Optimal Aminco-Bowman Spectrophotofluorometer Slit Sizes and Arrangements for the Analysis of Serotonin-O.P.T.

The need to measure subtile drug responses in discrete, small tissue areas of relatively low serotonin concentration[1] has imposed severe sensitivity requirements on fluorometric analyses since serotonin is frequently analyzed very near the detection limit. Recently, MAICKEL and MILLER[2] and MAICKEL, COX, SAILLANT and MILLER[3] have reported reaction of orthophthaldialdehyde (O.P.T.) with a number of 3-, 5- and 3-, 5-substituted indoles including serotonin; a 20-fold increase in serotonin standard fluorescence was obtained over conventional methods as a result of this reaction. No data, however, have been presented as optimizing fluorometer slit sizes and arrangements, yet by careful slit selection, detection sensitivity can often be greatly improved[4]. This paper presents experiments performed to select optimal sizes and arrangements of the Aminco-Bowman Spectrophotofluorometer (S.P.F.) slits specifically for analysis of the serotonin-O.P.T. complex. Selection was based on a trade-off study between S.P.F. resolution, sensitivity and primary scattering produced under various conditions. For the trade-off, greater weight was placed on the achievement of highest resolution and sensitivity since scattering is tolerable.

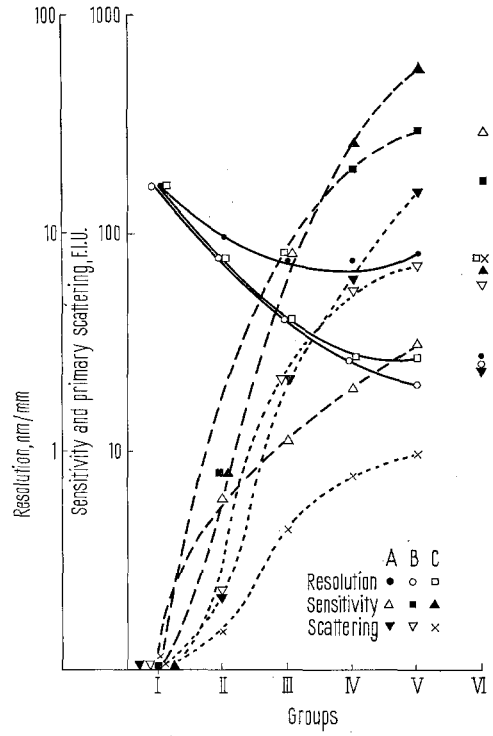
Materials and methods. Spectrophotofluorometer. The Aminco-Bowman S.P.F. experimental settings are indicated in the Table. Slit arrangements representing 5 degrees of resolution and sensitivity were used. Group I was set up for maximal resolution and lowest sensitivity, whereas Group V was set up for maximal sensitivity and lowest resolution. Each of the 5 groups was in turn studied in 3 degrees of scattering. Group VI is similar to V-B in slits 1–6; slit 7 was varied in 3 degrees of scattering to determine the optimal detector slit size.

Preparation of *o*-Phthaldialdehyde (O.P.T.)[5]. Dissolve 50.0 mg of $C_6H_4(CHO)_2$ in sufficient absolute ethanol to obtain exactly 100 ml; weighing must be performed in glass since O.P.T. attacks glassine paper. When stored in a glass stoppered bottle at 4 °C it is stable for at least one month.

Serotonin-O.P.T. specimens. 1.0 ml aliquots of a 620 ng/ml serotonin standard[6] in 0.1 *N* HCl were treated with 0.2 ml of 0.05 g/100 ml (w/v) O.P.T., 2.0 ml of 10 *N* HCl[3] and read. Specimens were activated at 360 nm wavelength and fluorescent emission recorded on a Honeywell solid state X-Y recorder which scanned from 200–800 nm wavelengths. Fluorescence intensity of the serotonin-O.P.T. samples was read at 470 nm wavelength[3].

