USE OF PYREX TEST & CULTURE TUBES AS SOLUTION CELLS WITH PFALTZ & BAUER PHOTOELECTRIC FLUOROPHOTOMETER

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ABSTRACT

Matched test tubes or culture tubes were used to advantage with the Pfaltz and Bauer photoelectric fluorophotometer in place of the plane sided cuvettes. No instrumental difficulties were introduced by use of these tubes. A reasonable degree of precision and reproducibility of results can be readily attained. In the case of the thiochrome method, a straight line relationship between thiamine concentration and the reading was maintained throughout the range of concentration normally encountered. There was no difficulty in obtaining a supply of suitably matched tubes which yielded consistent results over a considerable range of instrument sensitivity.

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INTRODUCTION

During a study of the application of the well-known thiochrome method (1, 2, 3, and 4) to a variety of fishery products, it became desirable to investigate the feasibility of using ordinary test tubes as solution cells in the cuvette chamber of the Pfaltz and Bauer photoelectric fluorophotometer. There are many practical advantages afforded by such a practice, especially when a considerable number of more or less routine assays are involved. The results of adapting the above-mentioned instrument to the use of test or culture tubes as cuvettes for fluorometric work are reported below.

CONSTRUCTION OF AN ADAPTER

Inasmuch as the regular construction of the instrument did not permit the use of tubes in the measurement of intensities of fluorescent radiation (5 and 6), an adapter was constructed which enabled such usage without alteration of the original form of the instrument.

The latter was fashioned from scrap material available in the laboratory, namely, a wooden spool and small pieces of sheet Bakelite and spring brass. Although a machinist could readily manufacture a more presentable and durable model, that described on page 9 served the purpose and continues to be serviceable.

In Figure 1 are shown drawings of three views of the tube adapter. Only the dimensions of the cover plate are critical, because the latter replaces the regular plate covering the cuvette chamber. The adapter accomodates the oval-shaped bacteriological culture tubes as well as ordinary chemical test tubes. The tubes are held in place by two spring brass clips bent to the desired shape from strips of brass about 50 mm. long, and set into two slots sawed in each side of the spool or "turret". Extension of the depth of the spring clips from 21 to about 30 mm. would, perhaps, insure greater permanency with respect to the precision of alignment of the tubes. However, the depth indicated has thus far proved adequate. In this work, the exclusion of exterior light was completed by covering the "turret", with tube in place, with a mailing cylinder of appropriate dimensions.

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Certain features of the adapter, not ususally found in such an accessory, are as follows:

1. The strong positive action of the brass spring clips not only insures precise and reproducible alignment of either round, or oval-shaped tubes, but also accomodates variations in outside diameter between 15 and 22 mm.

2. No part of the device extends into the cuvette housing to possibly effect interferences.

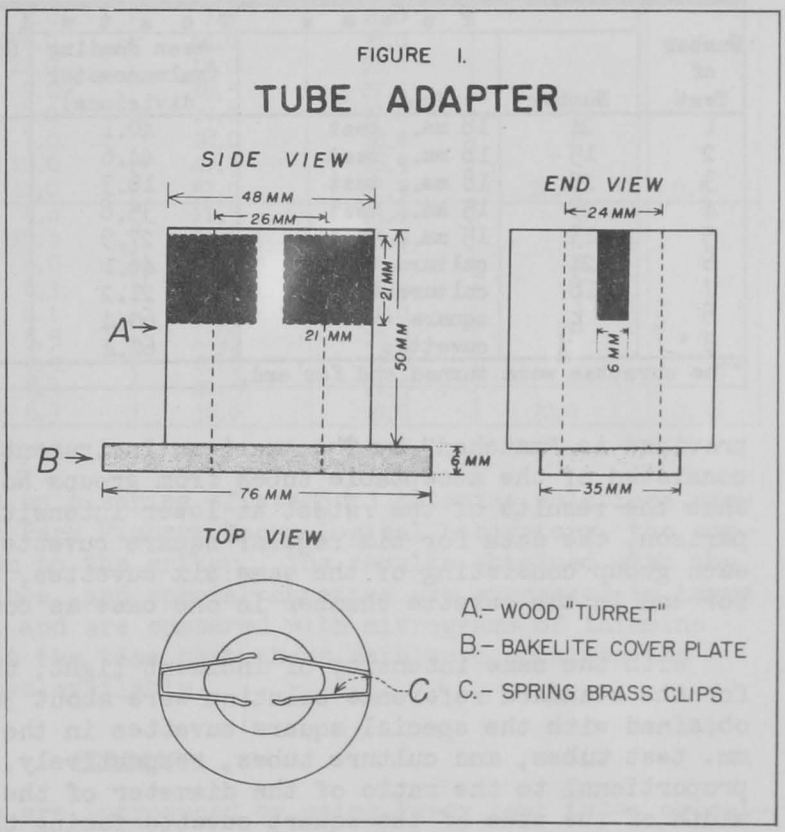
3. Successive measurements or a measurement of intensity of fluorescent radiation and transmitted light on the same solution are possible without disturbing the assembly; and, if desired, an identical surface may be readily presented to either photocell by simply rotating the tube.

