THE FLUOROMETRIC DETERMINATION OF O-PHTHALIC ACID INCLUDING THE ASSEMBLY OF A FLUOROMETER

Thesis for the Degree of M. S.

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THE FLUOROMETRIC DETERMINATION OF O-PHTHALIC ACID INCLUDING THE ASSEMBLY OF A FLUOROMETER

By

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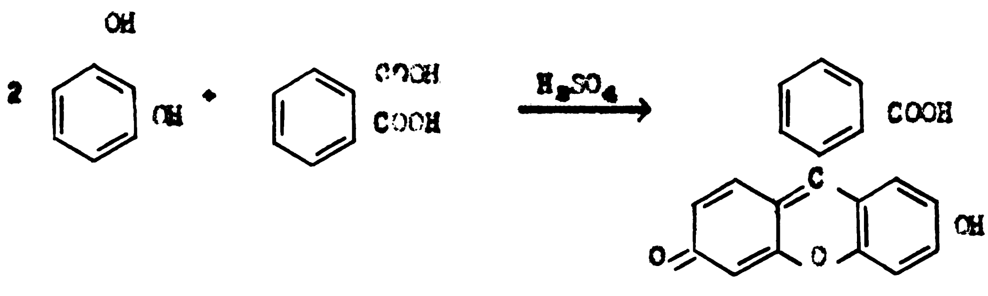
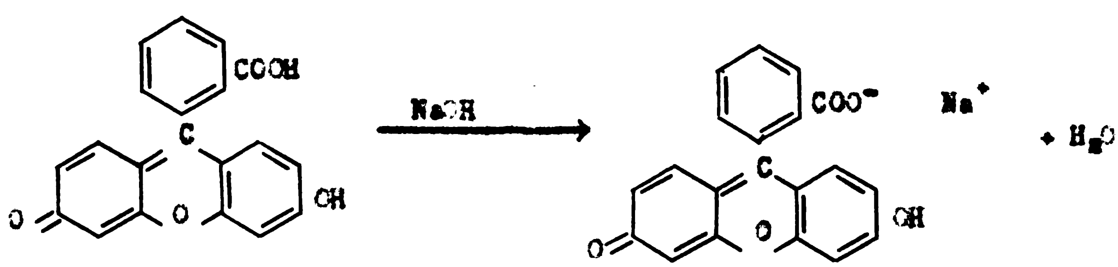
HISTORICAL

The condensation of 1,2-dicarboxylic acids with resorcinol to yield dyes of the fluorescein type has been recorded by Feigl (1), and the fluorescent compounds formed, as a result of these condensations, have been used for qualitative identification of 1,2-dicarboxylic acid structures. The reaction, according to Feigl, proceeds as follows:



I (Fluorescein)



II

A strong greenish-yellow fluorescence results on the addition of alkali to the reaction product (I) due to the formation of the ionic salt (II). This fluorescence is indicative of the presence of the 1,2-dicarboxylic acid structure.

Barr (2) has made a quantitative application of this type of reaction in developing a fluorometric method for the determination of succinic acid in apple tissue. Samples containing from 1 to 16 micrograms per 100 mls. were determined with a maximum deviation of plus or minus one microgram.

STATEMENT OF PROBLEM

The determination of mixtures of organic acids has long been a problem of considerable difficulty. The determination of such mixtures usually involves tedious azeotropic distillations (3, 4) or measurement of distribution between immiscible solvents (5, 6). Both of these methods are merely approximations and are not applicable to many systems.

The fluorometric determination of o-phthalic acid, by condensation with resorcinol, should permit its determination in the presence of other organic acids, excluding other 1,2-dicarboxylic acids. The application of such a method to a mixture of o-phthalic acid and benzoic acid is especially promising since benzoic acid is one of the major by-products in the industrial oxidation of naphthalene to o-phthalic acid.

The following discussion is the result of a study undertaken in an attempt to apply Feigl's qualitative fluorescence test on a quantitative basis, with respect to o-phthalic acid.

EXPERIMENTAL

1. General discussion

Since Barr (2) had succeeded in quantitatively applying Feigl's (1) spot test for 1,2-dicarboxylic acids to the determination of succinic acid, an attempt was made to apply his procedure to o-phthalic acid. Barr's procedure involved taking a 1.0 or 2.0 ml. aliquot of an aqueous solution of the acid and evaporating it to dryness at 70-100°C in an oven. The dry sample was moistened with 0.04 ml. of pure concentrated sulfuric acid and mixed thoroughly with 5-10 mgs. of freshly sublimed resorcinol. The mass was then heated at 125-130°C for one hour in an oven. The reaction residue was taken up in distilled water and quantitatively transferred to a 200 ml. volumetric flask and diluted to the mark. A 10 ml. aliquot of this solution was adjusted to the optimum pH and further diluted to 100 mls. This solution was then used for fluorescence measurements. Blanks were prepared in a similar manner, substituting an equal volume of distilled water for the aliquot of o-phthalic acid.

Considerable difficulty was encountered in obtaining reproducible results by this method. It appeared, that due to the insolubility of the o-phthalic acid in the concentrated sulfuric acid, complete reaction between the o-phthalic acid and resorcinol was not being effected. The procedure was, therefore, modified in order to assure completeness of reaction. This was accomplished by adding the concentrated sulfuric acid and resorcinol directly to the o-phthalic acid aliquot and then taking to dryness. In this manner a homogeneous reaction solution was obtained and fair reproducibility resulted.

A strong deep blue fluorescence was observed in all blank solutions prepared. The intensity of the blank fluorescence was so great, in most cases, that it accounted for 20-50% of the over-all fluorescence noted in the samples.

Deniges (7) has reported a qualitative test for the identification of resorcinol based on the strong blue fluorescence resulting on heating it with concentrated sulfuric acid. He postulated that the fluorescent species was some type of condensate of sulfuric acid and resorcinol, possibly a diester of sulfuric acid.

Since the large blank fluorescence seemed to result from reaction between sulfuric acid and resorcinol, the possibility of using other dehydrating agents in the condensation reaction was explored. Concentrated phosphoric acid, phosphorus pentoxide, acetic anhydride and anhydrous zinc chloride were tried. All of these reagents, with the exception of acetic anhydride, promoted the reaction between o-phthalic acid and resorcinol but also yielded the familiar deep blue blank fluorescence. The acetic anhydride apparently acted as an acetylating agent rather than as a dehydrating agent, since no fluorescence was observed from either the sample or blank solutions. The fact that anhydrous zinc chloride also effects the formation of the fluorescent blue blank species rules out ester formation and seems to indicate that the blank fluorescence results from either decomposition of the resorcinol or interaction of the resorcinol with itself.

None of the above reagents showed any particular advantage over sulfuric acid as the dehydrating agent and consequently all subsequent work was carried out employing sulfuric acid.

Since it seemed apparent that complete elimination of the blank fluorescence was impossible, an attempt was made to minimize it by running the reactions at reduced temperatures and pressures. The blank fluorescence was decreased by this procedure; however, incomplete reaction of the resorcinol and o-phthalic acid again resulted, as indicated by poor reproducibility of sample fluorescence intensity.

Although the large blank fluorescence could not be controlled by the above methods, it was subsequently found possible to decrease the contribution of the blank fluorescence to an insignificant amount by working in highly alkaline solutions. The data supporting this statement are presented and discussed in the following sections, 3 and 4, dealing with the effect of pH on sample and blank fluorescence.

2. Instrumentation and Filter Selection

The instrument used, throughout the course of most of this work, is the one whose construction and operating procedure are discussed in the appendix of this thesis. However, some data were compiled on the Lumetron Model 402-EF instrument.

