

## Fluorescence and Its Use as a Method of Testing and Analysis

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ONE of the results of the increasing popularity in recent years of so-called 'sun-ray' treatment has been the rapid development of improved methods of generating ultra-violet rays. This has placed in the hands of the scientific worker very efficient sources of such radiation, and it is therefore not surprising that other uses of this region of the spectrum should have followed in the wake of the above developments. One of the most interesting is the generation of a characteristic fluorescence in numerous substances, and this is now widely employed as a method of testing and analysis.

The range of ultra-violet radiation is usually

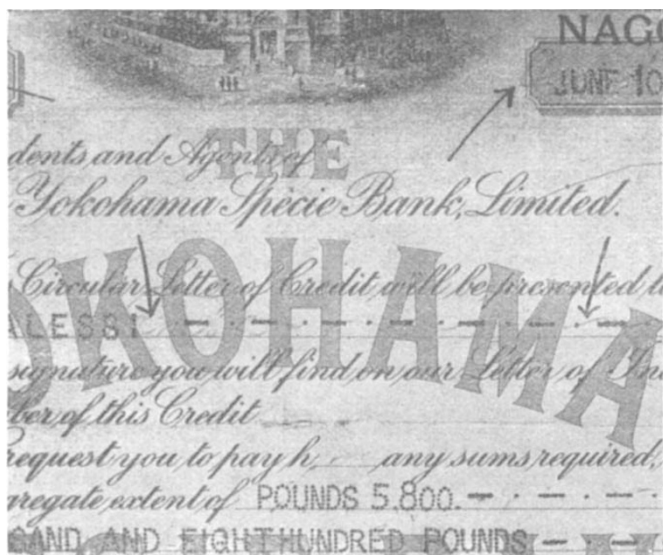


FIG. 1. Fluorescence photograph of a forged document. Arrows indicate original wording, which is not visible in daylight.

considered to extend from about 136 Å. to 4000 Å., but the principal rays used for obtaining fluorescence effects are confined to wave-lengths between about 2500 Å. and 3700 Å., and there is ample evidence that individual rays in this range are particularly effective. Similar selectivity is, of course, well known in connexion with work on ultra-violet therapy and on the photochemical activity of ultra-violet light.

### GENERATION OF ULTRA-VIOLET LIGHT

Methods of generating ultra-violet light are fully treated in works on light-therapy, and it is necessary here only to indicate some of the special requirements of fluorescence analysis.

Carbon and mercury arcs have both been used, but whilst the former is best adapted for fading tests on account of the similarity of its spectrum to that of sunlight, the latter has proved more popular where an intense ultra-violet radiation is

required with a minimum of visible rays. The character of the actual radiation emitted depends, however, on the type of lamp and on the working conditions. As is well known, the principle of the method is the production of an electric arc in the mercury vapour produced between two heated mercury electrodes. Mercury lamps vary considerably in design; some give a point-source and are particularly useful for spectroscopy and fluorescence microscopy, whilst others are designed to give a maximum luminous area. The unstable open forms of U- or H-tubes have now largely been replaced by completely enclosed types, one of the latest of which takes the form of an ordinary electric-lighting bulb containing a globule of mercury and two tungsten electrodes which serve both to heat the mercury and to carry the arc.

There are also rival claims between lamps operating in a vacuum and at atmospheric pressure. The latter have a great advantage in that they can be opened and cleaned, but on the other hand, with the former there is less necessity for such cleaning, owing to the absence of air. One well-known design consists of an evacuated quartz tube, with a reservoir of mercury at each end, into which protrude metallic leads connected to the electricity mains. When the lamp is tilted, the mercury runs across the floor of the vessel and short-circuits the two leads; the heat so generated produces mercury vapour and the arc then strikes.

Such lamps were at one time expensive and deteriorated rapidly, but recent models are cheaper both to buy and to run, and can eventually be regenerated. The changes which occur on ageing often produce

alterations in the spectral distribution of the radiation, and if these are not controlled from time to time discordant fluorescence results may be obtained.

During the last year or so, several 'lamps' which utilise the ultra-violet constituents of daylight have appeared. They are essentially darkened boxes fitted with a filter to remove visible rays, and are necessarily relatively inefficient. However, they are portable and comparatively cheap, and for some purposes are adequate.

### TECHNIQUE

The lamp is usually housed in a box, provision being made for viewing objects either by reflected or transmitted light through a filter which removes visible rays, the brilliance of which would obviously mask any fluorescence. Filters may be solutions of dyestuffs or coloured or coated glasses, and may be chosen so as to isolate almost any given range

of wave-lengths; nickel oxide glass ('Wood's glass') is, however, the best for most purposes. As already indicated, it is important to keep a control over both the quality and quantity of the radiations, and here again there is a wide choice of methods, of which the photoelectric cell and the use of the selective photochemical action of the rays on certain chemical reactions are the most promising.

