

either of these places. But of course the illiterate villager could hardly have brought or obtained it from these far off places which, as already stated, are about 250 and 560 miles away as the crow flies. Sir John Marshall and others have already suggested that in all probability the "Indus" culture extended to other parts of India. And there would seem to have been no more likely direction for its spread than into the fertile plains of the Jumna and the Ganges.

In my full paper I shall acknowledge all the help so generously given me by a

number of kind friends, but apart from those already named above I would like to record my special thanks to my assistant Mr. K. N. Kaul, M.Sc., for the excellent photographs here reproduced.

May 8, 1936.

Postscript.—During a second visit to Khokra Kot to-day a further collection of several thousand fragments of coin-moulds similar to those described above, was made.

Rohtak, 10th May, 1936.

B. S.

Fluorescence in Ultra-Violet Light as an Aid to Chemical Analysis.

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THE more spectacular applications of this method are now well known. Ultra-violet light now ranks as one of the "mystery rays" concerning which the man in the street is duly informed when a forgery or similar crime is "in the news", and numerous workers interested in the various ramifications of science applied to industry have put on record their belief as to the value of the method.

One aspect of the subject, however, does not appear to have received quite the attention it deserves, and this is the application of the method to what we may term ordinary chemical analyses, in order to distinguish them from the more or less specialised or empirical methods used in connexion with industrial work. In many cases these provide very sensitive and specific tests, some of which may be used quantitatively, and it is felt that once the possibilities are better known they will give the analyst yet another string to his bow.

The principle of the method of fluorescence analysis is now so well known that the shortest introductory description will suffice. In brief, substances which appear identical in ordinary daylight or artificial light may emit a characteristic fluorescence in ultra-violet light which not only enables them to be identified, but also supplies information regarding their nature and origin; hence the value of one of the applications of this method in industrial work, *e.g.*, the checking of samples against deliveries. This fluorescence may be so vivid as to be apparent even when minute quantities of material are present, and hence again its uses in criminological work, *e.g.*, for the detection of

forgeries and erasures, etc. In chemical analysis, however, the underlying principles are rather different and they may be summarised as follows:—

(1) Production or disappearance of fluorescence.

(a) Thus, two non-fluorescent substances (one a reagent and one the unknown) are caused to react so as to produce an end-product which fluoresces visibly even if the quantities involved are very small.

(b) Conversely the fluorescence of a substance or a reagent may be destroyed by the reaction. The obvious disadvantages of this method are that it is less sensitive and less specific than (a).

(c) Allied with (b) is the method based on the property of certain ions of inhibiting in a specific way the fluorescence of a substance without reacting with it chemically. Such inhibition may therefore be used as a test for the inhibiting substance, and if, as is usually the case, the minimum amounts necessary for the purpose are known, the method enables one to say whether the quantity present is over or above that amount.

(2) The change in the colour or intensity of the fluorescence of a substance may be used to indicate the end-point of a quantitative reaction in which it does not of necessity participate. In other words, the substance plays a part similar to that of the ordinary indicator used in volumetric analysis.

(a) The substance to be determined may be its own indicator (just as the disappearance of the colour of potassium permanganate indicates that an oxidation-reduction reaction is complete).

(b) The fluorescent substance is added to the reacting mixture. This is the usual procedure.

For the sake of completeness it should be mentioned that absorption spectrophotometry, using ultra-violet light, is a valuable aid to the identification and determination of many substances (especially organic compounds). The method hardly falls within the scope of this article, but it is completely analogous in principle with the use of the visible spectrum for the same purpose. It may also be mentioned that the light from the mercury arc is of great assistance in making certain determinations by colorimetric methods. The determination of bismuth, cadmium or antimony¹ by the sulphide method may be cited as examples.

APPARATUS AND TECHNIQUE.

Both apparatus and technique are very simple. The mercury arc lamp, which is the most popular source of ultra-violet light, may conveniently be used, and it is an advantage to choose a model which is supported or hung at such a height as to allow room for manipulation underneath it. This is a point worthy of consideration when volumetric reactions are being carried out, because the burette must be held over the titration-vessel and the latter must be in the full beam of the light. If, therefore, a lamp can be chosen with a window on the side instead of in the base, it is all to the good.

Visible light should of course be removed from the radiation by means of a Wood's glass filter, and in all cases the best results are achieved by working in a darkened room. The ultra-violet light provides sufficient illumination for manipulations, and it is even possible to read a burette with the usual degree of accuracy if the fluorescent quinine sulphate float described by the writer² is used; similarly, pipettes and other instruments may be rendered visible by smearing them with vaseline, which is brilliantly fluorescent.

Containers should be non-fluorescent and quartz is the ideal material. If the lamp has a window in the base it is best to use as a reaction vessel a basin or dish placed under it, because the light can then fall on the contents without striking the walls of the container. Some drop-reactions are conveniently carried out by the spotting method on non-fluorescent filter paper.

Reference must be made to the literature³ for further details of technique.

APPLICATIONS.