The primary filter was selected by checking a series of four filters to determine which one permitted passage of the most highly exciting band, relative to fluorescein solutions.

TABLE I

SELECTION OF PRIMARY FILTER

Filter Type	Fluorescein fluorescence intensity
Corning #5840	78
Pyrex #5874	55
Corning #5860	45
Lumetron Primary	56

As a result of these measurements, the Corning #5840 was selected for use as the Primary.

The secondary filter was selected by determining which of a group of green filters, permitted maximum transmission of the green fluorescein fluorescence.

TABLE II

SELECTION OF SECONDARY FILTER

Filter Type	Instrument Reading
Corning #4015	11.5
Corning #4010	4.0
Corning #4784	15.2
Corning #4084	9.0
Corning #4407	12.5
Corning #4308	16.0

The Corning filter #4015 was selected since its color most closely matched that of the fluorescence of fluorescein. The filters #4784, #4407 and #4308 transmitted more of the fluorescent light, but all had blue tinges which would seem to favor high transmission of the blue blank fluorescence also. Consequently, these were ruled out in favor of the filter #4015. In conjunction with this filter, the ultra-violet filter from the Lumetron instrument was used to prevent any scattered ultra-violet light from striking the photo cell.

3. Effect of pH on Sample Fluorescence

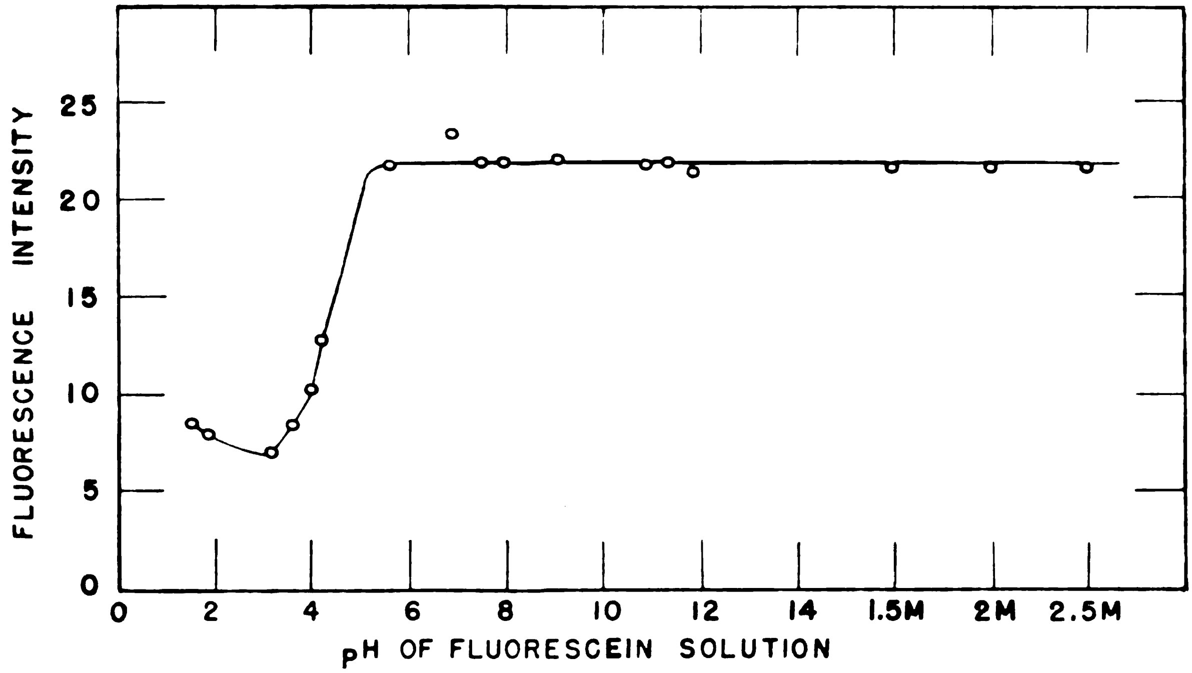
Since many fluorometric methods are highly sensitive to pH changes and demand quite rigid control of pH, a study was made of the effect of pH on the fluorescence intensity of fluorescein solutions, resulting from the condensation of o-phthalic acid with resorcinol. It was found that the reacted o-phthalic acid solutions showed an essentially constant fluorescence intensity in all solutions having a pH greater than 5.5 and even remained constant in solutions which were 2.5 M in sodium hydroxide. Graph I shows the over-all effect of pH on fluorescence intensity and indicates that close pH control is not necessary as long as the pH is greater than 5.5.

4. Effect of pH on Blank Fluorescence

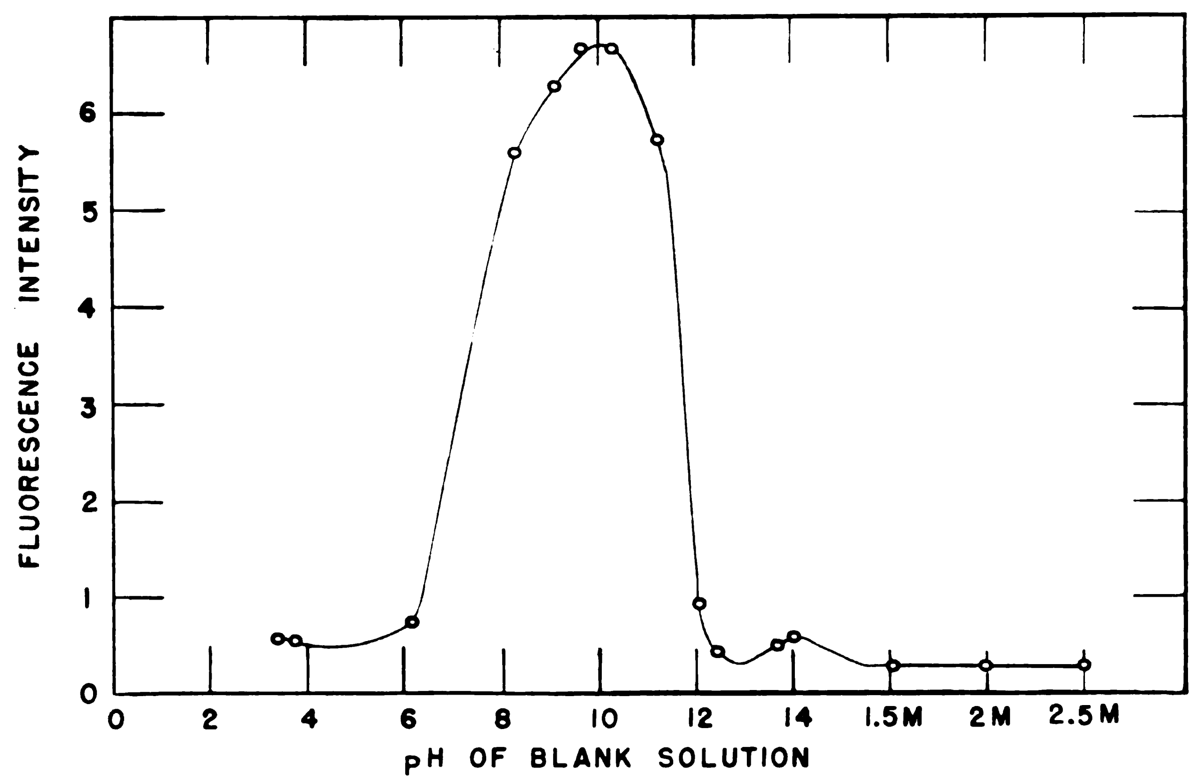
Graph II shows the behavior of blank solutions under conditions of varying alkali concentration. The graph indicates that minimum, constant, blank fluorescence is shown in strongly alkaline solutions, 1.5 M or greater in sodium hydroxide. Conversely, the sample shows a maximum and constant fluorescence intensity in strongly alkaline solutions. Therefore, it was decided to work in solutions which were 1.5 M or greater in sodium hydroxide. In this manner the blank fluorescence was suppressed to such an extent, that under normal conditions, it never exceeded 0.2 of a scale unit relative to distilled water as the zero fluorescence setting.

