution coefficients of gold (in trace amounts) increase almost linearly with hydrobromic acid concentrations from 0·5 to 5 N, reach nearly constant value at 5 and 6 to 7 N and decrease slightly with further increase in hydrobromic acid concentration. Distribution coefficients decrease with loading and with temperature. Apparent enthalpy change for the distribution reaction of gold between resin and solution is about 3 kcal. $mole^{-1}$. The absorption capacity of Dowex 50W × 8 [H^+] for gold (in 6 N hydrobromic acid), as determined from break-through graphs, increases considerably with the increase in gold concentration in the solution. Separation of both carrier-free and milligram amounts of platinum and gold can be achieved with 6 N hydrobromic acid *plus* 0·0035 M bromine solution at 15° C. Platinum is eluted in the solvent front and the gold, retained by the resin, is rapidly stripped off with acetylacetone. Statistical analysis of the results of the separations by use of tracers has proved that no systematic error is involved. Iridium, palladium and rhodium behave in a similar manner to platinum and can be separated from gold with this system. A possible mechanism of the adsorption of gold by cation-exchange resin, *i.e.*, the partition of undissociated bromoauric acid with the formation of a molecular (charge transfer) complex between the aromatic rings of resin network and complex acid, is suggested.

RAJMUND DYBCZYŃSKI and HANNA MALESZEWSKA

Department of Analytical Chemistry, Institute of Nuclear Research, Warszawa 91, Poland.

Analyst, 1969, **94**, 527–537.

Low-temperature Fluorescence of Some Bromide-ion Association Complexes in Hydrobromic Acid Glasses at −196° C

An examination has been made for the fluorescence of simple bromo-ion association complexes in hydrobromic acid glasses at −196° C. Strong fluorescence was observed for Sb(III), Sb(V), As(III), As(V), Bi, Ce(III), Pb, Tl(I), Sn(II) and U(VI), and the effects of variables such as hydrobromic acid molarity, time of irradiation, relationship between fluorescence intensity and metal-ion concentration were studied. With a standard commercial spectrofluorimeter with a slightly modified sample cell, these ions could be detected in hydrobromic acid at concentrations varying from 10^{-5} to 10^{-8} M. Copper(I) and tellurium(IV) also exhibit weak fluorescence.

G. F. KIRKBRIGHT, C. G. SAW and T. S. WEST

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Analyst, 1969, **94**, 538–542.

Thermogravimetry and Differential Thermal Analysis Studies on Potassium Titanyl Oxalate and Potassium Aluminium Oxalate and Related Oxalates

Thermogravimetry and differential thermal analysis studies are reported for potassium titanyl oxalate, potassium aluminium oxalate and the related oxalates, potassium, titanyl and aluminium oxalates. The thermal decomposition of these materials in dynamic atmospheres of air, nitrogen and carbon dioxide has been studied. The oxalates and their decomposition products have been characterised by chemical and X-ray analysis. The thermal decomposition results for the complex oxalates show distinct reaction stages, but the aluminium and titanyl oxalates are not clearly characterised under the conditions used in the present work.

The dehydration of the potassium titanyl oxalate starts at 70° C and proceeds in several stages until completion at 200° C. The anhydrous oxalate is not stable although only 1 per cent. of the original weight is lost between 200° and 275° C, but the decomposition is rapid above 275° C. The titanate formed as the final product is the result of the initial decomposition of the anhydrous complex. Potassium aluminium oxalate gives a stable anhydrous complex over the range 150° to 375° C, followed by a two-stage decomposition, with the bulk of the reaction complete at 575° C, and the end product is potassium aluminate, formed as a result of the initial decomposition.

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Analyst, 1969, **94**, 543–553.

An Investigation of the Performance of the Separated Air - Acetylene Flame in Thermal-emission Spectroscopy

The advantages of the application of a nitrogen-separated air - acetylene flame in the flame-emission spectroscopy of seventeen elements is described. In this flame the secondary reaction zone is separated by a stream of nitrogen flowing parallel to the flame to prevent access of atmospheric oxygen to its base. The low flame background and noise levels have been shown to result in improvement in the detection limits for fifteen elements. In addition, whereas molybdenum and vanadium atomic emission are not detectable in the interconal zone of the conventional flame, in the separated flame detection limits of 2 p.p.m. and 10 p.p.m. have been obtained in aqueous solution for molybdenum (379·8 nm) and vanadium (318·5 nm), respectively. Separation of much of the molecular-band emission from some elements with the secondary reaction zone may result in partial suppression of spectral interference from these elements on the atomic-line emission of the elements determined. The suppression of the interference of magnesium, which occurs through MgOH emission in the secondary zone, on the determination of iron is demonstrated as an example of this effect.

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Analyst, 1969, **94**, 554–562.

Performance of a Pre-mixed Oxygen-enriched Air - Acetylene Flame in Flame-emission Spectrophotometry

Improved sensitivities in flame-emission spectrophotometry have been obtained as a result of a simple modification to a Unicam burner - nebuliser system, which permits addition of oxygen to an air - acetylene flame. This gives enhancement factors of from 4 to 120 for the elements calcium, strontium, magnesium, barium, copper, silver, lead, iron, molybdenum, chromium, cobalt, nickel, manganese, vanadium, aluminium and lithium. The oxygen-enriched air - acetylene flame overcomes the interference effects of phosphate on calcium and magnesium, and partially eliminates the effect of aluminium on calcium.

J. F. CHAPMAN and L. S. DALE
Australian Atomic Energy Commission, Research Establishment, Lucas Heights, N.S.W., Australia.

Analyst, 1969, **94**, 563–568.

THE ANALYST

The Application of Landolt Reactions in Quantitative Catalytic Analysis

BY G. SVEHLA
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The place of Landolt reactions among quantitative catalytic methods is reviewed. By measuring the time lapse of the incubation period of a Landolt reaction (the reaction time) and plotting its reciprocal values *versus* catalyst concentration, a calibration graph can be obtained. The advantages of the use of Landolt reactions are as follows: there is no need for instrumentation and temperature control is simple. Theoretical backgrounds of the method are presented on a kinetic basis. During the analyses the uncatalysed and the catalysed reactions proceed simultaneously. The uncatalysed reaction can be examined separately if reaction time measurements are taken in the absence of the catalyst. Having obtained the velocity constant of the former, results of measurements of the reaction times of the simultaneous reactions can be evaluated to obtain the velocity constant of the catalysed reaction alone. The experimentally obtained calibration graph shows a linear correlation between reciprocal reaction time *versus* catalyst concentration; the rigorous kinetic examination, however, yields to a partly exponential correlation. By expanding the exponential member into a Taylor series and examining the error that arises by neglecting the non-linear members the simple linear correlation can be explained. Sensitivity, selectivity and precision of the methods are briefly discussed.

CATALYTIC or kinetic methods of chemical analysis are based on the well known fact that small amounts of catalysts are sufficient to initiate or accelerate certain chemical reactions. Thus some reactions take place or are accelerated only in the presence of the catalyst, and so the kinetic examination of the reaction can be used as a qualitative test for the substance. As the amount of catalyst required for the initiation or acceleration of the reaction is in most instances small, these qualitative catalytic tests may be extremely sensitive.

Besides qualitative tests, quantitative determinations can also be made on a similar basis. All these methods involve homogeneous catalysis in the dissolved phase, where the catalyst itself is involved in a reaction cycle, which will be called hereafter catalysed reaction. If the amount of catalyst is fairly large, the share of catalysed reaction in the over-all process will be high, and the originally slow reaction may become instantaneous. If, however, the amount of catalyst is decreased, we reach a region where the amount of catalyst has a measurable influence on the velocity of the over-all reaction, and by making kinetic measurements with various concentrations of the catalyst one can obtain a calibration graph, which can be used for quantitative analytical purposes.

Thus, quantitative catalytic analytical procedures are based on the measurement of reaction velocity, or a quantity associated with the latter. From the practical point of view this means that concentration and time measurements must be made simultaneously. The several published methods, which apply different techniques for this purpose, can be classified into two main groups.

The concentration can be chosen as the independent variable, and the time lapse from the mixing of the reagents until the concentration of a given reactant decreases or increases to a pre-determined value measured. These methods are often called chronometric procedures. In all instances two measurements must be made, one with a blank or known solution and the other with the unknown; from the two time values the concentration of the unknown can be calculated by using a calibration graph. These methods require special indication, in that it has to be known when the concentration of a species reaches a pre-determined value. Methods of indication will be dealt with later in more detail.

* A shortened version of this paper was presented as a lecture at the Joint Symposium on Limits of Detection of Analysis, Enschede, The Netherlands, 18th and 19th April, 1968.

All of the techniques, in which time is chosen as an independent variable, form the other group of quantitative catalytic methods. In these methods the reagents are mixed and the concentration readings taken after certain time intervals. From the concentration *versus* time graphs a suitable measure for the reaction rate can be obtained, and these, when plotted as a function of catalyst concentration, will form the calibration graph.

The application of Landolt reactions provides a new, simple sort of indication for chronometric procedures. Landolt[1] himself examined the reaction that takes place between sulphite and iodate ions in acidic medium. If the initial concentrations are such that iodate is in excess after mixing the reactants (iodate and sulphite) no visible change takes place for a while, but after the lapse of an incubation period, the colour of iodine appears. This latter process is immediate and is easily visible. The phenomenon is often termed the "Landolt effect," especially in the German literature.[2] The length of this incubation period, hereafter called reaction time, can be measured with a stop-watch.

If the volumes of reagents are measured accurately with pipettes or burettes, and the temperature is constant, time values are fairly reproducible. The coefficient of variation of time measurements can easily be kept below 1 per cent. A thermostat can be used to maintain temperature and no costly instruments, such as spectrophotometers or pH meters, are required.

Several other slow reactions exist, which can be transformed into processes showing the Landolt effect. These reactions are generally called Landolt reactions. The processes are mostly oxidation - reduction reactions involving halogens in various oxidation states, but acid - base and complexation reactions can also be converted into Landolt processes. Reaction time decreases if a catalyst is present. The analytical application of Landolt reactions is based on this principle; reaction times are measured in the presence and absence of the catalyst, and the concentration of the catalyst can be obtained from a calibration graph. Several methods have been worked out by various research schools in the past 10 years.[3 to 19]

Experimental procedure—

The advantage of this method is the simplicity of the experimental procedure for which only test-tubes, pipettes, glass mixers, a simple water thermostat and a stop-watch, although a wrist-watch could be used if necessary, are required. Two reagent solutions are prepared and placed in a thermostat; known amounts of one of the reagents, A, are transferred by pipette into clean, dry test-tubes that had previously been thoroughly washed, rinsed and carefully dried; traces of metal ions, *e.g.*, contaminations from the use of chromic acid - sulphuric acid, may cause serious interferences. A known volume of the test solution is added, the two solutions are mixed and placed in the thermostat and the stop-watch is started as a known volume of the other reagent, B, is introduced by means of a pipette. A glass stirrer should be gently used during and shortly after this addition to mix the reagents thoroughly. Immediately the Landolt effect occurs the watch must be stopped and the time readings, t, taken.

Reciprocal values of reaction times $\left(\frac{1}{t}\right)$ when plotted against the concentration of the catalyst give a linear calibration graph that can be used for the evaluation of analyses of unknown samples.

Theoretical backgrounds—

It is important to deal with the theoretical backgrounds of the method. Although research has been carried out on quantitative catalytic methods, which are quite widely used at present, the kinetic backgrounds of these procedures have not yet been elucidated. The main problem is to explain the particular shape of the calibration graph, *i.e.*, the linear correlation between reciprocal reaction time and concentration. The simplicity of this correlation is striking and suggests a rather simple explanation but, as pointed out below, this is not so. Some simple, rather formalistic explanations have already been made, but these cannot be regarded as serious attempts. A thorough kinetic study is required to explain the shape of the calibration graphs and to elucidate the other facts that emerge when research is made into these methods. The principle of the particular catalytic method, and the reactions involved, have to be taken into consideration when assessing the kinetic backgrounds. For the hydrogen peroxide - iodide Landolt reaction, as used for the determination

of molybdenum, we presented a kinetic explanation,[6,7] and in the present paper for the first time a general explanation is attempted, which is applicable to any catalytic method based on a Landolt reaction.

The theoretical basis of any catalytic method has to be explained in three separate steps. First the kinetics of the uncatalysed reaction have to be examined, then the catalysed reaction itself must be studied, which, however, proceeds simultaneously with the uncatalysed reaction if the catalyst is present, and when these studies are completed the explanation of the calibration graph can be attempted.

THE UNCATALYSED REACTION—

The uncatalysed reaction proceeds between two reactants, A and B. Although the stoicheiometry of this reaction might be quite complicated (*e.g.*, the reaction between halogenate and halogenide) it proceeds in several steps, one of which is usually rate determining. This involves one molecule of each of the two reactants, although hydrogen ions might also be involved (*e.g.*, the Dushman reaction). The rate-determining step can be written as

$$A + B + \nu H^+ \longrightarrow D + E \; (k_1') \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where D and E are the products and k_1' is the velocity constant. The reaction velocity can be described with the (differential) equation

$$-\frac{d\,[A]}{dt} = k_1'\,[A]\,[B]\,[H^+]^\nu \quad .. \quad .. \quad .. \quad .. \quad (2),$$

the square brackets representing actual (*i.e.*, ever-changing) concentrations, the over-all order of the reaction being $\nu + 2$.

In the application of Landolt reactions the hydrogen-ion concentration is kept constant by the application of buffers, thus $[H^+]$ is kept constant. Introducing the new velocity constant

$$k_1 = k_1'\,[H^+]^\nu \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

equation (2) can be written as

$$-\frac{d\,[A]}{dt} = k_1\,[A]\,[B] \quad .. \quad .. \quad .. \quad .. \quad (4)$$

leading to a pure second-order reaction. This reaction order has to be proved experimentally; a simple means for this will be discussed later.

To achieve the Landolt effect a third so-called retarding reagent, X, has to be added, which converts one of the reaction products, D, *e.g.*, iodine, back into one of the reactants, B, *e.g.*, iodide. The instantaneous reaction

$$D + X \longrightarrow B + F \quad .. \quad .. \quad .. \quad .. \quad (5)$$

thus proceeds. This means that the concentration of the reactant B is constant (until the occurrence of the Landolt effect). Denoting this as

$$[B] = \text{constant} = b_0 \quad .. \quad .. \quad .. \quad .. \quad (6)$$

equation (4) can be written as

$$-\frac{d\,[A]}{dt} = k_1 b_0 [A] = k_1''[A] \quad .. \quad .. \quad .. \quad .. \quad (7)$$

yielding a first-order equation. The integrated equation valid for first-order reactions can thus be applied. Let us denote the initial concentration of the reagent A as a_0. If x_0 moles of this reagent are consumed up to the time t (which is the reaction time), the correlation

$$k_1'' = k_1 b_0 = \frac{1}{t} \ln \frac{a_0}{a_0 - x_0} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

is valid. In the simplest case x_0 is equal to the initial concentration of the reactant X, or is correlated to this by the stoicheiometry of the reaction; a_0, x_0 and b_0, therefore, are defined by the composition of the reagents, and these are easily controllable experimentally.

All these hypotheses have, however, to be proved experimentally. Logically, the simplest way to do this would be to prepare solutions of different concentrations (thus varying a_0, x_0 and b_0), measuring the reaction time in each instance and calculating k_1 for each experiment. If the value of k_1 is really constant, the above interpretation can be accepted.

A suitable method for verifying the theory is to prepare only one pair of solutions (keeping a_0, b_0 and x_0 constant), but to measure the reaction times after diluting the mixture with known amounts of water. To a volume V_A of the solution of the reagent A, a volume V_{H_2O} of water is added and mixed. Now a volume V_B of the solution containing reagents B and X is added and the reaction time, t_n, taken. The degree of dilution, n, is defined by the volumes involved—

$$n = \frac{V_A + V_B + V_{H_2O}}{V_A + V_B} \quad .. \quad .. \quad .. \quad .. \quad (9).$$

Note, that while V_A and V_B are constant, V_{H_2O} is varied within a set of experiments. The actual initial concentrations of the reagents will therefore be $\frac{1}{n}$ times the original values, and equation (8) can be altered to

$$k_1 \frac{b_0}{n} = \frac{1}{t_n} \ln \frac{\frac{a_0}{n}}{\frac{a_0}{n} - \frac{x_0}{n}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10).$$

From this the k_1 constant can be expressed as

$$k_1 = \frac{n}{b_0 t_n} \ln \frac{a_0}{a_0 - x_0} \quad .. \quad .. \quad .. \quad .. \quad (11);$$

a_0, b_0, x_0 and n are known from the composition of the solutions and t_n is measured. The value of k_1 can be easily calculated and examined, to find whether or not its value is really constant with changing n.

Should the value of k_1 not be constant the hypothesis concerning the reaction order must be rejected. By analogous arguments expressions can be obtained for first or third-order reactions (note that k_1 was introduced as a second-order velocity constant) containing a_0, b_0, x_0, n and t_n in a less or more complicated expression. The actual work of calculation might be less or more complicated, but the constant can be found and the reaction order thus established. From the result the actual reaction mechanism can easily be reconstructed.

THE CATALYSED REACTION—

When the catalyst C is present, two simultaneous reactions will take place. One of these is the uncatalysed reaction, which was discussed in the previous section, and the other the catalysed reaction, in which the catalyst C has an important rôle. The over-all reaction equation will be identical with the uncatalysed process shown in equation (1), but the reaction takes place in more steps. In the first step the catalyst C reacts with one of the reactants, say A, in an instantaneous reaction—

$$A + C \longrightarrow P^* \quad .. \quad .. \quad .. \quad .. \quad .. \quad (12)$$

where the product P* can be anything, *i.e.*, one molecule or several species (* shows that an activated intermediate is formed), which now reacts with the other reactant, B—

$$P^* + B + \nu H^+ \longrightarrow D + E + C\ (k_2') \quad .. \quad .. \quad .. \quad (13),$$

thus the products D and E are formed and the catalyst is reproduced. Hydrogen ions might be involved in either reaction (13) or (14), or both; the important fact is that the over-all equation [sum of processes (12) and (13)] must be the same as the original reaction (1). The second step (13) is a slow reaction again, although faster than the original uncatalysed process.

Let us consider reaction (13) alone, although, as stated before, this reaction proceeds simultaneously with process (1). The velocity can again be measured by the rate of decrease in concentration of substance A, although it is not directly involved in the process. The rate equation can be written as

$$-\frac{d[A]}{dt} = k_2' [P^*] [B] [H^+]^\nu \quad .. \quad .. \quad .. \quad .. \quad (14).$$

Although this reaction shows a quite high order, this is reduced significantly by the special experimental circumstances applied. The concentration of the P* product is constant, and is equal to c_0, the analytical concentration of the catalyst, provided that the stoicheiometry of expression (12) is correct and the reactant A is present in large excess over the catalyst C, even at the instant of the Landolt effect. This is certainly true as far as analytical uses are concerned. If the stoicheiometry of the reaction is not as simple as that of (12), a constant factor comes between the concentrations [P*] and c_0, but this would not alter the picture. Thus, generally—

$$[P^*] = kc_0 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (15)$$

and within one experiment both k and c_0 are constant.

The concentration of the reactant B is also constant within one experiment, for reasons mentioned in the previous section, and will be denoted as b_0. If a buffer is used, the hydrogen-ion concentration is constant also. By using the symbol k_2 for the product of the following constants—

$$k_2 = k_2' \, k \, [H^+]^\nu \quad .. \quad .. \quad .. \quad .. \quad .. \quad (16)$$

and equation (14) can be written as

$$-\frac{d\,[A]}{dt} = k_2 \, b_0 \, c_0 \, .. \quad .. \quad .. \quad .. \quad .. \quad (17).$$

Thus, we have a zero-order reaction that proceeds with a constant speed. After integration we can express the decrease of concentration of A (x_2) during the time t as

$$-x_2 = k_2 \, b_0 \, c_0 \, t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18a).$$

The mechanism given in equations (12) and (13) is only one of the four possible combinations. The mechanism and kinetics of the other three combinations are discussed here briefly (the suffix o is omitted in these deductions)—

Combination (2)—The reactions

$$A + C \longrightarrow P^* \text{ (slow) } (k_3)$$

and $$P^* + B + \nu H^+ \longrightarrow D + E + C \text{ (fast)}$$

proceed. The rate equation is

$$-\frac{d\,[A]}{[A]} = k_3 \, [A] \, [C].$$

The reagent A is present in large excess over the catalyst, therefore its concentration is constant, $[A] = a$, and that of the catalyst is constant because of the fast reaction step, $[C] = c$. The rate equation

$$-\frac{d\,[A]}{[A]} = k_3 \, ac = \text{constant}$$

indicates a zero-order reaction. Integration leads to

$$-x_3 = k_3 act \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18b).$$

Combination (3)—The reactions

$$B + C \longrightarrow P^* \text{ (fast)}$$

and $$P^* + A + \nu H^* \longrightarrow D + E + C \text{ (slow) } (k_4')$$

proceed. The rate equation is

$$-\frac{d\,[A]}{[A]} = k_4' \, [A] \, [P^*] \, [H^+]^\nu.$$

Here $[A] = a$ is constant, because its concentration is much higher than that of D* (which is of the magnitude of [C]), $[P^*] = k_4''C$ is constant because of the fast step and $[H^+]^\nu = k_4'''$ is also constant because of the presence of the buffer. The rate equation therefore

$$-\frac{d\,[A]}{[A]} = k_4' a k_4'' c k_4''' = k_4 ac = \text{constant}$$

(where $k_4 = k_4' k_4'' k_4'''$) suggests a zero-order reaction, for which integration leads to

$$-x_4 = k_4 act \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18c).$$

Combination (4)—The reactions

$$B + C \longrightarrow P^* \text{ (slow) } k_5$$

and $$P^* + A + \nu H^+ \longrightarrow D + E + C \text{ (fast)}$$

proceed. The rate equation is

$$-\frac{d\,[A]}{[A]} = k_5\,[B]\,[C].$$

Here $[B] = b$ is constant because of the presence of the retarding reagent and $[C] = c$ is constant because of the fast step. The rate equation

$$-\frac{d\,[A]}{[A]} = k_5 bc = \text{constant}$$

suggests a zero-order reaction. Integration leads to

$$-x_5 = k_5 bct \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18d).$$

Equations (18b), (18c) and (18d) are equivalent to (18a) and could be used in deductions that are analogous to the one presented in the next part of the paper, where combination (1) is taken into account.

With the uncatalysed and catalysed reactions proceeding simultaneously the decrease in concentration of the substance A, denoted here by x_0, can be regarded as the sum of two concentration decreases; one as a result of the uncatalysed process (x_1), the other as that of the catalysed one (x_2).

$$x_0 = x_1 + x_2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (19).$$

x_0 is again equal to, or can be expressed by, the concentration of the retarding reactant X. At the same time the following two integrated kinetic equations will hold—

$$k_1 b_0 = \frac{1}{t} \ln \frac{a_0}{a_0 - x_1} \quad .. \quad .. \quad .. \quad .. \quad (20)$$

and

$$-x_2 = k_2 b_0 c_0 t \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18)$$

for the uncatalysed and catalysed reactions, respectively. From equations (18), (19) and (20) x_1 and x_2 can be eliminated and k_2 can be expressed as a function of a_0, b_0, c_0, x_0, t and k_1. [Expression (24) will hold when n is unity.] Thus, measuring the reaction time, t, knowing the initial (analytical) concentrations a_0, b_0, c_0 and x_0 and having determined k_1 previously, the value of k_2 can be obtained. By varying the concentrations of the reagents, further experiments can be made to determine if k_2 remains constant. The principle mentioned in the previous section is used and a_0, b_0, c_0 and x_0 are kept constant, but various dilutions (the dilution factor being n) are used. With such experiments the equations [analogous to (19), (20) and (18)] will hold—

$$\frac{x_0}{n} = \frac{x_1}{n} + \frac{x_2}{n} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (21)$$

$$k_1 \frac{b_0}{n} = \frac{1}{t_n} \ln \frac{\frac{a_0}{n}}{\frac{a_0}{n} - \frac{x_1}{n}} \quad .. \quad .. \quad .. \quad .. \quad (22)$$

and

$$-\frac{x_2}{n} = k_2 \frac{b_0}{n} \frac{c_0}{n} t_n \quad .. \quad .. \quad .. \quad .. \quad (23).$$

From these equations k_2 can be expressed as

$$k_2 = n \frac{\left\{a_0 - x_0 - \exp\left(\frac{t_n k_1 b_0}{n}\right)\right\}}{t_n b_0 c_0} \quad .. \quad .. \quad .. \quad (24)$$

where t_n is the time measured with the dilution factor n. If the value of k_2 is really constant with varying n the hypothesis about the reaction is verified.

THE CALIBRATION GRAPH—

If the k_1 and k_2 velocity constants are known (and this means that the kinetic background of the method is clear) we can attempt to explain the particular shape of the calibration graph, *i.e.*, the linear relationship (but not direct proportionality) between $\frac{1}{t}$ and c.

When the calibration graph is obtained, the initial (analytical) concentrations a_0, b_0 and x_0 are kept constant (and the suffix o will be abandoned from now on). Equations (18), (19) and (20) will hold for this set of experiments, with c and t being the variables. Let us eliminate x_1 and x_2 from these equations and express x as

$$x = a - a \exp(-k_1 bt) - k_2 bct \quad .. \quad .. \quad .. \quad .. \quad (25).$$

As t is in the exponent, expression (25) suggests a more complicated relationship than the one obtained experimentally.

The exponential member has to be examined more closely. By expanding into a Taylor series and collecting all the members that contain t with higher powers than 1 into a residium we obtain a linear expression for t. The expansion has to be made at a suitable medium value for t. If t is measured in minutes and the range is the usual 0 to 4 minutes, $t = 2$ is a suitable value yielding the simple equation—

$$T\,[\exp(-k_1 bt]_{t=2} = \exp(-2k_1 b) + 2k_1 b \exp(-2k_1 b) - k_1 bt \exp(-2k_1 b) + R \quad .. \quad (26).$$

If the remainder R is omitted, the equation becomes linear for t. The omission of R has to be justified. The error involved by this omission, expressed for the range $t = 0$ to 4 minutes is—

$$\Delta \text{ (per cent.)} = \frac{2k_1^2 b^2}{\exp(-2k_1 b)}\ 100 \quad .. \quad .. \quad .. \quad .. \quad (27).$$

Equations for reactions following different stoicheiometry or kinetics might be different; the expansion into Taylor series and the examination of the error can however be made easily, details of which are to be found in university mathematical texts.[20] This error (expressed as a percentage) has to be compared with the experimental error itself. If the error caused by "linearisation" is, for example, a few hundredths of 1 per cent., while the experimental error is a few per cent., this treatment is readily justified, as for the determination of molybdenum with the hydrogen peroxide - iodide reaction, published elsewhere.[8]

With $R = 0$, equations (25) and (26) can be combined and $\frac{1}{t}$ expressed as

$$\frac{1}{t} = c\left[\frac{k_2 b}{a - a\exp(-2k_1 b) - 2ak_1 b \exp(-2k_1 b) - x}\right] + \left[\frac{ak_1 b \exp(-2k_1 b)}{a\exp(-2k_1 b) + 2ak_1 b \exp(-2k_1 b) + x - a}\right] = c \cdot h + e \quad .. \quad (28).$$

Now all the quantities in the square brackets are constants and thus the linear correlation between $\frac{1}{t}$ and c is proved.

This theory has successfully been applied for the determination of molybdenum with the hydrogen peroxide - iodide reaction,[7] determination of copper with the peroxydisulphate - iodide reaction,[9] determination of vanadium with the bromate - iodide reaction[10] and determination of iron and molybdenum with the perborate - iodide reaction.[21]

SENSITIVITY, SELECTIVITY AND PRECISION

LIMIT OF DETECTION—

Sensitivity of the method can be judged from the limit of detection, *i.e.*, the lowest concentration of the substance that can be determined. Because of the fact that determination is made with the aid of the calibration graph, the regression line of $\frac{1}{t}$ over c has to be found and the $s\left(\frac{1}{t}c\right)$ standard deviation calculated. Details of such calculations are to be found in textbooks of mathematical statistics. By using Kaiser's definition[22] for

the limit of detection, we calculate first the minimum value of $\frac{1}{t}$, which is discernible from the standard deviation (the so-called "background noise," a term adopted mainly for instrumental techniques)—

$$\left(\frac{1}{t}\right)_{\text{min.}} = 3\sqrt{2}\, s\left(\frac{1}{t}c\right) \quad .. \quad .. \quad .. \quad .. \quad (29).$$

From this the limit of detection, *i.e.*, the $c_{\text{min.}}$ lowest concentration of the substance that can be determined is expressible as

$$c_{\text{min.}} = \frac{\left(\frac{1}{t}\right)_{\text{min.}} - e}{h} \quad .. \quad .. \quad .. \quad .. \quad (30)$$

where e is the intercept of the calibration graph with the $\frac{1}{t}$ axis and h is the slope. The limit of detection of catalytic methods based on Landolt reactions is generally of the magnitude of 0·1 to 1 μg ml^{-1}. When determining molybdenum with the hydrogen peroxide - iodide reaction[9] the limit of detection was 0·12 μg ml^{-1}, while that of vanadium determination with the bromate - iodide method[10] was 0·84 μg ml^{-1}.

SELECTIVITY—

The specificity and selectivity of these methods have to be examined experimentally, and detailed studies have to be made on the effect of other ions on the reaction time both in the presence and the absence of the catalyst. Selectivity can often be improved by the addition of a complexing agent to the system. Thompson and Svehla succeeded, for example, in making the determination of vanadium specific by the addition of larger amounts of citrate,[10] when not only the interfering ions were complexed but also the sensitivity of the method was improved. A suitable symbol to express the selectivity of a method is the "selectivity index" introduced by Belcher[23] and Betteridge.[24] This index shows the analytical principle (in the middle), the ion determined (superscript on right), the class of selectiveness (α, β, in superscript on left), the pH (subscript on left) and auxiliary complexation agents (subscript on right). Thus the vanadium determination based on the bromate - iodide reaction would have the selectivity index—

$${}_{2\cdot 2}^{\alpha}\text{Cat}^{\text{V}}_{\text{Citrate}}$$

α representing the best class of selectivity, *i.e.*, the reaction is specific only for one ion, *e.g.*, vanadium.

PRECISION—

The accuracy of analytical methods, based on the use of calibration graphs, is reported generally in a rather vague way, *i.e.*, some percentage error is presented in most cases. A more adequate way to express the reliability is to present the tolerance limits around the result with a given probability, say 95 per cent. If t is the average of N parallel time measurements the result can be given as[25]—

$$\frac{\frac{1}{t} - e}{h} \pm \frac{\tau(95\,M - 2)}{h}\, s\left(\frac{1}{t}c\right)\sqrt{\frac{1}{N} + \frac{1}{M} + \frac{\frac{\frac{1}{t} - e}{h} - \bar{c}}{SSD_{\text{c}}}} \quad .. \quad .. \quad (31).$$

In this expression e and h are the intercept and slope of the calibration line, τ $(95\,M - 2)$ is the value of the Student distribution function, with 95 per cent. probability and $M - 2$ degrees of freedom, $s\left(\frac{1}{t}c\right)$ is the standard deviation of the $\frac{1}{t}$ values of the graph. M is the number of points measured when obtaining the calibration graph, $\bar{c}$ is the mean of concentration values on the calibration graph (*i.e.*, the middle of the concentration range)

and SSD_c is the sum of square deviations of concentration values about $\bar{c}$. The quantities $e, h, s\left(\frac{1}{t}\right)\bar{c}$ and SSD_c are obtained by routine calculation of the least-square values of constants of the calibration line. The limits are narrowest if

$$\frac{\frac{1}{t} - e}{h} = \bar{c}$$

i.e., if the unknown concentration falls in the middle of the calibration graph. The same applies of any analytical method based on the use of a calibration graph. In the middle range of the calibration graph this tolerance is about 3 to 6 per cent. of the measured concentration for most of the catalytic methods involving Landolt reactions.

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A Catalytic Method for the Determination of Ruthenium

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A method is outlined for the determination of ruthenium based on its catalysis of the periodate oxidation of the tris(1,10-phenanthroline)iron(II) complex. This reaction has been used to detect 10^{-10} M ruthenium and to determine 10^{-9} to 2×10^{-8} M ruthenium, with a coefficient of variation of 4·8 per cent. The interference effects of various ions have been studied and the method is shown to have only three serious interferences, iridium, osmium and rhenium. The sensitivity attained compares favourably with other similar methods by which ruthenium can be determined.

It has been apparent for many years that kinetic information, concerning catalytic methods of analysis involving halates, is of increasing importance to the understanding of the processes involved, and might extend the versatility and usefulness of the methods themselves. There have been only a few attempts[1,2,3,4] made along these lines because of the complexity of the reactions involved. It was thought that by choosing a relatively simple system a useful foundation could be laid for future work. Reactions involving the vanadium catalysis of bromate oxidations of organic compounds have been considered[4] but these proved to be too complex in nature for the simple investigation sought. It has been reported[5] that ruthenium catalyses the periodate oxidation of the tris(1,10-phenanthroline)iron(II) complex, and besides offering a possible method for the catalytic determination of ruthenium it seemed that the catalysis might be caused by a simple sequence of oxidation - reduction reactions involving periodate, ruthenium and tris(1,10-phenanthroline)iron(II).[5] In the case of bromate oxidations, complexities are introduced by bromate oxidation of the bromide produced during the reaction, resulting in the formation of free bromine, which subsequently participates in the reactions. Such reactions are often auto-catalytic and exhibit induction periods. In the present instance no such complexity should be introduced as the periodate is reduced only to iodate.[6] The use of this reaction system for the determination of ruthenium will be described here and a report of the kinetics given at a later date.

The indicator, tris(1,10-phenanthroline)iron(II), was discovered by Walden, Hammett and Chapman[7] and is orange - red in the reduced form and pale blue in the oxidised, iron(III) form. It undergoes a reversible oxidation - reduction reaction. Tris(1,10-phenanthroline)-iron(II) is slowly decomposed by mineral acids[8] or by salts of other metals,[7] which form stable complexes with the ligand, for example, cobalt(II), nickel(II), copper(II), zinc(II) and cadmium(II). The indicator is most commonly used for cerium(IV) oxidations, but can be used successfully with other oxidants.[6,7]

Ruthenium has been determined by catalytic methods previously,[9,10,11,12] and these will be compared with the present results and also with some other techniques used in the determination of ruthenium.

Reagents—

Analytical-reagent grade reagents were used whenever possible.

Tris(1,10-phenanthroline)iron(II) sulphate, 5×10^{-4} M.

Potassium periodate.

Perchloric acid, 60 *per cent. w/w.*

Ruthenium solution, 10^{-4} M—A stock solution was prepared by dissolving the appropriate weight of ruthenium(III) chloride in water containing 0·5 ml of hydrochloric acid and diluting to 1 litre.

The solutions were prepared and standardised by the usually accepted procedures.[6] Glass-distilled water was used throughout.

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Experimental

Procedure—

A glass reaction vessel consisting of three limbs, each with a ground-glass stopper, was used to contain the reaction solutions. In the uncatalysed reaction the required volume of each reactant [perchloric acid, potassium periodate and tris(1,10-phenanthroline)iron(II)] was added by means of a pipette to a separate limb and the total volume made up to 100 ml by the addition of distilled water to one limb. In the catalysed reaction the ruthenium was added to the limb containing the periodate solution, and in the investigation of interferences the interfering species was also mixed with the periodate and ruthenium. The reaction vessel was maintained thermostatically in a water-bath at 25·0 ± 0·1° C for 30 minutes before use.

After mixing the three solutions, an aliquot of the reaction mixture was transferred rapidly to a 10-mm absorption cell maintained thermostatically, and the oxidation of the tris(1,10-phenanthroline)iron(II) was followed spectrophotometrically at 505 nm, by using a Hitachi - Perkin-Elmer 139 spectrophotometer in conjunction with a Honeywell Electronik chart recorder.

A suitable blank reaction, *i.e.*, uncatalysed reaction, is required. The ideal situation is when the rate of the blank reaction is negligible compared with the catalysed reaction and when the rate of the latter reaction is extremely sensitive to changes in concentration of the catalyst. A range of concentrations for each of the three reactants was investigated and an optimum concentration was chosen in each instance. A concentration of 5×10^{-5} M tris(1,10-phenanthroline)iron(II) gave a suitable absorbance, which could be monitored conveniently with the apparatus used. Perchloric acid in the concentration range 2×10^{-3} to 1·0 M was investigated. At the lower acid concentrations both the catalysed and uncatalysed reactions showed an induction period, and the catalysed reaction was relatively insensitive to the catalyst concentration. As the perchloric acid concentration was increased the induction period disappeared, and at higher acid concentrations the reaction was too fast to be observed. The optimum perchloric acid concentration was chosen as 10^{-1} M when there was a measurable rate but when no induction period was exhibited. The periodate concentration was fixed at 10^{-4}M, *i.e.*, twice the tris(1,10-phenanthroline)iron(II) concentration, as this ensured that the reaction could reach completion but that the reaction rate of the uncatalysed reaction was kept to a minimum.

It was found necessary to keep all three reactants separate before the final mixing, as pre-mixing of any two resulted in some reaction taking place during the thermostating period. Pre-mixing of the tris(1,10-phenanthroline)iron(II) and perchloric acid resulted in the slow decomposition of the complex,[8] while pre-mixing of the periodate and tris(1,10-phenanthroline)-iron(II) caused the complex to change from its orange - red colour to a pale green within the time of thermostating. Pre-mixing of the periodate and perchloric acid and allowing the mixture to stand for less than 20 minutes gave no appreciable change in rate, but pre-mixing for longer periods caused an increase in the rate, the extent of which varied with the time of pre-mixing.

Results and discussion

The periodate oxidation of tris(1,10-phenanthroline)iron(II) in the presence and absence of ruthenium can be formulated as in equation 1,

$$2\,IO_4^- + 2[Fe(1,10\text{-phen.})_3]^{2+} + 2H^+ \longrightarrow 2\,IO_3^- + 2[Fe(1,10\text{-phen.})_3]^{3+} + H_2O \qquad (1),$$

and enables the concentration of ruthenium to be determined in the range 2×10^{-8} to 10^{-9} M, with a coefficient of variation of 4·8 per cent. (for nine determinations) at a concentration of 5×10^{-9} M ruthenium. The reactions of periodate with other reduced species such as tris(α,α-dipyridyl)iron(II), diphenylaminesulphonate and Bordeaux B have been investigated but in no instance was the catalytic effect of ruthenium as sensitive as that with tris(1,10-phenanthroline)iron(II).