It is not possible to deal exhaustively with all the possibilities of the method, but the examples which follow have been chosen so as to indicate some of the most typical and important applications.

Inorganic Chemistry.—The conversion of fluorescein into eosin by the action of bromine provides a very sensitive test for this element (or for bromides) owing to the change in the nature of the fluorescence which results; this reaction is conveniently carried out on a filter-paper, the bromine being directed through a capillary tube on to a dried spot of fluorescein. Similarly the yellow fluorescence of resorufin may be destroyed by bromine although this test is also sensitive to chlorine.

The Gutzeit test for arsenic provides an interesting example of the use of fluorescence to increase the sensitiveness of a reaction, because colorations on the mercuric chloride paper corresponding with 0.01 to 0.001 mgrm. of arsenic are rendered visible, although they cannot be seen in ordinary light.

The vivid fluorescence of quinine sulphate is the basis of a number of useful tests involving, directly or indirectly, the formation of the sulphate ion. The detection of sulphites⁴ and sulphides⁵ will serve as examples, the substance under examination being heated with acid in a stream of carbon dioxide (to prevent premature oxidation), and the gas evolved being passed into bromine water or, if sulphides only are to be detected, into cold hydrogen peroxide. In each case a speck of quinine is added to the reagent, and the formation of a trace of sulphate causes it to fluoresce vividly. It has been found⁶ that dilutions so great as $1:0.5 \times 10^8$ of quinine sulphate has a visible fluorescence, and if the above reaction is carried out on the micro-scale, 0.25 and 0.1 mgrm. of sulphur as SO₂ and S, respectively, is detectable. It is of course important that the oxidising reagent should not fluoresce with quinine before the reaction

³ Cf. M. Haitinger, *Mikrochemie*, 1935, **16**, 321; Radley and Grant, *Fluorescence Analysis in Ultra-Violet Light*, 1935.

⁴ See J. Grant and J. H. W. Booth, *Analyst*, 1932, **57**, 514.

⁵ See J. Grant and H. P. Smith, *id.*, 1934, **59**, 749.

⁶ Grant, *loc. cit.*

¹ Cf. J. Grant, *Analyst*, 1928, **53**, 626.

² *Jour. Sci. Inst.*, 1932, **9**, 359.

starts, and although no difficulty is usually experienced with bromine water it may be necessary to test several supplies of hydrogen peroxide. Other uses of quinine sulphate are dealt with in the sections on organic substances and indicators.

Uranyl salts have a strong and characteristic fluorescence, but they cannot safely be used directly for this type of work because they are affected by the presence of other compounds. The work of Y. Volmar and V. Mathis⁷ has shown that of the inorganic ions Cl' , Br' , CN' , S'' , SCN' and FeCy_6''' will inactivate uranyl sulphate to an extent which varies inversely with the chemical equivalent, and that a definite minimum quantity is required to produce the required effect. The method may therefore be used to detect such ions, and under suitable conditions it is even possible to say whether more than the minimum quantity is present.

The *bead tests* yield additional information if they are inspected in ultra-violet light. Borax, sodium metaphosphate and calcium fluoride beads have all been used in this way, some of the most striking results being obtained with uranium salts (which give a yellow bead) and the rare earths; in the latter case cerium, samarium, niobium, terbium, thulmum and europium may be identified, even if present in very small quantities.

Among the *miscellaneous fluorescence reactions* of inorganic compounds may be mentioned the tetrahydroxy flavonal test for beryllium; the reaction of boric acid with fluorescein (sensitive to 0.02 mgrm.); the distinction of nitrites from nitrates by the red fluorescence they produce with certain dyes; the use of *o*-hydroxy quinoline, which forms fluorescent compounds with a number of metals (*e.g.*, zinc, magnesium or cadmium); and the dimethyl glyoxime test for rhenium.

Organic Chemistry.—The uses of quinine as an indicator of inorganic reactions have already been referred to, but its own fluorescence also enables it to be determined. This provides one of the best examples of this type of reaction in organic chemistry. The determination may be made by one of two methods according to the amount present, *viz.*—

(a) *Micro Methods.*—J. R. Nicholls⁸

matches the fluorescence of one of a series of standards containing known quantities of quinine in the presence of sulphuric acid, against that of the sample, and has obtained very accurate results for quantities of the order of 0.1 to 0.2 gm. per ml.

(b) *Macro Method.*—The writer⁹ has found that if the quinine is dissolved in a known amount of 0.01 *N* sulphuric acid, the excess may be back-titrated with 0.01 *N* alkali with an accuracy of 0.1 ml., the end-point being given by the change in the shade of the fluorescence. The advantages of the method are outlined below under the heading of fluorescent indicators.

There are a number of useful miscellaneous tests for organic compounds. Thus, a 10 per cent. solution of *p*-dimethyl amino-benzaldehyde is a general reagent for many *hydrocarbons*; thus, for example, it produces a red-brown, grey-blue, brilliant blue and deep-red fluorescence with benzene, anthracene, quinoline and quinone, respectively.