5. Use of Unsublimed Resorcinol

Both Feigl and Barr state specifically that freshly sublimed resorcinol should be used in the formation of the fluorescent species. Presumably a higher sample fluorescence and a lower blank fluorescence are obtained through use of freshly sublimed resorcinol. However, in a comparison of the results obtained using freshly sublimed resorcinol and unsublimed



GRAPH I. EFFECT OF pH ON SAMPLE FLUORESCENCE



GRAPH 2. EFFECT OF pH ON BLANK FLUORESCENCE

chemically pure resorcinol, no significant difference was observed under the conditions employed. The samples used in this study were 1.0 ml. aliquots of an o-phthalic acid solution containing 1.0 mg. per ml.

TABLE III

SUBLIMED RESORCINOL vs UNSUBLIMED

Freshly Sublimed		Unsublimed	
Sample Fluorescence	Blank Fluorescence	Sample Fluorescence	Blank Fluorescence
14.3*	0.12*	14.5*	0.21*
14.5	0.11	14.3	0.21
14.5	0.12	14.5	0.15
14.3	0.20	14.5	0.19
14.5	0.30	14.0	0.24
14.5	0.20	14.5	0.24
14.5	0.22	14.2	0.18
Ave. = 14.44	Ave. = 0.18	Ave. = 14.36	Ave. = 0.20

* Instrument readings

The slight difference noted between the average results obtained, is deemed insignificant because it is no greater than the average deviation from the mean in each case and is, therefore, assumed to be within the limits of reproducibility of the method.

The elimination of the sublimation procedure from the method does not appear to bring about any detrimental results and reduces the time required for the over-all procedure by approximately two and one-half hours; thereby, reducing by more than one-half the over-all time requirements of the procedure.

6. The Effect of Varying Quantities of Sulfuric Acid upon the Intensity of Fluorescence

In determining the optimum volume of concentrated sulfuric acid to use in effecting the condensation of o-phthalic acid and resorcinol; the other variables, oven temperature and time of reaction, were held constant. The oven was maintained at 125°C and the reaction period was taken as one and one-half hours. One ml. aliquots of an o-phthalic acid solution containing 1 mg. per ml. were used as samples.

TABLE IV

OPTIMUM VOLUME OF SULFURIC ACID

Volume H_2SO_4	0.01 ml.	0.05 ml.	0.10 ml.	0.15 ml.	0.20 ml.
Instrument Reading Sample I	14.2	14.5	13.5	11.8	8.28
Instrument Reading Sample II	12.0	15.0	13.0	11.5	9.12

Since the maximum fluorescence was attained using 0.05 ml. of concentrated sulfuric acid, volumes lying in the ranges 0.01-0.05 ml. and 0.05-0.10 ml. were checked in order to more closely approximate the optimum volume.

TABLE V

OPTIMUM VOLUME OF SULFURIC ACID

Volume H_2SO_4	0.02 ml.	0.03 ml.	0.04 ml.	0.06 ml.	0.07 ml.
Instrument Readings Sample I	14.8	14.8	14.5	14.2	13.0
Instrument Readings Sample II	14.5	14.5	14.5	14.0	14.0

The results show that an essentially constant, maximum fluorescence is obtained with all volumes of sulfuric acid in the range 0.02-0.06 ml. Therefore, 0.04 ml. of concentrated sulfuric acid was selected as the optimum volume, since it represents the middle of this range.

7. Determination of Optimum Reaction Time

In the evaluation of the optimum time of reaction the oven temperature was maintained constant at 125°C and 0.04 ml. of concentrated sulfuric acid was used. An o-phthalic acid solution containing 1.0 mg. of acid per ml. was again used for the sample preparation.

TABLE VI

OPTIMUM REACTION TIME

Reaction Time	1 hr.	1 hr.15 min.	1 hr.30 min.	1 hr.45 min.	2 hrs.
Instrument Reading Sample I	5.65	14.0	14.0	14.2	14.0
Instrument Reading Sample II	1.50	14.0	13.8	14.0	14.0

From the above data it is apparent that the reaction is complete after seventy-five minutes at 125°C. However, as a precautionary measure, ninety minutes was selected as the optimum time to preclude any possibility of incomplete reaction.

8. Determination of Optimum Oven Temperature

In a study of various reaction temperatures it was found that at temperatures below 120°C incomplete reaction resulted and at temperatures of 130°C or greater, complete reaction resulted but extraordinarily large blanks were observed. However, by maintaining the temperature at approximately 125°C complete reaction was still effected and the blank fluorescence was held to a minimum.

TABLE VII

OPTIMUM OVEN TEMPERATURE

	Temperature					
	105°C	110°C	125°C		130°C	
			Samples	Blanks	Samples	Blanks
Instrument Readings Sample I	6.45	11.1	14.2	0.22	14.2	0.59
Instrument Readings Sample II	7.15	13.5	14.5	0.21	14.0	0.58

9. Stability of Fluorescent Species in 2 N Sodium Hydroxide

Since the final dilution in the method is made with 2 N sodium hydroxide, the stability of the fluorescent species in this strongly alkaline solution was checked. It was found that after a period of three days the fluorescence intensity falls off by 0.3-0.5 units. This very slight decrease in fluorescence intensity, indicates that the species is quite stable, undergoing only a slight amount of decomposition.

10. Preparation of the Calibration Curve

The concentration range, over which the method is applicable, was determined. It was found that if quantities of o-phthalic acid less than 50 micrograms were reacted, it became difficult to obtain reproducible results. The high contribution of the blank fluorescence to the over-all fluorescence intensity, at low concentrations, is undoubtedly the cause of this lack of uniformity. Consequently 50 micrograms was designated as the lower concentration limit. The upper concentration limit was governed by the solubility of o-phthalic acid in water. The solubility of o-phthalic acid in water is approximately 0.54 gms. per 100 mls. Therefore, the upper concentration limit was taken as 5000 micrograms, although through use of a suitable solvent, the reaction could probably be applied to higher concentrations.

For the preparation of the calibration curve, thirteen solutions were prepared covering the range of 50 to 5000 micrograms. To assure that the fluorescence intensity value found for each concentration would represent a true average value, six samples were run at each concentration. The six samples were run over a period of three days, two being run each day. Each point on the curves, therefore, corresponds to an average fluorescence value of six samples.

