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Spectrophotometric Determinations of the Sulfonamides

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SPECTROPHOTOMETRIC DETERMINATIONS OF THE SULFONAMIDES.

By

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VITA

Paul William Brna was born in Greenwich, Connecticut, on January 23, 1914.

He was graduated from Greenwich High School, Greenwich, Connecticut in June, 1932.

The Bachelor of Arts degree in Chemistry and Zoology was conferred by Valparaiso University in June, 1938.

From 1938 to the present time the writer has been engaged as Chief Chemist at the Chicago Pharmacal Company, Chicago, Illinois. His work embodies Pharmaceutical Organic Analysis, and much of his time was spent in Organic Research. During the past four years he devoted part of his time to graduate study in Chemistry. He has served on the Research Committee of the American Pharmaceutical Manufacturers Association and is co-author of the following publications:

"Assay of Phenobarbital Tablets"-- December, 1941.

"Determination of Sulfa Drugs, Using Colorimetric or Fluorometric Methods"-- To be published, Fall of 1944.

"Development of Standard Methods For Rapid Ageing of Vitamin Preparation"-- To be published, Fall of 1944.

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HISTORICAL BACKGROUND

The spectrophotometric determination of the sulfonamide concentrations were performed by many workers in the field. The administration of sulfonamides were controlled by frequent determinations of the drug in the blood. Occasionally it was necessary to know the concentration of the drug in various body fluids such as the urine and cerebrospinal fluid. The technique was simple, requiring the use of a standard colorimeter, and was based on the method of Bratton and Marshall.[1] The method consisted in precipitating the protein of the fluid with trichloracetic acid, filtering, diazotizing the aryl amine in the filtrate with nitrous acid, adding ammonium sulfamate to destroy the excess nitrous acid and coupling the diazonium salt with N-(1-naphthyl)-ethylenediamine dihydrochloride. The color intensity of the azo dye solution was a measure of the sulfonamide content of the fluid. The undesireable features of this procedure are: the time for a single determination is exceptionally long, and

the color which was formed was stable for no longer than one hour, so that it was impossible to run a large number of samples simultaneously. The nitrogen bubbles which form after the addition of the ammonium sulfamate often lead to false readings.

Another quantitative method widely used was based on Werner's procedure.[2] Werner condensed the amino group with p-dimethyl aminobenzaldehyde, added 1 ml. of a 3 per cent solution of this reagent in 7 per cent v/v sulfuric acid to 9 ml. of the urine previously diluted one or two hundredfold, and measured the color intensity formed with those of similarly treated standards. Here, again, care was necessary; it was difficult to use this method for accurate work since the dye had a low tinctorial value. Werner observed that the presence of free trichloracetic acid interfered with the maximal production of color. Kuhnau[3] has reported that this effect was not a specific property of trichloracetic acid, since he estimated uliron (N-sulphanilyl-N^1 N^1-dimethylsulphanilamide)[4] in blood by

the addition of a trichloracetic acid solution of p-dimethylaminobenzaldehyde to a trichloracetic blood filtrate without previous neutralization of the blood filtrate. Morris[4] reports that this effect can be observed with a variety of acids including hydrochloric, sulfuric, p-toluenesulfonic and salicylsulfonic acids. It has also been reported that the total values obtained with these methods are subject to error resulting from the change in color intensity with small changes in the pH.[5]

It was the object of this study to develop a spectrophotometric method for the determination of the sulfonamides themselves, not in the blood or any of the body fluids. After much experimental work with diazotizations and coupling with other dyes, outside of those tried by Bratton and Marshall and Werner, it was decided to look into Doble and Geiger's[6] modification of Marshall, Cutting, and Emerson's original procedure, using alcohol as the protein precipitant and diphenylamine as the coupling reagent producing a yellow color. It was repor-

ted that upon using this method on blood and urine turbid solutions were obtained which could not be clarified by filtration and were not suitable for photelometric use.(7)

An effort was made to change this procedure and adapt the following: (a) trichloracetic and sulfuric acids were used in the diazotization with sodium nitrite, (b) alcohol was used as a solvent to prevent precipitation of the coupling reagents, and (c) p-aminoacetophenone with diphenylamine were used as the coupling reagents for the production of orange colored solutions.

Experimental Procedure

Reagents. 15 per cent trichloracetic acid; 1 N sulfuric acid; 95 per cent ethyl alcohol; 0.5 per cent sodium nitrite; p-aminoacetophenone previously recrystallized and purified in ethyl alcohol and made up into a 0.1 per cent solution in ethyl alcohol; and diphenylamine also recrystallized and purified with ethyl alcohol and made up into a 0.5 per cent solution in ethyl alcohol and stored in an amber bottle.