Among the reactions for *alcohols* mention may be made of the distinction of α - from β -naphthol by the green fluorescence obtained with a mixture of acetic and sulphuric acids in the presence of the latter (sensitivity 1 in 100,000) but not the former. Naphthoresorcinol is a reagent for glyoxal, and glycerol is frequently detected in food-stuffs by oxidation with bromine water to dihydroxyacetone which can then be made to fluoresce with a solution of β -naphthol in sulphuric acid.

Reactions are available for most of the *sugars*. Thus, a green fluorescence is produced with dextrose by β -naphthol, and with arabinose by zirconium oxychloride, while resorcinol in hydrochloric acid is a reagent both for fructose and sucrose (*e.g.*, in milk), a green and a red coloration being obtained, respectively.

Interesting reactions for *organic acids* are the orcinol test for malic acid (which is used as test for apple pulps in jam); the blue colour produced with $\beta\beta$ -dinaphthol in the presence of 1 part in 50,000 of tartaric acid; and the use of *o*-oxydiphenyl in sulphuric acid as a test for lactic acid. The list might be extended considerably to include amino-compounds, sterols, higher alcohols, etc. Mention should also be made of the detection and determination of certain *alkaloids* by the Volmar-Mathis method

⁷ *Bull. Soc. Chim.*, 1933, **53**, 385; **54**, 1266.

⁸ *Analyst*, 1934, **59**, 277.

⁹ *id.*, 1931, **56**, 653.

(*loc. cit.*), i.e., by observing the concentration necessary to inhibit the fluorescence of uranium salts.

Fluorescence Indicators.—As already pointed out these are now playing an increasingly important part in analysis. The advantages they offer over ordinary indicators are:—(a) increased sensitiveness owing to the high dilutions at which the fluorescence is visible; (b) they may frequently be used in turbid and/or coloured solutions; (c) owing to the fact that they are used in much smaller quantities than the ordinary indicators, the error normally involved in the latter case (owing to combination of the indicator with either of the reactants) is avoided. The writer has also found that if a little saponin is added to a particularly coloured or turbid solution and the titration is carried out in a conical flask, then the end-point may conveniently be shown by the change in fluorescence of the froth. Fluorescent indicators may be classified as follows:—

(a) **Acid-Alkali Indicators.**—These change in fluorescence according to the pH value of the medium in which they occur and they

may be chosen, just as with ordinary indicators, so that the end-point corresponds with any desired pH value. Quinine (pH 5.9–6.1 and 9.5–10.0) is the most familiar example, but it is now possible to cover the whole range of pH values. Thus eosin serves between 2.5 and 4.5; resorufin, 4.4–6.4; umbelliferone, 6.5–7.6; and coumarin, 10.0–12.0, etc.

(b) **Precipitation Reactions.**—Adsorption indicators are now used to indicate the end-points of these titrations (*e.g.*, silver nitrate against a halide), and if certain fluorescent substances (*e.g.*, Rhodamine) are used for the purpose an increased sensitiveness is obtainable.

(c) **Oxidation-Reduction Reactions.**—The fluorescence of certain substances (*e.g.*, resazurin and resorufin, see *supra*) is destroyed by the action of reducing or oxidising agents, and this serves as an end-point for the reactions concerned.

Further examples and details concerning all the above types of reaction are given in the literature (*cf.* Radley and Grant, *loc. cit.*³).

The Helium Content of the Atmosphere.

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“AIR is a physical mixture with the definiteness of composition of a chemical compound.” This was the conclusion reached by Francis G. Benedict of the Carnegie Institution (Washington) in 1912, after a series of very accurate analyses of the oxygen content of about 200 air samples which were taken during a period of 8 months at Boston and at several places over the ocean. The variations of the mean value (20.952%) were certainly less than $\pm 0.05\%$, in spite of all possible alteration in weather conditions, and although the experiments were made before, during and after the vegetative season. This constancy of the atmosphere's composition was later confirmed by A. Krogh (Copenhagen, 1919). Krogh states that the combustion of fuel and the respiratory exchange of organisms must cause a production of carbon dioxide which is similar in amount to the quantity of oxygen used up, while the assimilation of plants must diminish the carbon dioxide to an extent equal to the increase in oxygen; but that

all variations in the oxygen and carbon dioxide percentages due to combustion, respiratory exchange or assimilation should leave the “nitrogen” percentage practically unaltered. (Under “nitrogen” is understood nitrogen *plus* argon and the other rare gases.) The percentage of “nitrogen” (79.0215) is considered by Krogh to be a geophysical constant, which does not vary by more than $\pm 0.003\%$, if at all. As to the carbon dioxide content of the atmosphere, it is obvious that this can vary considerably and attain a multiple of its usual value where processes of combustion or respiration are going on in closed, or badly ventilated, rooms. On the other hand, in the open air the variations are very small, as can be gathered from the constancy of the oxygen percentage found by Benedict, and as has been directly proved by him and by J. S. Haldane in Oxford, who found that the deviations from the average value of 0.03% carbon dioxide never exceed 0.005%.

Although these careful analyses are quite convincing as far as they go we may never-