TABLE VIII

COMPILATION OF CALIBRATION CURVE DATA

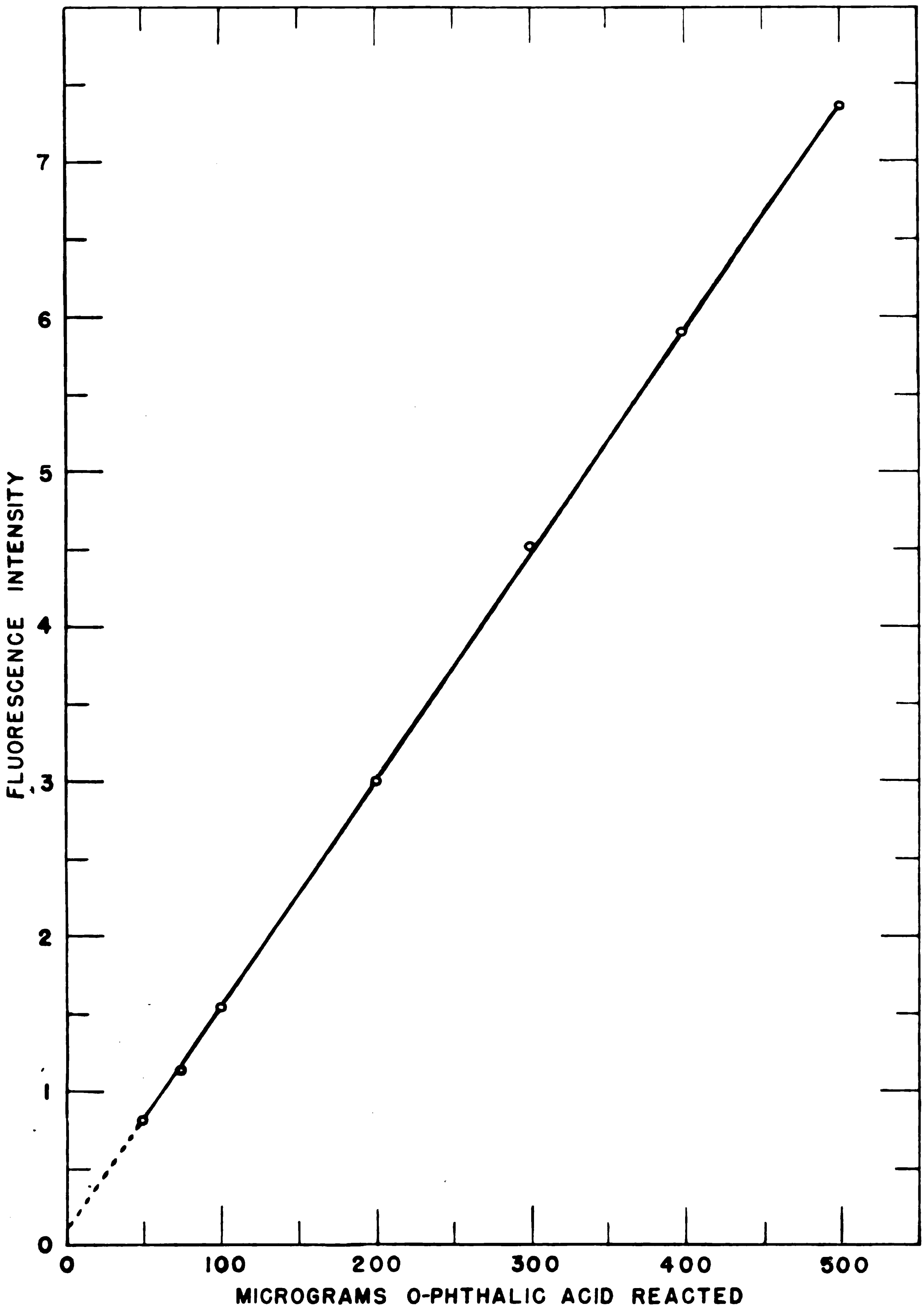
Amt. O-phthalic Acid Reacted	1	2	3	4	5	6	Average
50	0.800	0.850	0.770	0.850	0.825	0.770	0.827*
75	1.10	1.18	1.15	1.22	1.12	1.13	1.15
100	1.58	1.55	1.55	1.60	1.50	1.50	1.55
200	3.05	2.95	3.05	3.05	2.90	3.00	3.00
300	4.50	4.50	4.60	4.58	4.45	4.50	4.52
400	5.80	5.79	5.90	6.00	6.00	5.95	5.91
500	7.45	7.40	7.40	7.50	7.45	7.20	7.38
800	11.0	11.2	11.5	11.5	11.5	11.2	11.3
1000	14.5	14.0	14.5	14.0	14.5	14.2	14.3
2000	28.2	28.2	27.5	27.5	28.5	28.0	28.0
3000	40.5	40.5	40.0	40.0	42.0	41.5	40.8
4000	50.5	49.3	53.0	52.2	55.5	53.0	52.3
5000	58.5	62.3	64.5	63.2	65.0	64.5	63.0

* Average instrument readings

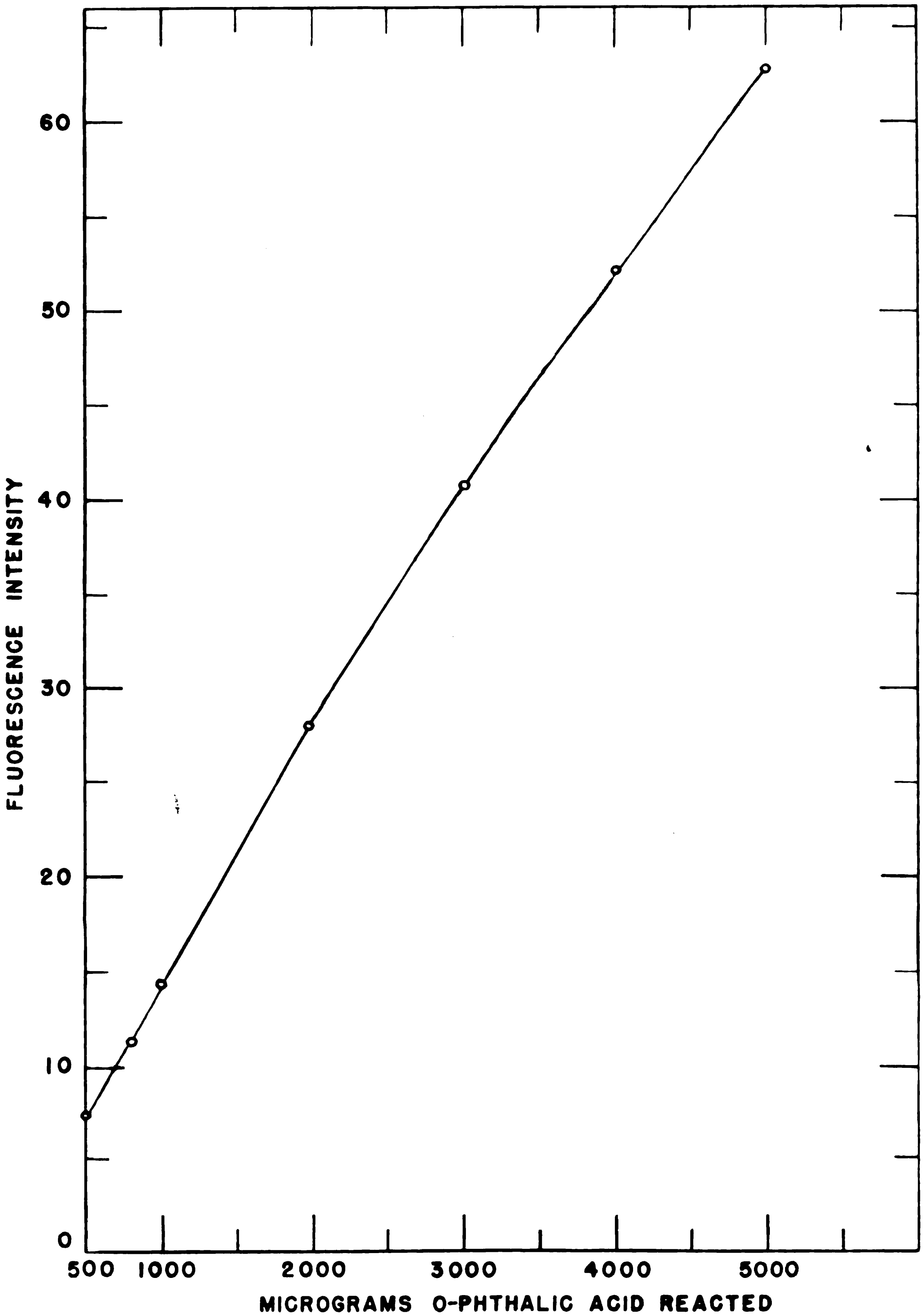
From the calibration curves (Graphs 3 and 4) it can readily be seen that a straight line relationship exists between fluorescence intensity and concentration up to approximately 2000 micrograms. Above 2000 micrograms the curve gradually falls off and no longer displays perfect linearity. However, the curve is sufficiently uniform in the range 2000 to 5000 micrograms that quite reliable results are obtained for samples falling in this range.