The effect of traces of ruthenium in the range 0 to 5×10^{-8} M on the rate of reaction (1) is shown in Fig. 1. It can be seen that the blank reaction in the absence of ruthenium is slow over the time of the experiment. Fig. 1 also shows that the fixed time method, used for obtaining a calibration curve, is well suited to the present system. After 10 minutes the absorbance changes relatively slowly and precise measurement of its value is possible. By using this method a linear dependence between ruthenium concentration and the reciprocal

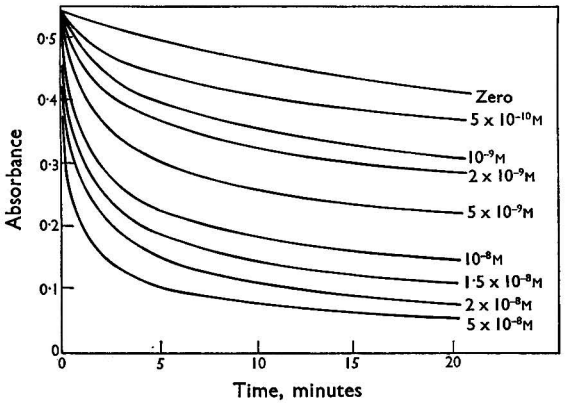

Fig. 1. Effect of traces of ruthenium on the rate of oxidation of tris(1,10-phenanthroline)iron(II) by potassium periodate. Decrease in absorbance at 505 nm caused by an initial concentration of 5×10^{-5} M tris(1,10-phenanthroline)iron(II) in the presence of 10^{-4} M potassium periodate, 0·1 M perchloric acid and varying concentrations of ruthenium from 0 to 5×10^{-8} M

of the absorbance after 10 minutes reaction time was obtained within the concentration limits 2×10^{-8} to 10^{-9} M. At higher ruthenium concentrations the linear dependence failed and the graph tended to become parallel to the ruthenium concentration axis. It was possible to detect ruthenium at 10^{-10} M but results in this region were not reproducible.

The effects of various cations, anions and complexing agents on the catalytic effect of ruthenium were investigated. Interference was defined to mean that the error in the determination of a known amount of ruthenium in the presence of foreign ions was greater than the coefficient of variation given above. A ruthenium concentration of 5×10^{-9} M was used to ascertain the effects of other cations and anions and, as the reagents themselves [tris-(1,10-phenanthroline)iron(II) and periodate] were present at concentrations of about 10^{-4} M, it was considered that 10^{-4} M represented a realistic maximum concentration level at which to study the effects of interferences. The results are summarised in Table I, in which interferences are also given a (+) or (—) symbol to designate enhancement or inhibition, respectively, of the catalytic effect of ruthenium. Where no interference is given then that particular ion does not interfere at concentrations up to 10^{-4} M.

TABLE I

EFFECTS OF INTERFERING IONS

Interfering ion	Interference level	Interference effect
V(IV)	10^{-4}	*
V(V)	10^{-4}	+
Fe(III)	10^{-4}	*
W(VI)	10^{-4}	*
Re(III)	5×10^{-7}	+
Os(VIII)	5×10^{-7}	+
Ir(III)	10^{-8}	+
CN^-	10^{-6}	—
SCN^-	10^{-6}	—
EDTA	5×10^{-6}	—
1,10-Phenanthroline	10^{-6}	—
Dimethylglyoxime	10^{-6}	—

* More complex and explained in the text.
\+ A positive error or enhancement.
— Inhibition.

No interference up to 10^{-4} M from: Na; Mg; K; Cr(VI); Mn(II); Co(II); Ni(II); Cu(II); Mo(VI); Rh(III); U(VI); Cl^-; NO_3^-; ClO_3^-; BrO_3^-; SO_4^{2-}; $H_2PO_4^-$; oxalate; tartrate; acetate; and 8-hydroxyquinoline.

Apart from iridium, osmium and rhenium, none of the other cations or anions presented serious interference, although at the interference levels given iron(III) and vanadium(IV) had unusual effects while tungsten(VI) formed a precipitate. Iron(III) caused an induction period to occur in the reaction, while vanadium(IV) caused an initial rapid decrease in the absorbance of the tris(1,10-phenanthroline)iron(II) followed by a slow increase until the initial absorbance was almost attained, followed finally by a slow decrease in the absorbance. The effect of iridium, the major interference, was complex. Iridium appeared to activate the catalytic effect of ruthenium as with a 10^{-8} M concentration present serious interference was observed, but in the absence of ruthenium the iridium seemed to have no catalytic activity at all. The enhancement from iridium could be avoided if either ruthenium or periodate were not pre-mixed with the iridium. As there is no requirement that the periodate and sample have to be pre-mixed in an analytical determination, this would probably provide a simple method of overcoming this interference.

The effects of some complexing agents were investigated at concentrations of 10^{-6} M in an attempt to find a simple method for masking the effects of osmium, rhenium and iridium and any further interferences that might subsequently be found and be of importance. The results are given in Table I and show that many of the more likely complexing agents which might be of general applicability, for example, cyanide, thiocyanate, EDTA and 1,10-phenanthroline, inhibit the catalytic effect of ruthenium itself and are, therefore, unsuitable for the purpose intended.

A reliable method for the separation of ruthenium in microgram amounts from other metals, and in particular from osmium, has been described[9] and might usefully be used in the present technique, if required. However, because of the high sensitivity of the present reaction, merely diluting the sample solution to reduce the ruthenium to the correct concentration level can in some circumstances be sufficient to eliminate the majority of the interference effects.

Feigl and Frankel[10] have reported a method for the detection of 0·5 μg ml^{-1} of ruthenium by its catalysis of the hypophosphite - nickel(II) reaction, and Kuznetsov[11] has also reported that 10^{-3} μg ml^{-1} of ruthenium can be detected by its catalytic effect on the reaction of sulphanilic acid with nitrate ion. Two previous methods have been proposed for the catalytic determination of ruthenium. Shiokawa[12] reported the determination of 0·7 to 6·0 μg ml^{-1} of ruthenium based on its catalysis of the chlorate - iodide reaction and Surasiti and Sandell[9] give a detection limit of 10^{-5} μg ml^{-1} of ruthenium by using its catalytic effect on the arsenic(III) - cerium(IV) reaction. Their most convenient working range was 5×10^{-3} to 5×10^{-4} μg ml^{-1}. In comparison with other catalytic methods for the determination of ruthenium, therefore, the method described here, which has a detection limit of 10^{-10} M ruthenium (approximately equal to 10^{-5} μg ml^{-1}) and a working range of 2×10^{-8} to 10^{-9} M (approximately equal to 2×10^{-3} to 10^{-4} μg ml^{-1}), is considerably more sensitive than all but one[9] of the earlier procedures and slightly more sensitive than that one. Of other techniques, polarography[13,14] and atomic-absorption spectrophotometry[15,16] permit determination of ruthenium down to concentrations of, at most, only 10^{-6} M, while spectrophotometric techniques, although theoretically capable of determinations at the 10^{-7} M level (when using 10-mm absorption cells), invariably fall short of this value.[17,18] The catalytic technique therefore offers considerable advantages in sensitivity over the other relatively inexpensive analytical methods and, additionally, in all cases outlined above except one,[9] the effect of interferences seems to be at a greater level than in the present method. The only other method of comparable sensitivity is that described by El Guebeley,[19] who uses the fact that hydrogen has a smaller overpotential on ruthenium than on mercury. At a suitable potential ruthenium is deposited from aqueous solution on to a small mercury electrode, the ruthenium then facilitates the discharge of hydrogen. The height of this catalytic wave is proportional to the ruthenium concentration. Ruthenium down to 5×10^{-10} M can be determined by this method.

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Separation of Gold from Platinum Metals on Cation Exchangers in Concentrated Hydrobromic Acid Solutions

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Separation of gold from platinum metals in the Dowex 50W × 8 [H^+] - hydrobromic acid *plus* bromine system was investigated. Distribution coefficients of gold (in trace amounts) increase almost linearly with hydrobromic acid concentrations from 0·5 to 5 N, reach nearly constant value at 5 and 6 to 7 N and decrease slightly with further increase in hydrobromic acid concentration. Distribution coefficients decrease with loading and with temperature. Apparent enthalpy change for the distribution reaction of gold between resin and solution is about 3 kcal. $mole^{-1}$. The absorption capacity of Dowex 50W × 8 [H^+] for gold (in 6 N hydrobromic acid), as determined from break-through graphs, increases considerably with the increase in gold concentration in the solution. Separation of both carrier-free and milligram amounts of platinum and gold can be achieved with 6 N hydrobromic acid *plus* 0·0035 M bromine solution at 15° C. Platinum is eluted in the solvent front and the gold, retained by the resin, is rapidly stripped off with acetylacetone. Statistical analysis of the results of the separations by use of tracers has proved that no systematic error is involved. Iridium, palladium and rhodium behave in a similar manner to platinum and can be separated from gold with this system. A possible mechanism of the absorption of gold by cation-exchange resin, *i.e.*, the partition of undissociated bromoauric acid with the formation of a molecular (charge transfer) complex between the aromatic rings of resin network and complex acid, is suggested.

As inferred from recent reviews[1,2] ion exchange, in contrast with extraction methods, has not currently been used for the separation of gold from platinum and the platinum metals. Anion exchange in halogen acid media offers little promise, as chlorocomplexes of gold and platinum are strongly absorbed by anion-exchange resins.[3,4,5] The elution of gold from the resin is difficult and usually not quantitative and, therefore, it is often necessary to ignite the resin.[6,7] An interesting possibility was indicated by Kraus, Michelson and Nelson[8] and Nelson, Murase and Kraus,[9] who found that gold is absorbed by sulphonic cation-exchange resin from concentrated hydrochloric acid solutions, but platinum shows negligible absorption. This was used further by Fu-Tszyun, Norseev, Khalkin and Tao-Nan,[10] who separated gold-199 from an irradiated platinum target, but no quantitative results were given for the accuracy and precision of the separation. The use of chlorinated solutions is a disadvantage, especially in routine work.

Gold is also absorbed by sulphonic cation exchangers from concentrated hydrobromic acid *plus* bromine solutions.[11,12] We have shown previously that carrier-free gold-199 can easily be separated from platinum with this system.[12] Gold retained on the ion exchanger is effectively and rapidly stripped from the column by elution with acetylacetone.

In this paper a detailed study of the separation of trace and milligram amounts of gold from platinum and other platinum metals is presented.

* Paper presented at the Second SAC Conference 1968, Nottingham.

Experimental

Ion-exchange resins—

Dowex 50W × 8 (sulphonic) and Amberlite IRC-50 (carboxylic) cation-exchange resins were used. Resin, of a fine mesh size, needed for column experiments, was prepared by the methods previously described,[13] and its particle size was determined microscopically. The water content of the air-dried resin was determined by drying it in an oven at 105° C to constant weight. The exchange capacity of strongly acidic groups, Z_S, and total exchange capacity, Z_C, were determined as described previously[13]; Z_S was 5·00 and 0·32 milli-equivalents g^{-1} of dry resin [H^+], and Z_C was 5·28 and 10·43 milli-equivalents g^{-1} of dry resin [H^+] for Dowex 50W × 8 and Amberlite IRC-50, respectively. Bed density, d_z, was 0·371 and 0·310 g of dry resin [H^+] ml^{-1} of the bed for Dowex 50W × 8 and Amberlite IRC-50, respectively.

Solutions and tracers—

Hydrobromic acid solutions (0·1 to 6 M) were prepared from about 7 M analytical-reagent grade hydrobromic acid; the higher concentration (about 8·7 M) was prepared by distilling 7 M hydrobromic acid. Appropriate amounts of bromine were added to each solution so that the final bromine concentration was 0·0035 M. Acetylacetone (chromatographic grade, obtained from T. Schuchard, München) was used without further purification.

Radioactive tracers were prepared by irradiating appropriate target materials in the Polish reactor EWA, at a flux of 10^{13} neutrons cm^{-2} s^{-1}, for about 20 hours.

Gold-198 was prepared by irradiating gold foil (spectrally pure grade), dissolving it in concentrated nitric acid - hydrobromic acid mixture (1 + 3) and evaporating three times with 7 M hydrobromic acid.

To separate platinum-191, -193*m*, -195*m* and -197 tracer, the irradiated platinum metal (spectrally pure grade) was dissolved in nitric acid - hydrobromic acid solution and evaporated three times with 7 M hydrobromic acid. Gold-199 was then removed on a column with Dowex 50W × 8 [H^+], as described elsewhere.[12]

Iridium-192 and -194 tracer were obtained by irradiating iridium nitrate prepared from iridium(IV) chloride, by Gilchrist and Wichers' method.[14] The irradiated target was then dissolved in nitric acid - hydrobromic acid solution and evaporated three times with 7 M hydrobromic acid.

Palladium-103, -109 and -111*m* tracer were prepared by irradiating palladium metal (spectrally pure grade), dissolving it in aqua regia and evaporating three times with 12 M hydrochloric acid. Silver-111 was then removed on a Dowex 1 × 8 [Cl^-] column from 8 M hydrochloric acid solution and palladium eluted with ammonia solution - ammonium chloride solution. The eluate was boiled with concentrated nitric acid and evaporated three times with 7 M hydrobromic acid.

Rhodium-105 tracer was prepared by irradiating ruthenium metal (spectrally pure grade), and isolating the carrier-free rhodium by Kobayashi's method.[15] The rhodium fraction was then evaporated three times with 7 M hydrobromic acid.

The radiochemical purity of tracers was monitored by half-life measurements and γ-ray spectrometry.

Procedure—

Distribution coefficients of gold were determined by batch-equilibration method. Accurately weighed amounts (about 0·2 g) of air-dried resin (0·10 mm $\leqslant \emptyset \leqslant$ 0·20 mm), the water content of which had been previously determined, were introduced into glass calibrated flasks, and 10-ml portions of hydrobromic acid solution of a given concentration were introduced into the flasks. Equal volumes (100 to 300 μl) of tracer solution of appropriate specific activity were added to each flask. For comparison the same amount of tracer solution was added to the other flask containing 10 ml of N hydrobromic acid and no resin. The flasks were then stoppered and left for 22 hours at room temperature, and were occasionally shaken. Aliquots of the solutions (2 ml) were then transferred into standard glass counting vessels (with filter-paper discs in the bottom) and evaporated to dryness under an infrared lamp. The counting rate was measured with a Geiger - Müller or a 1 × 1-inch NaI(Tl) scintillation counter, or both. Because of the relatively small amounts of ion exchanger used in equilibration no correction for the water adsorbed by the resin was introduced.

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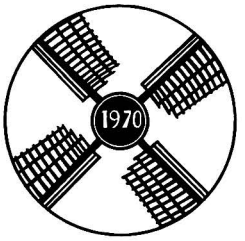

FIRST CIRCULAR

JOINT SYMPOSIUM ON ACCURATE METHODS OF ANALYSIS FOR MAJOR CONSTITUENTS

THE organising Committee has pleasure in inviting you to attend the Joint Symposium on Accurate Methods of Analysis for Major Constituents to be held at Imperial College of Science and Technology, London, on Friday and Saturday, April 3rd and 4th, 1970. The Symposium is organised under the auspices of the Society for Analytical Chemistry and the Analytical Section of the Koninklijke Nederlandse Chemische Vereniging.

SCIENTIFIC PROGRAMME

The scope of the symposium will cover modern developments in methods for the accurate determination of major constituents in materials arising in the metallurgical, inorganic, organic and biological fields. The emphasis will be on physico-chemical and instrumental rather than classical methods, although the latter will not be excluded. Contributions will be assessed in relation to current practice and accuracy attainable in a particular field or application. The subjects of the invited papers, to be read in the morning sessions, and the names of the speakers are as follows:

1. Dr. R. J. de Kock (Geleen): "Analysis of the micro-structure of Polyolefines."
2. Mr. B. Bagshawe (U.K.): "Metallurgical Analysis with Particular Reference to the Ferrous and Allied Industries."
3. Dr. R. F. Rekker (Haarlem): "The Analysis of Major Constituents in Products of Pharmaceutical Interest."
4. Dr. R. C. Chirnside (U.K.): "Major Constituents in Inorganic Systems—A Review of Current Techniques and Requirements."

Those wishing to submit papers (in English) are invited to send as soon as possible an abstract (of about 200 words) to one of the Joint Secretaries of the Scientific Committee:

Dr. F. J. Bryant, Society for Analytical Chemistry, 9–10 Savile Row, London W1X 1AF, England, *or*

Dr. H. L. Kies, Gebouw voor Analytische Scheikunde, Jaffalaan 9, Delft, The Netherlands.

The closing date for the submission of summaries will be Monday, November 17th, 1969. The summaries will be considered by the Scientific Committee and notice of acceptance or rejection will be sent to the authors by early January. For each paper 30 minutes will be reserved, including discussion. Facilities for projecting slides and films as well as overhead projection will be available.

Printed summaries of accepted papers will be provided for delegates, but the proceedings of the meeting will not be published. Authors are invited to submit full written versions of their papers whenever convenient (before the meeting if desired) to the Editor of "The Analyst" for publication. Such papers will be subject to separate refereeing. In consequence, acceptance of a paper for presentation at the meeting will not imply that it is suitable for publication in "The Analyst."

SOCIAL PROGRAMME

The Social Programme will include a Reception on April 2nd, an Official Symposium Dinner on April 3rd, a visit to a theatre on April 4th and a day tour, *e.g.*, to Hampton Court and Windsor on April 5th, returning by way of London Airport.

LADIES' PROGRAMME

There will be a special Programme for those not attending the Scientific sessions including visits to places of interest in London and its environs.

ACCOMMODATION

Accommodation will be provided in Halls of Residence at Imperial College and Queen Elizabeth College, London. The provisional charge for accommodation and meals from dinner on April 2nd to breakfast on April 5th will be £9. The price of the Symposium Dinner at Imperial College will be approximately £2 10s.

REGISTRATION FEE

The registration fee will be approximately £5.

SECOND CIRCULAR

A second circular, containing detailed information on the programme, registration, payment, accommodation, excursions, social events, etc. will be forwarded in January 1970, together with the final registration form. Those who wish to receive this Second Circular are requested to return the attached provisional application form before Monday, September 15th, 1969, since it may not be possible to reserve accommodation for those returning the form after this date.

ADDRESSES OF THE JOINT SECRETARIES OF THE ORGANISING COMMITTEE

Dr. R. Visser, Department of Chemical Technology, Technological University Twente, P.O. Box 217, Enschede, The Netherlands, *and*

Dr. D. I. Coomber, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London S.E.1, England.

The undersigned

SURNAME and CHRISTIAN NAME(S):
ADDRESS:
COUNTRY:
INSTITUTION or FIRM:

☐ wishes to receive all further notices of the Joint Symposium.

☐ intends to be present at this Symposium.

☐ intends to submit a paper on the subject of .....

.....

☐ intends to stay at the students' quarters for the nights following

☐ April 2nd ☐ April 3rd ☐ April 4th, 1970.

☐ is interested in a post symposium tour.

He (she) will be accompanied by persons.

Date: Signature:

(Please return to:— The Secretary,
The Society for Analytical Chemistry,
9–10 Savile Row,
London W1X 1AF,
England.

before September 15th, 1969)

Weight distribution coefficients (mmole g^{-1} of dry resin $[H^+]$ per mmole ml^{-1} of the solution) were calculated from—

$$\lambda = \frac{A_o - A_s}{A_s} \cdot \frac{v}{m}$$

where A_s and A_o are the count-rates of aliquots taken from the flasks with and without the resin, respectively; v is the volume of solution, ml; and m is the weight of the dry resin, g.

Column experiments were carried out with glass-jacketed columns of different dimensions, with glass-wool plugs at the bottom.[16] Water from a Höppler ultrathermostat was circulated through the jacket of the column to maintain constant temperature. The effluent was collected in drops on a moving paper-band impregnated with Plexi-glass solution in toluene. The drops were then automatically dried under infrared lamps, cut out from the band and the count-rate was measured with a Geiger - Müller or scintillation counter.

Detailed descriptions of the apparatus and procedure have been given in previous papers.[16,17]

In some experiments in which the quantitation of separation was examined, the predetermined volumes of effluent were collected in flat-bottomed test-tubes and the count-rate was measured with a 2 × 2-inch NaI(Tl) well-type scintillation counter.

Results

Distribution coefficients—

Distribution coefficients of gold, as determined by the batch method, are shown in Fig. 1. The weight distribution coefficients, λ_{Au}, for Dowex 50W × 8 are large at higher hydrobromic acid concentrations. At hydrobromic acid concentrations of less than 5 N, distribution coefficients decrease uniformly with decrease in acid concentration. The apparent rise of λ_{Au} at a hydrobromic acid concentration of less than, or equal to, 0·5 N has not been confirmed by column experiments. The peak elution volumes of gold for the 0·1, 0·5 and 1·0 N hydrobromic acid decreased uniformly with decrease in acid concentration. All of the curves were asymmetric and showed considerable tailing. Distribution coefficients for Amberlite IRC-50 are substantially lower than those for Dowex 50W × 8.

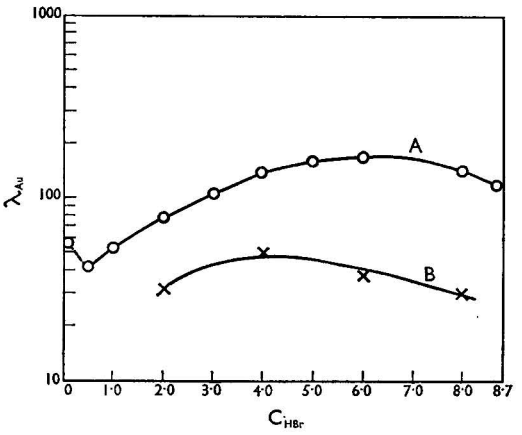

Fig. 1. Weight distribution coefficient of gold in the system: A, Dowex 50W × 8 $[H^+]$ - hydrobromic acid; and B, Amberlite IRC-50 $[H^+]$ - hydrobromic acid

Effect of temperature—

The effect of temperature on the elution of trace amounts of gold from the Dowex 50W × 8 column with 6 N hydrobromic acid is shown in Fig. 2. Weight distribution coefficients, λ_{Au}, shown in Fig. 2, were calculated from the equation[18]—

$$\lambda_{Au} = \frac{U_{max.} - (U_o + V)}{m} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where $U_{max.}$ is the peak elution volume; U_0 is the dead volume of the column, ml; V is the free volume of the resin bed; and m is the weight of the dry ion exchanger in the column, g. The plate heights, H, were obtained from the equation[18]—

$$H = \frac{L \cdot W^2}{8\,(U_{max.} - U_0)^2} \qquad (2)$$

where L is the column length; and W is the peak width for $M = 0{\cdot}368\ M_{max.}$ ordinate.

These values are also shown in Fig. 2. The rise in temperature has a favourable effect on elution kinetics, but the distribution coefficient of gold decreases markedly. Subsequent investigations on the separation of gold and platinum metals were, therefore, carried out at 15° C.

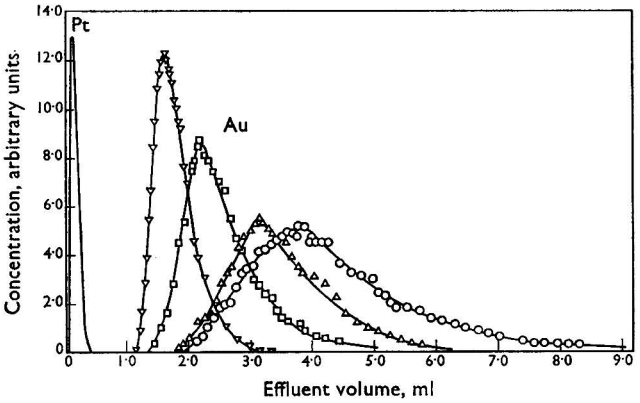

Fig. 2. Effect of temperature on elution of trace amounts of gold.

Resin: Dowex 50W × 8 [H+] (13 $\mu \leqslant \emptyset \leqslant 39\ \mu$). Bed dimensions: 2·50 cm × 0·0311 cm². Eluent: 6 N hydrobromic acid *plus* 0·0035 M bromine. Flow-rate: 0·56 to 0·60 ml cm^{-2} minute^{-1}

$\lambda_{Au(15)} = 137$	$H_{Au(15)} = 0{\cdot}209$ cm	○—○—○	15° C
$\lambda_{Au(30)} = 112$	$H_{Au(30)} = 0{\cdot}141$ cm	△—△—△	30° C
$\lambda_{Au(50)} = 76$	$H_{Au(50)} = 0{\cdot}115$ cm	□—□—□	50° C
$\lambda_{Au(75)} = 55$	$H_{Au(75)} = 0{\cdot}077$ cm	▽—▽—▽	75° C

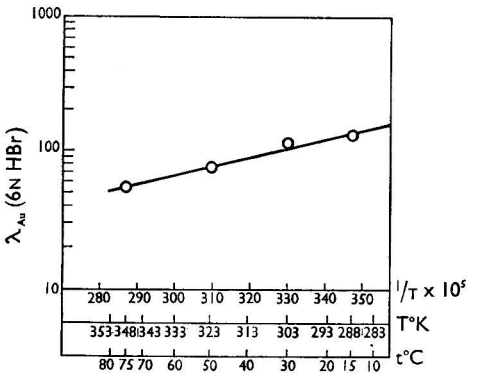

Fig. 3. Distribution coefficients of gold in the system: Dowex 50W × 8 [H+] - 6 N hydrobromic acid *plus* 0·0035 M bromine as a function of temperature. Points - experimental values; solid lines—calculated by the least squares method

The mechanism of absorption of gold by cation-exchange resin is not exactly known; it was impossible, therefore, to obtain an unambiguous equation for this reaction and to calculate the equilibrium constant. However, the distribution coefficient at constant hydrobromic acid concentration can be assumed to be constant, dependent only on temperature.

The enthalpy change for the distribution of gold between the Dowex 50W × 8 [H^+] and 6 N hydrobromic acid solution can then be calculated from the equation—

$$\Delta H = -2{\cdot}303\ \mathrm{R}\frac{d\log\lambda_{Au}}{d\left(\frac{1}{T}\right)} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

which, together with corresponding free-energy change—

$$\Delta G = -RT\ln\lambda_{Au} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

gave the respective entropy change—

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad .. \quad .. \quad .. \quad .. \quad (5).$$

Assuming that $\frac{d\Delta H}{dT} = \Delta C_p$ = a constant, distribution coefficients for the temperature range of 10° to 80° C were calculated from experimental results by the method of least squares.[16,19] The plot of λ_{Au} *versus* 1/T is shown in Fig. 3. The values of thermodynamic functions, in 10° intervals, are presented in Table I.

TABLE I

THERMODYNAMIC FUNCTIONS (APPARENT) FOR THE DISTRIBUTION OF GOLD BETWEEN DOWEX 50W × 8 [H^+] AND 6 N HYDROBROMIC ACID *plus* 0·0035 M BROMINE SOLUTION

Temperature, °K	283	293	303	313	323	333	343	353
ΔG, kcal.	−2·828	−2·824	−2·818	−2·806	−2·799	−2·785	−2·772	−2·756
ΔH, kcal.	−2·882	−2·951	−3·019	−3·087	−3·156	−3·224	−3·292	−3·361
ΔS, cal.°K^{-1}	−0·19	−0·43	−0·66	−0·90	−1·10	−1·32	−1·52	−1·71

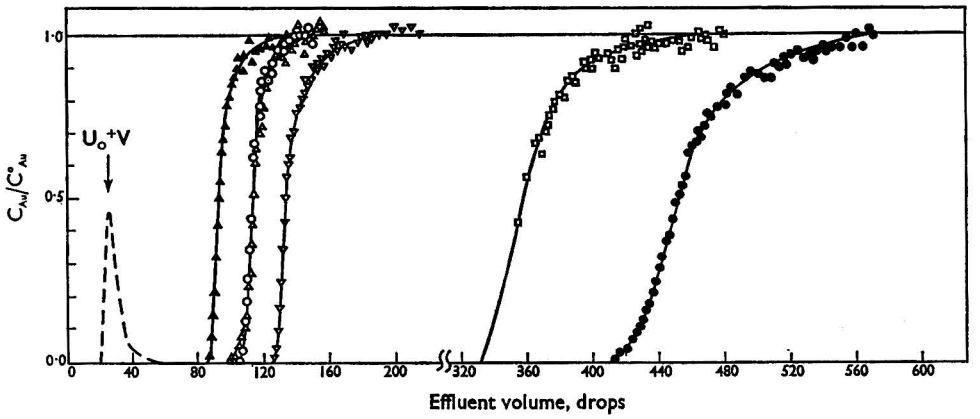

Fig. 4. Break-through curves for gold solutions in 6 N hydrobromic acid *plus* 0·0035 M bromine at 15° C, in dependence on gold concentration in the feed solution.
Resin: Dowex 50W × 8 [H^+] (13 $\mu \leqslant \emptyset \leqslant 39\ \mu$). Bed dimensions: 7·5 cm × 0·141 cm². Temperature 15° C. Flow-rate: 0·27 ml cm^{-2} minute^{-1}, if not indicated otherwise

●—●—● 0·125 mg of gold ml^{-1}
□—□—□ 0·25 mg of gold ml^{-1}
▽—▽—▽ 2·5 mg of gold ml^{-1}
○—○—○ 5·0 mg of gold ml^{-1}
△—△—△ 5·0 mg of gold ml^{-1} (flow-rate 0·135 ml cm^{-2} minute^{-1})
▲—▲—▲ 10·0 mg of gold ml^{-1}

EFFECT OF GOLD CONCENTRATION—

To determine the amount of gold that can be absorbed by cation-exchange resin, in the given conditions, a series of break-through experiments was carried out with various gold concentrations in the feed solution. The results are shown in Fig. 4. The sorption capacities, Z_{Au}, of Dowex 50W × 8 for gold are calculated from the equation—

$$Z_{Au} = \frac{[U - (U_0 + V)] \cdot C^\circ_{Au}}{m} \quad .. \quad .. \quad .. \quad (6)$$

where U is the volume of effluent at 50 per cent. break-through point ($C_{Au}/C^\circ_{Au} = 0{\cdot}5$); and C°_{Au} is the concentration of gold in the feed solution, and are given in Table II. As seen from Fig. 4, the break-through graphs are asymmetrical, indicating that the kinetics of distribution between the two phases becomes increasingly unfavourable as the loading increases. This effect is more pronounced at low concentrations of gold in the feed solution. It is characteristic that Z_{Au} is not constant and increases markedly with the increase in gold concentration in the solution.

TABLE II

SORPTION CAPACITY OF DOWEX 50W × 8 FOR GOLD IN CONTACT WITH 6 N HYDROBROMIC ACID *plus* 0·0035 M BROMINE SOLUTION WITH VARYING GOLD CONCENTRATIONS

Concentration of gold in solution, mg ml^{-1}	Z_{Au}	
	mg of gold g^{-1} of dry resin [H$^+$]	mmole of gold g^{-1} of dry resin [H$^+$]
0·125	5·88	0·0299
0·25	9·17	0·0466
2·5	30·6	0·155
5·0	44·9	0·228
10·0	70·5	0·358

The variation of the distribution coefficient of gold with loading is shown in Fig. 5. The distribution coefficient is constant up to a loading of about 0·05 mg of gold per gram of dry resin [H$^+$], and then slowly decreases with further increase in loading.

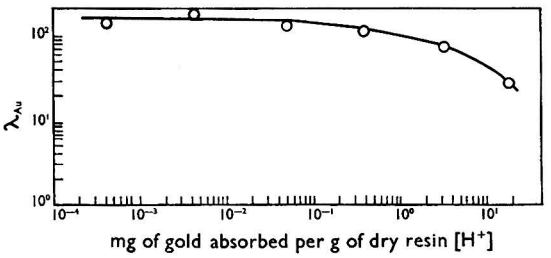

Fig. 5. Distribution coefficients of gold in the system: Dowex 50W × 8 [H$^+$] - 6 N hydrobromic acid *plus* 0·0035 M bromine as a function of resin loading

ANALYTICAL SEPARATIONS—

Micro amounts—As follows from the results presented above (for example see Fig. 2) trace amounts of gold are easily separated from platinum, which appears in the effluent in the solvent front. Elution of gold absorbed by the resin, with various concentrations of hydrobromic acid, is difficult and requires large volumes of eluting solution. The elution graphs show considerable tailing, especially pronounced at low hydrobromic acid concentrations. An attempt was made to use organic solvents for the elution of gold from the column. Acetylacetone, which is a powerful extractant for gold,[20] was the most suitable. The separation of trace amounts of gold from platinum, palladium, iridium and rhodium is shown in

Fig. 6. This method has previously been used for the isolation of carrier-free gold-199 from a neutron-irradiated platinum target,[12] and the completion of separation was confirmed by γ-ray spectrometry. As seen from Fig. 6, other platinum metals behave in a similar manner to platinum and can be quantitatively separated from gold with this system.

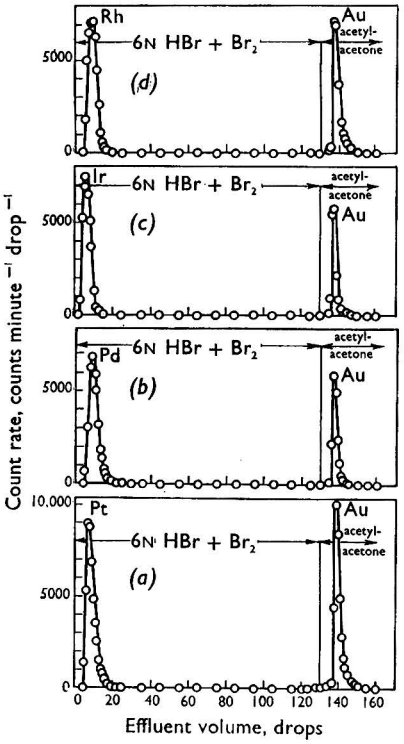

Fig. 6. Separation of submicrogram amounts of gold from small amounts (less than 50 μg) of platinum, palladium, iridium and rhodium.

Resin: Dowex 50W × 8 [H$^+$] (13 $\mu \leqslant \varnothing \leqslant 39\ \mu$). Bed dimensions: 2·50 cm × 0·0311 cm^2. Eluents: 6 N hydrobromic acid *plus* 0·0035 M bromine, and acetylacetone. Flow-rate: 0·29 to 0·37 ml cm^{-2} minute^{-1}. Temperature: 15° C

(*a*) Pt - Au
(*b*) Pd - Au
(*c*) Ir - Au
(*d*) Rh - Au

The amount of the component not absorbed by resin (platinum) has little effect on the separation, as shown in Fig. 7.

Macro amounts—From Table II it can be seen that the absorption capacity of Dowex 50W × 8 for gold is considerable, and macro amounts can be separated, provided appropriately large columns are used.

The resolution of mixtures containing milligram amounts of platinum and gold is presented in Fig. 8.

STATISTICAL EVALUATION OF THE METHOD—

To check the accuracy and precision of the method a series of 50-μl aliquots of platinum and gold tracers, containing about 350 μg of platinum and about 0·1 μg of gold, respectively, was mixed and separated on a 2·50 cm × 0·0311 cm^2 Dowex 50W × 8 column, as described above. The count-rates of platinum and gold fractions were compared with the count-rates of identical aliquots taken, with the same pipettes, from the same tracer solutions. The results are summarised in Table III.

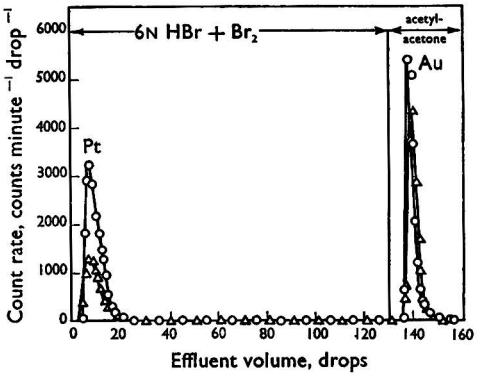

Fig. 7. Separation of milligram amounts of platinum from carrier-free gold-199.

Resin: Dowex 50W × 8 [H⁺] (13 $\mu \leqslant \varnothing \leqslant$ 39 μ). Bed dimensions: 2·50 cm × 0·0311 cm². Eluents: 6 N hydrobromic acid *plus* 0·0035 M bromine, and acetylacetone. Flow-rate: 0·67 ml cm⁻² minute⁻¹. Temperature: 15° C

○—○—○ 1 mg of platinum - carrier-free gold-199

△—△—△ 10 mg of platinum - carrier-free gold-199

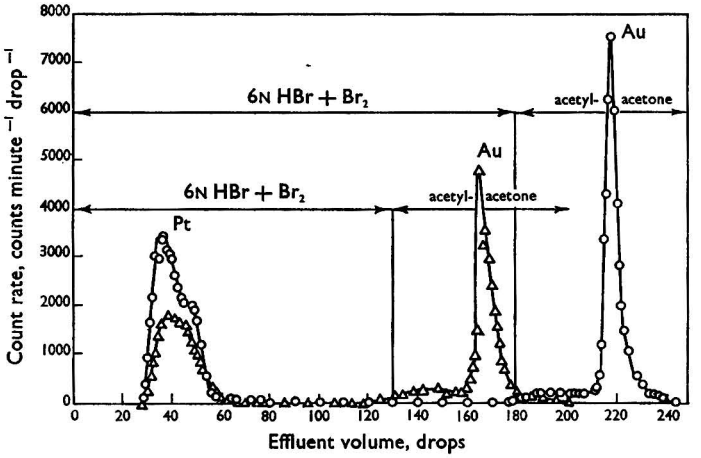

Fig. 8. Separation of milligram amounts of platinum and gold
Resin: Dowex 50W × 8 [H⁺]. Bed dimensions: 7·50 cm + 0·141 cm². Eluents: 6 N hydrobromic acid *plus* 0·0035 M bromine, and acetylacetone. Flow-rate: 0·14 ml cm⁻² minute⁻¹. Temperature: 15° C

○—○—○ 5 mg of platinum - 5 mg of gold
△—△—△ 10 mg of platinum - 10 mg of gold

Assuming that the possible errors in measuring the solution volume are negligible in comparison with the dispersion in count-rate caused by the statistical nature of radioactive decay (this assumption may not hold when pipetting small volumes of solution), the true value of a count number in a pre-determined time interval for a given aliquot (with 99 per cent. probability) is given by the expression, $N \pm 2{\cdot}58\sqrt{N}$, where N is the measured number of counts.

The amount "taken" is $N \pm 2{\cdot}58\sqrt{N}$, and the true amount "found" can be determined from the series of experimental results (see Table III) from the equation—

$$\mu = \bar{x} \pm t_\alpha \frac{s}{\sqrt{n}} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (7)$$

where μ is the true measured amount; $\bar{x}$ is the arithmetic mean of the sample; s is the standard deviation; t_α is the Student distribution parameter; and n is the number of experimental results.