Results. Results (Figure) indicated at (a) VI-C was eliminated from consideration based on poor resolution and sensitivity in relationship to VI-A and VI-B. Since resolution and scattering were similar in VI-A and VI-B, slit size VI-A (5 mm in position 7) was considered preferential based on sensitivity. (b) Groups I–IV were eliminated from consideration due to poor overall sensitivity and resolution and high scattering in comparison to Group V. (c) V-A was eliminated from consideration based on poor resolution and sensitivity in relationship to the other 2 arrangements in that group. V-C (5.0, 4.0, 5.0, 5.0, 4.0, 5.0 and 5.0 mm in positions 1–7, respectively) produced optimal conditions for reading of serotonin-O.P.T. from the standpoints of maximal sensitivity and resolution. Since greater resolution was produced by V-C over V-B, the increase in sensitivity of V-C over V-B was more than compensated for the slight increase in scattering between these two.

Discussion. The optimum Aminco-Bowman S.P.F. slit sizes and positions need to be determined for each substance (serotonin, catecholamines, histamine, etc.) on an individual basis. These are not standardized and their determination is an essential prerequisite for precise fluorometric detection, and for optimal S.P.F. sensitivity[4].



Spectrophotofluorometer resolution, sensitivity and primary scattering for slit positions and sizes tested. Resolution has been determined at 470 nm wavelength according to the following equation:

$$\text{Resolution} = \frac{\text{Peak width at half-height, nm}}{\text{Minimum slit width, mm}}.$$

Peak width at half-height (or half-intensity band width) is the span of wavelengths leaving the emission monochromator, each of which constitutes at least half as much energy as does the wavelength of maximal emission. Sensitivity is expressed in terms of fluorescence intensity (F.I.) produced at 470 nm wavelength at an instrumental attenuation of 3.0% full scale and a sensitivity of 40 sensitivity units. Primary scattering is expressed in terms of F.I. produced at 360 nm wavelength at similar attenuation and sensitivity settings.

[1] J. H. THOMPSON, Eur. J. Pharmacol. *2*, 329 (1968).
[2] R. P. MAICKEL and F. P. MILLER, Analyt. Chem. *38*, 1937 (1966).
[3] R. P. MAICKEL, R. H. COX JR., J. SAILLANT and F. P. MILLER, Int. J. Neuropharmac. *7*, 275 (1968).
[4] R. F. CHEN, Analyt. Biochem. *20*, 339 (1967).
[5] Mann Research Laboratories, 136 Liberty Street, New York (N.Y. 10006, USA), Lot No. 53369 was used for all experiments.
[6] Serotonin creatinine sulfate, Sandoz Pharmaceuticals, Inc., Route 10, Hanover (N.J. 07936, USA), Lot No. 511786 was used for all experiments.

Slit sizes and positions tested for optimization of spectrophotofluorometer resolution, sensitivity and primary scattering

Group	Slit position 1[a] Activation Monochromator	2[a] Cell	3[b] Cell	4[b] Cell	5[a] Cell	6[a] Emission Monochromator	7[c] Detector
I-A	1.0	0.5	1.0	1.0	0.5	1.0	5.0
B	1.0	0.5	3.0	3.0	0.5	1.0	5.0
C	1.0	0.5	5.0	5.0	0.5	1.0	5.0
II-A	2.0	1.0	1.0	1.0	1.0	2.0	5.0
B	2.0	1.0	3.0	3.0	1.0	2.0	5.0
C	2.0	1.0	5.0	5.0	1.0	2.0	5.0
III-A	3.0	2.0	1.0	1.0	2.0	3.0	5.0
B	3.0	2.0	3.0	3.0	2.0	3.0	5.0
C	3.0	2.0	5.0	5.0	2.0	3.0	5.0
IV-A	4.0	3.0	1.0	1.0	3.0	4.0	5.0
B	4.0	3.0	3.0	3.0	3.0	4.0	5.0
C	4.0	3.0	5.0	5.0	3.0	4.0	5.0
V-A	5.0	4.0	1.0	1.0	4.0	5.0	5.0
B	5.0	4.0	3.0	3.0	4.0	5.0	5.0
C	5.0	4.0	5.0	5.0	4.0	5.0	5.0
VI-A	5.0	4.0	3.0	3.0	4.0	5.0	5.0
B	5.0	4.0	3.0	3.0	4.0	5.0	3.0
C	5.0	4.0	3.0	3.0	4.0	5.0	1.0