The validity of the calibration curves was determined by preparing solutions of known concentrations of primary standard grade potassium acid phthalate and checking them against the calibration curves after reaction. Two stock solutions were prepared, one of which contained potassium acid phthalate equivalent to 4.19 mgs. of o-phthalic acid per ml. and another which was equivalent to 5 mgs. of o-phthalic acid per ml. These stock solutions were used to prepare six other solutions containing potassium acid phthalate equivalent, on acidification, to 83.7, 100, 419, 500, 837 and 1000 micrograms of o-phthalic acid per ml. One ml. aliquots of these solutions were reacted with resorcinol and their fluorescence intensities were measured. The amounts of o-phthalic acid, found by comparing these fluorescence intensity values with the calibration curves, corresponded quite favorably with the true amounts present. A maximum error of 3% was observed.

With the validity of the calibration curves being fairly well established, a group of unknown o-phthalic acid solutions were prepared and determined by application of the method. Four 50 ml. solutions of unknown o-phthalic acid content were prepared, without the knowledge of the analyst.



GRAPH 3. CALIBRATION CURVE (I)



GRAPH 4. CALIBRATION CURVE (2)

TABLE IX

VERIFICATION OF CALIBRATION CURVE

Fluorescence* Intensity	Micrograms o-phthalic acid found	Micrograms o-phthalic acid taken	Percent Error
1.29	81.8	83.7	-2.3
1.55	100.0	100.0	0.0
6.22	421.0	419.0	+0.6
7.48	515.0	500.0	+3.0
11.8	823.0	837.0	-1.7
14.1	975.0	1000.0	-2.5

* Average value of four samples

One ml. aliquots of these solutions were taken as samples. Four samples were drawn from each of the unknown solutions and their fluorescence intensities were measured after reaction. An average value of the intensity for each solution was then used to find its concentration from the calibration curves.

TABLE X

DETERMINATION OF UNKNOWN o-PHTHALIC ACID SAMPLES

Average Fluorescence Intensity	Micrograms Per Ml. Found	Milligrams Per 50 Ml. Found	Milligrams Per 50 Ml. Taken	Percent Error
2.13	139	6.95	7.00	0.7
3.39	227	11.4	11.0	3.6
8.47	585	29.3	29.0	1.0
15.4	1090	54.5	54.0	0.9

The maximum error found on this group of determinations was approximately 4%, which is in fair agreement with the maximum error observed when potassium acid phthalate solutions were checked against the calibration curve.

11. Application of the Method in the Presence of Various Contaminants

Feigl states that para dicarboxylic acids, of the terephthalic acid type, sometimes undergo condensation with resorcinol to yield fluorescent compounds. It was found on application of the method to an o-phthalic acid solution saturated with isophthalic and terephthalic acids, that no noticeable interference resulted from the presence of these acids. However, terephthalic acid has a very limited solubility in aqueous solution, 1.0 mg. per 100 mls., and consequently was probably present in such minute quantities that any contribution to the fluorescence intensity, made by it, was negligible.

In this evaluation a solution saturated with isophthalic and terephthalic acids and containing 500 micrograms of o-phthalic acid per ml. was used. Six 1.0 ml. samples were run and the average value of the fluorescence intensities of the six samples, was used in determining the concentration from the calibration curve. The concentration, as determined in this manner, was found to be 494 micrograms per ml. This 6 microgram deviation from the true value represents an error of 1.2%, which is considerably under the maximum error of 3.6% noted with pure samples.

Since benzoic acid and α-naphthoquinone are both formed, to a certain degree, in the industrial oxidation of naphthalene to o-phthalic acid, an attempt was made to apply the method in the presence of these contaminants.

Benzoic acid does not have any apparent effect on the method; however, the presence of α-naphthoquinone lends an orange-red color to the solutions which is accompanied by a slight decrease in fluorescence intensity.

TABLE XI

EFFECT OF VARIOUS CONTAMINANTS

Contaminant	Saturated With Benzoic Acid	Saturated With α-naphthoquinone	Saturated With Benzoic Acid and α-naphthoquinone
Micrograms o-phthalic acid taken	1000	1000	1000
Micrograms o-phthalic acid* found	990	975	950
Percent Error	1%	2.5%	5%

* Average of three samples

This effect was particularly noticeable in solutions saturated with both benzoic acid and α-naphthoquinone, where an error of 5% was detected. The method, therefore, has definite accuracy limitations in the presence of α-naphthoquinone due to color formation.

Many fluorometric methods involving the use of dehydrating agents, cannot be applied in the presence of sugars. Therefore, the effect of the presence of sugars on the method was determined. It was found that the method fails completely if sugars are present. The concentrated sulfuric acid preferentially chars the sugar and no green fluorescence, indicative of reaction between o-phthalic acid and resorcinol was noted.

12. Recommended Procedure

The reagents required for carrying out this determination are; chemically pure resorcinol, pure concentrated sulfuric acid and a solution of approximately 2 N sodium hydroxide.

An aqueous solution of o-phthalic acid should be used. Therefore, if a solid sample is to be determined, dissolve a weighed sample in a measured volume of water, using a small amount of sodium hydroxide to effect solution if necessary.

A 1.0 ml. aliquot of the aqueous sample solution is pipetted into a 5 ml. beaker and 0.04 ml. of pure concentrated sulfuric acid is added with a graduated one ml. pipet. Approximately 5 to 10 mgs. of chemically pure resorcinol is added and the mixture thoroughly stirred to promote solution of the resorcinol. The samples are then placed in an oven, at a controlled temperature of 125°C, for a period of one and one-half hours. At the completion of this reaction period, the samples are removed from the oven and allowed to cool to room temperature. The reaction products in the beaker are taken up in distilled water and quantitatively transferred to a 200 ml. volumetric flask. If a high concentration of o-phthalic acid was present in the sample, some difficulty may be encountered in dissolving the reaction products. However, addition of a small amount of dilute sodium hydroxide readily affects solution. Dilution to 200 mls. is made with distilled water and a 10 ml. aliquot of this solution is transferred to a 100 ml. volumetric flask. Dilution to 100 mls. is then made with 2 N sodium hydroxide.

The dilution procedures yield a final volume of 100 mls. which contains fluorescein equivalent to only one-twentieth of the original amount of o-phthalic acid reacted. Consequently, the possibility of eliminating one of the dilutions and making the method applicable to quantities of o-phthalic acid less than 50 micrograms was investigated. It was found that reducing the degree of dilution increased the intensity of the blank fluorescence, proportionately, much more than it did the sample fluorescence intensity. For example, when a 50 microgram sample was reacted and the reaction products directly diluted to 100 mls. with 2 M sodium hydroxide, the intensity of the blank fluorescence was increased approximately tenfold while the intensity of the sample fluorescence was only doubled, relative to the results observed under the conditions of the normal dilution procedure. This high contribution of the blank fluorescence under these conditions, makes reproducibility of the sample fluorescence intensity virtually impossible and in cases of low sample concentration, it completely blots out the green fluorescence of the samples and makes them appear blue on excitation. Therefore, it was concluded that the dilution procedures, as described previously, are essential if the contribution of the intensity of the blank fluorescence is to be held to an insignificant amount.