Instrument. All determinations were made with a

Coleman Universal Spectrophotmeter using a deep purple filter (PC-6) for making measurements in the region of 350-400 mmu; a purple filter (PC-4) of a special glass which substantially levels out the response of the photocell in the visible region, 400-650 mmu.; and measurements were made between 650-800 mmu. a red filter (PC-5) was substituted for the purple filter (PC-4) to remove stray light passed by the monochromator. All colors developed were compared against reagent blanks of the same age, in a pair of optically matched oblong cells of 20 ml. volume.

Calibration Curve. The calibration curves obtained in this study are actually the graphs, (I) and (II), which were run in distilled water containing the reagent blanks against which all colors formed, were compared.

The sulfanilamide and sulfathiazole solutions were made up as follows: pure sulfanilamide and sulfathiazole were prepared by recrystallization and purification from distilled water and alcohol respectively and thoroughly dried in an electric oven and desicator. 0.1000 gms. each of sulfanilamide and sulfathiazole were dissolved in

a liter of distilled water. Solutions equivalent to 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mgm. per cent were prepared by diluting 10, 20, 40, 80, 120, 160 and 200 ml. respectively to 200 ml. with distilled water. A milligram per cent is the same as micrograms per 0.1 ml.

To 1 ml. of each of the solutions in a 20 ml. oblong cell were added the reagents in the following order 3 ml. of 15 per cent trichloracetic acid, 0.5 ml. of 1 N sulfuric acid, 3 ml. of 95 per cent ethyl alcohol, 0.1 ml. of 0.5 per cent sodium nitrite. Mix the solution and allow to stand for 3 minutes to complete the diazotization. When this has been completed add 1.0 ml. of 0.1 per cent p-aminoacetophenone and 1.0 ml. of 0.5 per cent diphenylamine. Mix and allow to stand until the color fully develops.

The same procedure is followed from all of the concentrations or dilutions on each of the sulfonamides, except in the reagent blank in which 1 ml. of distilled water is used in place of the sulfonamide solution. The color produced for the reagent blank is a very pale pastel shade of

orange, and for the sulfonamide concentrations the colors ranged from pale orange to an intense golden orange.

All of the concentrations prepared were measured against the reagent blank and which were all of the same age. The wavelength dial was set from 360 mmu. to 780 mmu. using PC-6, PC-4, and PC-5 filters respectively. That is, the transmittance of the solutions was measured using the direct reading from the galvanometer scale. The procedure was repeated all the way through as a check on the readings of the galvanometer. At no time were there any variations in the readings.

The data covering mgm. per cent concentrations of the sulfanilamide using the reagent blank as a reference standard will be found in table I, and the same for mgm. per cent concentration of the sulfathiazole will be found in table II.

Temperature seemed to have no effect on any of the solutions, although each determination was run at room temperature.

Discussion of Results Obtained

Graph I shows the absorption curves for sulfanilamide. The sulfanilamide solutions show absorption peaks at 480 mmu. The sulfathiazole solution, in graph II, shows two absorption curves, one at 480 mmu. and the other at 550 mmu. An examination of tables I and II shows the order of agreement which may be expected in a series of analysis in which the ratios and the amounts varied. The procedure employed involves the assumption of a source of illumination of constant intensity. This assumption was checked by keeping the voltage of the battery constant which was readily performed by keeping the battery charger in operation during all determinations. It is quite obvious that if the observations were made with an instrument which did not maintain a constant voltage supply the readings on the galvanometer scale would be out of line.

Table III shows the per cent transmittance to be plotted against mgm. per cent concentration of sulfanilamide at 480 mmu., the absorption peak as shown on graph I. An examination of graph III

shows a linear curve from 0.5 mgm. per cent to 10 mgm. per cent concentrations, proving that these concentrations obey Beer's Law. The same applies to sulfathiazole where table IV shows per cent transmittance to be plotted against mgm. per cent concentrations at 480 mmu. and 550 mmu., the absorption peaks as shown on graph II. And an examination of graph IV shows linear curves for both wavelengths, which again shows Beer's Law to be true for those concentrations. Therefore, a quantitative estimation of each of the aforementioned sulfonamides is possible in the concentrations performed. These linear curves have been reported in the literature. (8) Lee, Hannay, and Hand report that Beer's Law is obeyed exactly to a concentration of at least 12 micrograms per determination. An examination of graphs III and IV shows that to be precisely true.