The procedure depends to a great degree on the nature of the sample. Useful information is often obtained if a little powder is blown on to moist filter-papers which have been treated with reagents, whilst solids in the mass are usually exposed on a fresh fracture which also may be spotted with reagents. Liquids are examined in non-fluorescent containers, preferably in open Petri dishes or in quartz test-tubes, and it is often an advantage with solutions to use a variety of solvents and dilutions. Capillary analysis, in which the 'zones' produced on a strip of filter paper suspended in the liquid or solution are examined in ultra-violet light, has proved very useful, notably in connexion with mixtures of alkaloids and dyes, and Danckwortt and Pfau<sup>1</sup> have even obtained semi-quantitative results in this way.

When, however, it is required to determine the composition of a mixture containing a fluorescent ingredient, the usual procedure is to match it against one of a series of known mixtures. The importance of working under strictly standardised conditions in all this work cannot be emphasised too strongly. The type of lamp, its age, the time which elapses after striking the arc, the filter, the temperature and humidity, etc., all determine the actual appearance of the fluorescence, and it is essential that each worker should evolve and adhere to his own conditions if apparent anomalies are to be avoided.

The use of fluorescent compounds as stains in microscopy and as indicators in titration work has greatly widened the range of application of the method. In the former case it is often possible to bring out fine details, for example, of plant structure, which are invisible in daylight, whilst the latter method may be used for coloured fluids, for example, for the titration of quinine<sup>2</sup> and of the acidity of wines, and for neutralisation titrations in extremely dilute solutions. Photographic methods require special technique and their applications are limited by the difficulty of

reproducing colour effects. They are, however, greatly used in criminological and museum work, for example, to provide evidence of falsification of documents (Fig. 1 is a photograph of a falsified document taken in ultra-violet light, in which the original writing is plainly visible, although not apparent in daylight).

#### APPLICATIONS

The applications of fluorescence are numerous and varied, and can only be briefly indicated here; they are discussed fully elsewhere<sup>3</sup>.

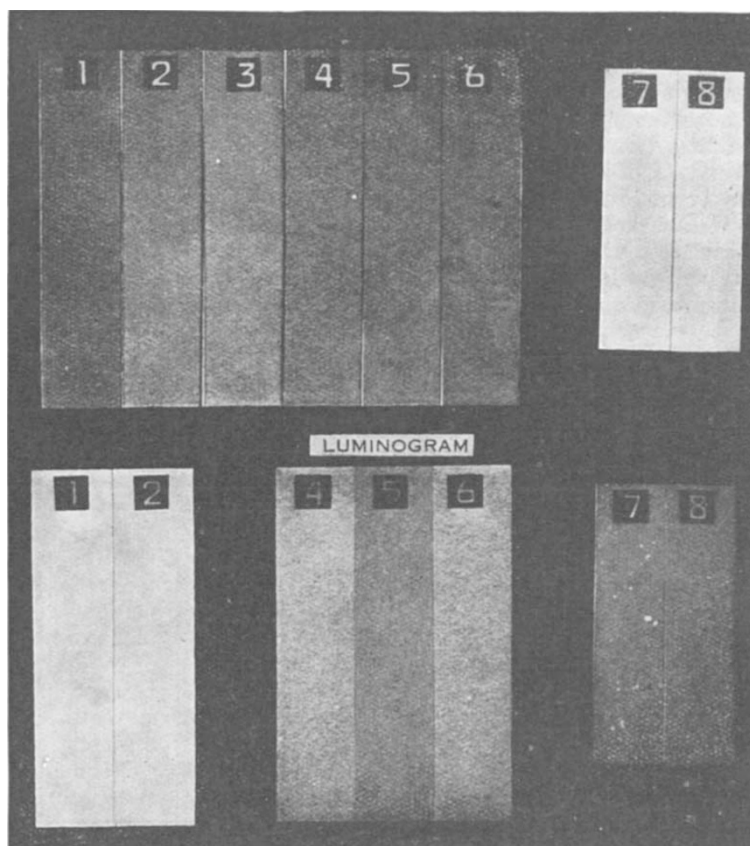


FIG. 2. Comparison of daylight and fluorescence photographs of strips of yellow paper.

The examination of chemical substances for purity is one of the best known. Many alkaloids, most dyestuffs and numerous inorganic and organic compounds give characteristic colours, and as these may depend on the origin, purity and method of manufacture, they form the basis of rapid testing methods, the importance of standardised conditions being always emphasised. Such methods have found application in connexion with paint pigments, drugs and all industries where dyestuffs are used. Thus in Fig. 2 specimens 1-4 are strips of dyed yellow paper which appear identical in daylight (top row) but vary considerably in colour and in intensity in ultra-violet light (bottom row); Nos. 5 and 6 are the same as



No. 4 after exposure to sunlight (1 week) and to ultra-violet light (2 hours), respectively, and comparison of No. 7 with No. 8 shows the effect of addition of wax size. Accelerated fading tests are best carried out with the carbon arc, which gives effects nearest to those of sunlight; in many cases the results from the mercury arc are definitely misleading and this lamp is more suitable for ageing tests, or for tests based on the use of the change in fluorescence on exposure (see Nos. 5 and 6 in Fig. 2) as a measure of stability.