The final solution, resulting from the dilution procedures, is used for the fluorescence intensity measurement. The fluorometer is set to give a zero reading with distilled water and the fluorescence intensity of the sample solution is read relative to this setting. If a null-point type instrument, such as the Lumetron model 402-EF, is used for the fluorescence intensity measurements, a standard solution must be used to obtain a "100" setting. A fluorescein solution, of appropriate concentration for the range being studied, is sufficiently stable for this purpose.

After the fluorescence intensity of the solution has been measured, the quantity of o-phthalic acid reacted originally, is found by comparison of the intensity value with the calibration curve.

Although no correction is applied for blank fluorescence, since it is deemed insignificant and is essentially constant at 0.1 to 0.2 units, it is a wise procedure to prepare a blank, as a control, for each group of samples determined.

The calibration curve is prepared by reacting o-phthalic acid solutions, of known concentrations, as described above and determining their fluorescence intensities. A plot of fluorescence intensities as ordinate and micrograms of o-phthalic acid reacted as abscissa then serves as a calibration curve.

The primary filter used in this determination is the Corning #5840 and the secondary filters are a green Corning #4015 filter and an ultraviolet filter which absorbs, primarily, the 365 millimicron mercury lamp emission.

SUMMARY

A fluorometric method has been presented for the determination of o-phthalic acid. Concentrations of o-phthalic acid ranging from 50 to 5000 micrograms per ml., can be determined in the presence of benzoic, iso-phthalic and terephthalic acids with an expected maximum error of approximately 4%. In the presence of α-naphthoquinone a slightly larger error, approximately 5%, is to be expected due to color formation resulting in internal absorption of fluorescent emission. The time requirement of the method is approximately two hours.

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<u>Assembly of a Fluorometer</u>

Assembling a simple, direct reading fluorometer requires the following basic parts; a direct reading electronic photometer, a mercury vapor lamp, a collimating lens, a ventilation fan and a constant voltage transformer for use in conjunction with the mercury vapor lamp. An optional additional part is an iris diaphragm which can be used to control the amount of light striking the photocell. With these parts available, the problem merely becomes one of constructing a suitable lamp housing and cell compartment.

The lamp housing and the cell compartment are constructed as separate units and are then joined together for use with the photometer. A photograph of the completely assembled fluorometer is shown in Figure 4.

The lamp housing, as diagrammed in Figure 1, is a sheet aluminum box of one piece construction. The housing is fitted with a removable top plate through which the mercury lamp is mounted. There are two circular openings in the housing; one on the front side, in which the lens is mounted, and one on the back side serving as a ventilation exit. The positioning of the aperture in which the lens is mounted, is critical in that it must coincide with the arc in the mercury lamp. The positioning of the ventilation exit is not critical and is governed only by the type of fan used.

The housing is constructed to accommodate a small interior electric fan. A small fan, of the type used in the Lumetron model 402-EF instrument, is well suited for this purpose. The instrument, as pictured in Figure 4, is equipped with an exterior draw type fan which also efficiently effects ventilation.

The cell compartment is a light tight box constructed of 3/16 inch aluminum plate and is equipped with a slide top. Figure 2 diagrammatically shows the cell compartment and also shows the manner in which the photocell search unit is attached to the compartment. The interior of the cell compartment is depicted in Figure 3. The cell guides, as pictured, are designed for use with cells of the type used in the Lumetron model 402-EF instrument. However, with slight modifications they could be used with other cell types.

The lamp housing and the cell compartment are joined together so that the ultra-violet light entrance aperture of the cell compartment and the lens aperture of the lamp housing coincide. The leads from the search unit are attached to the photometer and the lead from the mercury lamp is connected with a 115 volt output constant voltage transformer and the instrument is ready for use.

The photometer, used in the construction of this fluorometer, was the line operated Photovolt Electronic Photometer model 501-M. The photometer was equipped with the Photovolt "C" phototube which has maximum sensitivity in the range 300 to 600 millimicrons.

The model 501-M photometer has four different sensitivity ranges and this coupled with the use of an iris diaphragm, in conjunction with the phototube, makes the range of light intensities, measurable by it, almost unlimited.

The indicating meter is a microammeter scaled to read from 0 to 100 units. The four sensitivity ranges, in going from the least sensitive to the most sensitive setting, correspond to scale deflections to reading