Summary

A method for the determination of sulfonamides has been discussed that is based on the coupling of diazotized sulfonamides to p-amino-

acetophenone and diphenylamine. Changes in the procedure as proposed by Doble and Geiger in their modification of Marshall, Cutting, and Emerson's method have been used, thus cutting the time down to about 10 minutes for a determination. The colors developed were stable for 24 hours or more.

Suggestions For Future Work.

Work along this line could be extended to see whether Beer's Law will hold true for concentrations beyond 12 mgm. per cent. Investigations may also be carried on other sulfonamides as sulfapyridine, sulfadiazine, sulfaguanidine, etc. Another investigation may be carried on to find other dyes that might couple with the sulfonamides.

It would really be an achievement if someone would establish certain dyes or reagents which would be a specific for each individual sulfonamide in order that an operator could differntiate one sulfonamide from another.

TABLE I

PER CENT TRANSMITTANCE READINGS FOR SULFANILAMIDE AGAINST REAGENT BLANKS

λ	0.5	1.0	2.0	4.0	6.0	8.0	10.0 Mgm %
PC-6							
360	56.0	54.1	52.1	37.2	28.5	20.8	9.0
380	66.2	50.7	56.8	40.8	30.5	22.2	10.5
400	73.4	68.2	61.9	44.0	32.2	23.8	14.0
PC-4							
420	82.0	7.50	66.5	46.8	34.2	24.8	15.5
440	88.8	81.0	70.5	49.2	35.8	25.3	17.1
460	93.8	85.7	73.?	51.5	37.0	26.0	18.0
470	95.4	87.2	74.0	52.5	37.2	26.2	18.2
480	96.2	87.5	74.2	53.0	37.5	26.2	18.2
490	95.0	84.5	72.5	51.8	36.5	25.5	18.0
500	92.0	81.1	70.0	54.8	35.5	24.8	17.5
520	82.5	72.8	64.5	46.8	33.0	23.5	16.8
540	74.0	66.0	60.0	43.5	30.8	22.2	16.0
560	67.2	60.0	55.2	40.2	28.8	21.0	15.0

TABLE I
(Continued)

λ	0.5	1.0	2.0	4.0	6.0	8.0	10.0 Mgm %
PC-4							
580	59.2	53.5	49.5	36.8	26.8	20.0	13.8
600	52.5	47.0	45.0	34.2	25.2	18.8	12.8
620	46.8	42.2	40.5	31.5	23.5	17.8	11.8
640	42.0	37.5	36.2	29.2	22.5	17.0	11.0
PC-5							
660	37.3	33.5	32.2	27.2	21.2	15.8	10.2
680	33.2	30.5	28.8	25.0	20.0	14.8	9.5
700	29.5	27.0	25.8	23.2	19.0	13.8	8.8
720	25.8	24.3	23.2	21.2	17.8	13.0	7.9
740	23.2	22.2	21.4	19.8	16.5	12.5	7.5
760	22.2	21.0	19.8	17.2	15.5	11.8	6.8

TABLE II

PER CENT TRANSMITTANCE FOR MGM. PER CENT SULFATHIAZOLE

λ	0.5	1.0	2.0	4.0	6.0	8.0	10.0
PC-6							
360	46.9	45.5	40.5	30.0	18.0	8.0	5.5
380	49.9	48.0	42.0	31.0	19.8	10.5	7.3
400	52.5	50.0	43.5	32.1	21.2	12.5	9.5
PC-4							
420	54.8	52.1	44.9	33.4	22.3	14.5	11.7
440	58.0	54.2	46.0	34.5	24.1	16.0	12.5
460	60.0	55.5	47.5	35.5	25.8	18.0	14.0
480	61.0	56.8	48.8	36.2	27.0	20.0	14.8
500	59.5	56.0	47.5	35.2	26.5	18.5	13.5
510	59.8	56.5	48.8	35.0	26.0	18.0	13.2
520	60.5	57.5	50.5	37.5	26.8	19.2	13.5
530	63.5	60.5	53.5	39.0	28.2	20.5	14.0
540	69.5	65.0	56.0	40.5	29.5	22.0	15.5
550	72.5	67.1	57.2	41.6	30.5	22.2	16.2
560	72.0	66.5	57.0	41.2	29.8	22.0	16.0

TABLE II
(Continued)