Slit sizes in mm.
Principal functions: [a] Resolution and sensitivity; [b] scattering; [c] scattering and sensitivity.

Some investigators have reported the use of specific combinations of slit sizes and positions for individual fluorometric measurements; however, their effectiveness has not always been proven with published data. For the analysis of serotonin by BOGDANSKI et al.'s method[7], WISE[8] has used American Instrument Co. slit arrangement No. 5 (which is similar to that reported here) with the exception that a single polarizer was placed in position 4, whereas GLICK et al.[9] used slit arrangement No. 3. MAICKEL et al.[3] reported using slit sizes 3.0 mm in positions 1–6 and 1.0 mm in position 7 for analysis of serotonin-O.P.T.; however, this combination was apparently selected arbitrarily.

The slit sizes and positions selected here represent a trade-off between instrumental resolution, sensitivity and primary scattering. As can be seen, a compromise was needed, but the combination selected appears optimal from the standpoints of greatest instrumental resolution and sensitivity in the fluorometric measurement of the serotonin-O.P.T. complex[10].

Résumé. Une sélection des dimensions et des arrangements optimaux des fentes a été étudiée pour l'évaluation du complexe sérotonine-O.P.T. (*o*-phthaldialdéhyde) au moyen du spectrophotofluorimètre Aminco-Bowman. Les meilleures conditions ont été réalisées avec les dimensions de 5.0, 4.0, 5.0, 5.0, 4.0, 5.0 et 5.0 mm dans les positions 1–7 respectivement.

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[7] D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, J. Pharmac. exp. Ther. *117*, 82 (1956).
[8] C. D. WISE, Analyt. Biochem. *18*, 94 (1967).
[9] D. GLICK, D. VON REDLICH and B. DIAMANT, Biochem. Pharmac. *16*, 553 (1967).
[10] This research was supported in part by grants from the American Medical Association Education and Research Foundation and National Science Foundation to J.H.T. The authors wish to express their thanks to Mr. HARRY ALTHOUSE of Sandoz Pharmaceuticals, Inc., for the generous supply of serotonin creatinine sulfate.

CONGRESSUS

Israel

2nd International Symposium on Animal and Plant Toxins

in Tel Aviv 22 February – 1 March 1970

Under the auspices of the International Society of Toxicology and the Tel Aviv University the Symposium will be held in the Tel Aviv Hilton Hotel.
Correspondence for further information to be addressed to: Planning Committee, 2nd International Symposium on Animal and Plant Toxins, P.O.B. 16271, Tel Aviv (Israel).

France

La 21ème réunion annuelle de la Société de Chimie physique aura lieu

à Paris du 22 au 25 Septembre 1970.

Elle sera consacrée à une discussion sur le sujet de l'Etat de Transition réactionels: modèles généraux, systèmes hydrocarbonés.
Pour tous renseignements, s'adresser au Secrétariat Général de la Société de Chimie physique, 10, rue Vauquelin, F-75 Paris 5e (France).

CORRIGENDUM

J. W. Parker, Joan Steiner, Andrea Coffin, R. J. Lukes, Kathleen Burr and Laura Brilliantine: *Blastomitogenic Agents in Leguminosae and Other Families*, Experientia *25*, fasc. 2, p. 187 (1969). Because of an inadvertent reversal of code numbers the extract of *Trixis californica* which was reported as showing 38.6% transformation, was actually from a *Phaseolus* species. The *Trixis californica* seeds have subsequently been tested for activity and have produced no significant transformation.