THE MATCHING OF TUBES

Ordinary Pyrex chemical test tubes (18mm. in diameter) and oval culture tubes were tested in this work. Quinine sulphate dissolved in 0.1 N H_2SO_4 (0.2 microgram per ml.) was used as the standard reference solution. To minimize effects due to any instability of the quinine sulphate solutions to the exciting radiation (7), a fresh aliquot of the stock solution was used in each tube. To avoid "quenching effects" with temperature change (8 and 9), the reference solution was used at a reasonably constant room temperature (no significant "quenching effect" could be noted when the temperature of the solution was 22° ± 1° C.). Further, in selecting a group of matched tubes, the same procedure was routinely followed for taking readings. Thus, a uniform, minimum exposure to exciting radiation was maintained, such as was found to afford adequate protection of thiochrome solutions (6, 8).

FIGURE I.

TUBE ADAPTER



TEST TUBE OR CULTURE TUBE ADAPTER FOR PFALTZ AND BAUER FLUOROPHOTOMETER.

For the initial readings, the Pyrex brand trademark provided a convenient reference mark for the consistent positioning of the tubes. A distinguishing mark was permanently etched just beneath the trademark on an acceptable tube to serve both as a subsequent reference point, and as a means of identifying matched tubes. With special attention given to cleaning and general handling to avoid scratching, matched tubes

usually yielded reproducible results for an indefinite period. However, it was considered advisable to recheck the performance of tubes periodically or in the case of erratic results.

As an arbitrary standard for the selection of tubes, a variation of ± 0.5 scale division from the mean reading for a given lot of tubes was allowed when the absolute reading was about 50 percent of the total galvanometer scale. This limit of variation was proportionately reduced to ± 0.25 scale division for mean readings of 25 percent or less of the total scale. Tubes found acceptable at the former scale range were retested at the lower range with fresh standard solution, the lower range being obtained by reducing the intensity of incident light. Tubes acceptable at the higher range were usually also acceptable at the lower range of scale, as shown by the data following. Thus, a consistent response was usually obtained, over the range of scale commonly used in the regular assays, which includes a considerable range of instrument sensitivity, as well.

The data recorded in Table 1 are characteristic of those obtained for several lots of the different sizes and types of tubes. Group No. 3 consisted of tubes

Table 1 - Data on the Matching of Test, and Culture Tubes for Fluorometric Readings

Number of Test	Tubes Tested				Tubes Accepted	
	Number	Type	Mean Reading (galvanometer divisions)	Standard Deviation (galvanometer divisions)	Number	Percent of Total
1	24	18 mm., test	40.1	0.33	17	71.0
2	15	18 mm., test	44.6	0.46	11	76.0
3	25	18 mm., test	16.3	0.27	20	80.0
4	23	15 mm., test	35.8	0.43	14	60.5
5	13	15 mm., test	27.9	0.25	12	92.0
6	24	culture tubes	46.1	0.37	18	75.0
7	18	culture tubes	21.2	0.21	18	100.0
8 *	6	square cuvettes	60.4	1.09	-	-
9 *			60.4	0.80	-	-

*The cuvettes were turned end for end.

provided as "matched" by The American Instrument Company. Groups No. 5 and 7 consisted of the acceptable tubes from groups No. 4 and 6, respectively, and show the results of the retest at lower intensities, mentioned above. For comparison, the data for six regular square cuvettes are recorded as No. 8 and 9, each group consisting of the same six cuvettes, but with the latter turned end for end in the cuvette chamber in one case as compared with the other.

With the same intensity of incident light, the absolute readings obtained for the standard reference solution were about 38, 44, and 57 percent of that obtained with the special square cuvettes in the case of 15 mm.-test tubes, 18-mm. test tubes, and culture tubes, respectively. These values are approximately proportional to the ratio of the diameter of the respective type of tube to the width of the side of the square cuvette facing the photocell.

THE USE OF MATCHED TUBES IN THE THIOCHROME PROCEDURE

For the oxidation to thiochrome and preparation of appropriate blanks, according to the well-known thiochrome procedure (1, 2, 3, 4, and 10), 5 ml. aliquots of standard solutions of thiamine hydrochloride were used. The concentrations of the latter ranged from 0.05 to 0.4 microgram per ml.. Readings of the usual iso-

butyl-alcohol extracts of samples and blanks, using the previously mentioned types and sizes of tubes as solution cells, are recorded in Table 2. Data for the same series of standard solutions using the regular cuvettes are recorded for comparison.