λ	0.5	1.0	2.0	4.0	6.0	8.0	10.0
PC-4							
580	68.5	62.8	55.2	40.8	28.8	20.5	15.2
600	64.2	59.2	53.5	40.0	28.0	19.5	13.5
620	60.5	56.5	51.2	39.0	27.8	18.5	12.5
640	57.0	54.5	49.5	38.2	27.5	18.0	11.5
PC-5							
660	54.5	51.5	47.5	37.5	27.2	17.2	10.8
680	51.5	49.8	46.2	36.8	27.0	17.0	10.8
700	49.5	47.5	45.0	36.8			
720	48.0	46.2	44.0				
740	46.2	44.8	42.8				
760	45.2	44.0	42.5				

TABLE III

MILLIGRAM PER CENT CONCENTRATION OF SULFANILAMIDE AGAINST PER CENT TRANSMITTANCE AT 480 MMU WAVELENGTHS

480 Mmu.
96.2
87.5
74.2
53.0
37.5
26.2
18.2

TABLE IV

MILLIGRAM PER CENT CONCENTRATION OF SULFATHIAZOLE AGAINST PER CENT TRANSMITTANCE OF 480 AND 550 MMU WAVELENGTHS.

480 Mmu.	550 Mmu.
61.0	72.5
56.8	67.1
48.8	57.2
36.2	41.6
27.0	30.5
20.0	22.2
14.8	16.2

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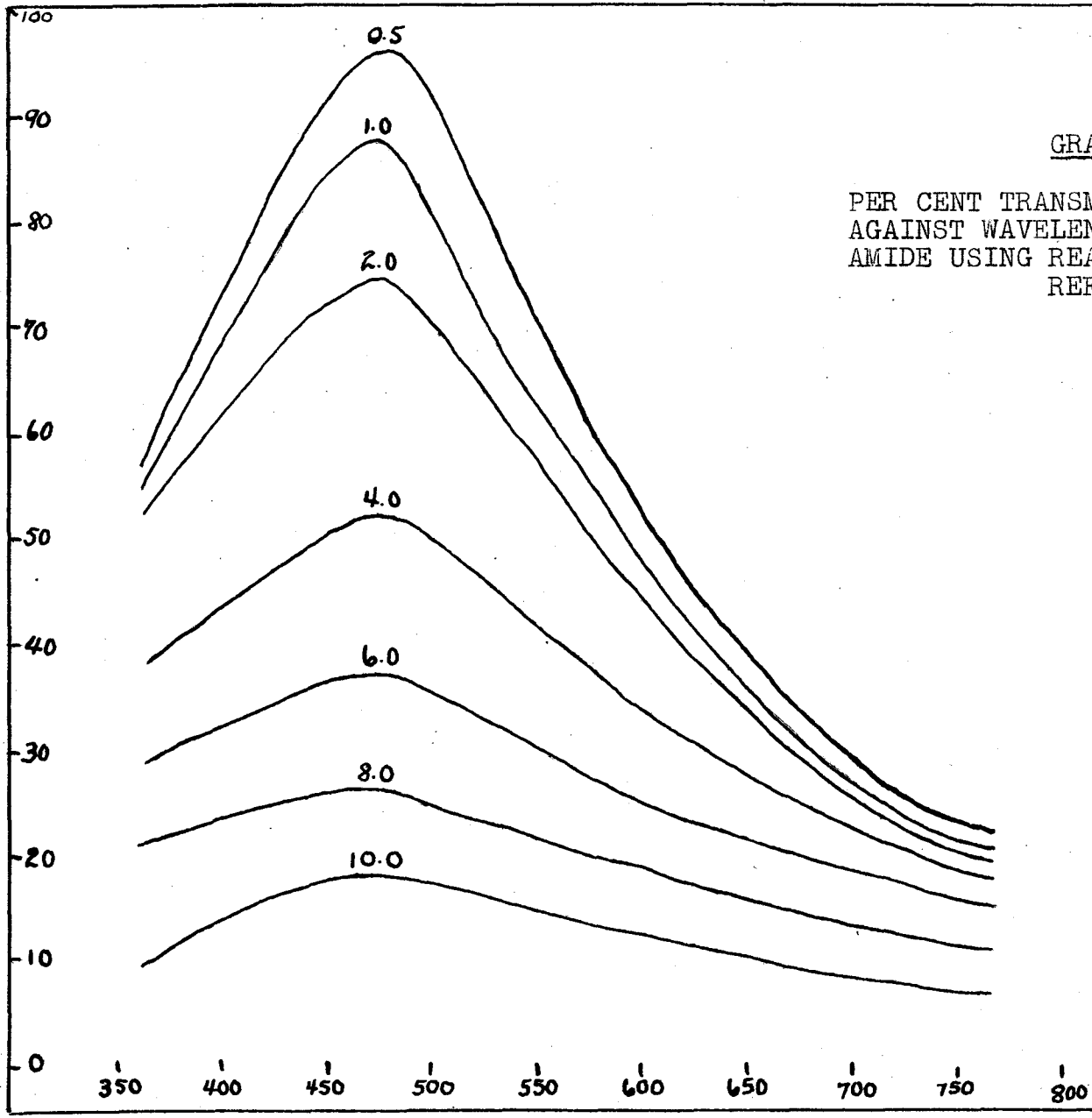
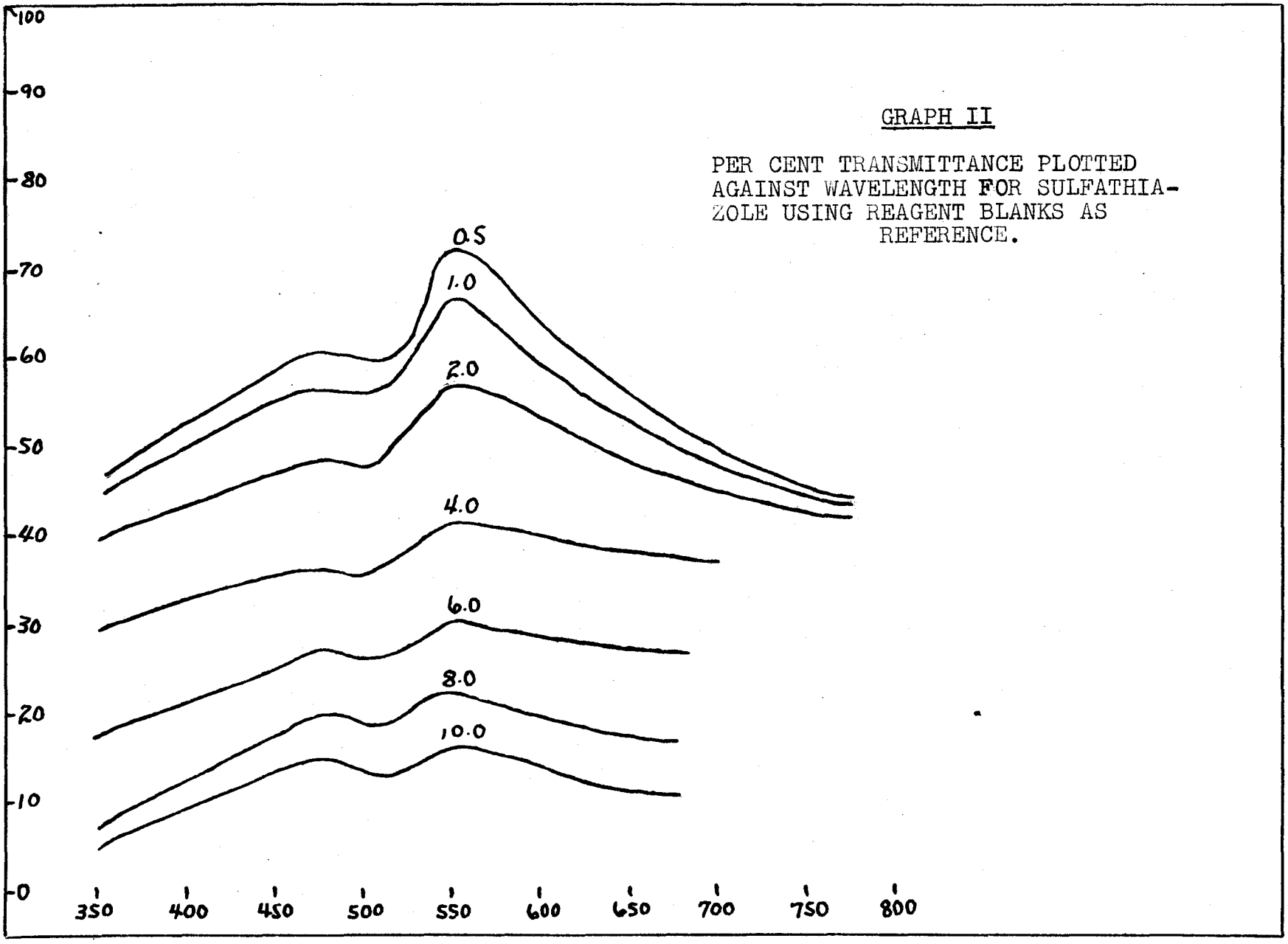


GRAPH I

PER CENT TRANSMITTANCE PLOTTED AGAINST WAVELENGTH FOR SULFANILAMIDE USING REAGENT BLANKS AS REFERENCE.