As can be seen from Table III, in each instance, the confidence interval, $\bar{x} \pm t_\alpha \frac{s}{\sqrt{n}}$ for the amount "found" (at a confidence level of 0·99) comprises entirely $N \pm 2{\cdot}58\sqrt{N}$, *i.e.*, the amount "taken." The method, therefore, does not involve any systematic error. The precision expressed in terms of relative standard deviation, s_r, is also good.

TABLE III

ACCURACY AND PRECISION OF THE METHOD

	Gold, counts	Platinum, counts
Amount taken	23,800 ± 397	75,840 ± 710
Amount found	23,570	75,030
	23,835	75,805
	23,910	75,950
	23,945	75,995
	24,860	76,030
	24,875	77,730
Arithmetic mean	24,165	76,090
Standard deviation	559	886
Relative standard deviation ..	0·023	0·012
True amount found	24,165 ± 919	76,090 ± 1460

DISCUSSION

The absorption of metals, which are known to form negative halide complexes, by cation-exchange resins has not been satisfactorily explained. Kraus, Michelson and Nelson[8] suggested that a reaction between an anionic complex, such as $AuCl_4^-$, and the organic network may account for this phenomenon. Titze and Samuelson,[21] who studied the sorption of iron(III) on various cation exchangers, similarly concluded that the sorption is caused by the affinity of the aromatic exchanger skeleton for iron(III) chloride complex. Fu-Tszyun, Norseev, Khalkin and Tao-Nan,[10] however, proposed a theory in which neutral sulpho groups of a cation exchanger, when in contact with concentrated acid or salt solution, acquire positive charge by addition of proton or metal ions.

None of these explanations seems convincing. One of the theories given[10] assumes, tacitly, the presence of undissociated sulphonic acid groups (which is probably not true), and even then fails to account for selective sorption of only some special complex anions. If electrostatic attraction between sulpho groups that had acquired positive charge and anions from the solution was a decisive factor, all anions (*e.g.*, platinum halide complexes) could also have been absorbed by the resin; this is not observed in practice. The entrance by co-ions into the resin phase is opposed by Donnan potential and requires simultaneous transfer of an equivalent amount of counter ions to preserve electrical neutrality. The concentration of electrolyte in the resin phase does not usually exceed that in external solution. In the case of complex anions, the parent acids of which are not completely dissociated, it is more reasonable to assume that partition of undissociated complex acid between the organic (resin) phase and aqueous solution is responsible for the high affinity of the cation exchangers towards some metals in concentrated hydrochloric acid and hydrobromic acid solutions. The dissociation constant $K_{diss.}$ of bromoauric acid, to our knowledge, has not been reported in the literature. Some values for similar compounds, *i.e.*, chloroauric acid are, however, available. Forsberg, Widell and Erwall[22] gave the value of $K_{diss.}$ as 1·35, while Kraus and Nelson[5] estimated it to be about 0·1. It is clear that chloroauric acid is not a completely dissociated acid, and this is probably also true for bromoauric acid.

Assuming that bromoauric acid is the only significant gold-containing species in the resin phase, and the respective species in the aqueous phase are bromoauric acid and the ion $AuBr_4^-$ we can write—

$$\bar{m}_{Au(total)} = \bar{m}_{HAuBr_4} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and

$$m_{Au(total)} = m_{HAuBr_4} + m_{AuBr_4^-} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

where m is the concentration, and barred symbols indicate resin phase.

Introducing the dissociation constant of bromoauric acid—

$$K_{diss.} = \frac{a_{H^+} \cdot a_{AuBr_4^-}}{a_{HAuBr_4}} = \frac{m_{H^+} \cdot m_{AuBr_4^-}}{m_{HAuBr_4}} \cdot \frac{\gamma_{H^+} \cdot \gamma_{AuBr_4^-}}{\gamma_{HAuBr_4}} \quad .. \quad .. \quad (10)$$

where γ is the activity coefficient, we obtain the following expression for the distribution coefficient of gold—

$$\lambda_{gold} = \frac{\bar{m}_{HAuBr_4}}{m_{Au(total)}} = \frac{\lambda_{HAuBr_4}}{\left(1 + K_{diss.} \frac{\gamma_{HAuBr_4}}{\frac{\gamma_{H^+} \cdot \gamma_{AuBr_4^-}}{m_{H^+}}}\right)} \quad .. \quad .. \quad .. \quad (11)$$

where λ_{HAuBr_4} is the distribution coefficient of bromoauric acid. From equation (11) the two extreme cases arising are—

(*i*)

$$K_{diss.} \frac{\gamma_{HAuBr_4}}{\gamma_{H^+} \cdot \gamma_{AuBr_4^-}} \gg m_{H^+}$$

i.e.,

$$\lambda_{gold} \simeq \frac{\lambda_{HAuBr_4} \cdot m_{H^+}}{K_{diss.} \frac{\gamma_{HAuBr_4}}{\gamma_{H^+} \cdot \gamma_{AuBr_4^-}}} \quad .. \quad .. \quad .. \quad .. \quad (12)$$

and (*ii*)

$$K_{diss.} \frac{\gamma_{HAuBr_4}}{\gamma_{H^+} \cdot \gamma_{AuBr_4^-}} \ll m_{H^+}$$

i.e.,

$$\lambda_{Au} \simeq \lambda_{HAuBr_4} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (13).$$

Even with these simplifications this theory agrees for the shape of log $\lambda_{Au} - m_{HBr}$ plot (see Fig. 1), *i.e.*, the initial linear rise of λ_{Au} with the increase in acid concentration [see equation (12)], and subsequent reaching plateau, corresponding to the situation given by equation (13). The decrease of λ_{Au} with the further increase in acid concentration (at $m_{HBr} > 7$ N) is probably caused by a decrease of λ_{HAuBr} as a result of electrolyte invasion of the resin. The electrolyte invasion, which can be high at high aqueous-phase concentrations,[23] makes the properties of both phases more alike.

The fact that the gold retained by the resin is difficult to elute with dilute acid solution but is rapidly stripped off with acetylacetone seems to confirm the mechanism for gold absorption by cation exchangers, which was outlined above.

The relatively high affinity of Dowex 50W × 8 for bromoauric acid might be caused by the formation of a charge-transfer complex between the aromatic rings of the resin network (donor) and bromoauric acid (acceptor).

The apparent enthalpy change for the distribution of gold between Dowex 50W × 8 [H^+] and 6 N hydrobromic acid solution is about 3 kcal. mole^{-1} (see Table I), *i.e.*, about the upper limit of analogous values for ordinary ion-exchange reactions (reference 16 and references contained therein). The entropy contribution to the free-energy change is small in comparison with the results for ion-exchange reactions.[13,16] The energy of formation of charge-transfer complexes[24] varies from decimal fractions of kilocalories to a few kilocalories mole^{-1} and so for gold ΔH lies well within this range.

The method for the separation of gold from platinum metals described in this paper is simple, rapid and quantitative and can be used for trace as well as milligram amounts. Separations should preferably be carried out at low temperatures (15° C), as the distribution coefficient, λ_{Au}, decreases with increase in temperature (see Fig. 3). The absorption capacity

of Dowex 50W × 8 for gold increases with the increase in gold concentration in the solution. Therefore, to separate given amounts of metals better results can be achieved with concentrated solutions, which are usually more convenient to use. As can be seen from the shape of the break-through graphs the absorption of gold is fairly rapid, and the chromatographic process is only slightly influenced by the changes in flow-rate (the two break-through graphs for $m_{Au} = 5$ mg ml^{-1} as shown in Fig. 4).

Statistical analysis of results has proved that the method does not involve any systematic error with reference to both platinum and gold. This was confirmed by counting the resin from the column after separation. No activity was found. This is of considerable importance, as the reduction of gold is usually the main obstacle in analysis. Evidence for reduction of gold and its irreversible adsorption by anion[3,6,7] and cation exchangers[25] is found in the literature. The use of hydrobromic acid *plus* bromine medium and acetylacetone elution ensures 100 per cent. recovery of gold equally well when it is present in carrier-free or milligram amounts. The work on separation of platinum metals and gold on various ion exchangers is in progress.

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Low-temperature Fluorescence of Some Bromide-ion Association Complexes in Hydrobromic Acid Glasses at −196° C

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An examination has been made for the fluorescence of simple bromo-ion association complexes in hydrobromic acid glasses at −196° C. Strong fluorescence was observed for Sb(III), Sb(V), As(III), As(V), Bi, Ce(III), Pb, Tl(I), Sn(II) and U(VI), and the effects of variables such as hydrobromic acid molarity, time of irradiation, relationship between fluorescence intensity and metal-ion concentration were studied. With a standard commercial spectrofluorimeter with a slightly modified sample cell, these ions could be detected in hydrobromic acid at concentrations varying from 10^{-5} to 10^{-8} M. Copper(I) and tellurium(IV) also exhibit weak fluorescence.

SEVERAL workers, *e.g.*, Randall[1] and Ohnesorge and Rodgers,[2] have described the occurrence of low-temperature fluorescence in solids and metal chelate compounds. The advantage of increased intensity of fluorescence at low temperatures for the chlorocomplexes of lead (−70° C) and thallium (−196° C) in frozen hydrochloric acid (glasses) has been used by others.[3,4,5] Generally, in these studies, a frontal illumination technique was used so that the fluorescence radiation from the surface of the sample was viewed along an axis that made an acute angle with the axis of the excitation radiation. We have examined several inorganic acids that form clear glasses at the temperature at which liquid nitrogen boils under atmospheric pressure (−196° C), to assess their suitability as media for low-temperature fluorescence, and have used the conventional right-angle excitation - fluorescence system in a simple sample cell with a commercial spectrofluorimeter. Both hydrochloric and hydrobromic acids form suitable glasses and give ion-association complexes with several metals that fluoresce strongly at this temperature. In a previous paper[6] we have discussed the behaviour of the hydrochloric acid system. This paper presents the results obtained with hydrobromic acid and fifty-eight elements. Strong fluorescence signals were obtained for antimony(III) and (V), arsenic(III) and (V), bismuth, cerium(III), lead, thallium(I), tin(II) and uranium(VI).

EXPERIMENTAL

APPARATUS—

An Aminco - Bowman spectrofluorimeter (American Instrument Co.) with low temperature attachment was used, as described elsewhere.[6] Sample tubes were made from precision-bore transparent silica tubing (Jensons Ltd., Hemel Hempstead) of length 20 cm, i.d. 3 mm and wall thickness 1 mm. A sample volume of 0·5 ml is sufficient to fill these tubes to a suitable depth for use in the instrument. Excitation and emission spectra were recorded on an XY recorder (Bryans Ltd., Mitcham, Surrey); 3-mm slits, corresponding to about 30-nm band pass were used in both monochromators.

REAGENTS—

Hydrobromic acid—Re-distilled, 48 per cent. general-purpose grade was used. The acid, when not re-distilled, exhibited a faint blue fluorescence at −196° C whereas the distilled acid showed virtually none.

Metal salts—Aqueous stock solutions, 10^{-2} M, of analytical-reagent grade potassium antimonyl tartrate, arsenic(III) oxide, bismuth nitrate, copper sulphate, lead nitrate, sodium arsenate, tin(II) chloride, thallium(I) sulphate and uranyl nitrate. Tellurium metal (Johnson, Matthey, Specpure), laboratory-reagent grade cerium(III) nitrate and antimony pentoxide were used as starting materials for the other ions that showed fluorescence.

The ions that exhibited no fluorescence were prepared from the purest materials available.

Results and discussion

A general survey of fifty-eight elements was made by preparing 10^{-3} M solutions of the elements in 6 M hydrobromic acid. A 0·5-ml aliquot of each was placed in a silica tube, which was cooled by immersing it in liquid nitrogen in the micro Dewar flask of the spectrofluorimeter. The glass thus produced was examined visually in the screening tests by placing it under a mercury-vapour discharge lamp and observing any fluorescence. The Dewar flask was then transferred to the spectrofluorimeter and the fluorescence signal was examined instrumentally by scanning each monochromator in turn, while maintaining the other at a suitable fixed position on the wavelength scale.

Under these conditions no fluorescence signal was observed for forty-six of the elements examined, *viz.*, Al, Ba, Be, Cd, Ca, Ce(IV), Cr(III), Co, Cu(II), Dy, Er, Eu, Gd, Ga, Au, Ho, In, La, Lu, Mg, Mn(II), Hg(II), Mo(VI), Nd, Ni, Nb, Pd(II), Pr, Ru, Sm, Sc, Se(IV), Ag, Sr, Ta, Tb, Th, Tu, Sn(IV), Ti(III) and (IV), V(V), Yb, Yt, Zn and Zr.

Fluorescence emissions were observed for twelve ions, *viz.*, Sb(III) and (V), As(III) and (V), Bi, Ce(III), Cu(I), Pb, Te(IV), Tl(I), Sn(II) and U(VI). Table I shows the visually observed fluorescence colours for these ions and the instrumentally recorded maxima for excitation and fluorescence. These maxima are uncorrected for instrumental parameters such as diffraction-grating efficiency at varying wavelengths, detector response and source-emission intensity.

Table I

Ions found to fluoresce in 6 M hydrobromic acid at −196° C

Ion	Colour of fluorescence	Excitation maximum, nm	Emission maximum, nm
Sb(III)	Red	360	586
Sb(V)	Red	360	586
As(III)	Faint red	356	584
As(V)	Faint red	356	566
Bi(III)	Blue	378	450
Ce(III)	—	250	350
Cu(I)	Faint blue	286	434
Pb	Blue	304	414
Te(IV)	Faint red	352	560
Tl(I)	—	270	410
Sn(II)	Orange	314	550
U(VI)	Green	327	494 strong 516 strong 540 weak 565 weak

The wavelengths of maximal excitation and emission for tellurium(IV) in hydrobromic acid are independent of the concentration of the acid. This is in marked contrast to the behaviour in hydrochloric acid, when the dependence is quite pronounced. The intensity of tellurium(IV) fluorescence is much weaker in hydrobromic acid than in hydrochloric acid. The fluorescence of copper(I) in hydrobromic acid is also very weak. For this reason the study of the analytical utility of low-temperature fluorescence of bromo-ion association complexes in hydrobromic acid was confined to the remaining ten ions.

Spectral characteristics—

Fig. 1 shows the excitation and emission spectra of the ten ions in 6 M hydrobromic acid at −196° C. These spectra are uncorrected for variations in detector sensitivity, lamp emission or grating transmission against wavelength. The relevant correction curves appear elsewhere.[7]

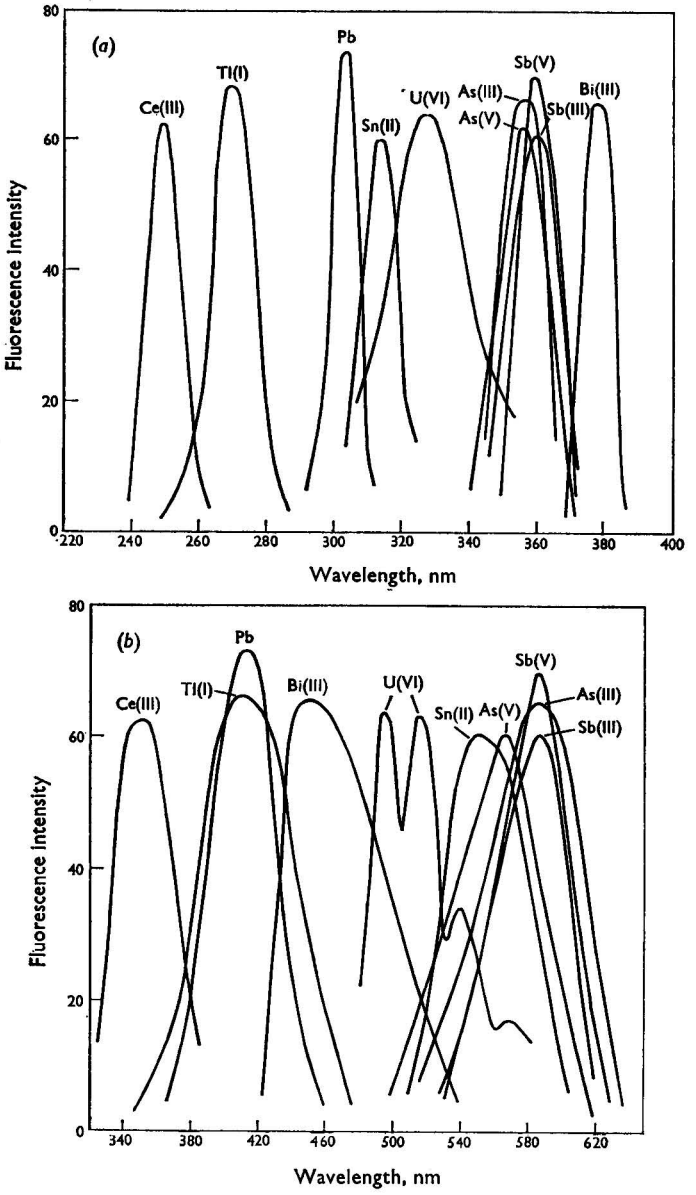

Fig. 1. Spectra: (*a*) excitation; and (*b*) emission in 6 M hydrobromic acid: Sb(III) concentration 4×10^{-5} M, sensitivity scale $\times 0{\cdot}03$; Sb(V) concentration 10^{-4} M, sensitivity scale $\times 0{\cdot}03$; As(III) concentration 2×10^{-3} M, sensitivity scale $\times 0{\cdot}01$; As(V) concentration 2×10^{-3} M, sensitivity scale $\times 0{\cdot}01$; Bi(III) concentration 4×10^{-4} M, sensitivity scale $\times 0{\cdot}1$; Ce(III) concentration 10^{-3} M, sensitivity scale $\times 0{\cdot}1$; Pb concentration 10^{-4} M, sensitivity scale $\times 1{\cdot}0$; Tl(I) concentration 10^{-3} M, sensitivity scale $\times 0{\cdot}3$; Sn(II) concentration 2×10^{-3} M, sensitivity scale $\times 0{\cdot}03$; U(VI) concentration 10^{-3} M, sensitivity scale $\times 0{\cdot}1$

HYDROBROMIC ACID CONCENTRATION—

Variation of hydrobromic acid concentration from 6 and 9 M did not affect the wavelengths of maximal excitation or fluorescence for antimony(III) or (V), arsenic(III) or (V), bismuth(III), cerium(III), lead, thallium(I) and tin(II). With uranium(VI) the maximal wavelength of excitation increases steadily from 327 nm in 6 M hydrobromic acid to 345 nm in 9 M hydrobromic acid. The wavelength of maximal fluorescence does not alter with acidity in this region, but the intensities of fluorescence are affected. Fig. 2 shows the effect of hydrobromic acid concentration on the intensity of fluorescence of the various ions under optimised conditions.

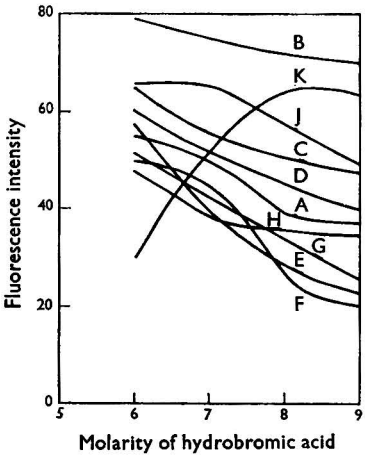

Fig. 2. Variation of fluorescence intensity with hydrobromic acid concentration: A, 4×10^{-5} M Sn(III); B, 10^{-4} M Sb(V); C, 4×10^{-3} M As(III); D, 2×10^{-3} M As(V); E, 4×10^{-4} M Bi(III); F, 10^{-3} M Ce(III); G, 10^{-4} M Pb; H, 10^{-3} M Tl(I); J, 2×10^{-3} M Sn(II); K, 10^{-3} M U(VI)

EFFECT OF TIME ON FLUORESCENCE EMISSION—

The effect of time on fluorescence intensity was studied under optimised conditions of excitation, acidity and so on, for the various ions by allowing the solution (*a*) to stand in darkness for 2 hours; (*b*) to stand under normal laboratory conditions (fluorescent strip-lighting) for 2 hours; and (*c*) to be irradiated continuously in the spectrofluorimeter cell for 1 hour.

TABLE II

EFFECT OF TIME ON FLUORESCENCE-EMISSION INTENSITIES

Solution, in 6 M HBr	Reduction in fluorescence intensity, per cent. Standing in darkness for 2 hours	Under laboratory lighting for 2 hours	Continuous irradiation for 1 hour
10^{-6} M Sb(III)	n.d.	n.d.	4
10^{-6} M Sb(V)	4	5	n.d.
10^{-4} M As(III)	n.d.	n.d.	n.d.
10^{-4} M As(V)	3	4	n.d.
10^{-6} M Bi(III)	4	12	4
3×10^{-6} M Ce(III)	2	10	7
10^{-6} M Pb	n.d.	n.d.	2
10^{-5} M Tl(I)	33	90	25
10^{-5} M Sn(II)	n.d.	n.d.	n.d.
3×10^{-6} M U(VI) (in 8 M HBr)	n.d.	n.d.	2

n.d. = Not detectable.

These experiments (see Table II) revealed that the only detectable difference occurred when thallium(I) was allowed to stand under normal laboratory fluorescent lighting for 2 hours. In this instance the fluorescence was almost completely destroyed, probably because of the oxidation of thallium(I) to thallium(III).[8]

ANALYTICAL CALIBRATION GRAPHS—

Table III shows the range of linearity of analytical graphs for each of the ten ions under the appropriately optimised conditions. No attempt was made to establish the upper limit of linearity. The limit of detection (defined as the concentration in μg ml^{-1}) required to produce a signal-to-noise ratio of unity is given in column 3. Because only 0·5-ml samples were used to produce the frozen glass, the absolute detection limits are as follows: Sb(III), 6×10^{-4} μg; Sb(V), 6×10^{-3} μg; As(III), $3{\cdot}7 \times 10^{-1}$ μg; As(V), $7{\cdot}5 \times 10^{-1}$ μg; Bi, 6×10^{-3} μg; Ce(III), $5{\cdot}6 \times 10^{-2}$ μg; Pb, 2×10^{-3} μg; Tl(I), 2×10^{-1} μg; Sn, $1{\cdot}2 \times 10^{-1}$ μg; and U(VI), 7×10^{-2} μg.

Even with re-distilled hydrobromic acid, a slight background fluorescence is obtained at the highest instrumental sensitivities. The thick-walled silica tubing itself exhibits a slight blank fluorescence at these settings.

TABLE III

CALIBRATION GRAPHS AND DETECTION LIMITS

Ion, in 6 M HBr	Concentration range of calibration graph	Limit of detection, μg ml^{-1}
Sb(III)	10^{-8} - 10^{-7} M	0·0012
Sb(V)*	10^{-7} - 10^{-6} M	0·012
As(III)	10^{-5} - 10^{-4} M	0·75
As(V)	2×10^{-5} - 10^{-4} M	1·50
Bi(III)	6×10^{-8} - 3×10^{-7} M	0·012
Ce(III)	8×10^{-7} - 10^{-5} M	0·112
Pb	2×10^{-8} - 4×10^{-7} M	0·004
Tl(I)	2×10^{-6} - 10^{-5} M	0·40
Sn(II)	2×10^{-6} - 10^{-5} M	0·24
U(VI) (in 8 M HBr) ..	6×10^{-7} - 4×10^{-6} M	0·14

* This detection limit is only approximate as the salt used in the investigation is of approximate composition.

CONCLUSIONS

With the exception of tin(IV), all of the elements that exhibit fluorescence as chloro-complexes in hydrochloric acid glasses at —196° C also exhibit fluorescence in hydrobromic acid glasses. Tin(II), which exhibits no fluorescence in hydrochloric acid glass, exhibits strong fluorescence in hydrobromic acid. Tin can, therefore, be determined in hydrobromic acid down to 0·12 μg as tin(II), whereas in hydrochloric acid the limit is 6 μg as tin(IV). The detection limits obtained for antimony, both as antimony(V) and antimony(III), are 10 and 100 times lower, respectively, in hydrobromic than in hydrochloric acid, when only antimony(III) fluoresces appreciably (detection limit 6×10^{-2} μg). Arsenic(III) and (V), which exhibit no fluorescence in hydrochloric acid glass, fluoresce strongly in hydrobromic acid at —196° C.

A comparison of the detection limits in hydrochloric and hydrobromic acid glasses shows that the former medium is more sensitive for bismuth, cerium, lead, thallium(I) and tellurium. Finally, it can be mentioned that because of the good separation between fluorescence and emission maxima it is possible to determine several of these elements simultaneously by suitable choice of excitation and emission wavelengths.

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Thermogravimetry and Differential Thermal Analysis Studies on Potassium Titanyl Oxalate and Potassium Aluminium Oxalate and Related Oxalates*

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Thermogravimetry and differential thermal analysis studies are reported for potassium titanyl oxalate, potassium aluminium oxalate and the related oxalates, potassium, titanyl and aluminium oxalates. The thermal decomposition of these materials in dynamic atmospheres of air, nitrogen and carbon dioxide has been studied. The oxalates and their decomposition products have been characterised by chemical and X-ray analysis. The thermal decomposition results for the complex oxalates show distinct reaction stages, but the aluminium and titanyl oxalates are not clearly characterised under the conditions used in the present work.

The dehydration of the potassium titanyl oxalate starts at 70° C and proceeds in several stages until completion at 200° C. The anhydrous oxalate is not stable although only 1 per cent. of the original weight is lost between 200° and 275° C, but the decomposition is rapid above 275° C. The titanate formed as the final product is the result of the initial decomposition of the anhydrous complex. Potassium aluminium oxalate gives a stable anhydrous complex over the range 150° to 375° C, followed by a two-stage decomposition, with the bulk of the reaction complete at 575° C, and the end product is potassium aluminate, formed as a result of the initial decomposition.

THE thermogravimetric (T.G.) decomposition of simple oxalates has been reported by Dollimore, Griffiths and Nicholson[1] and the differential thermal analysis (D.T.A.) by Ugai.[2] Many other papers, in which the thermal decomposition of a single oxalate is reported in detail, state that some oxalates decompose to the carbonate, while others decompose directly to either the metal or the oxide. The oxalates that decompose to the metal show a marked dependence on the atmosphere in which the decomposition is carried out, the metal usually being oxidised in air at the temperature of decomposition. Wendlandt, George and Krishnamurty[3] studied oxalate complexes of chromium, iron, cobalt and ruthenium, while in a recent publication details are given of the thermal decomposition of the oxalate complexes of iron.[4] A brief exploratory study on potassium titanyl oxalate was carried out by Dollimore and Nicholson.[5]

The two complex oxalates studied in this investigation are examples of two types: potassium titanyl oxalate, representing the class $K_2M(C_2O_4)_2$ (where M is a metal or, in this instance, the TiO group); and potassium aluminium oxalate, representing the class $K_3M(C_2O_4)_3$ (M in this instance is aluminium). In the present study of the thermal decomposition of complex oxalates, greater attention is paid to the atmospheric environment, and the residues investigated were prepared on the T.G. apparatus. Additional information on decomposition has been found to add to the previous report on the thermal behaviour of the basic aluminium oxalate by using X-ray diffraction data.[6]

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Method

Thermogravimetry—

Most T.G. results presented here were obtained on a modified Stanton thermobalance type TR1, but others were obtained by using an automatically recording vacuum balance.[7] In all experiments a 0·5-g sample contained in a platinum crucible, 3 cm deep and 2·5 cm diameter at the top, was used. The results were corrected for "buoyancy" by using this crucible with 0·5 g of α-alumina that had been previously heated to 1250° C. The linear rise in temperature was 4° C minute^{-1}, although in some instances other rates were used. The flow of gas was at a rate of 50 ml minute^{-1} downwards through a silica sheath inside the furnace. The gases used were dry air, dry nitrogen and dry carbon dioxide. A chromel - alumel or a platinum - platinum *plus* rhodium thermocouple was positioned immediately above the sample and used to measure the sample temperature. The nitrogen was described as "oxygen free." It was passed through a tube containing copper turnings at 450° C to remove the last traces of oxygen.

Differential thermal analysis—

The D.T.A. of all the materials in this work was carried out on a Netzsch differential thermal analyser, modified to give results on a potentiometric recorder. The sample consisted of 0·8 g of a 10 per cent. w/w mixture of the oxysalt in alumina that had previously been heated to 1250° C. The reference material was 0·8 g of the same alumina. Platinum sheath holders were used and the platinum - platinum *plus* rhodium thermocouples used in both holders were actually in direct contact with these powder mixtures. Both the sample and reference material, when placed in their respective platinum holders, were gently tamped down. A heating rate of 5° C minute^{-1} was used. The atmosphere was dynamic with a flow-rate and purification train the same as for the T.G. D.T.A. runs were made in dry air, oxygen-free dry nitrogen and dry carbon dioxide.

X-ray powder diffraction—

The residues for X-ray or chemical analysis were prepared on the thermobalance. When nitrogen was used the complex oxalate was heated at 4° C minute^{-1} until it reached the required temperature, then maintained at this temperature for half an hour. The furnace was then allowed to cool to room temperature with the balance mechanism remaining in operation to detect if oxidation of the sample was occurring. When the sample had cooled to room temperature it was stored under nitrogen in a sealed container until required. The X-ray analysis of all the residues was carried out with either a Newton-Victor generator and a 9-cm powder camera, or a Philips generator and a 11·48-cm camera with a copper target. The constituent compounds of a particular residue were identified by direct comparison with a photograph of a standard material and by comparing with results from the A.S.T.M. powder results file.

Chemical analysis—

Chemical analysis of the original complexes and residues was mainly confined to the determination of the oxalate content. This, in conjunction with the T.G. results, was considered sufficient in assessing the purity of the original complexes. In certain instances it was considered necessary to determine also the carbonate content and the alkali content of the residues.

Preparation and characterisation of the oxalates

Characterisation of potassium titanyl oxalate dihydrate, $K_2TiO(C_2O_4)_2.2H_2O$—

Commercially available analytical-reagent grade material was used. It was in the form of a white crystalline powder which, on chemical analysis for the oxalate content, gave a figure of 49·5 per cent. (calculated figure 49·7 per cent.). The d values calculated from the X-ray diffraction results are compared with those in the A.S.T.M. powder results file in Table I.

Preparation and characterisation of titanyl oxalate—

Titanium oxalate, $Ti(C_2O_4)_2$, does not exist, and all attempts to make it result in the formation of either a basic oxalate or a peroxo-oxalate.[8,9,10,11,12]

TABLE I

X-RAY DIFFRACTION RESULTS FOR POTASSIUM TITANYL OXALATE

$K_2TiO(C_2O_4)_2 2H_2O$

Material used in this study		A.S.T.M. results		Anhydrous complex used in this study	
d,Å	I/I_0	d,Å	I/I_0	d,Å	I/I_0
10·02	30	10·2	10	10·11	30
9·48	30	9·99	10	9·56	30
—	—	9·35	25	—	—
—	—	9·21	45	—	—
7·80	90	—	—	7·70	30
7·35	20	7·45	4	—	—
7·14	10	—	—	7·08	100
6·27	10	6·63	6	6·15	50
5·71	10	5·36	4	5·63	10
5·16	10	5·00	6	5·24	30
4·96	100	4·86	100	—	—
4·73	30	4·46	2	—	—
4·66	30	—	—	—	—
4·06	20	—	—	4·13	20
3·900	20	3·64	20	3·951	70
3·576	25	3·60	8	3·809	20
3·491	25	3·51	10	3·686	20
3·215	10	3·47	25	3·562	60
3·119	20	3·40	2	3·473	15
3·010	100	3·34	8	3·376	20
—	—	3·24	6	3·255	15
—	—	3·13	6	3·164	90
—	—	3·12	6	3·063	80
—	—	3·02	8	3·028	5
2·945	10	2·90	14	2·894	40
2·884	90	2·779	8	2·722	5
2·730	10	2·749	8	2·655	5
2·667	5	2·680	14	2·592	5
2·560	20	2·612	8	2·445	5
2·485	20	2·606	6	2·359	10
2·420	5	2·506	8	2·281	5
2·386	5	2·112	12	2·254	10
2·386	5	1·998	6	2·220	10

The exact formula of the material prepared depends on the method used. In the present method potassium metatitanate was first prepared, then titanic acid and finally the oxalate. Potassium hydroxide pellets (12 g) were weighed into a nickel crucible and 3 ml of water added. The mixture was fused by gently heating, and 6 g of titanium dioxide were added in portions while stirring. The crucible was placed in a furnace and heated to about 700° C for 30 minutes, then cooled in a desiccator. The titanate formed was a greyish brown crystalline mass, and 6·7 g of this material were slowly added to 50 ml of concentrated hydrochloric acid in a small beaker and heated to 100° C until all the titanate had dissolved. On cooling the solution a crystalline precipitate of potassium chloride was formed and this was filtered off. The filtrate was diluted with 25 ml of water and placed in a tap funnel, from which it was slowly run into a cold, well stirred mixture of 40 ml of 0·88 ammonia solution with 500 ml of water. The gelatinous precipitate of titanic acid formed was filtered off by suction and washed free from ammonia. The freshly prepared gel was added to 100 ml of a hot 10 per cent. solution of oxalic acid in which it dissolved completely. This solution was evaporated to about 25 ml and 100 ml of absolute ethanol added; a fine white precipitate of the oxalate was immediately formed, which was filtered off, washed with more ethanol and dried in a vacuum desiccator. The oxalate content was found by chemical analysis to be 47·9 per cent., the total weight lost on heating to 900° C was 55·0 per cent., and the water content was 28·7 per cent., corresponding to between $1\frac{1}{2}$ and 2 molecules of water of crystallisation ($TiO.C_2O_4.1{\cdot}75H_2O$).

PREPARATION AND CHARACTERISATION OF POTASSIUM ALUMINIUM OXALATE, $K_3Al(C_2O_4)_3.3H_2O$—

The complex was prepared by dissolving commercially available aluminium oxalate in a hot concentrated solution of potassium oxalate. The solution was filtered while hot to remove

any undissolved material and the filtrate left to crystallise overnight. Potassium aluminium oxalate crystallised from solution as a fine white powder, which was then filtered off and washed with cold water. The T.G. analysis of the air-dried complex indicated a water content of 11·2 per cent. (theoretical for 3 molecules of water of crystallisation, 11·69 per cent.). Chemical analysis for oxalate content was 57·15 per cent. (theoretical 57·11 per cent.). X-ray diffraction results for the hydrated and anhydrous complexes are given in Table IV.

PREPARATION AND CHARACTERISATION OF ALUMINIUM OXALATE—

This was available commercially, and is described as "basic" by the manufacturers. The samples used from various batches showed a constant weight loss of 64·2 per cent. when heated at 1250° C. The preparation[6] involved dissolving aluminium hydroxide gel in oxalic acid solution followed by recrystallisation from the resulting solution. The end product at 1250° C was shown by X-ray diffraction photographs to be α-alumina. The chemical analysis was reported to give 41·9 per cent. oxalate content and this agreed with analysis of the sample used in this investigation. From this information the formula $2Al_2(C_2O_4)_3.5Al(OH)_3.13H_2O$ was assigned to the original material. Dollimore, Dollimore and Perry[6] found that the X-ray powder diffraction gives a pattern similar to gibbsite but with many extra lines at higher d values. They consider aluminium oxalate as a three-dimensional array of Al^{3+}, $C_2O_4^{2-}$ and OH^- ions in a distorted gibbsite lattice.

RESULTS

POTASSIUM TITANYL OXALATE DIHYDRATE—

The T.G. results in nitrogen and air for potassium titanyl oxalate dihydrate were identical. The T.G. results in air and carbon dioxide at 4° C minute^{-1} are given in Fig. 1. Heating at 2° and 10° C minute^{-1} had no effect on the shape of the T.G. curve in nitrogen or air. A shift occurs towards a higher temperature for some of the reactions when the oxalate is heated in carbon dioxide, but this is to be expected, for in these reactions carbon dioxide and carbon monoxide are the product gases and the presence of carbon dioxide in the environmental atmosphere would tend to retard the completion of these reactions.

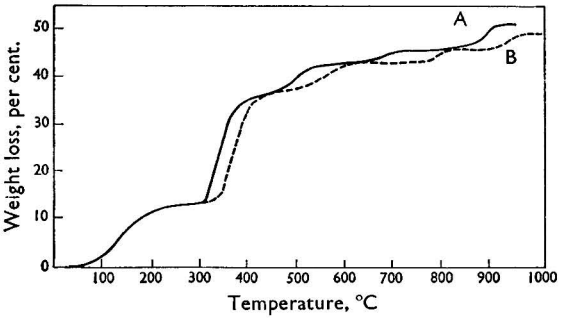

Fig. 1. T.G. analysis graph of potassium titanyl oxalate dihydrate: A, in air and nitrogen; B, in carbon dioxide

At about 70° C the potassium titanyl oxalate dihydrate begins to lose water, but the differential thermogravimetric (D.T.G.) curve indicates that the water is lost in a number of ill-defined stages. Dehydration is complete at 200° C. The anhydrous complex is not completely stable and 1 per cent. of the original weight is lost between 200° and 275° C. The oxalate then breaks down rapidly with a maximum reaction rate at 335° C. An inflexion in the curve just after this temperature suggests a two-stage process. An inclined plateau occurs between 390° and 470° C, with a weight loss from 35·6 to 36·2 per cent. At 475° C a further loss in weight occurs with a maximum rate at 500° C, leaving a residue at 42·5 per cent. weight loss. The material again loses weight at 620° C with a maximum rate at 650° C, leaving another plateau at 45·5 per cent. weight loss. At 825° C there is a final weight loss to 50·5 per cent. and this residue is stable at 1000° C, even for 6 hours.

The D.T.A. for potassium titanyl oxalate dihydrate in air, nitrogen and carbon dioxide is given in Fig. 2, together with corresponding D.T.G. results. The endothermic dehydration

peak is a compound one, thus confirming that the removal of water is not a simple one-stage process. All the other peaks are exothermic when the reaction is carried out in air. The main oxalate decomposition peak occurs with $\triangle T_{max.}$ at 335° C, and a second overlapping peak occurs with $\triangle T_{max.}$ at 360° C. Two other peaks are seen, one with $\triangle T_{max.}$ at 500° C, and the other at 760° C. Under atmospheres of nitrogen and carbon dioxide the main oxalate breakdown gives two similar endothermic peaks with $\triangle T_{min.}$ at 330° and 365° C. A shallow endothermic hump can be seen on the nitrogen curve at 500° C, but this is absent from the carbon dioxide curve.