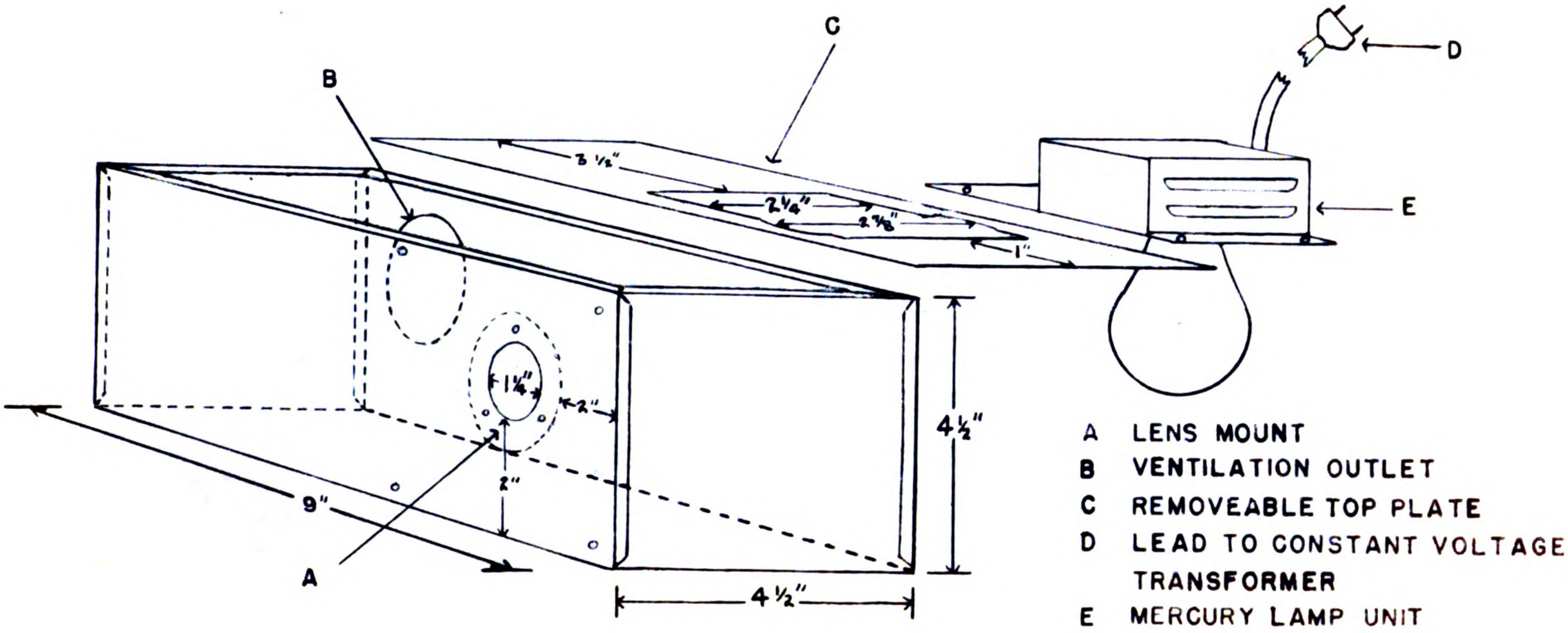
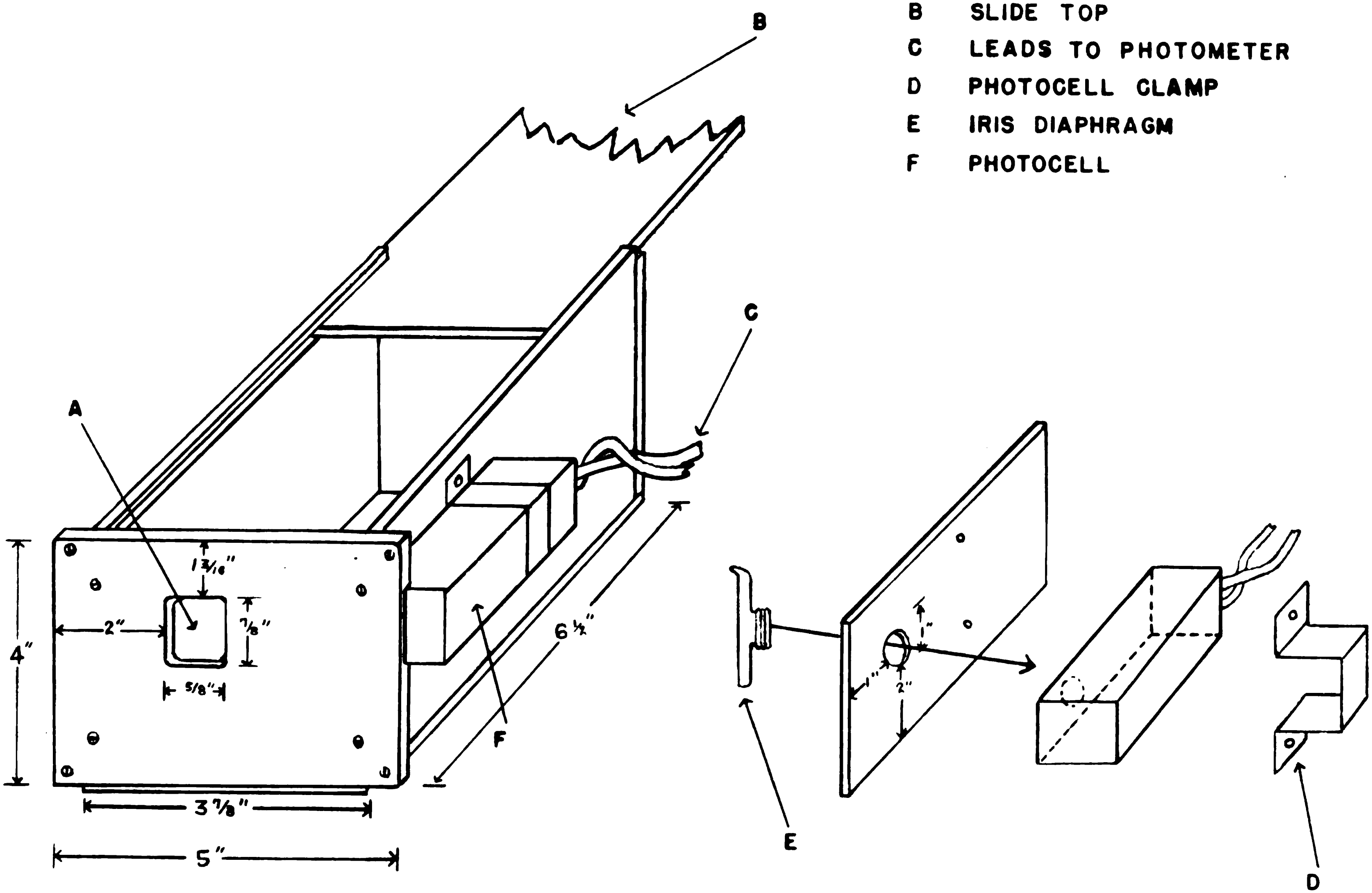


