Technical methods

A device for spectrofluorimetry

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Filter fluorimeters are of limited use in biological assay owing to their inability to discriminate between substances having similar fluorescent properties. This has led to the development of highly selective spectrophotofluorimeters (Udenfriend, 1962), which are, however, costly instruments. The Unicam SP 900 flame spectrophotometer[2] has a sensitive photomultiplier and high gain chopper amplifier, and it was thought that this might make a very effective secondary monochromator and detector, excitation being obtained from another spectrophotometer. Bartholomew (1958) constructed a spectrofluorimeter by using the monochromators of two Unicam SP 500 spectrophotometers. His coupling arrangements were relatively complex and required a special detector device as the photocells of the SP 500 are not sufficiently sensitive.

We have constructed a device for coupling an SP 500 spectrophotometer to an SP 500 flame spectrophotometer which enables fluorescent measurements to be made without affecting the original function of either instrument.

A surface aluminized mirror is placed at 45° to the light path of a SP 500 spectrophotometer (in the cuvette well). The beam is directed upward through the bottom of a cuvette held in a suitable holder.[3] The secondary beam emitted from the cuvette passes to the light entrance of the SP 900 (Figs. 1 and 2).

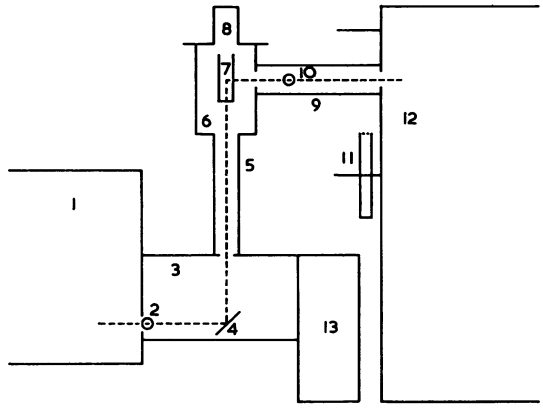

FIG. 2. *Diagram of the device (not to scale). The light path is shown as an interrupted line.*
1 *SP 500 spectrophotometer monochromator.*
2 *Silica lens.*
3 *Cuvette compartment of SP 500.*
4 *Front aluminized plane mirror.*
5, 9 *Light-tight chimneys.*
6 *Fluorescence cuvette housing.*
7 *Fluorescence cuvette (optical bottom surface).*
8 *Light trap.*
10 *Glass lens.*
11 *Burner of SP 900 flame spectrophotometer.*
12 *Body of SP 900.*
13 *Photocell compartment of SP 500 (not used).*

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[2]Unicam Instruments Ltd., Cambridge, England.
[3]A Unicam SP 505 fluorimetry attachment was adopted as the body of this device.

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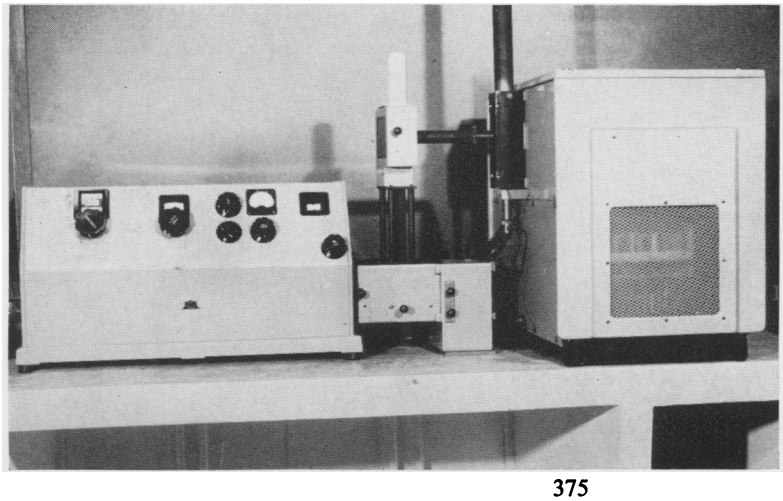

FIG. 1. *The device, showing its attachment to the SP 500 spectrophotometer (left) and the SP 900 flame spectrophotometer (right).*

The SP 500 is fitted with a silica lens which focuses the light beam in the centre of the cuvette compartment. This is used and the 45° aluminized mirror is placed at the focal point of this lens thus ensuring a reasonably compact light beam in the fluorescence cuvette. A glass lens is placed in the fluorescent light path to concentrate this beam; its omission causes a loss of about 50% sensitivity.