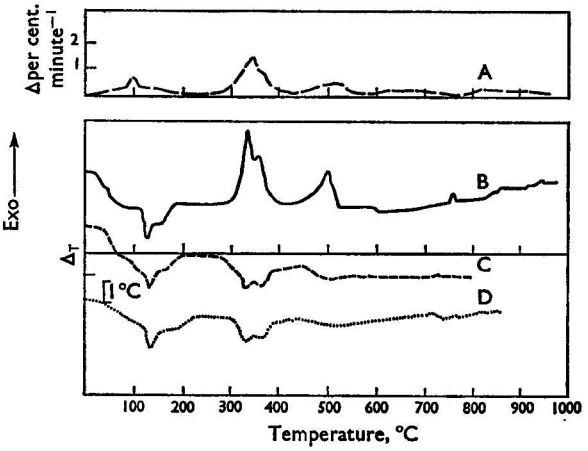

Fig. 2. D.T.A. and D.T.G. graphs for potassium titanyl oxalate dihydrate: A, D.T.G. in air; B, D.T.A. in air; C, D.T.A. in nitrogen; D, D.T.A. in carbon dioxide

Samples were prepared corresponding to the residue formed at various plateaux on the T.G. curve. Five samples were prepared, including one of the anhydrous complex. Table I gives X-ray analysis results for the anhydrous complex. The residues prepared at 450° and 600° C gave identical diffraction patterns. The residue at 1000° C showed lines that could be identified as potassium metatitanate. The residues were all mixtures and potassium carbonate could be identified in all of them. The residue formed at 450° and 600° C contained potassium oxalate. None of these residues contained any free oxides of titanium. The pattern from the sample heated to 1100° C could possibly be associated with that given in the A.S.T.M. index (I–1016) for potassium metatitanate. An attempt was made to identify some of the compounds in the sample prepared at 800° C by washing the residue in distilled water until all the soluble salts had been removed. Positive identification of the lines not belonging to potassium carbonate or oxalate was not possible. Chemical analysis results

TABLE II

POTASSIUM TITANYL OXALATE: CHEMICAL ANALYSIS OF RESIDUES

Temperature	Weight lost, per cent.	Oxalate, per cent.	Potassium carbonate, per cent.	K_2O, per cent.
250	12	54·95	0	0·0
450	35·5	28·34	4·88	0·0
600	41·5	3·4	59·52	0·5
800	45·5	0·0	50·67	13·33

on these residues are reported in Table II. A summary of the solid product of decomposition of anhydrous potassium titanyl oxalate is given in Table III. A comparison of this oxalate and the titanyl oxalate, also described in this paper, reveals that the loss in weight occurring between 450° and 600° C can in part be attributed to a change associated with the titanium ion rather than the potassium ion. This is expected as the T.G. analysis of the titanyl oxalate also shows a loss in weight between these two temperatures.

TABLE III

POTASSIUM TITANYL OXALATE: COMPOUNDS DETECTED IN RESIDUE

Temperature, °C	Compound detected in residue: Chemical analysis	X-ray analysis
450	$K_2C_2O_4$, K_2CO_3	$K_2C_2O_4$, K_2CO_3 and unidentified compounds
600	$K_2C_2O_4$, K_2CO_3, trace of K_2O	$K_2C_2O_4$, K_2CO_3 and unidentified compounds
800	K_2CO_3, K_2O	K_2CO_3 and unidentified compounds
1100	K_2CO_3, trace of K_2O	K_2CO_3 *plus* K_2TiO_5

TITANYL OXALATE HYDRATE—

The T.G. and D.T.A. of this material in air are shown in Fig. 3. It can be seen that a stable residue is formed between 340° and 500° C, and above 500° C this decomposes to another residue stable up to 1000° C. X-ray analysis results showed this to be rutile (TiO_2). An X-ray powder photograph of the residue below 500° C proved to be amorphous to X-rays. The D.T.A. results show peaks associated with each of the decomposition changes, their exothermic nature could be caused by gaseous reactions catalysed by the surface.

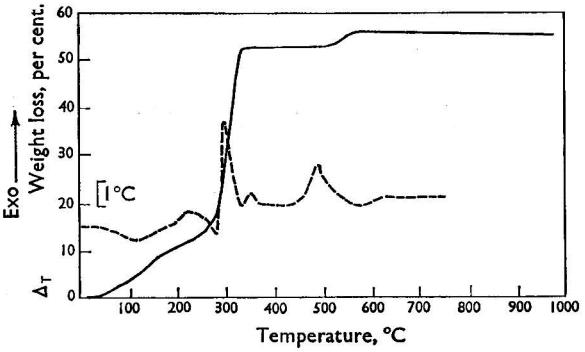

Fig. 3. T.G. and D.T.A. graphs of titanyl oxalate hydrate: T.G. is the continuous line; and D.T.A. the broken line

POTASSIUM ALUMINIUM OXALATE TRIHYDRATE—

The decomposition curves obtained in air and nitrogen are almost identical. The T.G. curve in air is shown in Fig. 4. Water is lost above 40° C, the rate increasing up to 100° C, and a stable anhydrous complex exists over the range 150° to 375° C. At 375° C the complex

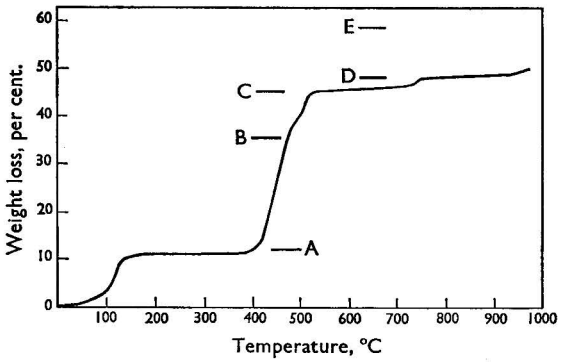

Fig. 4. T.G. graph of potassium aluminium oxalate trihydrate in air: A, anhydrous; B, $3K_2C_2O_4 + Al_2O_3$; C, $K_2CO_3 + Al_2O_3$; D, $2K_2CO_3 + K_2Al_2O_4$; E, $2K_2O + K_2Al_2O_4$

breaks down and a two-stage decomposition takes place, the inflexion being more marked in nitrogen than in air. The bulk of this reaction is complete at 475° C when 45·7 per cent. weight has been lost. This latter figure is high if it is assumed that the residue formed at this stage is a mixture of potassium carbonate and alumina (theoretical 44·2 per cent.). The inflexion in the decomposition curve occurs at about 38 per cent. and this is, again, above the figure of 35·06 per cent. calculated for the formation of an equimolecular mixture of potassium oxalate and alumina. The residue present at 575° C loses weight slowly up to 730° C in air and 775° C in nitrogen, then at these temperatures the residue decomposes more quickly to give a final stable plateau at 48·7 per cent. weight loss in air and 48·2 per cent. in nitrogen. These figures correspond to an equimolecular mixture of potassium carbonate, potassium oxide and alumina, and are also consistent with the formation of potassium aluminate detected in X-ray studies.

TABLE IV

X-RAY DIFFRACTION RESULTS FOR POTASSIUM ALUMINIUM OXALATE TRIHYDRATE AND THE ANHYDROUS COMPLEX

Potassium aluminium oxalate trihydrate		Anhydrous complex prepared by T.G. up to 300° C	
d,Å	I/I_0	d,Å	I/I_0
9·80	70	9·72	3
7·14	90	6·98	3
6·63	10	5·76	30
4·90	40	5·32	20
4·72	5	5·17	5
4·37	10	4·83	20
3·580	100	4·36	5
3·345	10	4·22	30
3·255	30	3·896	25
3·134	40	3·778	40
2·849	5	3·503	100
2·710	5	3·441	100
2·634	70	3·066	50
2·563	70	3·040	50
2·508	5	2·801	30
2·445	20	2·178	15
2·362	10	2·629	60
2·156	90	2·522	5
2·122	5	2·431	40
2·075	10	2·338	30
2·043	5	2·306	10
2·007	10	2·206	10
1·984	5	2·156	30
1·919	10	2·072	5
1·890	5	2·039	50
1·867	10	1·945	30
1·836	10	1·903	30
1·789	10	1·771	30

The D.T.A. results for the potassium aluminium oxalate trihydrate in air and nitrogen are given in Fig. 5, together with corresponding D.T.G. results. These show broad endothermic peaks corresponding to dehydration with $\triangle T_{\min.}$ at 100° C. A shoulder is present on the low temperature side of the peak. In air the next two peaks are exothermic with $\triangle T_{\max.}$ at 400° and 445° C, and can be identified with the oxalate decomposition reaction, as can the two endothermic peaks in nitrogen with $\triangle T_{\min.}$ at 410° and 460° C.

X-ray powder diffraction photographs were taken of residues from the heat treatment of potassium aluminium oxalate trihydrate on the T.G. unit at 200°, 420°, 600°, 800° and 1000° C in air, and 430° and 900° C in nitrogen (Table V). Potassium carbonate is present in all samples above 420° C and in none of the samples could any form of alumina be detected. Potassium aluminate could also be identified in samples above 420° C in air and was abundant in the sample heated to 1000° C. However, in nitrogen, only a trace of aluminate could be detected in the sample prepared at 900° C. The patterns at 420° C in air and 430° C in nitrogen were extremely complex, and potassium oxalate and potassium carbonate could be identified,

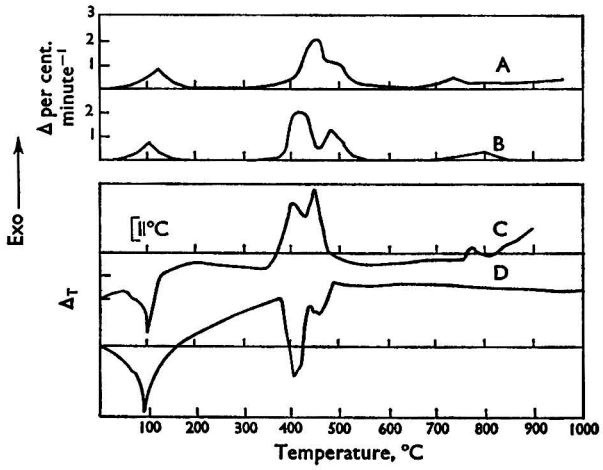

Fig. 5. D.T.A. and D.T.G. graphs for potassium aluminium oxalate trihydrate: A, D.T.G. in air; B, D.T.G. in nitrogen; C, D.T.A. in air; D, D.T.A. in nitrogen

with aluminate present in the sample prepared in air. As no new compounds were identified on heating from 600° to 800° C in air the loss in weight that occurs between these temperatures is probably caused by potassium carbonate and amorphous alumina undergoing a further solid-state reaction with the evolution of carbon dioxide and the production of more potassium aluminate.

TABLE V

POTASSIUM ALUMINIUM OXALATE TRIHYDRATE X-RAY DIFFRACTION ANALYSIS OF RESIDUES AND COMPOUNDS DETECTED IN RESIDUES

Heat treatment, °C	Weight lost, per cent.	X-ray findings
200 (air)	11·2	Hydrated complex Anhydrous complex
420 (air)	37·0	Complicated pattern includes K_2CO_3, $K_2C_2O_4$ and some aluminate ($K_2O.Al_2O_3$)
600 (air)	45·5	K_2CO_3 and trace of aluminate
800 (air)	48·0	K_2CO_3 and trace of aluminate
1000 (air)	52·5	K_2CO_3, strong lines of aluminate
430 (nitrogen)	37·5	Complicated pattern similar to that in air at 420° C, but no aluminate
900 (nitrogen)	48·5	K_2CO_3 and trace of aluminate

ALUMINIUM OXALATE—

The T.G. results in air reported by Broadbent, Dollimore and Dollimore[4] showed that the material lost weight from 50° C upwards, with the main decomposition complete by 425° C, but beyond 590° C conversion into alumina was not complete. In this investigation a slight inflexion was detected at about 250° C. This probably corresponds to a slowing down in the evolution of water from the sample and the start of the breakdown of the oxalate. The T.G. and D.T.A. results for this material are given in Fig. 6. The D.T.A. in nitrogen shows two endothermic peaks with $\triangle T_{min.}$ at 260° and 320° C, while in air two exothermic peaks are formed, the first with $\triangle T_{max.}$ at 285° C, and the second consisting of two overlapping peaks with $\triangle T_{max.}$ at 320° and 340° C. Broadbent, Dollimore and Dollimore[4] showed that when the oxalate is heated to 140° C the X-ray diffraction pattern contains lines that are caused only by gibbsite. At higher temperatures the gibbsite lines become faint and above

300° C there is a fairly intense pattern of boehmite (45 to 61 per cent. weight loss). The end product of heat treatment is α-alumina. This picture of non-stoicheiometry is unique among the metal oxalates, with the possible exceptions of iron(III) and chromium.

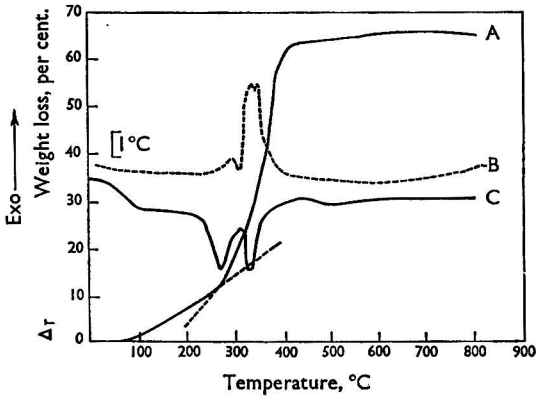

Fig. 6. D.T.G. and D.T.A. graphs for aluminium oxalate: A, T.G. in air and nitrogen; B, D.T.A. in air; and C, D.T.A. in nitrogen

Discussion

Dehydration and configuration of the complexes—

T.G. results are generally insufficiently sensitive to separate stages in dehydration, whereas D.T.A. can often pick out these separate stages as a series of endothermic processes in both nitrogen and air.

The water present in these complexes may be held either bonded to the metal ion or to the oxalate ion, or held as lattice water to satisfy a particularly stable crystal structure. Thermal analysis can provide information on this bonding, for if the co-ordinate link with the metal is operative, and if the thermal energy breaks the metal-to-oxygen bond between the metal and water molecule, then the dehydration process could be accompanied by breakdown of the oxalate ion. In the titanyl oxalates the metal ion is replaced by the TiO unit but otherwise this argument is valid. It should be noted that the complex oxalates contain stoicheiometric amounts of water, whereas the single oxalates contain non-stoicheiometric amounts of water and dehydration is accompanied by decomposition.

The complex potassium titanyl oxalate is octahedral, the titanium ion being surrounded by six oxygen ions: one single, one from a water molecule and four from the chelated oxalate ions. The D.T.A. curve shows that the dehydration occurs in two stages, but the second stage is not clearly defined. However, as there are only two molecules of water of crystallisation it could be that one of these is attached to the titanium by an ion-dipole bond.

The trisoxalato complexes of the tervalent metals (*e.g.*, Al^{3+}, Cr^{3+} and Fe^{3+}) have octahedral configurations and the water of crystallisation cannot be co-ordinated to the central metal ion. Ion-dipole bonding can take place, but it is more probable that the water is held as lattice water. A possible exception may be found in the hydrated sodium iron(III) oxalate but this is the subject of a further study.

Thermal decomposition of the anhydrous oxalato complexes—

The T.G. results indicate that stable anhydrous oxalato complexes of aluminium and titanyl can be formed. Table VI summarises the results obtained from the thermal decomposition of these two complexes. For reference the decomposition temperatures of the corresponding simple oxalates have been included, and all the temperatures have been taken from peak maxima on the D.T.A. curves. It should be remembered that for the simple oxalates dehydration could not be separated from the decomposition. It should also be pointed out that these temperatures do not represent thermodynamic decomposition temperatures but serve quite well for the comparisons of stability. The metal complex in each instance is more

stable than the single salt. This is not true of other complexes (*e.g.*, Co^{2+}, Ni^{2+} and Cu^{2+}). Some potassium carbonate is also found in the initial decomposition product in contrast to many other complexes which decompose as if they were mixtures of the simple metal oxalate and potassium oxalate.

TABLE VI

THERMAL DECOMPOSITION OF COMPLEX OXALATES OF ALUMINIUM AND TITANYL

	Complex	
	$K_3Al(C_2O_4)_3$	$K_2TiO(C_2O_4)_2$
Probable configuration	octahedral	octahedral?
Decomposition temperature, °C		
Complex	410	350
Simple oxalate	330	325
Product of initial decomposition in nitrogen	Al_2O_3 K_2CO_3 $K_2C_2O_4$	Unknown titanate No free titanium oxide K_2CO_3, $K_2C_2O_4$

The D.T.A. peaks in the region of oxalate decomposition are endothermic in nitrogen and exothermic in air. Experiments on a range of simple oxalates have indicated that the decomposition of the oxalate is endothermic, just as the decomposition of carbonate is endothermic. The only exceptions to this are the thermal decompositions of copper, mercury and silver oxalates. The exothermic character of the decomposition in air is then the result of further oxidation reactions. In most transition metal oxalates, and in the oxalates investigated here, the exothermic character arises from the oxidation of carbon monoxide by the air, catalysed by the oxide surface which is the product of the reaction, *i.e.*—

$$CO + \tfrac{1}{2}O_2 \rightleftharpoons CO_2$$

In some other oxalate decompositions free carbon is produced and this could be oxidised, but this reaction does not occur for the titanyl or aluminium oxalates in either the simple oxalate forms or as the potassium complexes. From these results it can be inferred that the exothermic peaks in air seen in the D.T.A. given in Figs. 2, 3, 5 and 6 are regions in which carbon monoxide is produced.

FORMATION OF COMPLEX OXIDES—

In both complex oxalates the thermal decomposition finally resulted in the production of a complex oxide. The details are summarised in Table VII. The complex oxide formation is easier in an atmosphere of air and there is no clear reason for this (see, for example, the aluminium complex oxalate).

TABLE VII

COMPLEX OXIDE FORMATION ON THERMAL DECOMPOSITION OF COMPLEX OXALATES

Complex	Products of initial decomposition in air	Complex oxide formation	Colour of complex	Probable formula	Detection
$K_3Al(C_2O_4)_3$	$K_2Al_2O_4$ K_2CO_3 $K_2C_2O_4$	Initial decomposition	White	$K_2Al_2O_4$	X-ray
$K_3Fe(C_2O_4)_3$	$K_2C_2O_4$ Fe_2O_3	600 to 700° C	Yellow - grey	$K_2Fe_2O_4$	X-ray
$Na_3Fe(C_2O_4)_3$	$Na_2C_2O_4$ Fe_2O_3	600 to 700° C	Yellow - grey	$Na_2Fe_2O_4$	X-ray
$K_2TiO(C_2O_4)$	K_2CO_3 $K_2C_2O_4$ Titanate	Initial decomposition	White	Titanate	X-ray

NOTE—Results for $K_3Fe(C_2O_4)_3$ and $Na_3Fe(C_2O_4)_3$ are taken from reference 4.

There are three ways in which the complex oxide can be formed: at the same time as the initial decomposition reaction; by solid-state reaction between the oxide of the central metal atom and potassium or sodium carbonate; or by liquid - solid reaction between molten

potassium carbonate (or sodium carbonate) and the metal oxide. In this last method it is probable that the potassium carbonate or sodium carbonate contains some K_2O or Na_2O.

For aluminium and titanyl complex oxalates the complex oxide appears as a result of the initial decomposition. This can be contrasted with the iron oxalate complexes (potassium and sodium) in which the second method is operative. There is a clear indication of a subsequent solid-state reaction in the aluminium oxalate complex decomposition in the temperature range 600° to 800° C in air. As no new compounds could be identified it can only be assumed that the loss in weight in this temperature range is caused by potassium carbonate and amorphous alumina undergoing a solid-state reaction with the evolution of carbon dioxide and the production of more potassium aluminate.

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An Investigation of the Performance of the Separated Air - Acetylene Flame in Thermal-emission Spectroscopy

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The advantages of the application of a nitrogen-separated air - acetylene flame in the flame-emission spectroscopy of seventeen elements is described. In this flame the secondary reaction zone is separated by a stream of nitrogen flowing parallel to the flame to prevent access of atmospheric oxygen to its base. The low flame background and noise levels have been shown to result in improvement in the detection limits for fifteen elements. In addition, whereas molybdenum and vanadium atomic emission are not detectable in the interconal zone of the conventional flame, in the separated flame detection limits of 2 p.p.m. and 10 p.p.m. have been obtained in aqueous solution for molybdenum (379·8 nm) and vanadium (318·5 nm), respectively. Separation of much of the molecular-band emission from some elements with the secondary reaction zone may result in partial suppression of spectral interference from these elements on the atomic-line emission of the elements determined. The suppression of the interference of magnesium, which occurs through MgOH emission in the secondary zone, on the determination of iron is demonstrated as an example of this effect.

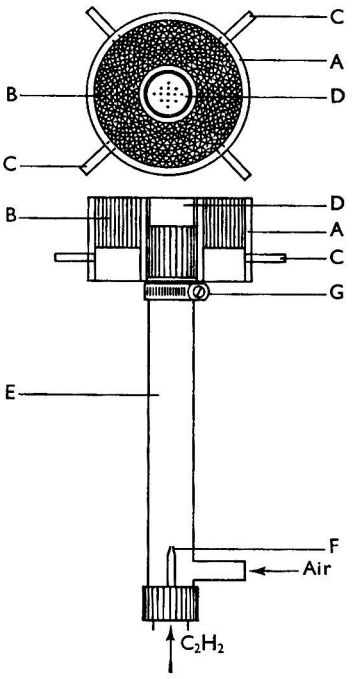
In earlier papers from this laboratory preliminary studies of the properties and use of the separated air - acetylene flame in atomic-fluorescence and thermal-emission spectroscopy have been reported.[1,2]

The secondary diffusion zone of a pre-mixed air - acetylene flame consists of carbon monoxide and hydrogen, produced in the primary zone, burning with the support of atmospheric oxygen. This zone can be separated and supported well away from the primary zone by a silica tube or a column of nitrogen or another suitable gas such as argon. Thus the hottest part of the flame immediately above the primary zone can be viewed spectroscopically free from the strong emission background of the secondary zone. As a result, the separated flame offers superior signal-to-background ratios in thermal-emission spectroscopy when nebulised metal-ion solutions are aspirated into the flame. Low noise levels are also obtained at the selected emission line for the metal under examination. Furthermore, in the interconal zone of the separated flame the carbon monoxide - hydrogen mixture is relatively reducing in nature because of the virtually complete absence of entrained oxygen. In a conventional flame, however, the fuel mixture is more oxidising because of the diffusion of atmospheric oxygen into this region. As a result molecular (metal) oxide and hydroxide-band emission from some elements, which is a frequent cause of spectroscopic interference in the determination of other metals, is considerably less troublesome in the separated flame. The benefits obtained from suppression of the flame background and minimisation of band emission from certain metals underline the potentialities of the separated-flame technique in thermal-emission spectroscopy.

This paper presents a comparison of the emission spectra of seventeen elements obtained in the interconal region of a nitrogen-separated air - acetylene flame with those obtained in a conventional air - acetylene flame. The detection limits are recorded for both flames and the effect of separation on the absolute atomic-line and molecular-band emission intensities is reported. The sensitive detection of atomic-line emission from molybdenum and vanadium, which could not be obtained in the conventional flame, is reported for the nitrogen-separated air - acetylene flame.

Experimental

A Unicam SP900A spectrophotometer was used in conjunction with a Servoscribe RE511 recorder to detect and measure radiation from the flame. The standard E.M.I. 9529B photomultiplier of the SP900A was replaced by an E.M.I. 9601B photomultiplier, as the latter is more sensitive in the ultraviolet region of the spectrum.



A = Brass shielding jacket
B = Coil of alternate layers of corrugated and flat cupro-nickel strip
C = Nitrogen inlet pipes
D = Stainless-steel burner head
E = Stainless-steel burner stem
F = Acetylene jet
G = Adjustable clip to retain shielding jacket at desired height

Fig. 1. Burner and shielding assembly

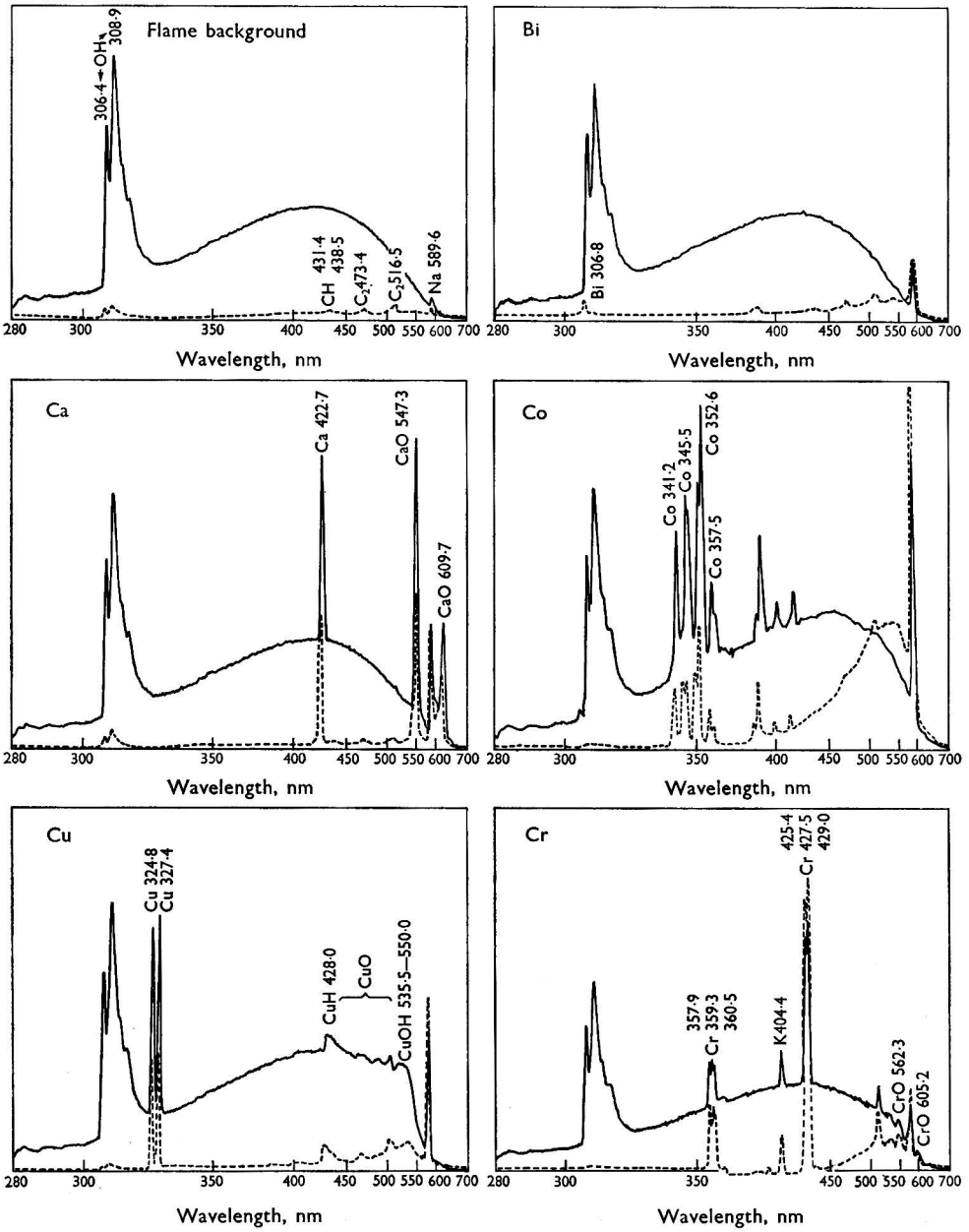
The burner and sheathing arrangement is shown in Fig. 1. A conventional cylindrical Unicam SP900 air - acetylene emission burner was clamped 7·5 cm from the monochromator entrance slit, so that the top of the stainless-steel burner was 3 cm below the centre of the monochromator slit. Separation of the flame was achieved by fitting a brass shielding jacket. The shielding jacket, A, contained a tightly wound spiral of alternate corrugated and flat cupro-nickel strip (0·1 mm thick, 2 cm wide), B. Nitrogen gas (oxygen-free grade) entered the jacket below the coil through four symmetrically placed inlet pipes, C, via a manifold from a 2 to 20 l minute^{-1} rotameter connected to a nitrogen cylinder. A nitrogen flow-rate of about 17·5 l minute^{-1} gave a stable separated flame with this burner assembly.

Stray radiation from the primary cones of the flame and from the "lifted off" secondary diffusion zone was prevented from entering the monochromator entrance slit by an asbestos screen with a horizontal slot 1·75 cm wide placed between the flame and the burner, level with the outside of the shielding jacket.

Samples of the metals investigated as their salts in aqueous solution were introduced into the flame via the Unicam indirect nebuliser, operating on air.

Results

The height of the top of the brass shielding jacket was varied from 1 cm above to 3 cm below the top of the burner. The flame separated well when the top of the shielding jacket was positioned between 1 cm above and 1·5 cm below the top of the burner head. The separation became slightly less efficient when the shielding jacket was moved further down the burner. With the jacket 1 cm above the top of the burner, radiation from the primary combustion zone of the flame was unable to fall on the entrance slit of the monochromator. In most instances the shielding jacket was positioned so that the top of the cupro-nickel coil lay in the same plane as the top of the burner, but for the molybdenum determinations



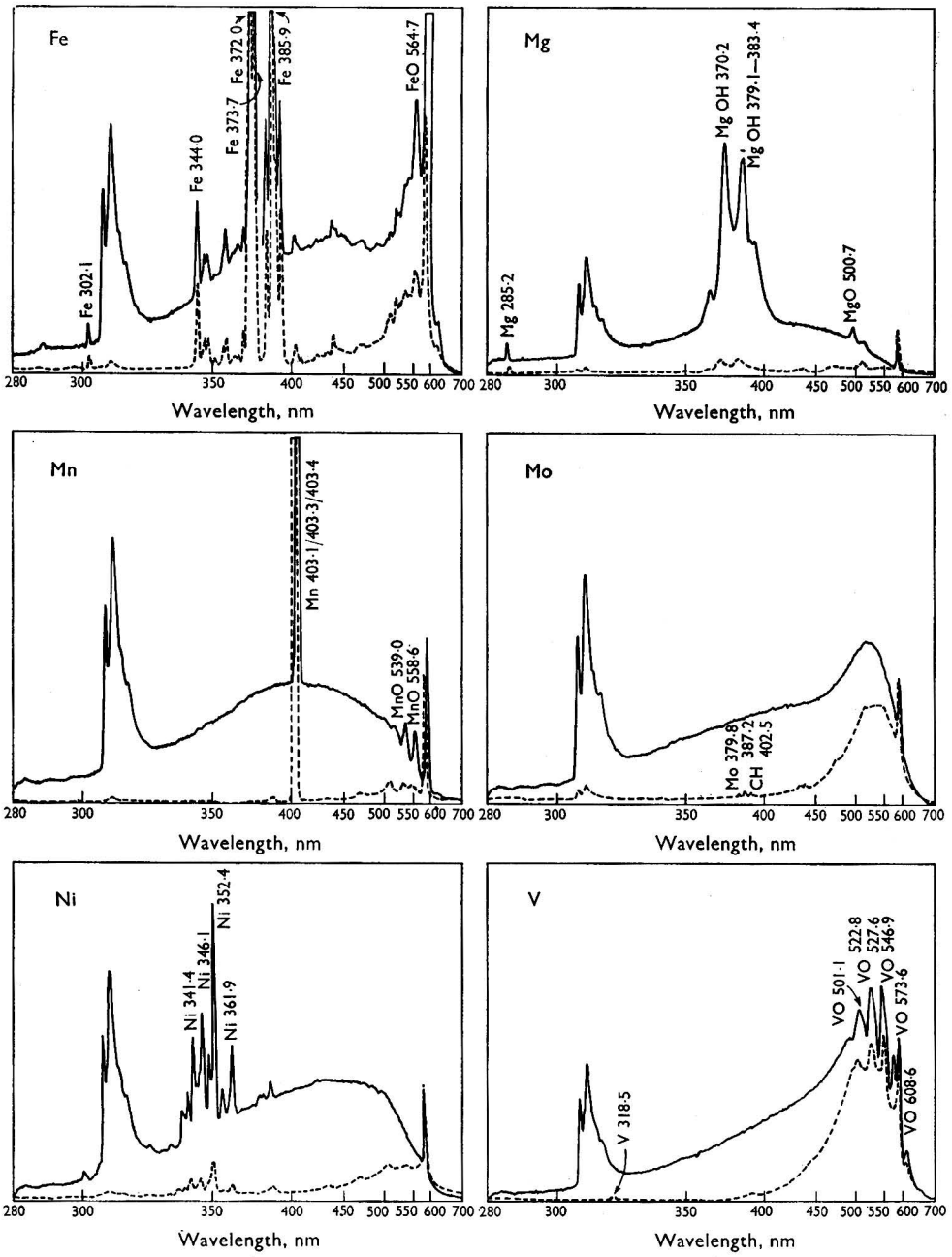


Fig. 2. Flame background and emission spectra for elements investigated in conventional (————) and separated (- - - - - -) air - acetylene flames. Air 4·4 l minute^{-1}; acetylene 1·7 l minute^{-1}; analysing slit width 0·04 mm (except for chromium, 0·035 mm; magnesium, 0·03 mm; and vanadium, 0·032 mm). The ordinate of the curve for the conventional flame should be multiplied by 5 to compare absolute emission intensities

it was raised about 1 cm to take advantage of the extra screening of the radiation from the primary cones. This is particularly advantageous in this instance, as the molybdenum line at 379·8 nm is rather close to the intense CH band head in the primary cones at 387·1 nm.

The emission spectra of the elements, Bi, Ca, Co, Cr, Cu, Fe, Mg, Mo, Mn, Ni and V, were recorded in both the normal and separated air - acetylene flames, and are shown in Fig. 2. To achieve maximum resolution, narrow monochromator slit widths were used (0·025 to 0·04 mm) and, consequently, relatively strong metal-ion solutions were used to facilitate observation of as many as possible of the analytically important atomic and molecular-band emissions for each element: 1000 p.p.m. solutions were used whenever possible.

Stable fuel-rich flames could not be supported in the separated mode with the conventional air - acetylene burner head because of the phenomenon of lift-off arising from the low burning velocity of such mixtures. For such flames an air - propane burner head was substituted to obtain a stable fuel-rich, separated air - acetylene flame.

As described elsewhere,[2] nitrogen shielding of the air - acetylene flame produces a dramatic suppression of the background emission of burning carbon monoxide and hydrogen in the interconal zone immediately above the primary cones. The results obtained here indicate that band emissions from oxide and hydroxide species from the elements investigated are also greatly reduced in the separated flame. The absolute atomic emission from the various elements is also reduced, however. This arises because the interconal region of the separated flame is necessarily cooler than the same region of the conventional flame. The relative magnitude of these reductions is shown in Table I. It is evident from these results that, except for barium and calcium, considerably greater reduction occurs in band emission than resonance-line emission for these elements.

TABLE I

EFFECT OF NITROGEN SEPARATION ON THE ABSOLUTE ATOMIC (LINE) EMISSIONS AND MOLECULAR (BAND) EMISSIONS IN THE AIR - ACETYLENE FLAME

Atomic emission			Molecular emission		
Element	Wavelength, nm	$\frac{I \text{ conventional}}{I \text{ separated}}$ (ratio)	Species	Wavelength, nm	$\frac{I \text{ conventional}}{I \text{ separated}}$ (ratio)
Barium	553·6	3·3	BaOH	488·8	3·2
Calcium ..	422·7	7·9	CaO(H)	554·7	4·5
Chromium ..	425·4	2·7	CrO	562·3	10
Copper	324·7	4·1	CuOH	ca. 560	12·5
Iron	372·0	4·0	FeO	564·7	11
Magnesium ..	285·2	20	MgOH	370·2	100
Manganese ..	403·3	3·4	MnO	558·6	18
Sodium	589·6	4·3	—	—	—
Vanadium ..	318·5	—	—	546·9	20

As a result, with the separated flame, a considerable improvement in signal-to-background ratio can be obtained when an element is determined at a strong resonance line (*e.g.*, iron at 372·0 nm), which is normally overlapped by the oxide or hydroxide-band emission of another element (*e.g.*, MgOH at 370 nm) and which normally causes serious spectral interference. Thus, with the separated flame, the detection limits and sensitivity of determination of many elements are improved by better signal-to-background ratios and signal-to-noise ratios. The selectivity of determination can also be improved by the secondary effect of partial suppression of spectral interference from molecular-band emission of other elements also present in the sample solution.

DETERMINATION OF MOLYBDENUM AND VANADIUM—

No atomic-line emissions from molybdenum at 379·8 and 319·4 nm, or from vanadium at 318·5 nm, were observed above the primary cones in the conventional air - acetylene flame, even with strong (1000 p.p.m.) test solutions and when the flame was made fuel-rich, but all of these lines were easily detected in the interconal region of the nitrogen-separated flame.

EFFECT OF ACETYLENE FLOW ON MOLYBDENUM AND VANADIUM EMISSION—

The variation of the intensity of the molybdenum and vanadium emissions in the separated flame with acetylene flow-rates at 379·8 and 318·5 nm, respectively, were studied by spraying 200 p.p.m. of molybdenum and 500 p.p.m. of vanadium solutions into the flame with a constant nebulising air pressure of 28 p.s.i. The results, shown in Fig. 3 (*a*), indicate

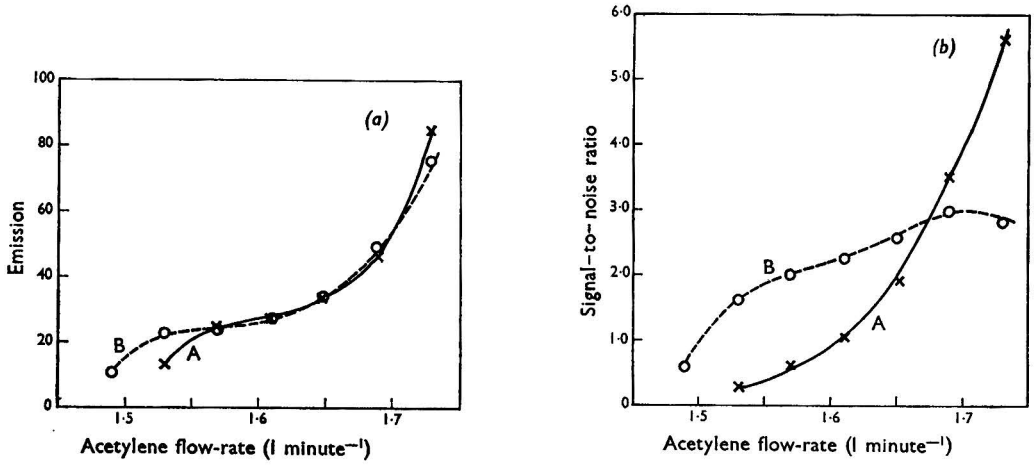


Fig. 3. Variation of (*a*) emission intensity and (*b*) signal-to-noise ratio for (B) molybdenum (at 379·8 nm) and (A) vanadium (at 318·5 nm) with acetylene flow-rate. Air 4·4 l minute^{-1}; analysing slit width 0·2 mm

clearly that the emission for both elements increases as the flame is made more fuel-rich, *i.e.*, more reducing. As shown in Fig. 3 (*b*), the signal-to-noise ratio for both elements became more favourable as the flame was made more fuel-rich. Consequently, the richest flame that could be used without "lift-off" was used for all further measurements with these two elements.