FIG. I LAMP HOUSING



A UV LIGHT ENTRANCE
B SLIDE TOP
C LEADS TO PHOTOMETER
D PHOTOCELL CLAMP
E IRIS DIAPHRAGM
F PHOTOCELL

FIG. 2 CELL COMPARTMENT

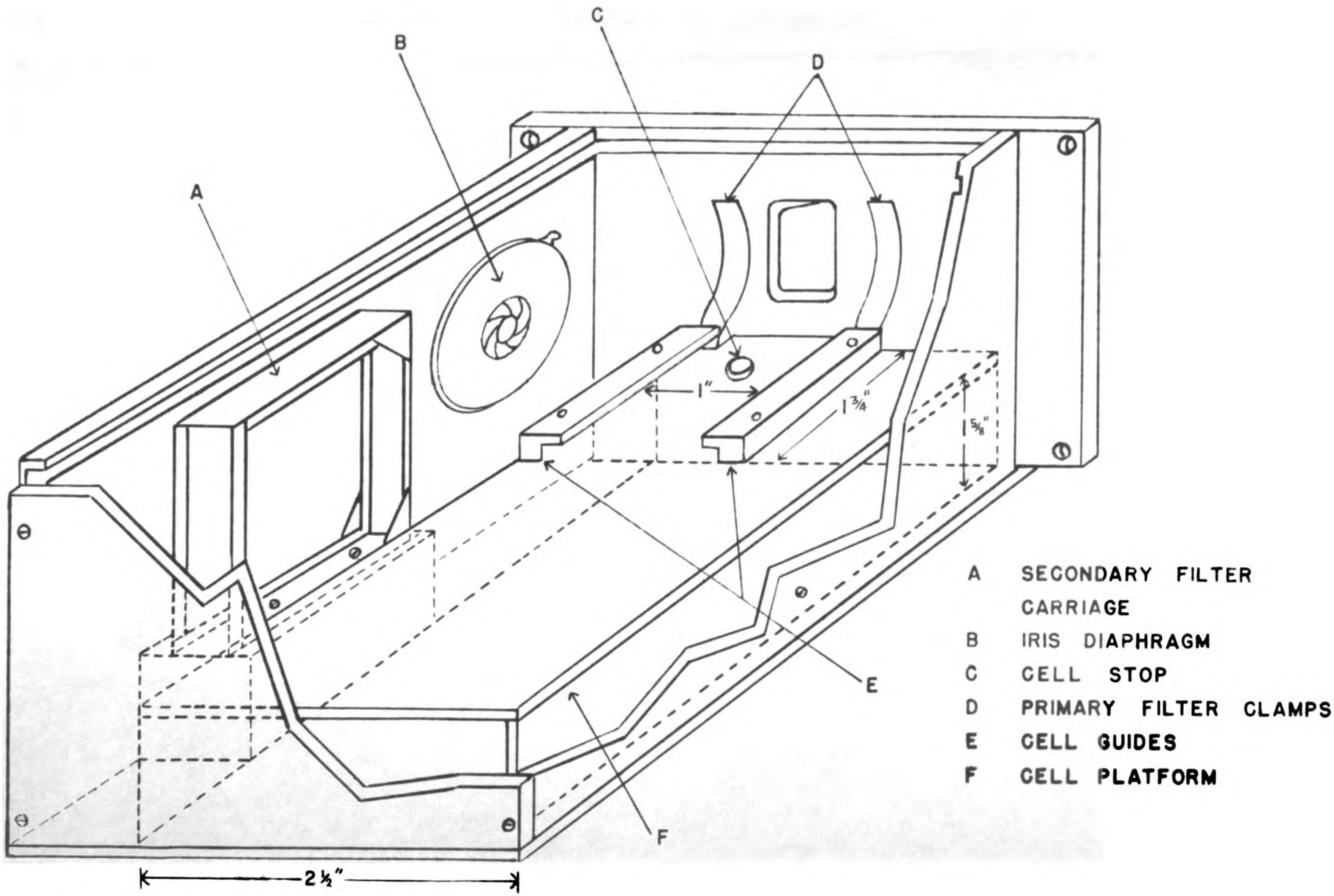


FIG. 3 INTERIOR OF CELL COMPARTMENT

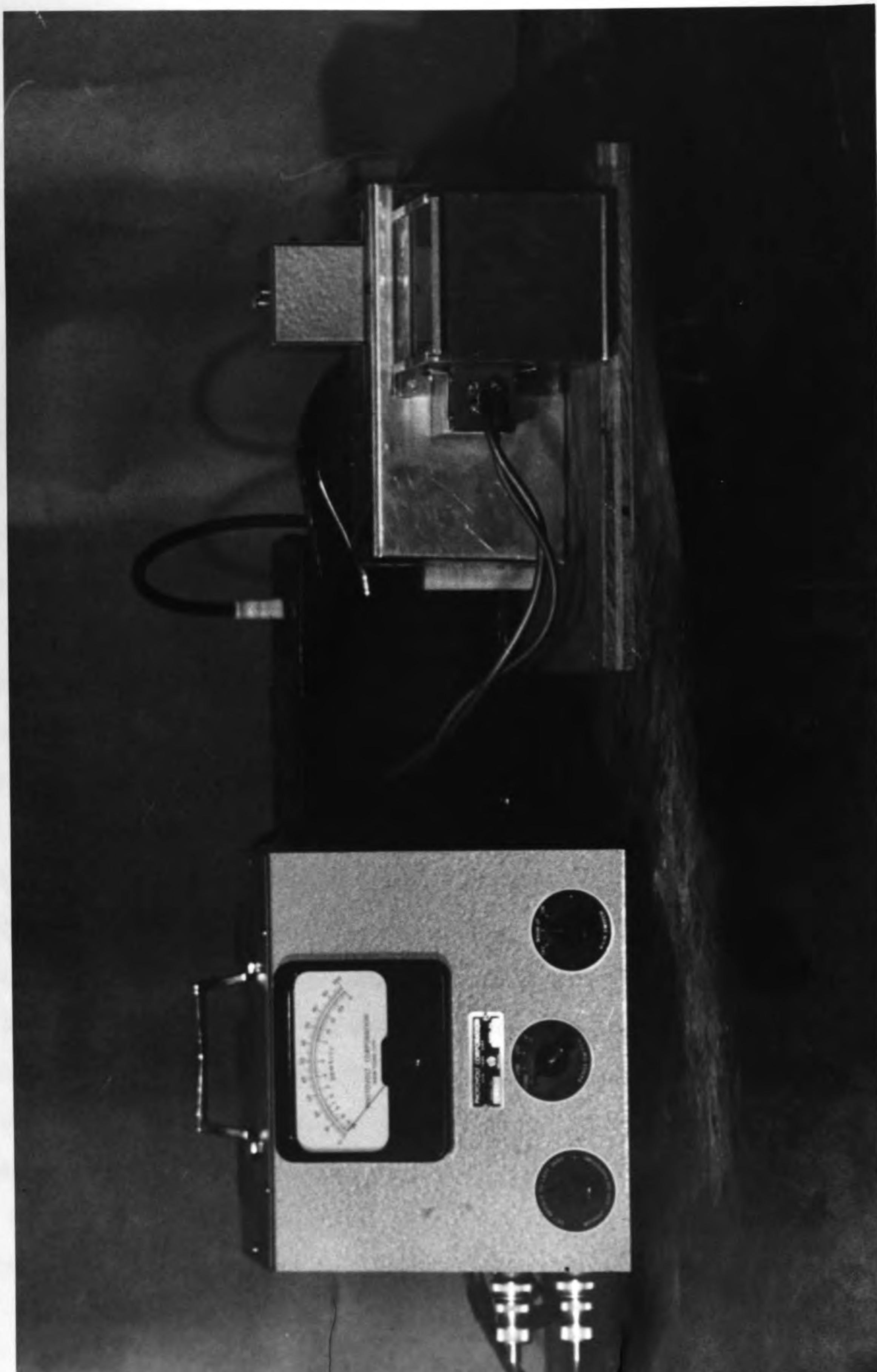


FIG. 4 FLUOROMETER ASSEMBLY

taken ratios of 1/10:1, 1:1, 10:1 and 100:1. Consequently, if the instrument is zeroed with a particular solution, for example distilled water, and the sensitivity ranges are adjusted so a 10:1 ratio exists between each range; intensities from 0 to 1000 scale units can be read accurately to three places, relative to the solution used in obtaining the zero setting.

Since the same degree of accuracy is obtained over the entire range of the instrument, no primary standard is necessary to obtain a "100" setting. Consequently the fluorescence intensity values cannot be spoken of as per cent fluorescence relative to a primary standard, but are only scale readings relative to distilled water as a zero fluorescence standard.

The procedure used in obtaining a fluorescence intensity reading is as follows. The photometer and the mercury lamp are connected to a power outlet and a period of 10 to 15 minutes is allowed for the photometer and lamp to warm up. A cuvette is then filled with the solution to be used as the zero fluorescence standard and the instrument is set to give a zero reading with this solution. The ratio of 10:1 between the four individual sensitivity ranges is obtained and the unknown sample is then placed in the cuvette and its fluorescence intensity read. The procedure for obtaining the zero reading must be carried out before each group of fluorescent samples is determined. However, the 10:1 ratio between sensitivity ranges, for a particular zero standard, is quite constant and only requires verification approximately every two weeks.

Further information, in regard to the mechanical procedure to be followed in obtaining the zero setting and adjusting the range ratios, can be obtained by consulting the Photovolt Bulletin number 305 (8) on the model 501-M electronic photometer.

THE FLUOROMETRIC DETERMINATION OF O-PHTHALIC ACID INCLUDING THE ASSEMBLY OF A FLUOROMETER

By

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AN ABSTRACT

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THESIS ABSTRACT

A fluorometric method has been developed for the determination of o-phthalic acid either alone or contaminated with various other carboxylic acids. The method involves the condensation of o-phthalic acid with resorcinol to form fluorescein. The condensation is effected through use of concentrated sulfuric acid and a reaction temperature of 125°C. A solution of the sodium salt of the fluorescein formed in this manner, yields an intense green fluorescence on excitation with ultra-violet light and the intensity of the fluorescence serves as a quantitative measure of the quantity of o-phthalic acid reacted.

Concentrations of o-phthalic acid ranging from 50 to 5000 micrograms per ml., can be determined in the presence of benzoic, isophthalic and terephthalic acids with an expected maximum error of approximately 4%. In the presence of α-naphthoquinone a slightly larger error, approximately 5% is to be expected, The time requirement of the method is approximately two hours.

A direct reading fluorometer was assembled, employing a Photovolt Electronic Photometer model 501-M, for use in this work. The appendix of the thesis, from which this abstract is drawn, contains instructions and diagrams describing the assembly of such an instrument.