Plant materials offer a wide scope for the method, particularly if fluorescence microscopy is employed. Examples are the differentiation of woods and of various strains of seeds, and the control of the composition of farm products such as fertilisers, poultry and cattle foods, dairy produce, etc. Fig. 3 shows the appearance in ultra-violet light of five rhubarbs (*R. Palmatum*, High-dried Flats, Rough Rounds, *R. Rhaponticum* and Canton, respectively), and is an example of the numerous applications of the method to plant drugs. The fluorescence produced by bacteria, fungi, animal organs, sera and biological fluids also lends itself to the method, and a considerable amount of work has been

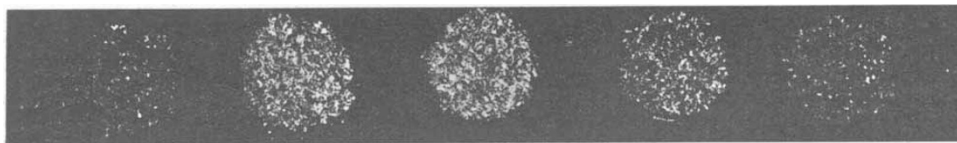


FIG. 3. Fluorescence photographs of five different rhubarbs.

carried out (notably by C. Dhéré<sup>4</sup>) on blood and the porphyrins.

Closely related to this are applications to medico-legal work, particularly from the point of view of identifying stains on garments and drugs in body fluids, and although the method is in its infancy, it has already met with some encouraging successes.

The method is also of considerable use in other branches of criminological work; erasures or alterations of written matter (see Fig. 1) and the detection of imitation water-marks are examples, and in a case recently submitted to me<sup>5</sup>, it was possible to differentiate easily between a genuine banknote and a particularly clever forgery from the fact that the dyestuff used to produce a blue design fluoresced with a green colour in one case but appeared black in the other.

Museum work should be mentioned in this connexion, since photographs in ultra-violet light of paintings, palimpsests and other documents frequently provide evidence of superimposition. The dyestuffs used on postage stamps are excellent indications of possible imitations in philately, and a complete technique has been evolved for the examination of geological specimens and fossils. Since ageing influences the appearance of sculpture materials, ivory, woodwork, etc., repairs and imitations may be detected.

The application to work involving the use of organic solvents and mineral or essential oils is particularly wide, as such materials usually glow vividly in ultra-violet light. Reference has already been made to the identification of dyestuffs and the use of fluorescent indicators, but it should also be mentioned that this work has provided useful information concerning the influence on fluorescence of chemical constitution.

In conclusion, the applications to the food industries merit a few words. Fatty foods fluoresce, the yellow colour of butter being modified by the presence of margarine or other foreign fats. A great deal of work has been carried out on milk, partly from the public health point of view and partly with the object of ascertaining the nature of the fluorescent constituent (lactochrome), which has now actually been isolated.

The sensitiveness of micro-tests for organic or inorganic substances may often be magnified if the test involves the production of a compound which fluoresces, and several aids to the detection of preservatives such as boric, benzoic and salicylic acids, as well as sulphites<sup>6</sup>, are provided in this way. Other work has been directed to the examina-

tion of spices, cheese, jams and bakery and fermentation products, and among the confectionery products special mention should be made of honey<sup>7</sup>, since applications of the methods of capillary analysis and absorption spectroscopy have yielded results which, it is claimed, enable a distinction to be made between honeys of different origins.

No mention has been made of applications of the method to the rubber, cellulose, paint, fuel or ceramic industries, or to sewage disposal and general organic and inorganic analysis, but numerous applications suggest themselves, and the general utility of the method (provided always that working conditions are standardised) is now recognised.

I am indebted to the British Hanovia Quartz Lamp Co., Ltd., for the loan of Fig. 1, and to Messrs. Chapman and Hall, Ltd., for Figs. 2 and 3 (from "Fluorescence Analysis in Ultra-Violet Light") which were kindly photographed for me by Col. W. R. Mansfield.

<sup>1</sup> P. W. Danckwortt and E. Pfau, *Analyst*, **52**, 707; 1927.

<sup>2</sup> J. Grant, *ibid.*, **56**, 653; 1931.

<sup>3</sup> J. A. Radley and J. Grant, "Fluorescence Analysis in Ultra-Violet Light" (Chapman and Hall, Ltd., 1933).

<sup>4</sup> C. Dhéré and others, *C. R.*, **179**, 351; 1924; **183**, 321; 1926.

<sup>5</sup> J. Grant, *Analyst*, **58**, 603; 1933.

<sup>6</sup> J. Grant and J. H. W. Booth, *ibid.*, **57**, 514; 1932.

<sup>7</sup> G. Orbán and J. Stitz, *Z. Unters. Lebensm.*, **56**, 467; 1928