ANALYTICAL MEASUREMENT OF MOLYBDENUM AND VANADIUM—

Stock solutions of 1000 p.p.m. of molybdenum, as ammonium molybdate, in distilled water and 1000 p.p.m. of vanadium, as ammonium metavanadate, in about 0·5 M nitric acid were prepared, and all further solutions were obtained by appropriate dilution of these with distilled water.

Linear calibration graphs were obtained for molybdenum at 379·8 nm over the range 10 to 100 p.p.m. (slit width 0·3 mm) and vanadium at 318·5 nm over the range 50 to 500 p.p.m. (slit width 0·3 mm). The limits of detection for molybdenum (signal-to-noise ratio 1) were found to be 2 p.p.m. at 379·8 nm and 20 p.p.m. at 319·4 nm. A detection limit of 10 p.p.m. was obtained for vanadium at 318·5 nm. When aqueous - ethanolic mixtures were aspirated the most fuel-rich separated flame that could be supported on the air - acetylene burner was less reducing than that obtained when aqueous solutions were used. Consequently, the detection limits for molybdenum were not improved. Stable, fuel-rich separated flames could be supported on the air - propane burner when aspirating aqueous - ethanolic solutions, and the following detection limits were obtained when aspirating 50 per cent. aqueous - ethanolic solutions: molybdenum (379·8 nm), 1 p.p.m.; and vanadium (318·5 nm), 5 p.p.m.

To the best of our knowledge atomic line emissions from these elements have not previously been reported above the primary reaction zone of a pre-mixed air - acetylene flame, although their atomic-line emissions have been reported in much hotter non-pre-mixed flames,[3,4,5] or under conditions of high background and noise in the primary reaction zone of the air - acetylene or air - hydrogen flame.[5,6]

DETECTION LIMITS FOR OTHER ELEMENTS—

The limits of detection of seventeen elements, Ba, Bi, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Pt, Sn, Sr, Tl and V, in the conventional and nitrogen-separated air - acetylene flames were measured in a manner similar to the molybdenum and vanadium determinations. The optimal acetylene flow-rates were determined in both flames for each element, and the detection limits given in Table II are those obtained at these acetylene flow-rates. Table II shows clearly that use of the separated flame leads to an improvement in the detection limit of about one order of magnitude in most instances in spite of the reduction in absolute emission of the atomic lines.

TABLE II

DETECTION LIMITS IN THE CONVENTIONAL AND NITROGEN-SEPARATED AIR - ACETYLENE FLAMES

Element	Wavelength, nm	Detection limits, p.p.m.	
		Conventional flame	Separated flame
Barium	553·6	1×10^{-1}	5×10^{-2}
Bismuth	306·8	20	2
Calcium	422·7	5×10^{-2}	2×10^{-3}
Chromium	425·4	8×10^{-2}	7×10^{-3}
Cobalt	352·6	3×10^{-1}	4×10^{-2}
Copper	327·4	1×10^{-1}	4×10^{-2}
Iron	372·0	5×10^{-1}	3×10^{-2}
Iron	344·0	4	5×10^{-1}
Lead	405·8	10	5×10^{-1}
Magnesium	285·2	1×10^{-1}	3×10^{-1}
Manganese	403·3	1×10^{-1}	1×10^{-2}
Molybdenum	379·8	not detected	2
Molybdenum	319·4	not detected	20
Nickel	352·4	3×10^{-1}	5×10^{-2}
Platinum	306·4	150	2
Strontium	460·7	2×10^{-2}	2×10^{-3}
Tin	284·0	20*	1*
Thallium	477·6	2×10^{-1}	4×10^{-2}
Vanadium	318·5	not detected	10

* With air - propane burner to support fuel-rich flames to produce tin atoms.

DRAUGHT SHIELD—

The nitrogen-separated air - acetylene flame should normally be used within the flame chimney or housing of commercial flame spectrophotometers. If, however, the flame is operated directly in front of the monochromator slit, without a protective housing, random draughts may cause some partial "unseparation" of the flame. Although no severe problems arise in this way when the nitrogen-separated flame is used within the flame housing of the SP900A spectrophotometer, a simple device may be used to overcome difficulties if this is not done. This consists of placing a large (12 cm long, 6 cm diameter) glass tube round the flame. The tube can be arranged to stand on four small feet on the outer rim of the brass shielding jacket, and an optically flat 1-cm diameter fused silica window attached to an aperture in the tube, with epoxy-resin cement, allows radiation from the interconal zone to reach the monochromator entrance slit.

Light reflections of the primary combustion cones from the inside of the glass tube can be prevented from entering the monochromator by the application of a matt black coating to the inside of the glass tube up to the level of the silica window. A stable separated flame can then be supported by using a slightly smaller nitrogen flow of 15 l minute^{-1}.

With this arrangement the flame noise level appears to be reduced still further, and a detection limit of 0·5 p.p.m. for molybdenum at 379·8 nm has been obtained in this way.

DISCUSSION

In addition to the reduction of flame background in the separated air - acetylene flame, the large reduction in interference from oxide-band emissions may prove extremely useful in the determination of many elements in alloys.

The intense MgOH band system between 360 and 410 nm is reduced in intensity about 200-fold on separation of the flame, and the flame background itself is also reduced by about two orders of magnitude in this region. The principal resonance lines of iron (372·0 nm), thallium (377·6 nm) and molybdenum (379·8 nm) are overlapped by this intense MgOH emission, and with the normal air - acetylene flame serious interference is avoided only by using other, less sensitive, lines which lie away from the magnesium hydroxide bands, or by preparing accurate reference solutions containing magnesium. Although separating the flame does not eliminate the interference completely, it can be reduced to an easily tolerable level by using a fuel-rich separated flame to reduce the intensity of the MgOH emission still further. Fig. 4 shows the effect of separating the flame on the ratios of the intensities of the iron

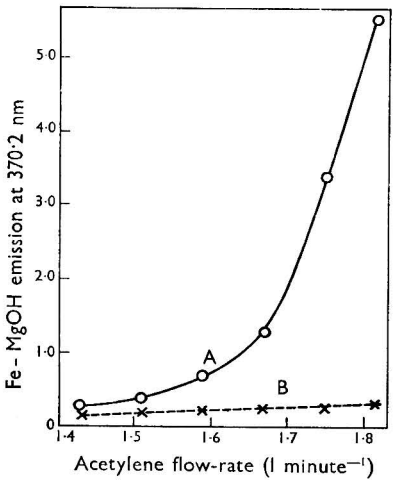


Fig. 4. Variation in Fe - MgOH emission at 370·0 nm with acetylene flow-rate in (B) conventional and (A) separated air - acetylene flames. Air 4·4 l minute^{-1}; analysing slit width 0·44 mm

atomic emission at 372·0 nm to that of the magnesium hydroxide molecular emission at the same wavelength. It is apparent that a considerable improvement in the spectral selectivity results from the use of a fuel-rich, nitrogen-separated flame.

The iron and manganese oxide band emissions interfere with the determination of sodium, particularly at trace concentrations. Hine, Crawford, Deutschman and Tipton[7] overcame this by using a fuel-rich flame to minimise oxide formation in the flame, but separation of the flame reduces the interference still further.

The decrease in temperature which occurs above the primary zone on separating the air acetylene flame leads to a reduction in the absolute atomic emission from all elements introduced into the flame to an extent that depends on the wavelength of the resonance emission if it is thermally controlled. The large reduction in the background of the flame enables this reduction in intensity of atomic emission to be compensated for quite easily, either by increasing the amplification of the signal from the photomultiplier, or by increasing the analysing slit width. The detection limits are generally improved by a factor of 10 in the separated flame, relative to the same signals in the unseparated flame, but magnesium is an exception, its limit of detection being three times better in the conventional (unseparated) flame. This anomaly is, however, easily explained. The flame background at 285·2 nm, the wavelength used for the magnesium emission measurements, is quite intense and the reduction in background and noise level should significantly improve the detection limit. The reduction in flame temperature on separation severely depresses the atomic emission of magnesium however, as the magnesium resonance line lies in the high excitation energy region at 285·2 nm (4·35 eV) and this effect leads to a reduction in the limit of detection. Increasing the analysing slit width to improve detection limits may, in some instances, re-introduce interferences from molecular-band emissions, especially when an instrument of only moderate resolving power, such as that used in this study, is used. We have found, however, that the use of the separated flame proves advantageous when interferences from banded molecular emissions must be minimised.

We are grateful to Aluminium Laboratories Ltd. (Banbury) for the provision of a grant to one of us (R.S.H.), and for the provision of the Unicam SP900A flame spectrophotometer used in this study.

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Performance of a Pre-mixed, Oxygen-enriched Air - Acetylene Flame in Flame-emission Spectrophotometry

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Improved sensitivities in flame-emission spectrophotometry have been obtained as a result of a simple modification to a Unicam burner - nebuliser system, which permits addition of oxygen to an air - acetylene flame. This gives enhancement factors of from 4 to 120 for the elements calcium, strontium, magnesium, barium, copper, silver, lead, iron, molybdenum, chromium, cobalt, nickel, manganese, vanadium, aluminium and lithium. The oxygen-enriched air - acetylene flame overcomes the interference effects of phosphate on calcium and magnesium, and partially eliminates the effect of aluminium on calcium.

The application of high temperature pre-mixed flames to flame-emission analysis has recently received a great deal of attention because of the high sensitivity obtained for a wide range of elements. Interferences caused by stable compound formation have been eliminated or reduced because the temperature is sufficiently high to induce dissociation of the compounds. Higher sensitivity is also achieved by the dissociation of stable complexes of the elements, resulting in an increased free-atom population in the flame.

Much of the early work on high temperature flames was directed towards their use in atomic-absorption spectrophotometry. Amos and Thomas[1] used oxygen - nitrogen - acetylene flames in the investigation of the absorption of aluminium, and Amos and Willis[2] were able, by using nitrous oxide - acetylene and oxygen - nitrogen - acetylene flames, to extend the range of elements detectable by the atomic-absorption technique to include refractories such as beryllium and titanium. They also found that, with the nitrous oxide - acetylene mixture, interferences such as aluminium in magnesium and phosphate in calcium determinations were eliminated.

In flame-emission analysis, D'Silva, Kniseley and Fassel[3] described an air-diluted, pre-mixed oxy-acetylene flame and, recently, Fassel and Golightly[4] reported detection limits for sixty-seven elements with a similar fuel-rich, oxy-acetylene pre-mixed flame. Pickett and Koirtyohann[5] used the nitrous oxide - acetylene pre-mixed flame in emission analysis and reported excellent sensitivities for thirty-four elements. They also studied the interference effect of aluminium on calcium for several flame-burner combinations, and showed that the interference was substantially reduced with the nitrous oxide - acetylene flame compared with other flames. More recently, Dagnall, Thompson and West[6] recorded strong emissions from zinc, cadmium and lead with nitrous oxide - hydrogen mixtures.

Kirkbright, Semb and West,[7] in their work on separated flames, added oxygen to a shielded air - acetylene flame to restore the depressed sensitivity brought about by the decrease in temperature in the interconal zone. With this system, a 20-fold increase in calcium emission was observed when the oxygen was added.

We have been investigating the performance of an unshielded oxygen-enriched, pre-mixed air - acetylene flame to determine its general usefulness in flame-emission analysis. This has been achieved by a simple and inexpensive modification to a commercial pre-mixed burner - nebuliser system, similar to that described by Kirkbright, Semb and West. With this flame, temperatures of up to 2500° K have been produced, resulting in significant improvement in sensitivity for a range of elements. Some common interference effects associated with the air - acetylene flame have also been studied with the oxygen-enriched air - acetylene flame.

EXPERIMENTAL

BURNER - NEBULISER ASSEMBLY—

The unit used was the burner and fog chamber from an SP900 Unicam flame spectrophotometer. Oxygen was fed via a flow-rate meter and needle valve into the compressed air line at a point just before the nebuliser. Air and oxygen were mixed in the nebuliser and fed, with the aerosol, into the fog chamber. In this system, the aerosol - oxidant and fuel were mixed in the burner stem.

INSTRUMENT ARRANGEMENT—

Light from the flame was focused by a spherical lens on to the slit of a 0·5 M Ebert plane grating monochromator (Jarrell-Ash Co.) that had a reciprocal linear dispersion of 1·6 nm per mm (first order 30,000 lines per inch grating). Radiation from a 2-cm length of the flame that extended from the tip of the primary reaction zone was monitored on an E.M.I. 6256B photomultiplier (S-13 cathode) operating at 1000 V. The output of the photomultiplier was fed to a d.c. microvoltmeter and displayed by a 10-mV chart recorder. Slits were maintained at 30 μm.

OPERATING CONDITIONS—

The burner was lit with an acetylene flow of 0·22 litre per minute, with the air pressure at 18 p.s.i. (corresponding to 2·5 litres per minute). The oxygen was turned on and both acetylene and oxygen flow-rates were increased to the maximum settings. We used up to 0·8 litre per minute of oxygen and 1·1 litres per minute of acetylene without ignition of the gas mixture inside the burner. With a higher oxygen flow there was a tendency for the gas mixture to blow back, which was signalled by the acetylene flow becoming erratic.

Solution flow-rates were 4 ml per minute (aqueous) and 1·5 ml per minute (ethanolic), and no increased rate of nebulisation was observed when oxygen was added. Air and acetylene flow-rates were calibrated at their supply pressures. Oxygen flow-rates were determined with the system operating under a pressure of 18 p.s.i.

FLAME TEMPERATURE MEASUREMENT—

The two-line method of temperature measurement, as described by Broida and Shuler[8] and Winefordner, Mansfield and Vickers,[9] was used to determine the electronic temperature of the flame for various oxygen and acetylene flow-rates, with a constant air pressure of 18 p.s.i. For a given oxygen flow-rate the acetylene was varied to give maximum intensity for the iron 373·713 and 373·487 nm lines. Table I gives the range of temperatures obtained, the precision for each measurement being about $\pm 20°$ K.

TABLE I

FLAME TEMPERATURES FOR VARIOUS OXYGEN AND ACETYLENE FLOW-RATES

Air constant at 2·5 litres per minute

Oxygen flow-rate, litres per minute	Acetylene flow-rate, litres per minute	Temperature, °K
0	0·22	2150
0·12	0·36	2230
0·32	0·63	2330
0·54	0·86	2380
0·80	1·01	2470

RESULTS

SENSITIVITY ENHANCEMENT—

Enhancement factors were obtained for the elements Ca, Sr, Mg, Ba, Cu, Ag, Pb, Fe, Mo, Cr, Co, Ni, Mn, V, Al and Li by aspirating ethanolic solutions (20 per cent. v/v) of the metals. The net signal for a given element at zero oxygen was compared with the signal when the oxygen flow-rate was 0·8 litre per minute and the acetylene flow-rate 1·1 litres per

minute, as all elements exhibited greatest sensitivity at these settings. The enhancement factors and the corresponding detection limits are given in Table II.

TABLE II

SENSITIVITY ENHANCEMENTS EXPRESSED AS DETECTION LIMITS (μg PER ml) FOR THE PRE-MIXED OXYGEN-ENRICHED AIR - ACETYLENE FLAME

Element	Wavelength, nm	Detection limits*	
		Air - acetylene	Oxygen-enriched air - acetylene†
Silver	328·1	0·5	0·05 (10)
Aluminium	396·1	60	0·5 (120)
Barium	553·6	0·05	0·005 (10)
Calcium	422·7	0·01	0·0005 (20)
Cobalt	352·9	1	0·1 (10)
Chromium	425·4	0·1	0·005 (20)
Copper	327·4	1	0·1 (10)
Iron	372·0	1	0·2 (5)
Lithium	670·8	0·005	0·001 (5)
Magnesium	285·2	0·5	0·05 (10)
Manganese	403·1	0·1	0·025 (4)
Molybdenum	379·8	1000	30 (33)
Nickel	352·5	4	1 (4)
Lead	405·8	20	2 (10)
Strontium	460·7	0·004	0·0004 (10)
Vanadium	437·9	140	2·5 (55)

* Based on 2 × standard deviation of the background noise level.
† Enhancement factors in brackets.

The detection limits obtained with the oxygen-enriched flame are included with those obtained by Fassel and Golightly (oxy-acetylene) and Pickett and Koirtyohann (nitrous oxide - acetylene) in Table III.

TABLE III

DETECTION LIMITS (μg PER ml) FOR OXYGEN-ENRICHED AIR - ACETYLENE, OXY-ACETYLENE* AND NITROUS OXIDE - ACETYLENE† FLAMES

Element	Wavelength, nm	Detection limits		
		Oxygen-enriched air - acetylene	Oxy-acetylene	Nitrous oxide - acetylene
Silver	328·1	0·05	0·3	0·02
Aluminium	396·1	0·5	0·2	0·01
Barium	553·6	0·005	0·05	0·002
	455·4	—	0·03	—
Calcium	422·7	0·0005	0·005	0·0001
Cobalt	352·9	0·1	—	—
	345·4	—	1	0·05
Chromium	425·4	0·005	—	0·005
	357·9	—	0·1	—
Copper	327·4	0·1	0·2	0·01
Iron	372·0	0·2	0·7	0·05
Lithium	670·8	0·001	0·001‡	0·00003§
Magnesium	285·2	0·05	0·2	0·005
Manganese	403·1	0·025	0·1	0·005
Molybdenum	379·8	30	0·03	—
	390·3	—	—	0·1
Nickel	352·5	1	0·6	—
	341·4	—	—	0·03
Lead	405·8	2	—	0·2
	368·4	—	3	—
Strontium	460·7	0·0004	0·004	0·0002
Vanadium	437·9	2·5	0·3	0·01

* Reference 4.
† Reference 5.
‡ E.M.I. 6255B photomultiplier.
§ 1P28 photomultiplier.

INTERFERENCE EFFECTS—

We investigated the interference effects of phosphate on calcium and magnesium when the oxygen and acetylene flow-rates were varied, the oxygen flow being set first and the acetylene then adjusted to give maximum signal. Fig. 1 shows that the phosphate interference on calcium is removed when the oxygen flow-rate is 0·6 litre per minute. In the test solution the calcium-to-phosphorus ratio was 1:50, and when this interference was investigated at a ratio of 1 : 350 no depression of the calcium intensity was observed. Similarly, the phosphate interference on magnesium was removed at these oxygen and acetylene settings.

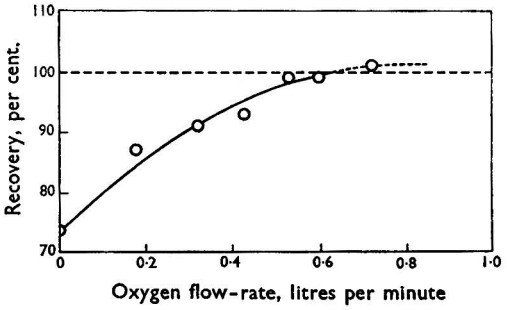

Fig. 1. Recovery of 10 p.p.m. of calcium in the presence of 500 p.p.m. of phosphorus for various oxygen flow-rates

The effect of aluminium on calcium was studied, but the temperature of the flame was apparently not high enough to remove this interference completely. Fig. 2 shows the partial removal of the interference, suggesting that much higher temperatures are required to dissociate the calcium - aluminium complex significantly. We found that an aluminium-to-calcium ratio of 2:1 caused no interference when operating at the maximum settings of oxygen and acetylene.

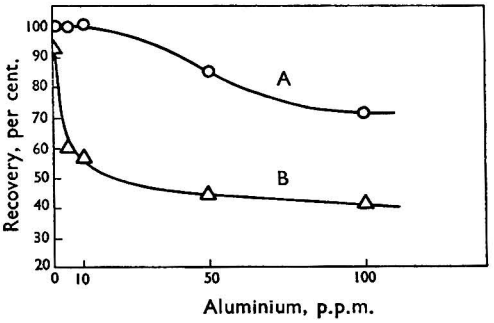

Fig. 2. Interference of aluminium in the determination of 5 p.p.m. of calcium. Comparison of A, oxygen-enriched air - acetylene flame with B, air - acetylene flame

To demonstrate the removal of interferences in the determination of calcium, we analysed N.B.S. Sample 120a (phosphate rock). By using air - acetylene, the calcium oxide content was found to be 31·2 ± 0·9 per cent., but with oxygen enrichment the result obtained was 48·5 ± 1·5 per cent. The provisionally certified value for this material is 50·3 per cent. of calcium oxide, and analysis by X-ray fluorescence[10] and plasma jet excitation (L. S. Dale, unpublished work) gave values of 48·1 ± 1·0 and 48·5 ± 2·5 per cent., respectively.

Discussion

The explosion-free operation of the oxygen-enriched pre-mixed flame is probably the result of the relatively low burning velocity of the gas mixture. From the dimensions of the burner and the experimentally determined gas flow-rates, the gas velocity was calculated to be about 180 cm per second. The burning velocity of the mixture must be less than this value and, according to Amos and Willis,[2] is 160 cm per second for an air - acetylene flame. Therefore, the addition of oxygen at the maximum flow-rate of 0·8 litre per minute (equivalent to 32 per cent. of oxygen with respect to air) does not appear to increase the burning velocity of the mixture significantly. Minor blow-backs occurred when the flame was operated at oxygen settings above 0·8 litre per minute but caused no damage to the equipment. The system was operated for extended periods at the maximum safe flow-rates of oxygen and acetylene, and the only observed deterioration in its performance was the increased rate of deposition of solids on the burner head.

The maximum measured temperature of the flame is about 2500° K. Higher temperatures can be achieved with modified burner heads that permit operation at higher gas flows, but this possibility was not investigated.

Significant sensitivity enhancement for several elements was achieved by using this flame at the maximum temperature (see Table II). The increased sensitivity for the individual elements studied depends on the excitation potential of the neutral-atom resonance line, the ionisation potential of the element and the stability of the monoxide of the element formed in the flame. Sensitivity is limited for those elements with excitation potentials greater than 3·5 eV (including silver, copper, magnesium and lead) because their lowest excited levels are not sufficiently populated.

Sensitivity is also limited for those elements of low ionisation potential. The enhancement for potassium was tested at the higher temperature, but no improvement in sensitivity was observed. Although an increase in intensity would be expected from thermal considerations, the number of neutral potassium atoms is reduced by ionisation (by about 33 per cent. at 2470° K). An observed enhancement for potassium in the presence of a large excess of rubidium supports this explanation. Ionisation at this temperature is low (less than 1 per cent.) for all elements except barium (about 9 per cent.).

The population of neutral atoms in the flame can also be reduced by the formation of stable monoxides of the elements. This is illustrated by the poor sensitivity for aluminium, vanadium and molybdenum in the normal air - acetylene flame, the dissociation energies of their monoxides being 5·94, 6·33 and 5·03 eV, respectively.[11] The increased sensitivity for these elements with the oxygen-enriched flame can be attributed to the partial monoxide dissociation at the higher temperatures, which results in an increase in free atom population. As the excitation potentials of their atomic lines are less than 3·5 eV, higher sensitivity is obtained resulting from the atoms liberated by this dissociation. The increased sensitivity may also be caused by free atoms produced by reduction of the monoxides by the flame. An increase in CN emission (band heads at 387·14 and 388·34 nm) has been observed in the inner cone of the flame, and the increase in sensitivity for these elements may be caused by the reduction of the monoxide by CN radicals. It is perhaps significant, on the basis of this increase in sensitivity, that the ratio of intensities of CN 387·14 nm and CH 387·20 nm is 0·5 in the air - acetylene flame and 5 in the oxygen-enriched flame. The high sensitivity for chromium is probably caused by the lower dissociation energy of its monoxide, although Kirkbright, Semb and West[7] have suggested that the chromium 425·4 nm line may be enhanced by an energy-transfer mechanism involving excited CH radicals.

The excitation conditions of the oxygen-enriched flame are inadequate for detecting refractory elements such as boron, silicon, zirconium and hafnium, and no significant intensity measurements have been recorded for them. They form extremely stable oxides in the flame, the monoxides having dissociation energies of 8 eV, or more.

A significant feature of the oxygen-enriched flame is its ability to overcome interference effects encountered with the ordinary air - acetylene system, the interference of phosphate on calcium being removed at 2320° K. The calculated ionisation of calcium at this temperature is 0·4 per cent. When using hotter flames of nitrous oxide - acetylene or oxy-acetylene, with temperatures about 3000° K, calcium is about 20 per cent. ionised. Ionisation leads to a decrease in sensitivity because of the depopulation of the neutral-atom ground state, and it is usually necessary to add an excess of a low ionisation element, such as rubidium,

to reduce this effect and to avoid interference from variable small amounts of elements with low ionisation potentials, such as sodium and potassium. By using the pre-mixed oxygen-enriched air - acetylene flame we have found that for the removal of some interferences, such as phosphate on calcium and magnesium, temperatures in the vicinity of 2300° to 2350° K (measured) are sufficient. As higher temperatures can lead to appreciable ionisation effects, particularly with alkaline earth elements, the use of lower temperatures obviates the need to add ionisation suppressors, with the subsequent advantage of less sample preparation, particularly by way of dilution when the element to be determined is present in low concentration. A similar observation has been made by Fleming[12] in the investigation of interferences of such elements as aluminium, silicon and zirconium in the atomic absorption of magnesium. He used an air - nitrous oxide - acetylene flame and showed that most interferences are removed with 23 per cent. of nitrous oxide. Thus, high temperatures of about 3000° K may not be necessary to overcome many of the interferences encountered in flame-emission analysis.

Although no extensive investigations of the use of the oxygen-enriched air - acetylene flame for atomic absorption has been made, indications are that sensitivities and freedom from interference effects are similar to those recorded in emission.

The oxygen-enriched flame can be operated equally well with either aqueous or ethanolic solutions. This is an advantage over the air-diluted oxy-acetylene flame, which requires 50 per cent. v/v ethanolic solutions to maintain its stability. A comparison of the oxygen-enriched flame with the higher temperature oxy-acetylene and nitrous oxide - acetylene flames is difficult to undertake because of variations in detection limit definition and instrumentation. Compared with the air - acetylene flame, the oxygen-enriched flame provides substantial improvement in sensitivity, and the modification should prove to be a valuable supplement to flame spectrophotometers with similar burner - nebuliser systems.

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The Flame-photometric Determination of Sodium in High Purity Water

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In connection with an investigation of the performance of sodium-responsive glass electrodes, an independent method was required for determining the sodium content of water (0 to 50 μg of sodium per litre). A flame-photometric technique was used for this purpose; details of the technique and its performance are given. Special attention was given to the problem of accurately calibrating the flame photometer and, to this end, a new technique has been evolved.

The need to determine very small concentrations of sodium (0 to 50 μg per litre) in samples of water from the steam - water circuit of power stations has been discussed by Webber and Wilson.[1] In the same paper, they described an investigation of the use of sodium-responsive glass electrodes for such determinations. As part of that work, an independent method of determining the sodium content of aqueous solutions was required to determine the extent of any deviations of the electrode response from a Nernstian-type equation. Flame photometry appeared to be a suitable technique, and this paper gives details of the technique used and of the tests made of its performance.

Calibration of the flame photometer—

The normal method for calibrating a flame photometer involves setting the zero of the instrument to correspond with the water used to prepare calibration standard solutions. This method introduces bias whenever the water contains an appreciable concentration of sodium relative to that of a sample. For our work with sodium-responsive glass electrodes, we desired to make unbiased determinations at a concentration of about 1 μg of sodium per litre. It seemed improbable that we could guarantee to prepare water containing less than 0·1 μg of sodium per litre, and approximate estimates obtained from the electrode technique indicated contents of 1 to 2 μg per litre. Accordingly, we found it necessary to consider this source of bias in detail.

Let I_T = intensity of light received by the detector of the flame photometer,

I_S = intensity of light received by the detector and caused by sodium coming from the solution being aspirated,

I_C = intensity of light received by the detector and caused by sodium from all sources (*e.g.*, fuel gases) other than the solution being aspirated,

I_B = intensity of light received by the detector and caused by background radiation from the flame,

C_w = concentration of sodium in the water used for setting the zero of the instrument,

C_s = concentration of sodium in the solution being aspirated, and

$\frac{dI_S}{dC_s} = k$, the slope of the calibration graph, which can be determined conventionally.

Now
$$I_T = I_S + I_C + I_B \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1),$$

therefore
$$I_T = k.C_s + I_C + I_B.$$

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Thus, the zero setting of the instrument corresponds to an intensity $k.C_w + I_C + I_B$, and this intensity is, in effect, subtracted from the intensity for each sample. The observed intensity, I_T, for a sample is, therefore, given by

$$I'_T = k\,(C_s - C_w).$$

The apparent concentration, C'_s, of the sample is, therefore,

$$C'_s = I'_T/k = C_s - C_w,$$

and the result is negatively biased by an amount equal to C_w.

To prevent this bias, it is necessary to determine either $(I_C + I_B)$ or C_w. In the first instance, one would set the zero to correspond with $(I_C + I_B)$; in the second, the zero would be set to correspond with $(I_T - kC_w)$. In one method[2] proposed to overcome this error it appears to be assumed that I_C has a zero value; subtraction of the background intensity from the sodium intensity thus gives kC_w. This assumption seemed unjustifiable to us, and experimental results confirmed this for our conditions. In a second method[3] used to estimate $(I_C + I_B)$ the zero is set to correspond with the intensity obtained when no solution is aspirated into the flame. This technique suffers from the drawback that non-aspiration of water into the flame is likely[4] to lead to an increase of the temperature of the flame. Such an increase will, in general, lead to an increase in the intensity of radiation from sodium atoms, even though the number of sodium atoms in the flame remains constant. Thus, the measured intensity will differ from that obtained when water containing no sodium is aspirated, and an error will result. As this technique of using a "dry" flame appeared to be convenient, we sought a means of correcting for error caused by the difference in the temperatures of "wet" and "dry" flames.

With small concentrations of sodium, the intensity of any of its spectral lines is directly proportional to the number, N_e, of sodium atoms in the excited energy level for that line, *i.e.*,

$$I_C + I_S = aN_e$$

where a is a parameter for a given line and apparatus that varies with flame temperature. N_e is given by a form of the Boltzmann equation

$$N_e/N_0 = (P_e/P_0)\exp(-E_e/kT)$$

where N_0 = number of sodium atoms in the ground state,

P = statistical weight factor for the energy levels involved in the spectral transition,

E_e = excitation energy of the excited state, *i.e.*, $3{\cdot}36 \times 10^{-19}$ J for the sodium doublet at 590 nm,

k = Boltzmann's constant, *i.e.*, $1{\cdot}38 \times 10^{-23}$ J °K^{-1}, and

T = absolute temperature of the flame, °K.

For temperatures about 2000° K, only an extremely small proportion of sodium atoms are in states other than the ground state.[4] N_0 is, therefore, essentially constant and equal to the total number of sodium atoms in the flame.

The ratio of the numbers of atoms in the excited state at two temperatures, T and T', is equal to the ratio of intensities received by the detector and caused by sodium. This ratio, R, is given by—

$$R = \frac{I_C + I_S}{I'_C + I'_S} = \frac{N_e}{N'_e} = \frac{\exp(-E_e/kT)}{\exp(-E/kT')} \quad .. \quad .. \quad .. \quad (2)$$

and R can, therefore, be calculated for any two temperatures. Thus, if the temperatures of the "wet" and "dry" flames are determined, multiplication of the intensity, I'_c, for the "dry" flame by R will give the intensity, I_c, corresponding to the same amount of sodium in the "wet" flame.

This calculation on the effect of temperature applies only to the sodium radiation and not to the background radiation at the same wavelength. Therefore, before making the temperature correction, it is necessary to eliminate the background contribution to the observed intensity at the wavelength of the sodium doublet. This can be achieved by measuring the intensity of the background on either side of the sodium doublet, and subtracting the mean value, I_B, from the observed intensity. This is equivalent to re-writing equation (1) in the form

$$I_T - I_B = I_S + I_C.$$

By this technique, estimates of $(I_S + I_C)$ (from the "wet" flame) and $R.I'_C$ (from the "dry" flame, for which $I_S = 0$) can be obtained. The concentration, C_s, of sodium in the solution used for the "wet" flame is then given by

$$C_s = \frac{1}{k} \cdot I_S = \frac{1}{k}\left[I_S + I_C - I_C\right],$$

therefore

$$C_s = \frac{1}{k}\left[(I_T - I_B)_{wet} - R\,(I_T - I_B)_{dry}\right] \quad .. \quad .. \quad .. \quad (3).$$

Equation (3) can be used to estimate the true sodium content of any solution. In particular, it can be used to estimate C_w, so that the total sodium concentrations of calibration standard solutions are known. This approach was used in the present work, as described below.

Experimental

The reagents, apparatus and technique used were exactly as described under Method, except when otherwise stated.

Effect of aspirated water on the flame temperature—

The temperatures of "wet" and "dry" flames were measured by the sodium D-line reversal technique.[5] For this, sodium must be introduced into the flame, and a dilute solution of sodium chloride was therefore aspirated for the "wet" flame. For the "dry" flame, a carbon rod, which had been impregnated with sodium chloride, was placed in the flame. The rod reduced the flame temperature; to measure this reduction, the temperature of the "wet" flame was also determined when the carbon rod was placed in it. The results obtained are given in Table I.

Table I

Temperatures of "wet" and "dry" flames

Flame conditions	Mean flame temperature, °K*
"Wet" flame without carbon rod	2183 (±14)
"Wet" flame with carbon rod	2102 (±3)
"Dry" flame with carbon rod	2147 (±4)

* The figures in brackets are the 95 per cent. confidence limits.

From these results, the temperature of the normal "dry" flame (*i.e.*, no carbon rod present) was calculated to be 2230° K. Substitution of the appropriate temperatures in equation (2) gave a value for R of 0·78 (±0·02).

Calibration graph and precision—

Replicate analyses of a series of standard solutions were made; a summary of the results is given in Table II, which indicates that the calibration graph departed only slightly from linearity at 50 μg of sodium per litre. If linearity were assumed, resulting errors were much smaller than the standard deviation for concentrations below 10 μg per litre.

Table II

Calibration results (0 to 50 μg of sodium per litre)

Sample	Mean* peak height above background, mm	Peak height *minus* 0·78 × "dry" flame peak height, mm	Within-batch standard deviation, μg of sodium per litre
"Dry" flame	10·7	—	1·0
Blank	11·6	3·3	1·4
Blank + 10 μg of sodium per litre	31·1	22·8	1·6
Blank + 50 μg of sodium per litre	122·2	113·9	2·4

* Each mean is based on twenty-four determinations.

To check the relationship between response and concentration more fully at low concentrations, a series of solutions was analysed. The results, which are shown in Table III, confirm both the attainable precision and also the expected linearity of the calibration graph for these concentrations.

TABLE III

CALIBRATION RESULTS (0 TO 12 μg OF SODIUM PER LITRE)

Concentration of sodium added to water, μg per litre	Mean* peak height, mm	Mean peak height after subtraction of the blank, mm	Within-batch standard deviation, μg of sodium per litre
0	15·0	—	0·9
2·5	22·1	7·1	0·8
5·0	28·1	13·1	1·1
7·5	33·0	18·0	0·7
10·0	42·1	27·1	0·6

* Each mean is based on four determinations.

METHOD

REAGENTS—

Air—Pass compressed air through a Millipore filter (pore size 0·8 μm), held on a porous polythene disc, before it enters the photometer.

Propane—Filter in the same way as for air.

Water—Re-circulation (in an all-plastic system) of distilled water through a laboratory-scale, mixed-bed de-ionisation column is a convenient means of preparing water containing about 2 μg or less of sodium per litre. Use this water to prepare standard sodium solutions and for washing apparatus.

Standard sodium solution A—Dry sodium chloride (analytical-reagent grade) in an oven at 250° to 350° C for 1 to 2 hours. Weigh 0·254 g of the dried sodium chloride, dissolve it in water and dilute with water to 1 litre in a calibrated flask. Store in a stoppered polythene bottle. This solution was found to be stable for at least 6 months.

1 ml of solution A ≡ 100 μg of sodium.

Standard sodium solution B—Dilute 10 ml of solution A with water to 1 litre in a calibrated flask. Store in a stoppered polythene bottle. This solution was found to be stable for at least 2 months.

1 ml of solution B ≡ 1 μg of sodium.

APPARATUS—

Polythene bottles—All samples and standard solutions should be stored in polythene bottles reserved solely for this purpose.

Clean all containers by soaking them for several days in de-ionised water. Check that they are adequately clean by filling them with de-ionised water, setting aside overnight, and then analysing the contents of each bottle as described under Procedure.

Flame photometer—All work was carried out with a Unicam SP900 flame spectrophotometer fitted with a motorised wavelength-drive and an air - propane burner. The instrument response was displayed on a 10-mV chart recorder.

Use a polythene container of about 25-ml capacity for holding the solution while it is being aspirated into the instrument. When not in use, fill the container with de-ionised water, and cover it. A polythene sample tube with a hinged lid was found to be convenient.

PROCEDURE—

Sample collection—Much care is required to prevent contamination of the sample during its collection and analysis. The concentration of sodium in samples has been found to be stable for many days, but it is prudent to analyse samples without undue delay after their collection.

Analysis of samples—After lighting the flame and adjusting the gas pressure, aspirate de-ionised water into the flame for 30 minutes before making any determinations. This period allows the instrument to warm up, and allows the nebuliser, expansion chamber and burner to be cleaned by the de-ionised water. During this warming-up period, and throughout the time required by the subsequent batch of analyses, the controls of the instrument (apart from the zero control) must remain exactly as they were set in Preparation of calibration graph.

At the end of the warming-up period, remove the de-ionised water from the capillary of the nebuliser, switch on the recorder-chart drive, and set the wavelength control to 610 nm.

When a steady reading is recorded, switch on the wavelength drive and record the spectrum of the "dry" flame until a wavelength of 580 nm is reached. Rinse the polythene sample holder with the sample and then aspirate the sample. Again, record the spectrum from 610 to 580 nm. Finally, remove the sample from the capillary of the nebuliser and record the spectrum of the "dry" flame from 610 to 580 nm.

All solutions to be analysed are treated exactly as described above. At least one solution of known sodium content should be analysed in each batch of determinations to check the constancy of the calibration graph. The preparation of such solutions is described under Preparation of calibration graph.

Calculation of results—Draw a straight line through the background on either side of each peak on the recorder chart, and measure the vertical height of the maximum of the peak above this line. Calculate the average peak height, H_D, of the "dry" flames on either side of the solution peak. Calculate the corrected peak height of the solution by subtracting $0{\cdot}78\ H_D$ from the measured peak height of the solution. From this corrected peak height and the calibration graph, calculate the concentration of sodium in the sample.