Table 2 - Data on the Assay of Pure Thiamine Solutions, Using Test, and Culture Tubes as Solution Cells

Solution Cell	Thiamine in Sample in Micrograms	Total Reading (galvanometer divisions)	Blank Reading (galvanometer divisions)	Net Reading (galvanometer divisions)	Galvanometer Divisions Per Microgram (calculated)	Mean Reading Per Microgram (galvanometer divisions)	Standard Deviation
Special cuvette	0.5	33.0	6.8	26.2	52.4		
	0.5	38.3	11.5	26.8	53.6		
	0.6	40.0	7.0	33.0	55.0		
	0.9	55.5	7.5	48.0	53.0		
	1.0	65.0	9.9	55.1	55.1		
	1.5	80.0	9.0	72.0	52.0	53.5	1.27
Culture tubes	0.25	18.4	9.2	9.2	36.8		
	0.25	18.2	9.2	9.0	36.0		
	0.5	24.3	6.0	18.3	36.6		
	0.5	32.5	13.2	19.3	38.6		
	1.0	44.1	9.2	34.9	34.9		
	1.0	48.7	10.0	38.7	38.7		
	1.0	48.8	11.5	37.3	37.3		
	1.5	73.0	17.5	55.5	37.0	36.9	1.35
18-mm. test tubes	0.25	14.0	7.0	7.0	28.0		
	0.5	22.0	8.0	14.0	28.0		
	0.5	24.0	10.0	14.0	28.0		
	0.6	25.5	9.0	16.5	27.5		
	1.0	34.0	7.0	27.0	27.0		
	1.0	35.0	7.0	28.0	28.0		
	1.5	52.7	10.0	42.7	28.5		
	2.0	67.0	10.0	57.0	28.5	28.0	0.5
15-mm. test tubes	0.5	19.5	8.0	11.5	23.0		
	0.5	28.8	17.2	11.6	23.2		
	0.9	25.5	6.0	19.5	21.6		
	0.9	26.3	6.3	20.0	22.0		
	0.9	25.0	6.1	18.9	21.0		
	0.6	18.0	5.5	12.5	20.8		
	1.0	30.0	7.2	22.8	22.8		
	1.0	29.8	8.3	21.5	21.5		
	1.5	36.3	6.3	30.0	20.0	22.0	0.98

In Table 3 are recorded data on a series of standard thiamine solutions prepared by a member of the College Park Fishery Technological Laboratory, the concentrations of which were unknown to the writer. The results obtained with the matched 15-mm. tubes, culture tubes, and special cuvettes are expressed in terms of micrograms of thiamine found, and are compared with micrograms of thiamine present in 5 ml. of solution. At the time that these data were obtained the matched 18 mm.-test tubes were not available.

SUMMARY

No instrumental difficulties are introduced by using Pyrex test tubes or culture tubes as solution cells with the Pfaltz and Bauer photoelectric fluorophotometer for fluorometric measurements (5 and 6). It also appears evident that a reasonable degree of precision and reproducibility of results can be readily attained; and that, in the case of the thiochrome assay, a straight line relationship between thiamine concentration and reading is maintained throughout the range of concentration normally encountered. It is further apparent that there is no particular difficulty in obtaining a supply of suitably matched tubes which yield consistent results over a considerable range of instrument sensitivity.

The oval-shaped culture tubes provided both the convenience of test tubes, and a greater sensitivity in terms of galvanometer deflection per microgram of thiamine. Therefore, this type of tube was used chiefly in subsequent work involving a considerable number of assays of extracts of fishery products. The regular cuvettes were used to check the results of an appropriate number of these assays.

Table 3 - Assay of "Unknown" Concentrations of Thiamine, Using Test, and Culture Tubes as Solution Cells (Micrograms Thiamine per Aliquot)

Sample	Amount Present	Amount Found*		
		Special Cuvettes	15-mm. Test Tubes	Culture Tubes
A	1.50	1.51	1.50	1.52
B	0.90	0.95	0.94	0.92
C	1.10	1.09	1.09	1.12
D	1.90	1.92	1.92	1.91
E	0.60	0.60	0.60	0.60
X	0.70	0.69	0.70	0.70

*The values are the averages of the results of at least four assays, in each case.

From these latter results, together with those summarized in this article, it was concluded that, at least for practical purposes, matched test tubes may be used to advantage with the Pfaltz and Bauer instrument for fluorometric assays in place of of the plane-sided cuvettes. The desirability of having available a number of the latter for control and calibration purposes is, of course, obvious.

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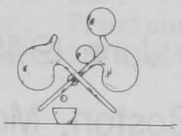

FLOUNDER BIRDS

2 pounds flounder fillets
1 teaspoon salt
1/8 teaspoon pepper

Bread stuffing
4 tablespoons melted butter or other fat
3 slices bacon (optional)

Remove skin from fillets. Sprinkle both sides with salt, pepper. Place a small ball of stuffing on each piece of fish. Roll fish around stuffing and fasten with toothpicks or skewers. Place rolls on a well greased baking pan. Brush tops with melted fat and lay ½ slice of bacon on the top of each. Bake in a moderate oven 350° F. for 25 to 35 minutes depending on size. Remove carefully to a hot platter, take out fastenings, garnish and serve hot. Serves 6.

Sole or other small fillets may be used for the above recipe.