The SP 900 flame spectrophotometer was used as the secondary monochromator and detector because it has been specially designed to have excellent light-gathering properties, and is fitted with a very sensitive and stable chopper amplifier.

Excitation from the hydrogen lamp fitted to the SP 500 was not effective so the unmodified instrument could only be excited by the tungsten lamp and can therefore be used for the fluorescence assay of substances that have maximum absorption above about 360 millimicrons. A xenon arc lamp could be used in place of the hydrogen lamp and this would extend the range into the ultra-violet region. The SP 900 has two detectors: a Mazda 27m3 photomultiplier for 250-680 millimicrons and a red sensitive photocell (with additional amplification) for 680-1,020 millimicrons. It can therefore accept any fluorescent beam likely to be encountered in normal use.

Sensitivity of the instrument was tested by using riboflavine in 0·2 M phosphate buffer. With the gain of the SP 900 set to near maximum and the slits of both instruments fully open[4], it was possible to detect 0·001/μg./ml. riboflavine with ease. The ultimate sensitivity was much better than this but there was some drift and instability on the galvanometer and accurate readings could not be taken. Free 11-hydroxy-corticoids in normal plasma (Mattingly, 1962) were easily measured.

The effect of varying the slits of both instruments was studied using a strong (1 p.p.m.) solution of riboflavine (Fig. 3). At the wavelengths concerned, it appears that sensitivity is good at slits of 1 mm. or more. When either set of slits is narrower than 1 mm. the slopes of the curves become steep. A comparison of curves 1 and 3 shows that it is better to have wide slits on the primary and narrower ones on the secondary side than vice versa since, for example, with the primary slits set at 2 mm. (curve 1),

[4]At an excitation wavelength of 470 millimicrons, the band width is then about 20 millimicrons; at a fluorescent wavelength of 536 millimicrons, it is about 30 millimicrons.

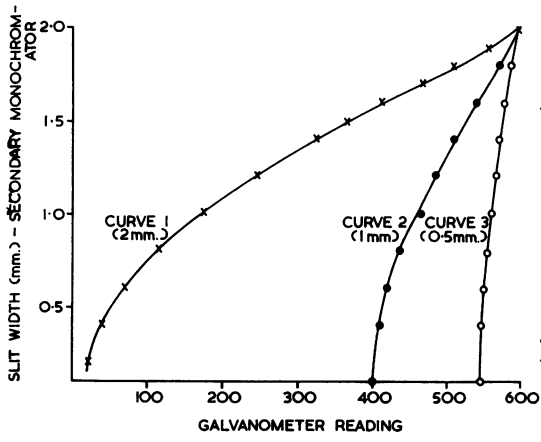

FIG. 3. *The effect of varying the slits of both monochromators on the function of the fluorimeter.*
The galvanometer scale has been expanded by adjusting the 'zero' controls, the 'gain' being kept constant.
The settings of the primary monochromator (SP 500) are given in parentheses.

there is a galvanometer deflection of 35 divisions for a 0·2 mm. difference in secondary slit width between 0·4 and 0·6 mm., while with the primary slit set at 0·5 mm. (curve 3), there is only a 10 division deflection of the galvanometer for a similar difference between 1·8 and 2·0 mm. in the secondary slits.

The advantages of this device are that it can be made at very small cost, fitted in a few moments, and does not affect the normal function of the spectrophotometers used.

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Mattingly, D. (1962). *J. clin. Path.*, **15**, 374.
Udenfriend, S. (1962). *Fluorescence Assay in Biology and Medicine*, p. 62. Academic Press, Inc., New York and London.