Preparation of calibration graph—

Preparation and analysis of standard solutions—Collect about 3 litres of de-ionised water in a polythene aspirator. Add 5, 10, 15, 20 and 25 ml of standard sodium solution B to a series of pre-weighed polythene bottles. Dilute the contents of each bottle with de-ionised water from the aspirator until the total contents weigh 500 g, and mix each solution thoroughly. These solutions contain 10, 20, 30, 40 and 50 in each instance *plus* x μg of sodium per litre, where x μg per litre is the sodium content of the de-ionised water. These solutions were found to be stable for at least 10 days.

While aspirating the 50 *plus* x μg per litre solution, adjust the controls of the instrument so that the recorder deflections for the peak and background correspond approximately to 80 and 5 per cent., respectively. Analyse these five solutions and the de-ionised water as described under Analysis of samples. Repeat this series of determinations at least once and then again as required until the calibration graph is defined with the required precision.

Measure the recorded spectra as described under Calculation of results. Plot the corrected peak heights against the concentration of sodium added to the de-ionised water; the concentration of sodium in the de-ionised water is then equal to the negative intercept on the concentration axis. Calculate the total sodium concentration of each of the six solutions, and draw the calibration graph as corrected peak height against total sodium concentration.

Discussion of the method

The results in Tables II and III show that, in the concentration range 0 to 10 μg of sodium per litre, the standard deviation of analytical results was about 1 μg per litre. This precision was adequate for our purpose, and may simply be improved by replicate analyses.

Bias in the analytical results depends principally on the correctness of the factor R used to correct the "dry" flame intensity, and on the magnitude of the sodium peak height for the "dry" flame. Calculations showed that R varied between 0·73 and 0·84 for "wet" flame temperatures between 2083° and 2283° K, and differences between "wet" and "dry" flames of 37° to 57° K. The uncorrected peak heights for "dry" flames were normally equivalent to less than 10 μg of sodium per litre. Thus, use of a value for R of 0·73 instead of 0·78 would cause a change in the determined sodium content of a solution equal at most to 0·5 μg per litre. The errors involved in the measurement of our flame temperatures were considerably smaller than the range of 200° K assumed in the above calculations. Therefore, we conclude that the technique of "dry" and "wet" flame measurements described in this paper is capable of giving results with a bias of less than 0·5 μg per litre. Such an error was tolerable for our purpose.

Other instruments may require different values for the correction factor, R. However, the calculations above indicate that, provided the sodium content of the "dry" flame is small (equivalent to less than 10μg of sodium per litre), considerable variations in flame temperature do not cause changes in R producing errors larger than 1 μg per litre in the final analytical result.

Bias arising from the various possible types of interference was not investigated. The method is intended only for the analysis of high purity water, and it was considered that interference effects would be negligible.

The proposed method is of particular value in providing an independent estimate of the sodium content of solutions used for calibrating sodium-responsive glass electrodes as described by the authors.[1]

This paper is published by permission of the Central Electricity Generating Board. Thanks are given to Dr. L. Brealey for helpful advice on suitable instrumental conditions for the flame-photometric determination of sodium, to Dr. D. L. Thomas of these laboratories for carrying out the temperature measurements of flames, and to Mrs. E. A. Wheeler for experimental assistance.

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Lung Tissue Hydrolysates: Studies of the Optimum Conditions for the Spectrophotometric Determination of Hydroxyproline

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Two procedures developed earlier by the authors for the spectrophotometric determination of hydroxyproline have been tested on hydrolysates of dried formalin-fixed and ethanol-fixed lung tissues. The effects of dilution of the hydrolysates and of the presence of various amounts of sodium chloride have been studied, as well as several methods commonly used to remove competing amino-acids or other interfering materials from hydrolysates before the spectrophotometric determination of amino-acids. Several amino-acids reported to cause interference in other spectrophotometric methods for hydroxyproline determination have a negligible effect in the procedures used in the present study.

Lung tissue hydrolysis with varying concentrations of hydrochloric acid, sulphuric acid and sodium hydroxide, under various conditions, has been studied. No reduction in hydroxyproline yield was observed when the acid hydrolyses were prolonged for several days. A simple procedure is described, in which hydrolysis takes place overnight at 105° C in a polypropylene tube enclosed by a glass tube fitted with a spring-loaded stopper; this procedure has given good results over a long period.

THE metabolism of collagen has been studied in several fields of work, as it is a major constituent of connective tissues. In pneumoconiosis research, studies have been made of the relationship between the collagen content of pneumoconiotic lungs and the amount and type of dust present. Hydroxyproline has often been used as an index of collagen, as the latter is the only protein containing high concentrations of this amino-acid; two improved spectrophotometric procedures for hydroxyproline determination have recently been developed by the authors.[1]

One of these procedures has been successfully used by Koevoet[2] for urine hydrolysates. Another adaptation, by Firschein and Shill,[3] was, however, sensitive not only to components of urine hydrolysates, but also to those of gelatin hydrolysates, because of changes in the recommended conditions.[1] Their remedy was a preliminary chromatographic separation. Chromatography was also recommended by Stegemann and Stalder as part of their improved procedure.[4] They overcame the sensitivity of their procedure to salt by evaporation rather than neutralisation of the hydrochloric acid used for hydrolysis. The lack of organic solvent at the oxidation stage makes the widely used method of Prockop and Udenfriend[5] sensitive not only to salt but also to chloramine-T concentration, and hence to materials that compete for the chloramine-T. In effect, the optimum chloramine-T concentration has to be determined afresh for each matrix.[6] The sensitivity to salt was overcome in the original procedure[5] by saturation of the solution with potassium chloride and, in later modifications, by removal of cations by chromatography and of hydrochloric acid by evaporation[6]; these latter authors still mistakenly assume that the chloramine-T oxidation product is the same as that given by peroxide.

In view of the sensitivity of the above and other methods to components of tissue hydrolysates, the effect of such components on the procedures of Bergman and Loxley[1] has been tested in the present work. An adaptation of these procedures designed for the routine analysis of urine hydrolysates will be described elsewhere.

The conditions of hydrolysis used by previous workers to liberate hydroxyproline from different tissues have varied considerably. In the present work the effect of such variations on the yield of hydroxyproline has been studied, especially with respect to formalin-fixed pneumoconiotic lungs.

In experiments to liberate lung dusts by tissue digestion,[7] it has been shown that a preliminary extraction of the tissue with a lipid solvent is necessary before a homogeneous hydrolysate can be produced with mineral acid. The present work examines the effect of such extractions on the yield of hydroxyproline.

Under most conditions acid hydrolysis of lung tissue gives rise to humin, a coal-like material that could provide a surface on which hydroxyproline might be catalytically decomposed or irreversibly adsorbed. As the presence of an optimum concentration of tin(II) chloride has been shown to minimise humin production,[7] the effect of tin(II) chloride in the hydrolysis medium on the hydroxyproline yield has been studied.

Experimental

All of the chemicals used were of analytical-reagent grade when available. Absorbances were measured on a Cary, Model 11, recording spectrophotometer, or a Hilger SP500 instrument.

Preparation of lung powders—

Whole lungs fixed in formalin or ethanol were cut into small pieces, minced and dried to constant weight at 105° C in a vacuum oven (usually for 2 days). The dried lung was ground in an end-runner mill until more than 99 per cent. passed through a British Standard 100-mesh sieve (mesh size 152 μm). The lung powder was equilibrated with atmospheric moisture and stored in a sealed bottle. When a sample was taken for analysis, a second sample was taken for moisture determination. The latter was heated at 105° C at atmospheric pressure overnight (18 hours), cooled for 20 minutes in a desiccator and the weight loss measured. All values for hydroxyproline concentration were expressed in terms of dried lung material.

Hydrolysis techniques—

Hydrolyses with various concentrations of sodium hydroxide and sulphuric acid and with 5·65 N hydrochloric acid were carried out in polypropylene centrifuge tubes; 0·5 g of dried lung powder, or an equivalent amount of other material to be hydrolysed, was placed in a polypropylene tube with 10 ml of solution, and the tube placed in a 50-ml standard-taper glass test-tube fitted with a spring-loaded stopper and a PTFE sleeve. The glass tube, shielded in a metal box, was then heated for the appropriate time (about 18 hours in the recommended procedure) in an oven at 105° C and allowed to cool. The hydrolysate, and any distillate in the glass tube, were washed into a 200-ml volumetric flask and made up to volume. This solution was then centrifuged to remove humin and any dust present in the tissue, and the supernatant liquid stored at room temperature in a glass bottle. Some hydrolyses were carried out with 11·3 N hydrochloric acid in standard-taper glass centrifuge tubes fitted with plastic stoppers and clamped in metal frames. In addition, other hydrolyses, with 5·65 N hydrochloric acid, were carried out in sealed-glass ampoules heated in an autoclave at 140° C.

Preparation for the spectrophotometric determination—

Aliquots for the spectrophotometric procedures of Bergman and Loxley[1] should have pH values between 2 and 6 to avoid overloading the oxidation-stage buffer, and the procedure recommended for hydrolysates is to add sufficient alkali to neutralise only about 90 per cent. of the acid. The rapid procedure A is best suited to hydroxyproline contents of between 5 and 40 μg per ml of aliquot and overnight procedure B to contents of between 2 and 15 μg per ml. The neutralisation and dilution of the hydrolysates was adjusted accordingly. With the concentrations of hydroxyproline normally found in lungs, the following procedure was adopted.

For 0·5 g of lung hydrolysed with 10 ml of 5·65 N hydrochloric acid and diluted initially to 200 ml, a 20-ml aliquot was taken for the rapid procedure A and 1·0 ml of 5·1 N sodium hydroxide added before dilution to 50 ml in a calibrated flask. A 10-ml aliquot was taken for the overnight procedure B and 1·0 ml of 2·6 N sodium hydroxide added before dilution

to 100 ml. These partially neutralised and diluted solutions of hydroxyproline were less stable than the original solutions, and were kept for not more than 1 or 2 days before 1-ml aliquots were taken for the spectrophotometric procedures. Hydrolysates of low hydroxyproline content would need to be diluted to a lesser extent in order that the hydroxyproline concentrations in the final aliquots fall within the desired ranges; these aliquots would, therefore, contain higher concentrations of other tissue components and partially neutralised acid. In consequence, hydrolysates were diluted to various degrees, and the effect on the colour yield was examined.

REAGENTS FOR THE SPECTROPHOTOMETRIC DETERMINATION—

Use analytical-reagent grade reagents when available.

Chloramine-T oxidant solution—Dissolve 0·35 g of chloramine-T in 5 ml of distilled water. Immediately before use mix this solution with 20 ml of the following buffer solution.

Buffer solution—Dissolve 57 g of sodium acetate trihydrate, 37·5 g of trisodium citrate dihydrate and 5·5 g of citric acid monohydrate in about 500 ml of distilled water. Then add 385 ml of isopropyl alcohol and dilute the mixture to 1 litre with distilled water. This solution is stable indefinitely.

Ehrlich's reagent (*A*)—Dissolve 19·25 g of *p*-dimethylaminobenzaldehyde in 44·5 g of perchloric acid (sp.gr. 1·54). Dilute this solution to 250 ml with isopropyl alcohol immediately before use.

Ehrlich's reagent (*B*)—Dissolve 12·0 g of *p*-dimethylaminobenzaldehyde in 30 g of perchloric acid (sp.gr. 1·54). Dilute this solution or 150 ml of reagent A to 250 ml with isopropyl alcohol immediately before use.

The blank values of Ehrlich's reagents A and B increase with time.

COLOUR DEVELOPMENT PROCEDURES

Procedure A—To a 1-ml aliquot of the test solution in a 6 × 1-inch test-tube are added 2 ml of analytical-reagent grade isopropyl alcohol followed by 1 ml of the oxidant solution. The contents of the tube are mixed and then allowed to stand for 4 minutes; 13 ml of Ehrlich's reagent A are then added. The solution is mixed, then heated in a water-bath at 60° C for 25 minutes, cooled with running tap water for 2 to 3 minutes and then diluted to 50 ml in a volumetric flask with isopropyl alcohol.

Procedure B—To a 1-ml aliquot of the test solution in a 50-ml reagent bottle are added 2 ml of analytical-reagent grade isopropyl alcohol, followed by 1 ml of the oxidant solution. The contents of the bottle are mixed and then allowed to stand for 4 minutes; 13 ml of Ehrlich's reagent B are then added. The bottle is stoppered and left overnight in a thermally insulated box, together with the other solutions in the batch as well as standards and blanks.

In both procedures the absorbances of the coloured solutions are measured in a spectrophotometer at 558 nm. The molar absorptivity should be about 90×10^3 litres cm^{-1} $mole^{-1}$ with both methods.

Stock solutions of hydroxyproline in water were found not to be stable over long periods, even when kept at a pH of 3, as suggested by Stegemann.[8] However, a 400 μg per ml solution of hydroxyproline in 10 per cent. isopropyl alcohol was found to be stable indefinitely. Just before each series of determinations, standard solutions containing 40 μg per ml, for procedure A, and 10 μg per ml, for procedure B, were prepared by dilution with water. Periodically, and whenever a new batch of reagent was started, the reagent blanks and linearity of the "standard graph" of absorbance against micrograms of hydroxyproline were checked. Hydroxyproline was determined in a series of standards covering the ranges 5 to 40 μg per ml for procedure A and 2 to 20 μg per ml for procedure B.

Since publication of the two procedures,[1] the overnight procedure B, has been the more extensively used with hydroxyproline solutions and hydrolysates, because of its greater convenience in routine use. This experience has led to a modification of Ehrlich's reagent for the overnight procedure. Reduction of the perchloric acid concentration at the colour development stage from 0·60 to 0·55 M was desirable to give optimum incubation times of about 20 hours at 20° C and about 17 hours at 22° C. The aldehyde concentration at the colour development stage was also reduced from about 0·40 to about 0·24 M to give a 2 to 3 per cent. increase in colour yield (see Concentration of oxidation and colour development reagents and Fig. 2).

Results and discussion

Choice of hydrolysis technique—

The effect of different concentrations of hydrochloric acid and sodium hydroxide, and various durations of the hydrolysis, on the yield of hydroxyproline was investigated, by using gelatin as a test material. The results for hydrolysis at a temperature of 105° C are shown in Fig. 1. Increased concentrations of acid and alkali both gave increased rates of hydrolysis. The hydrolyses with alkali gave the higher rates, but the hydroxyproline yields obtained with these hydrolyses under the optimum conditions were 2 to 3 per cent. lower than those obtained with the acid hydrolyses. Similar results were obtained for dried lung material, irrespective of whether it had been originally fixed in formol - saline or in ethanol.

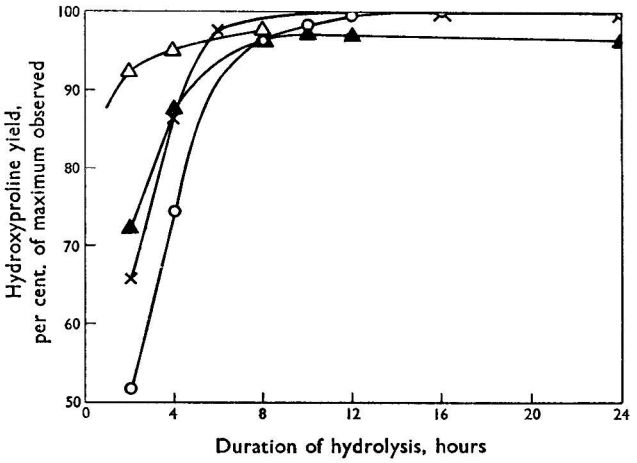

Fig. 1. Variation with time of hydroxyproline yield from the hydrolysis at 105° C of gelatin with concentrated and dilute hydrochloric acid and sodium hydroxide: O, 5·65 N hydrochloric acid; X, 11·3 N hydrochloric acid; ▲, 6 N sodium hydroxide; and △, 10 N sodium hydroxide

Several authors have found that the yields of hydroxyproline from various tissues decrease on extended hydrolysis with 5·65 N hydrochloric acid. No such losses of hydroxyproline were observed in the present work when the acid hydrolyses were prolonged to 6 days. Pure hydroxyproline solutions were treated with 5·65 N hydrochloric acid at 105° C in polypropylene and glass tubes for periods of up to several weeks; no losses of hydroxyproline were observed. A possible explanation for the decrease observed by other workers is that hydroxyproline reacts with a tissue component that is not present in significant amount in lungs. Such a reaction might be catalysed by glass surfaces etched by repeated hydrolysis. Polypropylene tubes have been used for routine hydrolysis in the present work, although no adverse effects from etched glass tubes have been found.

The hydroxyproline yields obtained by hydrolysing gelatin and lung tissue with 5·65 N hydrochloric acid for 18 hours (overnight) at 105° C and for 4 hours in an autoclave at 140° C were compared. No significant differences were observed. The former procedure is preferable, however, because of its use of "dead time" and its greater convenience.

The production of humin during hydrolysis of lung tissue with 11·3N hydrochloric acid at 60° C is less than with 5·65 N hydrochloric acid at 105° C.[7] Compared with the yields at 105° C with 5·65 N acid, only 40 per cent. of the hydroxyproline had been liberated after 18 hours with 11·3 N acid at 60° C and, even after 7 days, only 95 per cent.

Sulphuric acid, 6 and 12 N, was used instead of hydrochloric acid to hydrolyse some lungs with a view to using the hydrolysates for the determination of nitrogen by the Kjeldahl method. However, hydrolysis with sulphuric acid proved unsatisfactory, as greater amounts

of humin were formed, and the hydroxyproline yields were about 4 per cent. lower than those given by hydrochloric acid. In addition, when sulphuric acid hydrolysates were diluted to a lesser extent than normal, because the hydroxyproline contents were low, sodium sulphate crystallised out at the colour development stage.

The addition of tin(II) chloride during hydrolysis with 5·65 N hydrochloric acid resulted in pale yellow hydrolysates rather than the normal dark brown, indicating that less humin had been formed. As the amount of tin(II) chloride was increased to 1 g, however, the colour yield in the spectrophotometric determination decreased to 85 per cent. This decrease was probably caused by the tin(II) chloride catalysing fading of the final colour.

When lungs containing more than about 10 per cent. of lipids were hydrolysed with acid, a solid black crust formed and remained over the hydrolysate, even after 7 days at 105° C. When the lipids were removed by extraction with ethanol - ether before hydrolysis, no crust was formed, but significant amounts of hydroxyproline have on occasion been removed by the extraction, although these could be completely recovered by hydrolysis of the dried extract. To test whether this lipid crust might trap hydrolysable material, the lipids were held in solution over the hydrolysing acid during hydrolysis, and the hydroxyproline yields were compared with those obtained in the presence of the crust. To hold the lipids in solution in this way, 5 ml of dekalin or diethyl carbitol were added to the lung powders together with the hydrolysing acids, but no beneficial effects were observed.

In view of the foregoing results, hydrolysis with 10 ml of 5·65 N hydrochloric acid for about 18 hours at 105° C was adopted as the standard procedure for subsequent work. It is interesting to note that over 80 per cent. of the authors of recent publications on tissue hydrolysis have also used about 6 N hydrochloric acid.

Concentration of oxidation and colour development reagents—

For pure hydroxyproline the colour yields obtained with the two spectrophotometric procedures of Bergman and Loxley[1] were shown to be relatively independent of the concentration of chloramine-T at the oxidation stage. In the present work this factor was investigated for a series of hydrolysates, as they could contain material that would react with the chloramine-T in competition with the hydroxyproline. However, no appreciable variation in colour yield was found by varying the chloramine-T concentration, and the concentration of 12·5 mM, adopted for pure hydroxyproline, was used for hydrolysates also.

The effect on the colour yield of the concentration of *p*-dimethylaminobenzaldehyde at the colour development stage was studied for procedure B at a perchloric acid level of 0·55 M, with pure hydroxyproline and with hydrolysate solutions. A lung with a low hydroxyproline content was used for preparing the hydrolysates, to exaggerate the tissue effect. Fig. 2 shows that there is an optimum concentration of aldehyde between 0·2 and 0·3 M for both types of solution.

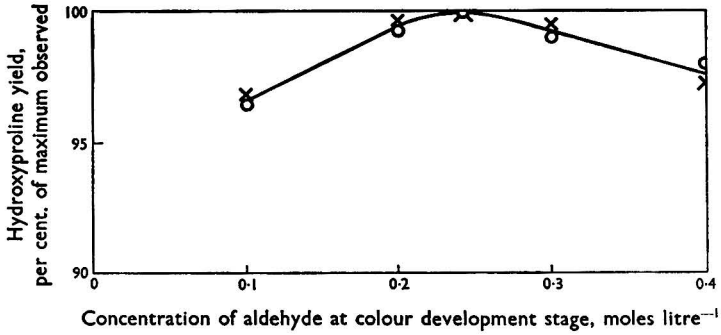

Fig. 2. Variation of colour yield in overnight procedure B with aldehyde concentration (0·055 M perchloric acid) for hydroxyproline alone and for a lung hydrolysate: O, 40 μg of hydroxyproline alone; and ×, 40 μg of hydroxyproline from 5 mg of lung tissue

COMPARISON OF RESULTS FROM THE COLORIMETRIC PROCEDURES—

The hydrolysates obtained by the standard procedure from forty-four lungs (containing from 0·6 to 4·7 per cent. of hydroxyproline) were analysed for their hydroxyproline contents by both the rapid procedure A and overnight procedure B. When the 1-ml aliquots for the two procedures contained the same amount of hydrolysed tissue (0·5 mg), forty-two of the lungs gave pairs of results that agreed within experimental error. The average difference of the forty-two pairs was 0·05 μg and the standard deviation $\pm$0·06 μg. Two lungs (see Table II) gave results in which the yield by procedure B was consistently 3 to 5 per cent. lower than that by procedure A, despite alterations in the conditions. The factor for this difference has not been identified.

TABLE I

COLOUR YIELDS, MEASURED BY COLORIMETRIC PROCEDURES A AND B, OF HYDROXYPROLINE ADDED TO LUNG HYDROLYSATES

	Concentration in aliquot, μg per ml, of		Colour yield of added hydroxyproline by				
Lung No.	Hydrolysed tissue	Added hydroxyproline	Procedure A		Procedure B	
1	200	4·0	99·3	99·3	97·3	97·9
2	200	4·0	99·5		99·8	
3	200	4·0	99·5		97·8	
4	200	4·0	98·8		96·5	
1	200	20·0	100·0	99·7	100·0	99·9
2	200	20·0	98·9		99·8	
3	200	20·0	100·1		99·9	
4	200	20·0	99·8		99·9	
1	1000	20·0	99·0	97·8	98·5	97·8
2	1000	20·0	98·3		97·8	
3	1000	20·0	98·5		97·5	
4	1000	20·0	95·5		97·5	
		Average values	99·0		98·5	

EFFECT OF THE DEGREE OF DILUTION OF HYDROLYSATES—

With an increase in the concentration of hydrolysed tissue in the final aliquot (resulting from dilution of the hydrolysate to a lesser degree than normal), there was a decrease in the hydroxyproline yield with both methods of colour development. The reduction was only about 2 per cent. for each milligram of hydrolysed tissue in a 1-ml aliquot, and was sufficiently small to be ignored. Confirmation that the tissue effect was small was provided by experiments in which measurements were made of the colour yield from hydroxyproline added to aliquots containing different amounts of hydrolysed lung. The results for four lungs are shown in Table I. Dilution of gelatin hydrolysates to a lesser degree than normal did not cause reductions in colour yield. With tissues of low hydroxyproline contents, necessitating large tissue contents in the final aliquots, an extrapolation to zero tissue content could be made from two results at different tissue concentrations.

When a tissue hydrolysate is diluted to a lesser degree than normal, the final aliquot contains higher concentrations of the other tissue components, but it also contains higher concentrations of acid or partially neutralised acid, unless the acid has been removed by, for example, evaporation in a vacuum desiccator. The procedure recommended for the present spectrophotometric procedures is to add sufficient sodium hydroxide to neutralise 90 per cent. of the acid, and thereby produce sodium chloride. Other spectrophotometric procedures are sensitive to the presence of sodium chloride. For example, sodium chloride at a concentration of 1·5 M has been found to give colour yields as low as 80 per cent. in methods in which the oxidation of hydroxyproline takes place in alkaline solution, and Woessner,[9] who used the slightly acidic chloramine-T introduced by Stegemann,[8] as oxidant, obtained a yield of 93·7 per cent. in the presence of 1·5 M sodium chloride. The colorimetric procedures used in the present work have already been shown to be unaffected by a concentration of 0·1 M sodium chloride in the aliquot.[1] The procedures have now been tested with 1·5 M sodium chloride in a 10 μg per ml solution of hydroxyproline. Even at this high

chloride concentration, colour yields of 98·5 per cent. by procedure A and 97·2 per cent. by procedure B were obtained. The inhibiting effect of sodium chloride takes place at the oxidation stage, and the stabilising effect of the conditions used in the present work for chloramine-T oxidation accounts for the high yields in the presence of high concentrations of sodium chloride.

Experiments have also been carried out to examine the effect of a 90 per cent. concentration of neutralised acid on the hydroxyproline colour yield from hydroxyproline solutions and from hydrolysates. With the 10 μg per ml hydroxyproline solution, the colour yield decreased as the 90 per cent. neutralised acid content increased until, at a chloride concentration of 0·6 M (0·54 M sodium chloride *plus* 0·06 M hydrochloric acid), the colour yield for procedures A and B was about 97 per cent. Similar effects were observed with hydrolysates.

To examine the separate effects of tissue concentration and partially neutralised acid concentration on the oxidation and colour development stages, procedure B was carried out on hydrolysates in which these concentrations were varied independently, sometimes before and at other times after the oxidation stage. The results show that the total effect of increased tissue concentration on the hydroxyproline colour yield was a combination of a slight increase in colour yield, caused by factors operating at the oxidation stage, and a reduction in colour yield, caused by factors operating at the colour development stage. Variations in the partially neutralised acid concentration affected only the oxidation stage.

EFFECT OF ADSORBENTS COMMONLY USED TO REMOVE INTERFERING COMPONENTS IN OTHER COLORIMETRIC PROCEDURES—

Various adsorbents have been used to remove interfering components from tissue hydrolysates before the determination of hydroxyproline by various spectrophotometric methods. Although no major interferences were encountered in the present work, activated charcoal ("Nuchar," as received), anion-exchange resins, chromatographic alumina and silica gel were tested for their effect on the recovery of hydroxyproline from pure solutions and from hydrolysates at various levels of acidity. In all instances, the hydroxyproline colour yield was decreased; the effect increased with increasing acidity and with increasing amounts of adsorbent. Of the adsorbents tested, charcoal had the greatest effect per unit weight in removing colour and odour, as well as hydroxyproline, from hydrolysates. Our findings of the adverse effects of adsorbents have been confirmed by Stanley, Dawson, Field and Glicksman.[10]

The effect observed with hydrolysates, whereby aliquots containing higher concentrations of hydrolysed tissue gave reduced colour yields, was thought to be caused by a component, not present in gelatin, interfering with the colour development. None of the adsorbents removed the effect, however. It is suggested that separation methods should be developed for, and tested with, interfering components as they are encountered.

COMPETING AMINO-ACIDS—

Several spectrophotometric methods for determining hydroxyproline have shown interference from the amino-acids proline, tyrosine and tryptophane. In both the rapid procedure A and overnight procedure B, these amino-acids gave coloured solutions with molar absorptivities of 70, 40 and 180 litre cm^{-1} $mole^{-1}$, respectively, in contrast to a molar absorptivity of 90,000 litre cm^{-1} $mole^{-1}$ given by hydroxyproline. No colour was obtained with urea, or with the amino-acids glycine or alanine, even in concentrations 100 or 1000 times larger than the hydroxyproline concentrations normally used. The effect on the hydroxyproline colour yield of these five amino-acids, in concentrations up to ten times greater than the hydroxyproline concentration, was investigated by the method of standard additions. The colour yield was complete, within experimental error. The effect of urea in even greater excess was investigated because the determination of free hydroxyproline present in small amounts in urine was known to be of interest. An aliquot containing 2 μg of hydroxyproline and 2 mg of urea per ml of solution gave a hydroxyproline colour yield of 92·8 per cent. In another experiment, a colour yield of 90 per cent. was obtained from hydroxyproline added to hydrolysed urine (1 μg per ml). This value for the colour yield could be explained almost entirely by the concentration of urea in the urine. The background colour was allowed for by an additional blank in which Ehrlich's reagent was added to the aliquot before the oxidation solution was added.

In the spectrophotometric method of Prockop and Udenfriend,[5] the presence of 2 mg of alanine almost completely inhibited colour formation with 10 μg of hydroxyproline. For tissue hydrolysates low in hydroxyproline, therefore, these authors suggested a procedure that involved the use of additional chloramine-T (which reduced the colour yield considerably in their method) and the addition of alanine (which partly reversed this effect). With this arrangement, any alanine and similar materials contained in the tissue had little effect on the colour yield. There have also been other methods in which amino-acids such as alanine have been used to swamp the procedure in an attempt to make it insensitive to tissue hydrolysate concentration. Most of the effects of amino-acids on these hydroxyproline determinations appear to be associated with the relative instability of intermediates at the oxidation and colour development stages. With the high isopropyl alcohol concentrations in the present procedures, the presence of 2 mg of alanine at the oxidation stage resulted in a hydroxyproline colour yield of about 97 per cent. for the standard oxidation time of 4 minutes.

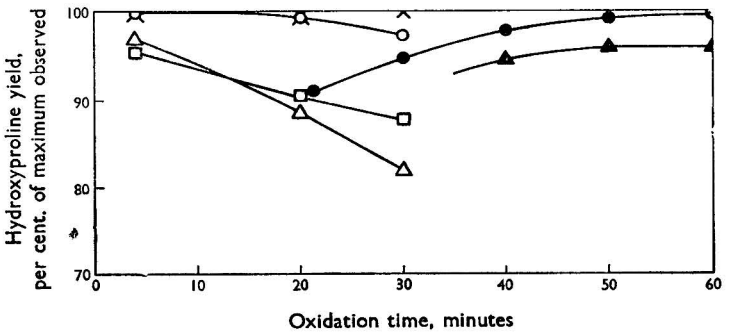

Fig. 3. Variation of colour yield with oxidation time at 20° and 0° C for hydroxyproline alone and in the presence of alanine, and at 20° C for lung hydrolysate alone and in the presence of alanine. Oxidation of 10 μg of hydroxyproline: ●, alone at 0° C and ○, at 20° C; ▲, with 2 mg of added alanine at 0° C and △, at 20° C; ×, from 500 μg of tissue at 20° C; and □, from 500 μg of tissue with 2 mg of added alanine at 20° C

As alanine appeared to be reacting with the chloramine-T in Prockop and Udenfriend's method, it was thought that alanine could be reducing the colour yield in the present procedures by slowing down oxidation of the hydroxyproline. It was therefore added in various amounts to pure hydroxyproline solutions and to tissue aliquots, the oxidation time was varied and the corresponding colour yield recorded. The results are shown in Fig. 3. With hydroxyproline alone, the colour yield decreased by about 1 per cent. in proceeding from the standard oxidation time of 4 minutes to 20 minutes. When 2 mg of alanine had been added, the colour yield for 4 minutes was about 97 per cent., while that for 20 minutes had decreased even further to less than 90 per cent. Two lung hydrolysates were tested with aliquots containing 0·5 mg of hydrolysed tissue. In the absence of added alanine, the colour yield increased slightly as the oxidation time was increased from 4 to 20 minutes. When 2 mg of alanine had been added, however, the colour yield for 4 minutes was about 95 per cent., and that for 20 minutes was again about 90 per cent. Alanine, or the product of its reaction with chloramine-T, appeared to be slowly destroying the hydroxyproline oxidation product.

To slow down the oxidation so that the early stages could be studied, this stage was carried out for various periods at 0° C with hydroxyproline alone and in the presence of alanine. The results are also shown in Fig. 3. Although the colour yield is reduced by the presence of the alanine, the shapes of the two curves are similar.

The above differences between lung hydrolysates and alanine alone may well explain why the use of alanine in the method of Prockop and Udenfriend[5] has only limited success in preventing interferences.[6,10]

COLOUR FADING CAUSED BY ULTRAVIOLET LIGHT—

The fading of the coloured solution produced from pure hydroxyproline had been shown to be accelerated by ultraviolet light, even that produced by standard fluorescent strip lights.[1] In the present work, coloured solutions were produced from hydrolysates and from a pure hydroxyproline solution, and their responses to fluorescent light were compared. Solutions from both sources were exposed to fluorescent light for a period of 4 hours in soda-glass bottles and in vitreous silica cuvettes, and others were stored for the same period in a darkened cupboard. A comparison of the degrees of fading in the soda-glass bottles and the darkened cupboard indicated that the soda-glass bottles provided complete protection against the fading effect of the light. On the other hand, the solutions in the cuvettes faded markedly, those produced from hydrolysates fading to 96 per cent. and those from pure hydroxyproline to 90 per cent. of their original absorbances.

LOW-TEMPERATURE COLOUR DEVELOPMENT—

Although the optimum values of *p*-dimethylaminobenzaldehyde and perchloric acid were found to vary for colour development at room temperature (20° C) and at 60° C, the courses of colour development were similar, even although the speeds were very different.[1] This indicated that time and temperature were complementary, thus giving a flexible method. The overnight colour development at room temperature was found to be a convenient routine between the working days of the week, but it left the week-ends as "dead time." However, it was thought that the use of a temperature lower than 20° C, with its consequently slower colour development, might allow colour development to be completed satisfactorily in about 65 hours, *i.e.*, over the week-end. Colour development in a refrigerator at 4° C was investigated, as well as a combined routine of colour development, partly at 4° C and partly at room temperature. The optimum values of aldehyde and perchloric acid were found to be the same as those recommended for the overnight procedure B.

When the course of a colour development at room temperature was interrupted by a period at 4° C there were two effects. As the rate of colour development was considerably slowed down at the lower temperature and then speeded up again at room temperature, the total time spent at room temperature before the maximum colour was reached was not much affected by the period spent at 4° C. The absorbance of this maximum colour was, however, lower than that obtained with a colour development carried out entirely at room temperature. For instance, a colour was developed for 6 hours at room temperature, followed by 11 hours at 4° C, and was allowed to continue its development at room temperature. The absorbance reached a maximum after a further 10 hours, but the yield was only about 90 per cent. of that obtained with a normal room temperature procedure. To reach the maximum colour yield in about 65 hours, entirely at 4° C, the perchloric acid concentration needed to be double the concentration normally used for the overnight procedure B; it was found that the colour yield was only about 80 per cent. of that obtained at room temperature.

REPRODUCIBILITY OF RESULTS—

Over a period of about 5 years the 40-μg standards for procedure A gave an average molar absorptivity of $89{\cdot}3 \times 10^3$ litre cm^{-1} $mole^{-1}$, with a coefficient of variation of $\pm 1{\cdot}5$ per cent. (20 results). With procedure B, modified as described under Experimental, the 10-μg

TABLE II

REPRODUCIBILITY OF REPEATED HYDROXYPROLINE DETERMINATIONS ON SEVERAL HYDROLYSATES FROM EACH OF THREE LUNGS

	Lung No.	Hydroxyproline content, per cent.	Coefficient of variation, per cent.	Number of hydrolyses	Number of determinations
Procedure A	1	4·69	±3·0	4	10
	2	1·44	±3·8	5	12
	3	0·631	±2·8	6	19
Procedure B	1	4·65	±3·6	3	6
	2	1·36	±3·8	5	15
	3	0·604	±4·5	7	28

standards gave an average molar absorptivity of $91{\cdot}5 \times 10^3$ litre cm^{-1} $mole^{-1}$, with a coefficient of variation of only $\pm 2{\cdot}1$ per cent. (18 results), despite the fact that temperature regulation was achieved by the use of an insulated box rather than by thermostatic control.

During the course of studies on pneumoconiotic lungs, three lungs were hydrolysed several times over a period of 1 year, and the hydrolysates analysed several times by procedures A and B. The results are shown in Table II. Lungs Nos. 2 and 3 had been chosen because they showed large differences between procedures A and B.

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Factors Influencing the Colorimetric Determination of Nitrite with Cleve's Acid

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The determination of nitrite with Cleve's acid has been investigated; the influence of composition of reagents and several experimental conditions have been evaluated. It is shown that provided the standards used to prepare the calibration graph and samples are treated in a similar way, then the choice of conditions may largely be left to the operator. A recommended procedure is given.

Nitrogen-containing compounds are widely distributed throughout nature and are found in most biologically active materials. Nitrite, an intermediate state in the nitrogen cycle, is found in soils, waters and effluents, and in some food products. Trace amounts of nitrites in potable waters may indicate organic pollution, while relatively large amounts of nitrites are frequently added to industrial cooling waters to inhibit metallic corrosion. Additionally, nitrite is often a convenient parameter by which, following a reduction step, to determine nitrate.[1]

Sawicki, Stanley, Pfaff and D'Amico[2] have compared many spectrophotometric methods for determining nitrite. In most of these methods the nitrite concentration is determined following the formation of a red azo dye. The Griess-Ilosvay procedure,[3] in which diazotised sulphanilic acid is coupled with 1-naphthylamine, has been preferred by many workers. The advantages of this method are its good sensitivity, wide range, freedom from interference, rapidity and convenience. Unfortunately, 1-naphthylamine, because of its carcinogenic properties, is now regarded as hazardous, even for laboratory workers.[4] A recent Statutory Instrument[5] further emphasises the need for care in the use of such substances.

Several alternative coupling reagents were suggested by Professor Boyland of the Chester Beatty Research Institute, and were examined in this laboratory. Of these, Cleve's acid, 1-naphthylamine-7-sulphonic acid, was found to be most satisfactory from the analytical point of view, and a method for the determination of nitrite involving the use of this reagent was published by Crosby.[6] Opinion of medical workers suggests that the possible hazards from the use of Cleve's acid are negligible,[7,8] and there is no evidence to indicate that the sulphonic acid group is removed during metabolic processes. It would appear that the loss of carcinogenic activity is associated with the increased solubility of the sulphonic acid derivative compared with the free base.[8]

Experience in several laboratories with Cleve's acid reagent showed that for amounts of nitrite above that normally found in drinking water the experimental conditions needed to be more clearly defined. It was also desirable to relate composition and performance of the samples of Cleve's acid used in the various laboratories.

It will be shown that, providing the standards and samples are treated in the same way, the method can be used under a variety of laboratory conditions to suit the requirements of the individual analyst.

Experimental

Reagents—

Sulphanilic acid solution—Dissolve 0·5 g of analytical-reagent grade sulphanilic acid in a solution containing 30 ml of analytical-reagent grade glacial acetic acid and 120 ml of water. Store in a brown bottle.

Cleve's acid solution—Dissolve 0·1, 0·2 or 0·5 g of Cleve's acid in 120 ml of water, warming on a water-bath. Filter the solution, cool and add 30 ml of glacial acetic acid. Store in a brown bottle.

Standard nitrite solution I—Dissolve 0·493 g of analytical-reagent grade sodium nitrite in water and dilute to 1 litre in a calibrated flask.

1 ml of solution ≡ 100 μg of nitrogen, present as nitrite.

Standard nitrite solution II—Dilute 10 ml of standard nitrite solution I to 1 litre in a calibrated flask. Prepare immediately before use.

1 ml of solution ≡ 1 μg of nitrogen, present as nitrite.

PROCEDURE—

In each experiment the required amounts of standard nitrite solution II were added to a series of 50-ml calibrated flasks and the volume of each adjusted to about 40 ml; 2 ml of sulphanilic acid solution were added to each and then, after the prescribed interval for diazotisation, 2 ml of the Cleve's acid solution under investigation were also added. At the end of the coupling time, the absorbance of the solutions was measured with a Unicam SP600 spectrophotometer in 10-mm cells and at a wavelength of 525 nm, with water in the reference cell.

RESULTS

EXAMINATION OF MATERIALS—

The commercial product known as Cleve's acid is principally 1-naphthylamine-7-sulphonic acid, with varying proportions of the 1,6-isomer and sometimes also small amounts of the 1,5- and 1,8-isomers. The product used by Crosby[6] was imported and thought to be essentially the 1,7-isomer, and the performance of this reagent (A) was compared with two other samples of the material. One of these (B) was of laboratory-reagent grade, available from B.D.H. Ltd., and likely to be the reagent at present in use in analytical laboratories. A third sample (C), from a British manufacturer, was privately obtained.

Each of these products was examined by infrared spectroscopy, which showed that A and B were samples of the free acid, while sample C was in the form of the sodium salt. Quantitative measurements enabled the ratio of 1,6- to 1,7-isomers to be determined and the results are given in Table I. A further sample of each material was ashed at 600° C as a check on the presence of neutral salts.

TABLE I

EFFECT OF COMPOSITION OF CLEVE'S ACID REAGENT

With 0·2 g of Cleve's acid per 150 ml of solution

Description	Composition	Absorbance value for 10 μg of nitrogen
Cleve's acid A	1,6-isomer absent 1,7-isomer 95 per cent.	0·60
Cleve's acid B	1,6-isomer 40 per cent. 1,7-isomer 60 per cent.	0·57
Cleve's acid C	1,7-isomer (Na salt) 90 per cent.	0·60
Pure 1,6-isomer	—	0·51
Pure 1,7-isomer	—	0·61
Pure 1,5-isomer	—	0·14
Pure 1,8-isomer	—	0·59

The above reagents, together with the purest available samples of 1,5-, 1,6-, 1,7- and 1,8-isomers, were used separately to prepare calibration graphs under optimum conditions (see below). The absorbance values for 10 μg of nitrogen are given in Table I.

Hence, as regards the composition of the reagents, it can be concluded that contamination of the 1,7-isomer with moderate amounts of 1,6- and 1,8-isomers is not a serious disadvantage because, under similar conditions of test for a given nitrite content, they give an absorbance of at least 80 per cent. of the 1,7-isomer; that it is immaterial whether the reagent is present as the free acid or as its sodium salt; and that contamination with more than small amounts of the 1,5-isomer should be avoided.

EFFECT OF REAGENT CONCENTRATION—

The variation of absorbance with nitrite concentration for different solution concentrations of the samples of Cleve's acid A, B and C is shown in Table II.

TABLE II

EFFECT OF CONCENTRATION OF CLEVE'S ACID REAGENT

Nitrite, μg of nitrogen	Cleve's acid A, g per 150 ml			Cleve's acid B, g per 150 ml			Cleve's acid C, g per 150 ml		
	0·1	0·2	0·5	0·1	0·2	0·5	0·1	0·2	0·5
1·0	0·063	0·059	0·064	0·048	0·067	0·058	0·060	0·060	0·062
2·5	0·150	0·153	0·160	0·133	0·153	0·150	0·148	0·156	0·157
5·0	0·298	0·306	0·316	0·267	0·290	0·294	0·290	0·308	0·314
7·5	0·440	0·454	0·464	0·397	0·434	0·434	0·425	0·454	0·466
10·0	0·580	0·596	0·614	0·525	0·572	0·572	0·562	0·598	0·608

The effect of reagent concentration is small within the range 0·1 to 0·5 g of Cleve's acid per 150 ml of solution, but the absorbance values obtained with samples A and C are noticeably higher than those with sample B. This reflects the greater proportion of the 1,7-isomer in these two samples. Indeed, it can be seen in Table I that the depression of the absorbance value of sample B is proportional to the increase in the amount of the 1,6-isomer present. A concentration of 0·5 g of Cleve's acid per 150 ml of solution is close to the limiting solubility of Cleve's acid in water, and although a concentration of 0·1 g per 150 ml of solution would suffice for most purposes, it is recommended, in view of the possible uncertain purity of the reagent, that a solution containing 0·2 g of Cleve's acid per 150 ml of solution should be used in any standard method.

TABLE III

EFFECT OF DIAZOTISATION TIME AND COUPLING TIME

Coupling time, minutes	Cleve's acid A, diazotisation time			Cleve's acid B, diazotisation time			Cleve's acid C, diazotisation time		
	Zero	10 minutes	20 minutes	Zero	10 minutes	20 minutes	Zero	10 minutes	20 minutes
20	0·464	0·557	0·566	0·442	0·515	0·544	0·464	0·545	0·579
40	0·529	0·596	0·606	0·500	0·560	0·573	0·537	0·595	0·600
60	0·535	0·599	0·606	0·512	0·563	0·573	0·548	0·602	0·606
100	0·541	0·600	0·606	0·512	0·565	0·573	0·548	0·602	0·606

EFFECTS OF TIMES OF DIAZOTISATION AND COUPLING—

The separate effects of time of diazotisation and of coupling were investigated by using three solution concentrations of the three samples of reagent. The results for the 0·2 g per 150 ml solutions of the three reagents are given in Table III, while a graphical comparison of the results for Cleve's acid A is shown in Fig. 1.

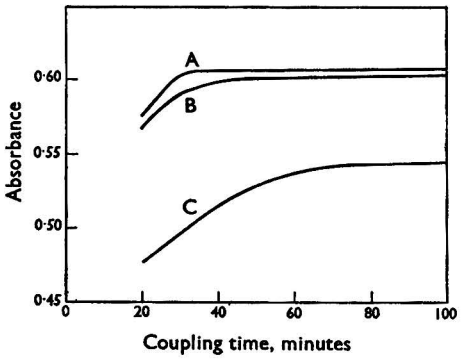

Fig. 1. Effect of diazotisation time and coupling time on absorbance of solution containing 10 μg of nitrogen as nitrite. Diazotisation time: graph A, 20 minutes; graph B, 10 minutes; and graph C, zero

If a reagent concentration of 0·2 g per 150 ml is chosen, then the optimum conditions are 20 minutes for diazotisation, followed by 40 minutes for coupling. However, by using a more concentrated reagent solution (0·5 g per 150 ml) the time of diazotisation can be reduced to 10 minutes and that for coupling to 20 minutes.

TABLE IV

EFFECT OF TEMPERATURE

Temperature, °C	Absorbance values for 10 μg of nitrogen
15	0·595
20	0·606
25	0·608
30	0·608

EFFECT OF TEMPERATURE—

Table IV shows the variation of absorbance with temperature for a solution containing 10 μg of nitrogen, as nitrite. The reagent solution used consisted of 0·2 g of Cleve's acid A per 150 ml and the reaction times were 20 minutes for diazotisation and 40 minutes for coupling.

It can be seen that provided the solution temperature is between 20° and 30° C further strict control is unnecessary.

CONCLUSION

A study of the results shows that provided the experimental conditions used for the preparation of the calibration graph and for the examination of samples are exactly the same, then the choice of these conditions may, to a large extent, be left to the operator. However, for most types of sample, suitable optimum conditions would be obtained with a reagent solution containing 0·2 g of Cleve's acid per 150 ml of solution, a diazotisation time of 20 minutes and a coupling time of 40 minutes.

Our thanks are given to I.C.I. Ltd., Dyestuffs Division, for the supply of the samples of pure 1-naphthylamine-6-sulphonic acid and 1-naphthylamine-7-sulphonic acid, and to the Government Chemist for permission to publish this paper.

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The Qualitative Analysis of Dilute Aqueous Solutions of Thiols and Thioethers by Thin-layer Chromatography

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The preparation of solid derivatives from thiols and thioethers was investigated in order to discover a method of concentrating extremely dilute solutions of these compounds and to analyse them by thin-layer chromatography.

It was found that thiols could be separated from thioethers by reaction with phenylmercury acetate and a method was developed for the analysis of mixtures of thiols by the thin-layer chromatography of the phenylmercury mercaptides formed by this reaction.

A method used by earlier workers for the preparation of dialkylsulphinimines from thioethers and chloramine-T was improved to make it suitable for use with microgram amounts of thioethers, and the derivatives were separated successfully by thin-layer chromatography.

EARLIER workers collected thiols and thioethers by precipitation from solutions of mercury(II) cyanide and mercury(II) chloride, respectively, and decomposed the precipitated mercaptides and addition compounds to prepare other derivatives. Thiols were converted into 2,4-dinitrophenyl thioethers,[1] and thioethers were allowed to react with chloramine-T to give sulphinimines.[2] The derivatives were then analysed by paper chromatography.

These methods when tested to determine their suitability for use in research on food flavours were found to be inadequate for the following reasons. The aspiration of vapours from aqueous solutions into 3 per cent. w/v solutions of mercury(II) cyanide or chloride in the manner described by Folkard and Joyce[1] failed to give precipitates of mercaptides or mercury(II) chloride - thioether addition compounds when only a few micrograms of thiol were present, presumably because such small amounts of mercury derivatives are soluble in water. The conversion of thiols into 2,4-dinitrophenyl thioethers proceeded satisfactorily only when the amount of reagents did not exceed three times the theoretical amounts which, in the absence of weighable precipitates, were unknown. Excessive losses occurred in decomposing micromolar amounts of mercury derivatives.

Attempts to analyse mixtures of mercury mercaptides and mercury(II) chloride - thioether addition compounds by thin-layer chromatography failed.

Sulphinimines could be formed directly by the addition of chloramine-T to dilute aqueous solutions of thioethers, but a reagent was required that would react quantitatively with thiols in extremely dilute aqueous solutions to give derivatives that could be separated by thin-layer chromatography.

Alkyl and arylmercury salts seemed likely to have the required properties. Sachs[3] prepared compounds of the type RHgSR′ by reactions between alkyl or arylmercury salts and sodium mercaptides in ethanol or acetone. Karash[4] prepared analogous compounds from alkylmercury hydroxide or its halides and mercapto-fatty acids.

Several organomercury salts such as *p*-mercuribenzoic acid[5] have been used as reagents for the titrimetric or spectrophotometric determination of thiol groups in proteins, but the use of the products of these reactions for the separation and identification of thiols has not been reported previously.

Experimental

Phenylmercury mercaptides—

Phenylmercury chloride, ethylmercury chloride, methoxyethylmercury chloride and ethoxyethylmercury chloride were tested as reagents for thiols and thioethers.

The methoxy and ethoxy compounds were unsuitable because they were insufficiently soluble, even in ethanol. Phenylmercury chloride and ethylmercury chloride were insoluble in water but reacted with thiols in ethanolic solution. The thiols did not react completely, however, even in the presence of an excess of reagent. No reaction occurred with thioethers and little, if any, with disulphides.

The ethylmercury mercaptides and phenylmercury mercaptides were placed on silica-gel plates; the chromatograms were developed with a mixture of 1 volume of ether and 9 volumes of light petroleum (b.p. 40° to 60° C), and the spots were located by spraying with dithizone solution.

The chromatographic properties of the ethylmercury and phenylmercury derivatives were similar. In both instances the ethanethiol and hexanethiol derivatives were separated easily.

This method seemed promising but the insolubility of the reagents in water and the failure of the reaction to proceed to completion were serious disadvantages. Phenylmercury acetate was tried because it is appreciably soluble in cold water (about 0·4 per cent.). Solid phenylmercury acetate was added to about 0·01 M aqueous solutions of ethanethiol and hexanethiol. As the reagent dissolved the solution became turbid and the odour disappeared. The derivatives were extracted with ethyl acetate and examined by thin-layer chromatography as before. In addition to spots of mercaptan derivative a small spot at R_F 0·2 appeared in all chromatograms, including reagent blanks, and seemed to be caused by an impurity in the reagent. The reagent remained at the origin.

Phenylmercury acetate was added to an aqueous solution of ethanethiol, which reacted completely, and the water was distilled off under reduced pressure; the distillate was tasteless and gave no reaction with dithizone.

Hydrogen sulphide is known to be formed during the cooking of many foods. Precipitates, varying in colour from yellow through red to black, formed when hydrogen sulphide was mixed with different portions of phenylmercury acetate solution. These were presumably complex addition compounds. The supernatant solutions gave chromatograms containing only the spot at R_F 0·2, which was larger than that produced by an extract from an untreated solution of phenylmercury acetate.

It was also found that this spot was considerably enlarged if the solutions of phenylmercury acetate were heated.

The use of plates with extremely thin coatings gave a 5 to 10-fold increase in sensitivity, so that a spot containing $2{\cdot}5 \times 10^{-4}$ μmoles could be detected. Thin plates also gave separate spots when the solvent front had moved only 5 cm.

Preparation of sulphinimines—

Leaver and Challenger[2] described the preparation of dialkylsulphin-*p*-toluenesulphonimines from thioethers and chloramine-T and their separation by paper chromatography.

They prepared sulphinimines from aqueous solutions of thioethers by saturating the solution with chloramine-T and extracting the reaction products with chloroform. In the present work dichloromethane was used instead of chloroform because it is less toxic and showed less tendency to form emulsions with saturated solutions of chloramine-T.

This procedure extracted an appreciable amount of chloramine-T which gave a large spot at the origin of the chromatograms, but it was found that both the reagent and the sulphinimines were retained if the extract was passed through a column of alumina and that the sulphinimine alone could then be eluted with ethanol.

When small volumes of millimolar solutions of thioethers were used good thin-layer chromatograms were obtained, but when larger volumes of more dilute solutions were used, spots consisting of decomposition products and other impurities from the chloramine-T appeared. Experiments with a 0·2 per cent. aqueous solution of ethyl hexyl thioether showed that the sulphinimines could be obtained virtually free from impurities without any noticeable reduction in the yield by adding only enough chloramine-T to form a 1 per cent. solution. Blank experiments with saturated solutions of chloramine-T indicated that the amount of impurities increased rapidly with time.

THIN-LAYER CHROMATOGRAPHY OF SULPHINIMINES—

Both alumina and silica gel gave good separations but silica gel gave smaller spots.

The chromatograms were developed with mixtures of ethyl acetate and light petroleum (b.p. 60° to 80° C). The R_F values of the derivatives increased with increasing molecular weight, hence if maximum separation of the higher thioethers were of primary interest increasing proportions of light petroleum were used.

The alkyl-*p*-toluenesulphonimidosulphin-*p*-toluenesulphonimines produced by the reaction between thiols and chloramine-T[6] were examined to discover whether they could be used for the analysis of mixtures and to what extent they interfered with the identification of thioethers. Several solvent systems were tried but none could separate the derivatives of different thiols, and in all solvent systems the R_F values of thiol derivatives were much higher than those of the ethyl hexyl thioether derivative. This method could, therefore, be used to separate thiols as a class from thioethers. Earlier authors, who used paper chromatography, located the spots on their chromatograms by spraying them with acidified potassium iodide solution followed by heating to 80° C in an oven until spots of iodine appeared. This method was tried with thin-layer chromatograms, but the spots sometimes failed to appear and often faded rapidly.

When fluorescein was added to the spray reagent the spots appeared immediately without heating as white or pale green on a green background. Under ultraviolet light the spots appeared dark against a bright green fluorescent background. Spots containing 0·1 μmole, which were hardly visible by daylight, were clearly visible under ultraviolet light.

Extremely thinly coated plates bound with calcium sulphate could not be used because the aqueous spray caused the adsorbent layer to disintegrate, but when starch was used as the binder the plates could be sprayed with acidified potassium iodide and blue spots appeared on heating; this procedure gave a 100-fold increase in sensitivity.

METHODS

PREPARATION OF PLATES FOR THIN-LAYER CHROMATOGRAPHY

Normal coating—Shake 100 g of the Kieselgel G (for thin-layer chromatography, as supplied by E. Merck A.G.) with 200 ml of distilled water for 90 seconds and pour into the reservoir of a commercial plate spreader set to give a coat 500 μm thick. Coat the plates, allow them to stand at room temperature for 10 minutes and then place them in an oven at 100° C for 1 hour. Transfer the plates to a desiccator, allow them to cool and store in the desiccator.

Very thin coating, bound with calcium sulphate—Shake 10 g of silica gel (Machery Nagel GHR) with 30 ml of distilled water for 90 seconds and pour into the reservoir of a plate spreader set to give a coat 100 μm thick. Coat the plates and allow them to stand for at least 10 minutes before placing them in the oven. Heat at 100° C for 1 hour.

Very thin coating, bound with starch—Shake a mixture of 9·5 g of silica gel (Machery Nagel Kieselgel N-HR) and 0·5 g of soluble starch (Lintner's) with 30 ml of distilled water until well mixed and pour into the reservoir of a plate spreader set to give a coat 100 μm thick. Proceed as described above.

THIN-LAYER CHROMATOGRAPHY OF MIXTURES CONTAINING THIOLS

REAGENTS—

Phenylmercury acetate—Microanalytical-reagent grade.

Developing solvent—One volume of ether to 9 volumes of light petroleum (b.p. 40° to 60° C).

Spray reagent—Add 1 drop of concentrated hydrochloric acid to 50 ml of a 0·1 per cent. w/v solution of dithizone in ethyl acetate.

PROCEDURE—

Add an excess of phenylmercury acetate to the solution. If any compounds other than thiols that may be present are of no interest, extract with ethyl acetate and evaporate the extract to a suitable concentration. The evaporation or distillation of excess of solvent must be carried out at temperatures not exceeding room temperature. If it is necessary to preserve compounds of other classes, distil the aqueous solution containing phenylmercury acetate

to dryness under reduced pressure (not greater than 2 torr). The design of the still will, of course, depend upon the volatility of the compounds present. Dissolve the residue in ethyl acetate.

Spot the ethyl acetate solution on the plate and develop with the ether - light petroleum mixture in the usual manner. Remove the plate from the tank, allow the solvent to evaporate and spray. Phenylmercury mercaptides will appear as orange spots. The background is often grey or brown at first.

Analysis of thioethers by thin-layer chromatography

Reagents—

Aluminium oxide—Brockman activity I.
Chloramnie-T.
Ethanol, 96 per cent.—Analytical-reagent grade.
Dichloromethane—Analytical-reagent grade.
Developing solvent—Ethyl acetate, which may be diluted with up to 50 per cent. of light petroleum if necessary to improve the separation of higher thioethers.
Spray reagent—To 50 ml of a 1 per cent. w/v aqueous solution of potassium iodide add 2 drops of concentrated hydrochloric acid. If 500-μm plates bound with calcium sulphate are to be used, add 2 drops of a saturated aqueous solution of fluorescein (sodium salt). This reagent must be prepared afresh daily.

Procedure—

Add about 100 per cent. excess of chloramine-T to the thioether solution. If the approximate concentration of thioethers is not known, add sufficient reagent to remove any odour of thioether or, alternatively, add sufficient to give a 1 per cent. w/v solution. Allow the mixture to stand for 10 minutes and extract with three portions of dichloromethane. A few drops of ether and a little sodium sulphate may be added to prevent the formation of emulsions.

Pass the extracts through a column containing 1 g of aluminium oxide. Reject the dichloromethane and elute the sulphinimines with 5 ml of ethanol.

Spot the ethanolic solution of sulphinimines on to silica plates prepared in the manner described above and develop the chromatogram in the usual way. When the development is complete, remove the plate from the tank and wait until the solvent has evaporated completely before spraying.

Spray starch-bound plates with acidified potassium iodide solution and heat in an oven at 80° to 100° C until the spots appear. Spray other plates with the fluorescein reagent and view them under ultraviolet light. The spots will appear dark on a green fluorescent background.

RESULTS AND DISCUSSION

The methods developed in this work make it possible to separate thiols and thioethers from each other and from other classes of compounds, and to prepare simultaneously derivatives that can be used to identify individual compounds by thin-layer chromatography. If only one of the two classes is of interest the other can be ignored because there is no mutual interference.

The well known difficulty of preparing pure specimens made it impossible to determine whether disulphides react with phenylmercury acetate, but if they do so it is only to a small extent. Disulphides react with chloramine-T to give thiol derivatives.

These methods have been applied successfully to the extremely dilute solutions obtained by the distillation of volatile flavouring constituents from foods and provide a simple method of concentrating such solutions.

The original compounds can be liberated from phenylmercury mercaptides by dithizone and from sulphinimines by tin and hydrochloric acid.

The R_F values of phenylmercury derivatives of some normal alkanethiols were determined and are as follows: methanethiol 0·50; ethanethiol 0·58; propanethiol 0·64; and hexanethiol 0·72.

Thiols and thioethers in solution together were analysed with one portion of solution. The thiols were first converted into their phenylmercury derivatives, thioethers were

then removed by distillation under reduced pressure and chloramine-T was added to the distillate and the sulphinimines were isolated in the manner described above.

The order of separation achieved for the lower thioethers is demonstrated by the R_F values, which were determined with ethyl acetate: dimethyl sulphinimine 0·08; diethyl sulphinimine 0·12; ethyl propyl sulphinimine 0·18; dipropyl sulphinimine 0·31; methyl hexyl-sulphinimine 0·35; and ethyl hexyl sulphinimine 0·39.

Both sets of R_F values were determined with 500-μm plates, but the thinner plates give similar values.

As dithizone reacts with phenylmercury mercaptides to give the orange phenylmercury dithizonate, it is probable that the mercaptides could be separated, either on preparative plates or small columns and determined colorimetrically. This possibility will be investigated later.

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Chlorobenzoic Acids and Derivatives: Analysis of Mixtures by Thin-layer Chromatography*

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Thin-layer chromatographic (silica gel G) R_F values were obtained for the three isomeric monochlorobenzoic and two dichlorobenzoic acids. The *p*-bromophenacyl esters of benzoic, *o*-, *m*- and *p*-chlorobenzoic, and 3,4-dichlorobenzoic acids were prepared and R_F values obtained. The esters of benzoic, *m*-chlorobenzoic and 3,4-dichlorobenzoic acids were separated from the ternary mixture by thin-layer chromatography. When 2,4-dinitrophenylhydrazine in hydrochloric acid solution reacted with the esters spotted at the origin, subsequent thin-layer chromatography gave characteristic two-spot patterns for each, and this facilitated recognition of the type of mixture of monochlorobenzoic acids. An explanation is given of the formation of two hydrazones from each ester.

In an attempt to detect and identify components in mixtures of benzoic, mono- and dichlorobenzoic acids obtained when benzoic acid is chlorinated in the presence of a transition metal salt, the technique of thin-layer chromatography was applied. Previously, several non-halogenated aromatic acids,[1,2,3,4] including benzoic acid,[4] had been separated and identified by thin-layer chromatography, and the method had successfully been applied to chlorinated cresols and xylenols[5] and substituted tri-iodobenzoic acids.[6] In addition, thin-layer chromatography was used to separate and identify the *o*-, *m*- and *p*-chloronitrobenzenes.[7]

In this paper procedures are described for the identification of benzoic, *o*- and 3,4-dichlorobenzoic acids as well as *m*- and *p*-chlorobenzoic acids; the latter two, however, are not separated from one another. As the acids were close together on the chromatograph two varieties of derivative were synthesised. The first were the *p*-bromophenacyl (PBP) esters and the second were the 2,4-dinitrophenylhydrazones of the PBP esters (DP) formed *in situ* by reaction between the PBP esters and 2,4-dinitrophenylhydrazine (DNP) in hydrochloric acid solution. Thin-layer chromatography of the former gave enhanced separations of benzoic and *o*-chlorobenzoic acids from *m*-, *p*- and 3,4-di-chlorobenzoic acids, while the latter derivatives gave twin-spot patterns that, for *o*- and *p*-chlorobenzoic acids, were unique and made possible a "fingerprint" identification of those acids alone and in mixtures.

Experimental

Reagents—

Dioxan—This was dried over calcium hydride.

2,4-Dinitrophenylhydrazine (DNP)—This was Eastman "white label" grade and, as supplied, had a melting-point in the range 198° to 201° C (decomposes) (corrected). Recrystallisation from dioxan[8] gave red crystals with melting-point 200·5° to 201·0° C (decomposes) (corrected). Both non- and recrystallised DNP gave DP products with identical chromatographic behaviour. A DNP solution was prepared by stirring a 0·50-g sample with 100 ml

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of 2 N hydrochloric acid and allowing it to stand overnight; the yellow supernatant liquid was filtered and stored for use during the subsequent 2 to 3 days.

p-*Bromophenacyl esters*—These were prepared by a standard procedure[9] with the following changes: 100 to 400 per cent. more water and 100 to 300 per cent. more 95 per cent. ethanol were used during the esterification step because of the low solubilities of the sodium salts.

Other solvents, aromatic acids, and the *p*-bromophenacyl bromide were of commercially available, reagent-grade quality and were used without further purification.

CHROMATOGRAPHIC PROCEDURE—

Coat 20 × 20-cm glass plates with a 0·25-mm layer of silica gel G. Allow the plates to air-dry for 30 minutes, then dry in an oven at 110° to 115° C for 30 minutes and store in a desiccator over calcium chloride.

Fill a developing tank (about 21 × 9 × 20 cm) to a depth of 1 cm with the appropriate solvent—

Type of compound	Solvent	Equilibrium procedure
Acid	Benzene - dioxan - glacial acetic acid (90 + 25 + 4 v/v) (A)	I
Ester	Xylene saturated with formamide[5] (B), chloroform or benzene	I
Hydrazone	B or benzene	II

Procedure I—Place cover on tank and allow it to stand overnight.

Procedure II—Same as I but remove cover for 10 minutes just before development. After setting the plate in the tank, replace the cover. The degree of saturation of the atmosphere surrounding the chromatoplate has a significant effect on the R_F values of the hydrazones formed *in situ*. When the solvent was benzene, procedure II gave the best separations, and a description of the other procedures is given in the Discussion; corresponding R_F values appear in Table III.)

Dissolve the acids, esters or mixtures of each in anhydrous ether - benzene - light petroleum (4 + 3 + 1 v/v) at 30° to 60° C and concentrations of 15 to 30 mg ml^{-1}. Apply 20 to 60-μg amounts to the adsorbent layer at 1·5 cm from the bottom of the plate by micropipette.

Prepare hydrazones of the PBP esters *in situ* as follows: apply 60 to 150 μg of ester to the layer by successive application of small volumes of solution to the same spot area, permitting the solvent to evaporate between each application. Then apply DNP solution on top of the ester spots in 1 to 5 applications of 5 × 10^{-4} ml each. Allow the plate to stand for 10 to 15 minutes in the desiccator to ensure complete reaction. (Yellow hydrazones appear almost immediately and most parts of the reactions may be assumed to be instantaneous.)

At room conditions, place the plate in the tank and allow development to proceed until the solvent front has advanced 15 to 18 cm. Remove the plate from the tank and place it in a fume hood for 30 minutes to remove volatile solvents. (When solvent system A is used, following the air-drying, heat the plate in an oven at 110° to 115° C for 1·5 to 2 hours to complete removal of the acetic acid.)

The spots were made visible as follows.

Acids—Spray the plate with either a 0·1 per cent. solution of bromocresol green in butanol or a 1 per cent. solution of bromocresol green in 90 to 95 per cent. ethanol. Both procedures give yellow spots on a background that is initially blue and becomes green on standing.

PBP esters—Spray the plate with 0·25 to 0·50 per cent. DNP in 2 N hydrochloric acid; yellow spots on a white background are obtained.

DP hydrazones—The yellow spots on a white background do not require to be made visible. However, the less intense spots are rendered more easily visible by illumination with an ultraviolet lamp fitted with a long-wave filter. Thus viewed, the spots appear brown on a blue background.

Results and discussion

Chlorobenzoic acids—

Table I lists the R_F values obtained for the three mono- and two di-chlorobenzoic acids chromatographed by solvent system A, which gave the largest differences in R_F value of the solvents tested. However, this solvent failed to separate the *o*- from the *m*- and *p*-isomers in a ternary mixture.

PBP esters—

Conversion of the chlorobenzoic acids into the PBP esters and subsequent thin-layer chromatography gave considerable improvement in separation. As is seen from Table II, development with benzene separates five compounds into three groups. The first contains benzoic and *o*-chlorobenzoic acids; the second contains the *m*- and *p*-isomers; 3,4-dichlorobenzoic acid forms an ester that separates cleanly from the other four derivatives. Thus, in a mixture containing the major products from chlorination of benzoic acid by many procedures (benzoic, *m*- and 3,4-di-chlorobenzoic acids) complete separation can be achieved.

Table I

R_F values for benzoic acid and some chlorobenzoic acids by thin-layer chromatography on silica gel G

Solvent equilibration procedure I, solvent system A, development time 90 minutes

Acid	$R_F \times 100$
Benzoic	80
o-Chlorobenzoic	76
m-Chlorobenzoic	78
p-Chlorobenzoic	79
2,4-Dichlorobenzoic	75
3,4-Dichlorobenzoic	78

Table II

R_F values for the *p*-bromophenacyl esters of benzoic and some chlorobenzoic acids by thin-layer chromatography on silica gel G

Solvent equilibration procedure I

	$R_F \times 100$		
Solvent	Chloroform	B	Benzene
Development time, minutes	60	70	65
Acid			
Benzoic	—	—	40
o-Chlorobenzoic	72	38	43
m-Chlorobenzoic	73	44	51
p-Chlorobenzoic	73	42	49
3,4-Dichlorobenzoic	—	—	57

DNP hydrazones of the PBP esters—Thin-layer chromatography of the reaction products formed *in situ* gave two spots for each ester treated with DNP. In each reaction, the faster component had a more intense spot. Fig. 1 shows the characteristic patterns obtained by thin-layer chromatography of these derivatives of benzoic and four chlorobenzoic acids. The relative positions of upper and lower spots for each provide useful clues to their identification, with the exception of *m*-chloro and 3,4-dichlorobenzoic acid DP derivatives, which displayed similar chromatographic behaviour with the two solvents tried. R_F values are given in Table III.

The effects on resolution of varying the degree of saturation with solvent in the developing tank atmosphere may be noted by study of R_F values for benzene in Table III. (Equilibration procedure III: the tank was lined with Whatman No. 1 filter-paper and allowed to stand overnight with solvent. Procedure IV: as II except that the cover was removed for 5 minutes before use.) The following are some differences in R_F value between *o*- and *m*-chlorobenzoic acid derivative analogues: Procedure II gave 8 while IV and III gave 7 and 6, respectively, for the upper spots; for lower spots, II gave 5 and the other two procedures (III and IV) each gave 3. A similar superiority in separation was exhibited by Procedure II for the *m*- and *p*-isomers: 2 and 3 for upper and lower spots, respectively, while the average difference for each of the other procedures was 1 for both upper and lower spots.

TABLE III

R_F VALUES FOR THE TWO MAJOR PRODUCTS OF THE *in situ* REACTION BETWEEN DNP AND THE *p*-BROMOPHENACYL ESTERS OF BENZOIC AND SOME CHLOROBENZOIC ACIDS BY THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G

Acid	Solvent	Solvent equilibration procedure	$R_F \times 100$ Spot position upper	$R_F \times 100$ Spot position lower	Development time, minutes
Benzoic	B	I	35	19	145
o-Chlorobenzoic	B	I	33	19	145
m-Chlorobenzoic	B	I	42	24	145
p-Chlorobenzoic	B	I	43	23	145
3,4-Dichlorobenzoic	B	I	44	25	145
Benzoic	Benzene	II	58	36	145
o-Chlorobenzoic	Benzene	II	54	37	145
m-Chlorobenzoic	Benzene	II	62	42	145
p-Chlorobenzoic	Benzene	II	64	39	145
Benzoic	Benzene	III	29	17	70
o-Chlorobenzoic	Benzene	III	29	19	70
m-Chlorobenzoic	Benzene	III	35	22	70
p-Chlorobenzoic	Benzene	III	36	21	70
3,4-Dichlorobenzoic	Benzene	III	35	21	70
Benzoic	Benzene	IV	51	30	160
o-Chlorobenzoic	Benzene	IV	49	32	160
m-Chlorobenzoic	Benzene	IV	56	35	160
p-Chlorobenzoic	Benzene	IV	57	33	160
3,4-Dichlorobenzoic	Benzene	IV	56	36	160

Lower spot patterns arising from the three binary and the ternary mixtures of *o*-, *m*- and *p*-isomers of the DP derivatives were studied over the binary mixture composition range of from 1:3 to 3:1 and of 1:1:1 for the ternary mixture. Fig. 2 shows a chromatoplate on which the composition of binary mixtures was 1:1. Each mixture gave rise to a spot pattern that provides a clue to identification of its components, but this was not so for the upper spot patterns in which the *o*- + *m*- could not be distinguished from the *o*- + *p*-derivative mixtures.

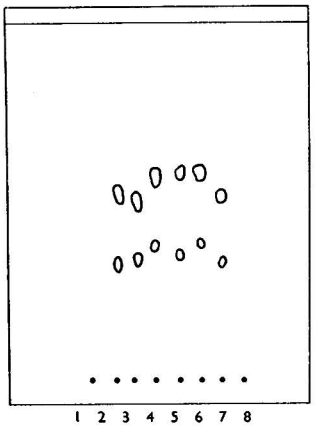


Fig. 1. Thin-layer chromatographic plate (silica gel G) of the *in situ* formed DNP hydrazones of the PBP esters of (2 and 7) benzoic, (3) *o*-, (4) *m*-, (5) *p*-, and (6) 3,4-dichlorobenzoic acids developed with benzene. (1 and 8) reagent - DNP in hydrochloric acid solution

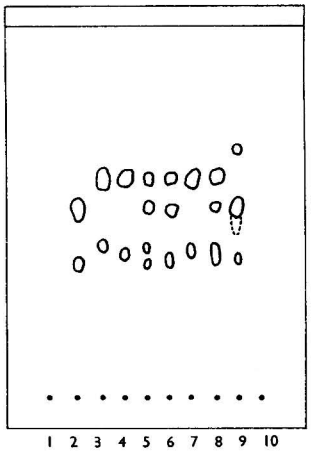
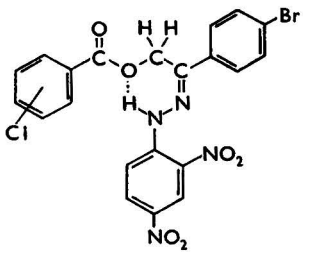


Fig. 2. Thin-layer chromatogram of *in situ* formed DNP hydrazones of PBP esters of some chlorobenzoic acids: (2) *o*-; (3) *m*-; (4) *p*-; (5) *o*- + *m*- (1:1); (6) *o*- + *p*- (1:1); (7) *m*- + *p*- (1:1); (8) *o*- + *m*- + *p*- (1:1:1); (9) recrystallisation fraction from (8) (see text); (1 and 10) DNP in hydrochloric acid solution. Adsorbent, silica gel G; solvent, benzene

A low intensity spot (uppermost spot, position 9, Fig. 2), suspected to result from hydrolysis of the PBP ester, was occasionally observed on these plates.

Formation of two products when the PBP esters are treated with DNP in acid medium on the thin-layer chromatographic adsorbent can be explained by reference to the two geometrical isomers of the DNP hydrazones, *syn-* and *anti*-isomers, **I** and **II**, are shown. The possibility for internal hydrogen bonding for one isomer is also indicated. The structures shown result from reaction of the DNP with the ketonic oxygen. Hydrazones are formed only slowly from esters with a parent alcohol higher than ethyl.[10] Thus there would seem to be only a slight possibility for the formation of hydrazone isomers by reaction of DNP with the π-bonded oxygen of the ester group.



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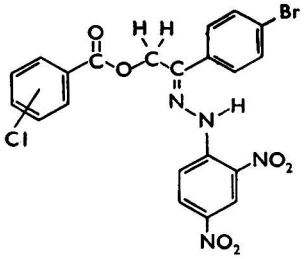

II

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Reversed-phase Extraction Chromatography with Paper and Columns Supporting an Extractant Selective for Copper*

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Supports treated with a commercial mixture of substituted oximes (Lix-64) are shown to separate copper effectively from several selected cations, namely, iron(II), iron(III), cobalt, nickel, manganese(II), chromium(III), molybdenum, tungsten and vanadium(IV), the eluents being ammonium sulphate, ammonium chloride, sodium chloride or ammonium sulphate *plus* ammonium hydrogen difluoride solutions.

The R_F spectra of the various ions are presented, when eluted on paper treated with the extractant, as functions of the pH of the eluting ammonium sulphate or ammonium chloride solutions.

Representative separations are reported, either on paper or on cellulose powder columns treated with the extractant.

The use of organic extractants as the stationary phases for the separation of inorganic substances has often proved to be a powerful analytical tool. The selectivity features of many organic compounds studied for liquid - liquid extraction processes, coupled with the multi-stage character of chromatography, make it possible to obtain many interesting separations. Such a technique, which may be termed reversed-phase extraction chromatography,[1] has rapidly gained popularity during the last 12 years, and more than three hundred works on the subject have been published.

Liquid - liquid extraction techniques are now currently used for the recovery of copper from industrial waters.[2,3] Mixtures of extractants, selective for copper, are available on the market, their use being patented and their chemical composition kept secret by the manufacturer. One of these mixtures is a liquid compound sold under the trade name of Lix-64. It is defined by the manufacturer as a water-soluble mixture of substituted oximes, which form water-insoluble complexes with metallic cations, principally copper; its peculiar feature is the reported ability to extract copper from aqueous solutions having pH values down to 1·4 and to allow for the easy separation of copper from iron(III).[3,4] The α-hydroxyoximes (and the other mixture of oximes available on the market, Lix-63[5]) are able to extract copper only from solutions with pH values higher than 3.[5,6] The 2-hydroxybenzophenoximes extract copper at pH values as low as 1·4, and effectively separate it from iron(III).[7] In this instance, however, the rate of copper extraction is slow, but it can be increased by the addition of α-hydroxy aliphatic oximes without any serious effect on the iron(III) - copper separation ability of the original aromatic compound; this feature is still unexplained, and is reported in a patent owned by the firm that produces Lix-64[7]; we believe, therefore, that Lix-64 consists of one of such mixtures, probably 2-hydroxy-5-dodecylbenzophenone oxime and 5,8-diethyl-7-hydroxy-6-dodecanone oxime.

We have used Lix-64 as the stationary phase in reversed-phase extraction chromatography on paper and on columns. The satisfactory results obtained are reported in the present work.

Experimental

Lix-64 is produced by General Mills (Kankakee, Illinois, U.S.A.), and was used as supplied. Specimens from three different stocks were used, no difference among stocks being found for our purposes. All other chemicals were of analytical-reagent grade, mainly produced by C. Erba (Milan).

* Part of this work was included in a paper presented at the Second SAC Conference 1968, Nottingham.

For paper experiments, Whatman No. 1 CRL/1 type paper was used. It was impregnated with Lix-64 by dipping for 1 minute in 20 per cent. solutions of the extractant in cyclohexane, previously equilibrated with twice their volume of a solution with the same composition as the eluent to be used, its pH being always 3 as preliminary experiments had shown that the pH of equilibration had no effect on the R_F values obtained.

After drying with a current of warm air, the paper was ready for elution. The spots of the various ions were applied by using about 0·02 ml of 0·1 N solutions. The elutions were carried out in a glass device described in previous work[8]; the positions of the spots of the various metals were detected by means of suitable spray reagents,[9,10] except for iron(III) and copper, which form coloured complexes (brown - grey and dark yellow - green, respectively) with the extractant and are readily visible on the treated paper.

Columns were made with Whatman standard grade cellulose powder: 20 g of cellulose, previously dried in an oven at 40° C for 1 hour, were mixed with 20 ml of Lix-64 diluted with 70 ml of cyclohexane. The slurry was stirred for about 60 minutes, and then filtered through paper. The treated cellulose was pressed between two filter-paper sheets and was kept in an oven at 40° C for 2 hours; it was finally crushed in a mortar and packed into glass tubes to obtain the required column beds.

The effluents were collected in fractions by means of suitable apparatus, and the various fractions were analysed by colorimetric techniques for the ions of interest.

Results and discussion

Paper-chromatographic experiments—

The reaction responsible for the extraction of copper with Lix-64 cannot be exactly defined because of the uncertain composition of the extractant itself. It can be affirmed, however, that the over-all process is a sort of cation exchange, and the extraction strongly depends on the acidity of the aqueous solution.[4] Therefore, the effect of the pH value of the eluent on the R_F values of selected cations spotted on paper treated with Lix-64 was investigated.

Copper, iron(II), iron(III), cobalt, nickel, manganese(II), chromium(III), molybdenum(VI), tungsten(VI) and vanadium(IV) were the ions considered. As most results reported in the literature for the extraction with Lix-64 are referred to ammonium sulphate solutions,[4] 0·5 M solutions of this salt were first used as the eluents, their pH being varied by the addition of ammonia solution or sulphuric acid.

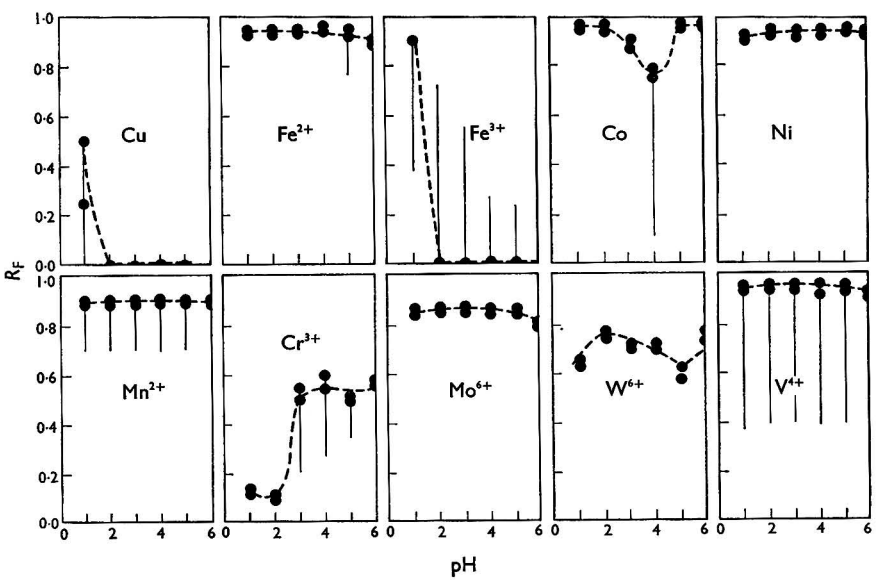

Fig. 1. R_F values for selected ions eluted with 0·5 M ammonium sulphate solutions at different pH on paper treated with 20 per cent. Lix-64 in cyclohexane. Vertical lines refer to tails and streaks encountered

The R_F values obtained are shown in Fig. 1 as functions of the pH of the eluting solutions. It appears that many tailing and streaking phenomena take place only, as shown later, when ammonium sulphate solutions are used as the eluent.

The results given in Fig. 1, however, are comparable with those obtained by liquid - liquid extraction. When Lix-64 solutions are contacted with ammonium sulphate solutions of pH 2, it is reported[4] that copper is "strongly extracted," iron(III) "slightly extracted," molybdenum and vanadium(IV) "very slightly extracted" and, finally, tin, iron(II), cobalt and nickel are not extracted at all. The agreement with the chromatographic results is apparent, except for iron(III), which in our method is rather firmly retained by the stationary phase. When 0·5 M ammonium chloride solutions were used as the eluent, sharply defined spots were obtained for the ions tested, exceptions being the streaks again found for iron(III). The results obtained are given in Fig. 2. A comparison with Fig. 1 shows that the general behaviour of each ion is similar in both instances: nevertheless, when chloride is used instead of sulphate, a lower retention is displayed by the stationary phase for tungsten and especially for chromium at a low pH. Cobalt and copper, on the other hand, are retained slightly more strongly when chloride is used.

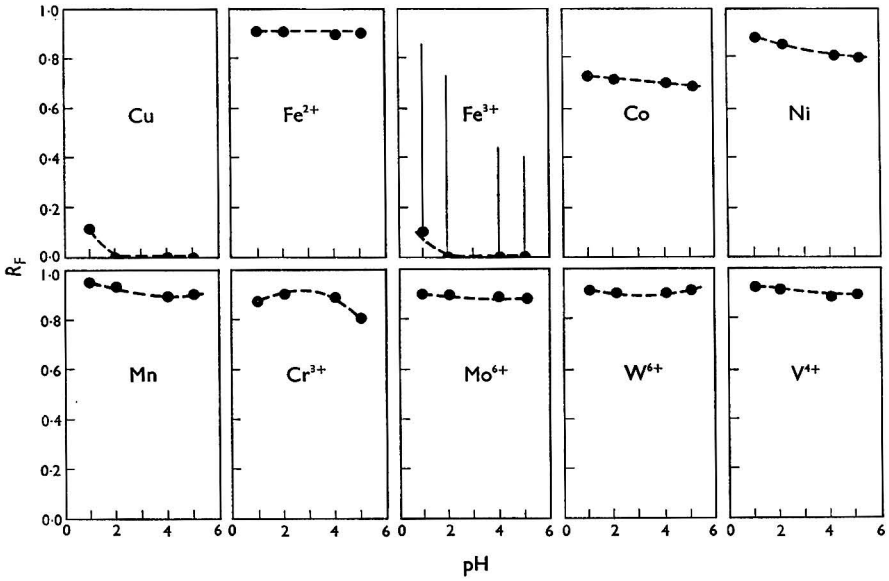

Fig. 2. R_F values for selected ions eluted with 0·5 M ammonium chloride solutions at different pH on paper treated with 20 per cent. Lix-64 in cyclohexane. Vertical lines refer to streaks encountered

We do not attempt to explain the differences found between the two sets of results but make the following observations. The rather noticeable tailing effects found with ammonium sulphate may be imputed to the slight solubility of the adducts in the aqueous phase. Although these complexes are practically insoluble, as the literature on liquid - liquid extraction shows, under conditions in which the ratio of the aqueous to the organic phase is extremely high, as in chromatography, a slight leaching cannot be excluded. Furthermore, partial solubility of the adducts in ammonium sulphate was confirmed by other experiments described elsewhere.[11]

An interesting feature is found in Fig. 1 for cobalt, which displays peculiar behaviour when the pH of the eluent is 4. Analogous behaviour has been reported for zinc when extracted from sodium sulphate solutions by 5,8-diethyl-7-hydroxy-6-dodecanone oxime[6]; in this example, maximum extraction (which does not exceed 5 per cent.) is reported at a pH of about 6·5. It is worth mentioning that cobalt does not show this phenomenon when eluted on Lix-64 with ammonium chloride solutions (Fig. 2).

Some experiments were carried out with 0·05 M solutions of ammonium sulphate, ammonium chloride and sodium chloride. The R_F values obtained were practically identical with those found with the more concentrated solutions, several tailing effects being again found when ammonium sulphate was used. Sodium chloride solutions gave the same results as ammonium chloride.

It is apparent that when Lix-64 treated paper is eluted with solutions of pH $\geq$ 2, copper can be effectively separated from all the other ions tested except iron(III). To achieve the iron(III) - copper separation, small amounts of ammonium hydrogen difluoride were added to the ammonium sulphate eluting solutions, on the basis of indications reported for liquid - liquid extraction.[4] The best results were obtained with 0·5 M ammonium sulphate solutions 0·18 M in ammonium hydrogen difluoride; although the pH of this solution is about 3·8, iron moves with the eluent front, but copper does not move from the starting point. The same results were obtained with more dilute eluting solutions, provided that the same ratio was maintained between sulphate and difluoride concentrations.

COLUMN-CHROMATOGRAPHIC EXPERIMENTS—

The first column made with cellulose powder treated with Lix-64 was 12 mm in diameter and 27 cm long. Once the column was prepared with the dry powder, it was eluted with 1 litre of 0·5 M ammonium sulphate solution at pH 3, both to condition the column itself and to drain away the excess of organic phase. The capacity for copper of the total bed was 176 mg, as obtained by loading the column from a 1 mg ml^{-1} copper solution in 0·5 M ammonium sulphate at pH 3. To work out the best conditions for the recovery of retained copper, 2 mg of copper were loaded on the column from 2 ml of 0·5 M ammonium sulphate solution, and were eluted with sulphuric acid at various concentrations. The best results were obtained with 2 to 4 M sulphuric acid; most of the copper was recovered as a sharp peak, tailing, however, being unavoidable because of the excessive length of the column itself.

Several separations were then performed with 0·5 M ammonium sulphate solution eluent at pH 3. When the column was loaded with 2 mg of copper and 10 mg of any one of the ions not retained by Lix-64 (*i.e.*, all the ions tested except iron(III)), the elution peak of the unretained ion had its maximum falling between 17 and 19 ml of effluent. The volume of the feeding solution was 6 ml: all peaks obtained were satisfactorily sharp, except that for cobalt, which showed a slight tendency to tail.

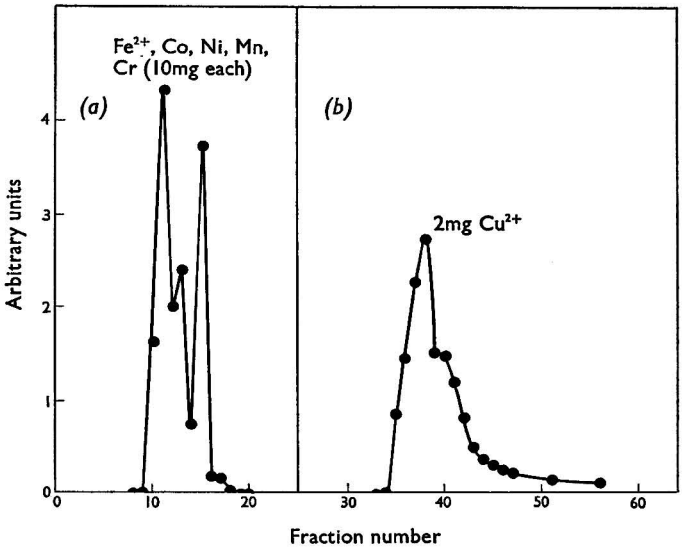

Fig. 3. The separation of copper from iron(II), cobalt, nickel, manganese and chromium on columns of cellulose powder treated with Lix-64: (*a*) 0·5 M ammonium sulphate at pH 3; (*b*) 2 M sulphuric acid. Column dimensions, 12 × 270 mm; flow-rate, 1 ml minute^{-1}. The effluent was collected in 2-ml fractions

When copper was separated from mixtures of several ions, clear separations were again obtained. A representative separation is shown in Fig. 3. The volume of the feeding solution was 10 ml; as indicated in Fig. 3, the amounts involved were 2 mg for copper and 10 mg for each of the other cations, namely iron(II), cobalt, nickel, manganese and chromium. The eluent was 0·5 M ammonium sulphate at pH 3. Only copper was retained, and all other ions were completely recovered within the first 40 ml of effluent; copper was then eluted with 2 M sulphuric acid. The eluent flow-rate was in both instances 1 ml minute^{-1}.

In addition to ammonium sulphate, 0·5 M ammonium chloride and 0·5 or 0·05 M sodium chloride solutions were used as the eluents, and good results were also obtained.

A shorter column was then prepared, 13 mm in diameter and 3 cm long. After the preliminary elution to drain the excess of extractant, the capacity for copper was determined as described for the longer column; a capacity of 24 mg of copper was found for the whole bed, in agreement with the results obtained with the latter column.

With the shorter column, copper was separated from iron(III), the eluent for iron being 0·5 M ammonium sulphate containing 1 per cent. of 0·18 M ammonium hydrogen difluoride. A representative separation is shown in Fig. 4: copper is eluted with 3 M sulphuric acid, and is totally recovered within 20 ml of effluent as in this instance the tail of its peak is rather small because of the shortness of the column.

As for the longer column, eluent compositions other than that already reported were used, namely 0·5 M ammonium chloride *plus* 0·18 M ammonium hydrogen difluoride and 0·05 M sodium chloride *plus* 0·018 M ammonium hydrogen difluoride; effective separations of copper from iron(III) were obtained in all instances.

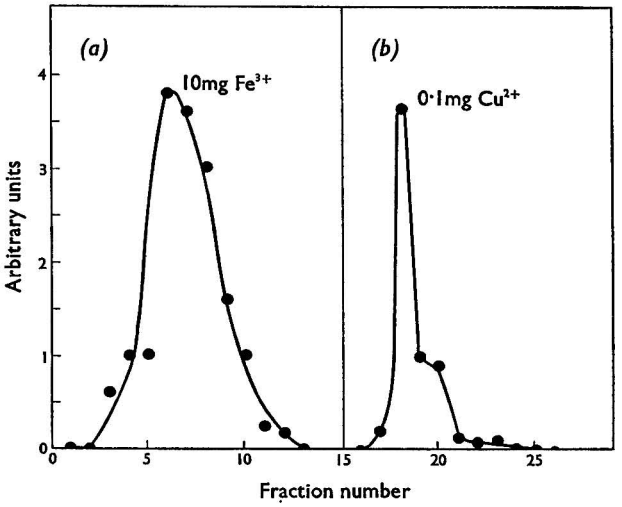

Fig. 4. The separation of copper from iron(III) on columns of cellulose powder treated with Lix-64: (*a*) ammonium sulphate - 0·18 M ammonium hydrogen difluoride; (*b*) 3 N sulphuric acid. Column dimensions, 13 × 30 mm; flow-rate, 1 ml minute^{-1}. The effluent was collected in 2-ml fractions

Figs. 3 and 4 demonstrate that good separations of copper from other ions are obtainable with columns having Lix-64 as the stationary phase. The column capacity for copper is rather high (6 mg of copper per cm^3 of bed), so that appreciable amounts of copper can be isolated. Copper is fully retained by the stationary phase from solutions with pH higher than 2 as a narrow, dark yellow - green band that is not displaced nor broadened even when several litres are percolated through the column.

Very short columns can therefore be used, from which copper is recovered as a narrow untailed peak. Some experiments have been carried out on beds 2 to 3 mm long, backed on the sintered-glass disc of Gooch crucibles: microgram amounts of copper were completely

retained from 10 to 50 ml of ammonium sulphate solutions, and were recovered within 3 to 5 ml of sulphuric acid. Even thick filter-paper discs treated with the extractant, which are easier to prepare and handle than thin cellulose powder layers, are able to retain copper from remarkably large volumes of solution, especially when 0·05 M sodium chloride is used. The use of treated thick filter-paper discs for the isolation of copper has found a useful application in the colorimetric determination of copper in dilute solutions.[11]

Conclusions

The use of Lix-64 as the stationary phase in reversed-phase extraction chromatography on paper and on columns allows copper to be effectively separated from several other ions, such as cobalt, nickel, manganese(II), chromium(III), iron(II) and iron(III).

Several advantages are obtained over the traditional analogous separation by means of anion-exchange columns.[12]

Copper is most strongly retained of all the above mentioned ions, iron(III) included; the difference between the distribution coefficient of copper and those of the other ions is large, so that sharper separations can be obtained; the eluent composition is not restricted to rather concentrated halide or halogen acid solutions; copper can be retained by short columns, when it can be recovered in small volumes of effluent.

The authors gratefully acknowledge the laboratory work of Mr. G. Cosmai and Mr. G. Marchisi.

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Book Reviews

POWDERED VEGETABLE DRUGS. AN ATLAS OF MICROSCOPY FOR USE IN THE IDENTIFICATION AND AUTHENTICATION OF SOME PLANT MATERIALS EMPLOYED AS MEDICINAL AGENTS. By BETTY P. JACKSON, Ph.D., B.Pharm., B.Sc.(Lond.), F.P.S., F.L.S., and DEREK W. SNOWDON, B.Pharm.(Lond.), M.P.S. Pp. viii + 203. London: J. & A. Churchill Ltd. 1968. Price 65s.

This atlas, produced by the principal lecturer and the senior lecturer in Pharmacognosy at the School of Pharmacy, Sunderland, is the first to be published in the English language since Greenish and Collin's "Anatomical Atlas of Vegetable Powders," which was published in 1904 and has been out of print for some time.

Although microscopy is less highly rated than formerly, it is still a useful analytical technique for the identification and authentication of plant materials. Cannabis, for example, is easily and quickly recognised by microscopy. This atlas describes ninety-four vegetable powders, arranged in their morphological groups, currently used as medicinal agents. The drawings of each powder (all by the authors and based on $\times 500$ magnification) are given 1, or in a few cases 2, whole page plates and the facing pages give detailed accounts of the vegetable fragments, emphasising the diagnostic characters by which each powder can be recognised, attention being given to the characteristics that distinguish the different powders within their morphological groups. The distinctive characters of each powder have been portrayed with accurate detail and, also, with a clarity of drawing not always found in such illustrations. A useful appendix of the microscopic mountants used in the preparation of the powders prior to drawing is provided.

Little can be found amiss with this book, except perhaps the omission of such a widely used drug as senna pod. The authors have excluded all such drugs which rarely appear on the market in the powdered or much broken form. However, this is a minor detail and should not detract from such an admirably produced book. The book is to be highly recommended to practising analysts, pharmacologists and students of pharmacy, particularly those specialising in pharmacognosy, in fact it should become a standard reference book in all analytical laboratories. The authors' hopes that this atlas will become a useful successor to Greenish and Collin should be fully realised.

M. J. R. Moss

LES MÉTHODES ANALYTIQUES DANS LES RECHERCHES SUR LE MÉTABOLISME DES MÉDICAMENTS. By JEAN HIRTZ. Pp. x + 364. Paris: Masson & Cie. 1968. Price Fr. 98.
Monographies de Pharmacie

Most students realise how far the science known, since the sixteenth century, as pharmacy has travelled since the apothecary "in tattered weeds with overwhelming brows went culling of simples" for his "needy shop where a tortoise hung, an alligator stuffed and other skins of ill-shaped fishes." Although it was not until 1721 that the term pharmacology appeared to describe the study of the effects of drugs—which are by no means simples—thoughts of this must have been in certain minds earlier, witness Lear's "Give me an ounce of civet, good apothecary, to sweeten my imagination" and, later, Tennyson's "What drug can make a withered palsy cease to shake?" Few scientists are now unaware of the incidence of the fundamental disciplines from mathematics, synthetic organic and physical chemistry and biochemistry to biology and pharmacology on that of pharmacy. The part played by that handmaiden of nearly all these disciplines—analytical chemistry—has been slow in receiving such wide recognition; rather it appears to have been silently taken for granted. Yet it is among the most exacting of disciplines, calling for high skill of hand and brain from its devotees. This comes out nowhere more clearly than in the present volume, which is devoted to the study of the work of the analyst in finding precisely what happens to the drug in the animal, mainly human, body, rather than what happens to the body itself. This study may be said to have first started 100 years ago with the fate of quinine, and during the last quarter of a century has increased logarithmically as has the number of drugs. While the metabolism of drugs is a matter of pharmacology, its elucidation is a matter for the analyst, on whose findings are based the knowledge of the mode of action and of removal of what is, in most cases, a foreign substance, which findings often point the way to the synthesis of new drugs with desired pharmacological action.

The volume is essentially a book of reference for the analyst engaged in this field; it does not enter into practical details (to which there are ample references), but surveys the relevant literature

and outlines the methods and techniques that have been used to identify and determine the products of drug metabolism. In this respect it is a veritable mine of information, the literature being covered up to January, 1967, with 1044 references to about 350 drugs and 650 metabolites. The volume is unique in that no other work covering the field has appeared since, although there have been volumes on the chemistry of drug metabolism and detoxication mechanisms, they give no indication of the techniques utilised to obtain the results. All modern techniques are covered, including the various forms of chromatography, radioactive isotope labelling, separating, characterising and determining metabolites in biological media. There are twenty chapters dealing with drugs on the basis of their structural chemical relationships independently of their activity. Some of these chapters, taken at random, deal with phenolic acids, amines, phenothiazines, azepine derivatives, sulphonamides, heterocyclic compounds, alkaloids and antibiotics. The volume is well indexed—an essential in this type of book; there are indexes of compounds by name and by empirical formula, and of authors. Interesting features are the collection of the formulae of compounds discussed on each page at the bottom of the page, which serves to show up the structural relationship very readily, and the printing of all drug names in heavy type. Altogether a most useful book, truly what T. H. Huxley called one of the counters of science. J. I. M. JONES

MODERN ASPECTS OF MASS SPECTROMETRY. Edited by ROWLAND I. REED. Proceedings of the Second NATO Advanced Study Institute of Mass Spectrometry on Theory, Design and Applications, held July, 1966, at the University of Glasgow, Glasgow, Scotland. Pp. xii + 389. New York: Plenum Press. 1968. Price $20.

This book is the proceedings of the second NATO Study Institute of Mass Spectrometry, which constituted a summer school of lectures, at an introductory level, presented in conjunction with experimental courses on the subject. These NATO instructional seminars (the two to-date have been held at Glasgow University, Scotland) are undoubtedly valuable to newcomers to mass spectrometry, and of assistance to those who have moderate experience in the field but who wish to expand their interest or to explore further applications. However, it is questionable whether collecting together the manuscripts of the lectures that illustrate the course into a symposium proceedings provides us with a book of any depth.

The scientist is often caused to reflect on the huge volume of published material and on the difficulty of selecting material of most value to himself. This is perhaps exceptionally true in the field of mass spectrometry and it is, therefore, disappointing that the present book has little to say that is really new, despite its title, which might lead us to expect a comprehensive review of recent instrumentation and applications.

To an unfortunate extent some of the chapters are extended versions of manufacturers' technical information. There are important notable exceptions: the paper by J. E. Collin, of the University of Liége, is a good review and presents a sound discussion of the theory of excitation and ionisation processes; the paper by R. C. Svedberg is a valuable description of Knudsen-cell vaporisation techniques in conjunction with mass spectrometry, and recognises the limitations as well as important applications of the method; the two chapters by A. Macoll, and by J. H. Beynon and A. R. Fontaine, cover comprehensively the mechanisms of dissociation and re-arrangement of organic molecular ions, but both deal with the subject matter without sub-headings and are sometimes difficult to follow. Quantum-mechanical aspects of ionisation and of dissociative fragmentation are covered in two papers that deserve mention, by G. R. Lester and M. E. Wacks, although the latter does not draw the reader's attention to serious doubts about the validity of the RPD method (since the work of Anderson, Eggleton and Keesing) for simulating mono-energetic electron beams.

On the whole the book is uncritical, and is totally so in its experimental sections. Little attempt is made to guide the reader in the choice of instrument or ionisation method most suitable to his particular analytical problem. For example, gas-chromatographic measurements are described in successive papers in which a sector-field instrument, a Dempster-type small mass spectrometer (in conjunction with infrared measurements) and a quadrupole mass filter are used; no comparison is made of the suitability of each instrumental method for this application.

The book is free from significant errors, and the printing is satisfactory although the reproduction of diagrams and photographs is unusually poor. There is important information to be found in the text, although it is not material that is inaccessible elsewhere. On balance, the book is too expensive. D. J. FABIAN

ANALYTICAL REACTION GAS CHROMATOGRAPHY. By VIKTOR G. BEREZKIN. Translated from Russian. Translation Editor L. S. ETTRE. Pp. x + 193. New York: Plenum Press. 1968. Price $12.50.

This well produced volume is a translation of the original paperback monograph, which was reviewed earlier (see *Analyst*, 1967, **92**, 786). It contains, in addition, a foreword especially written by the author for the English Edition, a few more references, a subject index and a 5-page supplementary chapter on selective, chemically active sorbents in gas chromatography in which, among other matters, recent work on the use of silver salts in polar solvents for separating hydrocarbons is briefly reviewed. The original introduction has been re-written in part, clarified and expanded.

The translation is, on the whole, excellent and there are various improvements in the text. Thus in many instances when the original text shows merely a number reference to literature cited at the end of the chapter, the translation gives also the authors' names in the text itself. The index (subjects only) is a useful addition, but is far from comprehensive.

The appearance of the English-language edition of the work should help to focus attention on possible applications of composite schemes of gas chromatography and chemical reactions in the field of chemical analysis. It is unfortunate that the price has to be so high. G. S. SMITH

ADDITION POLYMERS: FORMATION AND CHARACTERIZATION. Edited by DEREK A. SMITH, Ph.D., M.Sc., F.R.I.C., F.I.R.I., F.N.C.R.T. Pp. viii + 492. London: Butterworth & Co. (Publishers) Ltd. 1968. Price 110s.

Dr. Smith and his colleagues at the National College of Rubber Technology have written a generally useful account of present-day methods of polymer characterisation, which should prove interesting to many polymer scientists. In addition, much of the material can be recommended to undergraduates specialising in polymer studies. The treatment of the mechanism and kinetics of addition polymerisation is not presented at a very advanced level and, furthermore, amounts to less than a quarter of the whole work. As the book is primarily concerned with polymer characterisation, the restriction to so-called addition polymers appears to be arbitrary.

The impression left after reading this volume is of the uneven treatment of the various topics; the reviewer has, however, to concede that the editor has taken the precaution of neutralising this line of criticism by boldly stating in his preface that "no attempt has been made to equalise the depth of treatment in the various chapters." This decision, it seems to this reviewer, leaves the completed work as a miscellany of review articles whereas it could have been made into a standard text in its field.

Some errors that could mislead a student are noted here. For instance, kinetic chain length is inversely proportional, not to initiator concentration (p. 37), but to the square root of that quantity. On the same page, reactions II and IV are incorrectly printed which, taken in conjunction with the curious description of these steps as radical-forming reactions (p. 38), could well confuse someone unfamiliar with copolymerisation. The misprint in equation 2.23 is similarly unfortunate. The treatment of sedimentation equilibrium (section 5.6) does not give a correct picture of the status of this technique as far as synthetic high polymers are concerned. The reference to a fluorine n.m.r. analysis of a sample of polyvinyl trichloroacetate (p. 316) seems a careless slip, and it is surely not a good example to students to present a table of rate constants (p. 34) without stating their units.

To list other errors, most of which are unlikely to mislead seriously, would be unforgivably fault-finding; better to mention the virtues. The fine summary of chain branching in polyethylene, the short sections on solid-state polymerisation and on the practical significance of the molecular-weight distribution are all to be welcomed. Best of all is the succinct chapter on Industrial Aspects of Addition Polymerisation written by Dr. Smith. The set of numerical problems that follows the chapter on molecular-weight determination pleased this reviewer; could not the editor have persuaded his collaborators to have followed this example?

In short, then, a curate's egg. G. J. HOWARD

MICROWAVE SPECTRAL TABLES. Volume IV. POLYATOMIC MOLECULES WITHOUT INTERNAL ROTATION. By MARIAN S. CORD, JEAN D. PETERSEN, MATTHEW S. LOJKO and RUDOLPH H. HAAS. National Bureau of Standards Monograph 70. Pp. xii + 418. Washington, D.C. 20402: U.S. Government Printing Office. 1968. Price $5.50.

One can best describe this work in the authors' own words: "This volume contains data on the microwave spectra of 166 polyatomic molecules incapable of exhibiting internal rotation.

These data are based upon a systematic search of the literature up to January 1961 and include some information of later dates."

Read with the full title one can see that these tables are of partial coverage and somewhat out of date. Nevertheless, they are fairly cheap and most gas microwave experts will be happy to have a major list of transition frequencies, and their interpretation, on their own shelves and will thank the compilers for their hard work clearly presented. D. H. WHIFFEN

LA THERMO-ANALYSE. By MIREILLE HARMELIN. Pp. 121. Paris: Presses Universitaires de France. 1968. Price F. 3.30.

Following a brief introduction, Mademoiselle Harmelin presents seven chapters describing different thermal analysis techniques, *viz.*, thermogravimetry (it is encouraging to see this term used rather than "thermogravimetric analysis"), differential thermal analysis, evolved gas analysis, dilatometry, equilibrium diagrams and thermal analysis, thermomagnetic analysis and thermometric titrations. Clearly, this list is not intended to be comprehensive, although it is puzzling not to find even a passing reference to differential scanning calorimetry, microcalorimetry or electrothermal analysis.

Although in the introduction, Mademoiselle Harmelin says that the object of the book is to describe the principles of the techniques, one detects a natural bias for her own specialist fields in that the chapters on thermogravimetry and differential thermal analysis are the longest. To conclude, therefore, that T.G. and D.T.A. are the most important techniques would only be partially true, since, for example, evolved gas analysis is one of the most rapidly advancing techniques of thermal analysis and yet claims a chapter of only 3 pages.

Throughout the book reference is made to numerous workers, who are classified only by name and year—a method that makes further reading a tedious business.

While in the chapter on thermogravimetry, the application of this technique to kinetic studies is oversimplified, it is gratifying to see several examples of automatic determinations, which is a sadly neglected field of investigation.

There is no true index, only a table of chapter headings and sub-headings. A useful bibliography is also included. C. J. KEATTCH

ADVANCES IN X-RAY ANALYSIS. VOLUME 11. Edited by JOHN B. NEWKIRK, GAVIN R. MALLETT and HEINZ G. PFEIFFER. Proceedings of the Sixteenth Annual Conference on Applications of X-Ray Analysis held August 9–11, 1967. Pp. xii + 499. New York: Plenum Press, 1968. Price $22.50.

Once again the proceedings of a Denver Conference have been published in book form. The central theme was quantitative methods in X-ray spectrometric analysis, and most of the nineteen papers presented on X-ray fluorescence were on this topic.

Of the remaining twenty-one papers included in the volume, sixteen are on a wide ranging miscellany of topics in X-ray diffraction, four are on electron-microprobe analysis and one is about the detection of rare earth impurities in yttrium oxide and gadolinium oxide by X-ray excited optical fluorescence.

Papers on the X-ray fluorescence analysis of metals indicate that the basic operations are now regarded as routine, and that effort has been concentrated on improved methods of handling observed data to obtain more accurate results. Developments have been such that, in the Foreword, Dr. Pfeiffer, a co-Chairman at the Conference, states that most elements that are present in amounts greater than a few parts per million can be determined with accuracies rivalling those attainable by wet methods.

The commendably high standard of presentation that has previously characterised the series has been maintained in this volume. E. A. KELLETT

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The Flame-photometric Determination of Sodium in High Purity Water

In connection with an investigation of the performance of sodium-responsive glass electrodes, an independent method was required for determining the sodium content of water (0 to 50 μg of sodium per litre). A flame-photometric technique was used for this purpose; details of the technique and its performance are given. Special attention was given to the problem of accurately calibrating the flame photometer and, to this end, a new technique has been evolved.

H. M. WEBBER and A. L. WILSON

Central Electricity Research Laboratories, Cleeve Road, Leatherhead, Surrey.

Analyst, 1969, **94**, 569–574.

Lung Tissue Hydrolysates: Studies of the Optimum Conditions for the Spectrophotometric Determination of Hydroxyproline

Two procedures developed earlier by the authors for the spectrophotometric determination of hydroxyproline have been tested on hydrolysates of dried formalin-fixed and ethanol-fixed lung tissues. The effects of dilution of the hydrolysates and of the presence of various amounts of sodium chloride have been studied, as well as several methods commonly used to remove competing amino-acids or other interfering materials from hydrolysates before the spectrophotometric determination of amino-acids. Several amino-acids reported to cause interference in other spectrophotometric methods for hydroxyproline determination have a negligible effect in the procedures used in the present study.

Lung tissue hydrolysis with varying concentrations of hydrochloric acid, sulphuric acid and sodium hydroxide, under various conditions, has been studied. No reduction in hydroxyproline yield was observed when the acid hydrolyses were prolonged for several days. A simple procedure is described, in which hydrolysis takes place overnight at 105° C in a polypropylene tube enclosed by a glass tube fitted with a spring-loaded stopper; this procedure has given good results over a long period.

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Analyst, 1969, **94**, 575–584.

Factors Influencing the Colorimetric Determination of Nitrite with Cleve's Acid

The determination of nitrite with Cleve's acid has been investigated; the influence of composition of reagents and several experimental conditions have been evaluated. It is shown that provided the standards used to prepare the calibration graph and samples are treated in a similar way, then the choice of conditions may largely be left to the operator. A recommended procedure is given.

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Analyst, 1969, **94**, 585–588.

The Qualitative Analysis of Dilute Aqueous Solutions of Thiols and Thioethers by Thin-layer Chromatography

The preparation of solid derivatives from thiols and thioethers was investigated in order to discover a method of concentrating extremely dilute solutions of these compounds and to analyse them by thin-layer chromatography.

It was found that thiols could be separated from thioethers by reaction with phenylmercury acetate and a method was developed for the analysis of mixtures of thiols by the thin-layer chromatography of the phenylmercury mercaptides formed by this reaction.

A method used by earlier workers for the preparation of dialkylsulphinimines from thioethers and chloramine-T was improved to make it suitable for use with microgram amounts of thioethers, and the derivatives were separated successfully by thin-layer chromatography.

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Analyst, 1969, **94**, 589–593.

Chlorobenzoic Acids and Derivatives: Analysis of Mixtures by Thin-layer Chromatography

Thin-layer chromatographic (silica gel G) R_F values were obtained for the three isomeric monochlorobenzoic and two dichlorobenzoic acids. The *p*-bromophenacyl esters of benzoic, *o*-, *m*- and *p*-chlorobenzoic, and 3,4-dichlorobenzoic acids were prepared and R_F values obtained. The esters of benzoic, *m*-chlorobenzoic and 3,4-dichlorobenzoic acids were separated from the ternary mixture by thin-layer chromatography. When 2,4-dinitrophenylhydrazine in hydrochloric acid solution reacted with the esters spotted at the origin, subsequent thin-layer chromatography gave characteristic two-spot patterns for each, and this facilitated recognition of the type of mixture of monochlorobenzoic acids. An explanation is given of the formation of two hydrazones from each ester.

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Analyst, 1969, **94**, 594–598.

Reversed-phase Extraction Chromatography with Paper and Columns Supporting an Extractant Selective for Copper

Supports treated with a commercial mixture of substituted oximes (Lix-64) are shown to separate copper effectively from several selected cations, namely, iron(II), iron(III), cobalt, nickel, manganese(II), chromium(III), molybdenum, tungsten and vanadium(IV), the eluents being ammonium sulphate, ammonium chloride, sodium chloride or ammonium sulphate *plus* ammonium hydrogen difluoride solutions.

The R_F spectra of the various ions are presented, when eluted on paper treated with the extractant, as functions of the pH of the eluting ammonium sulphate or ammonium chloride solutions.

Representative separations are reported, either on paper or on cellulose powder columns treated with the extractant.

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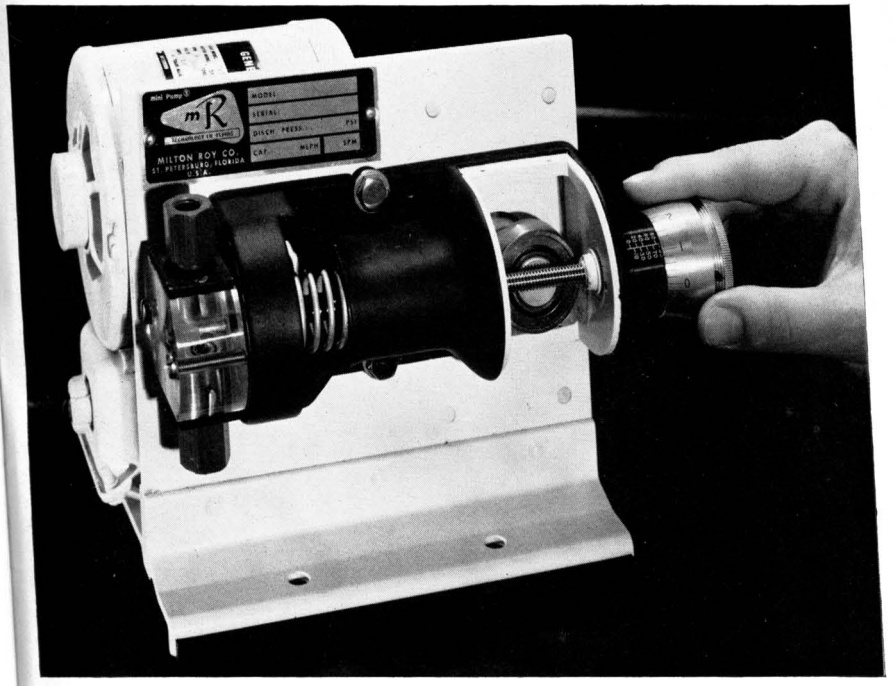
Analyst, 1969, **94**, 599–604.



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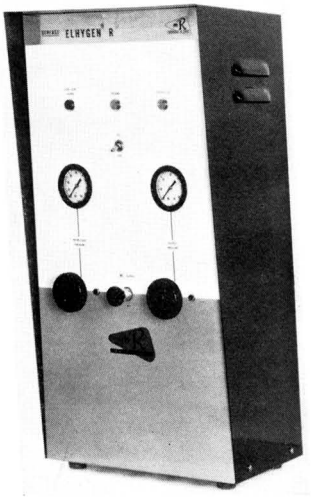

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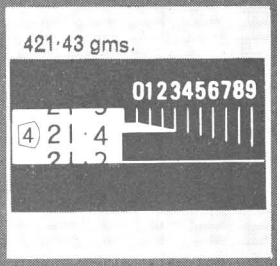

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