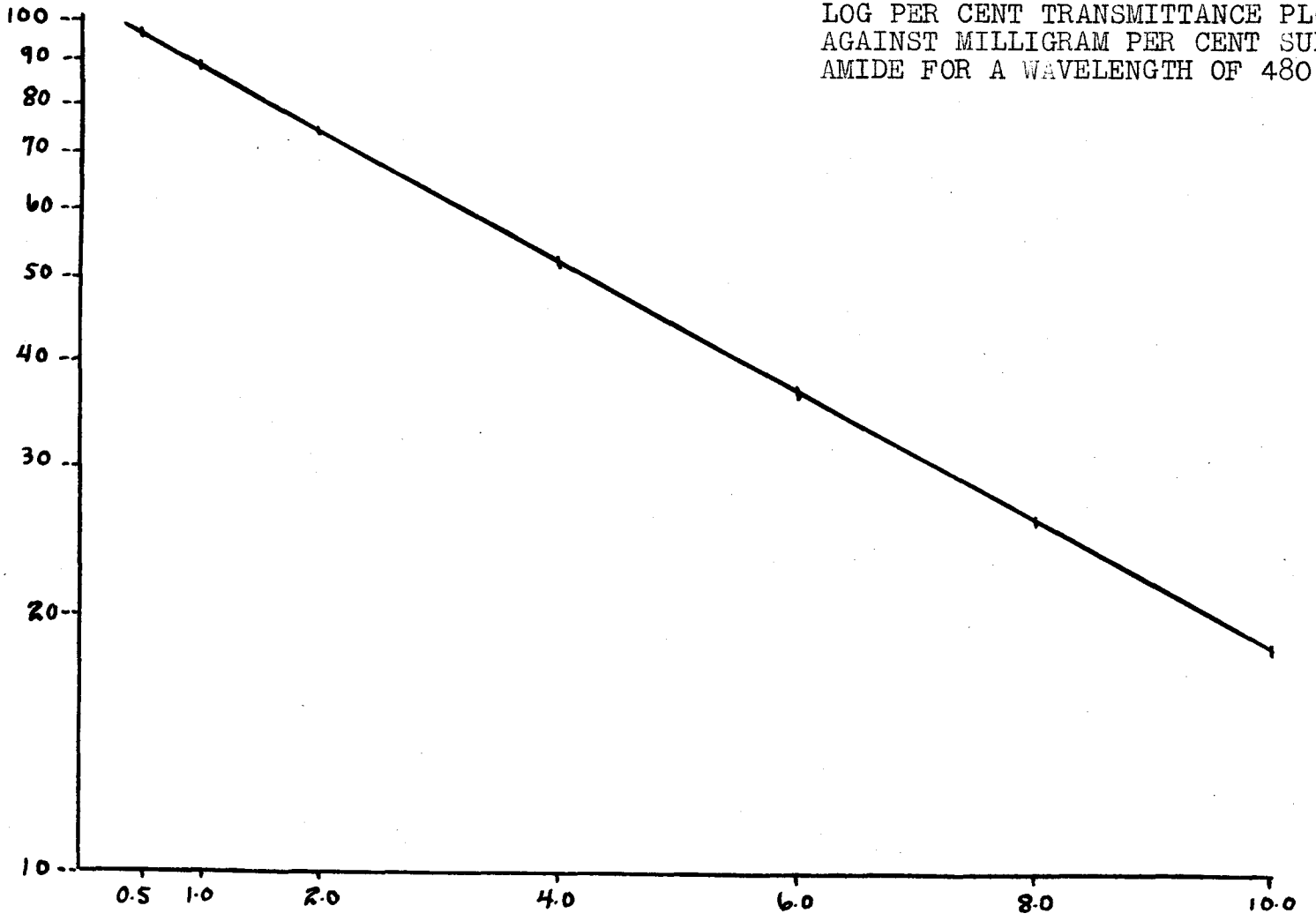


GRAPH II

PER CENT TRANSMITTANCE PLOTTED AGAINST WAVELENGTH FOR SULFATHIAZOLE USING REAGENT BLANKS AS REFERENCE.

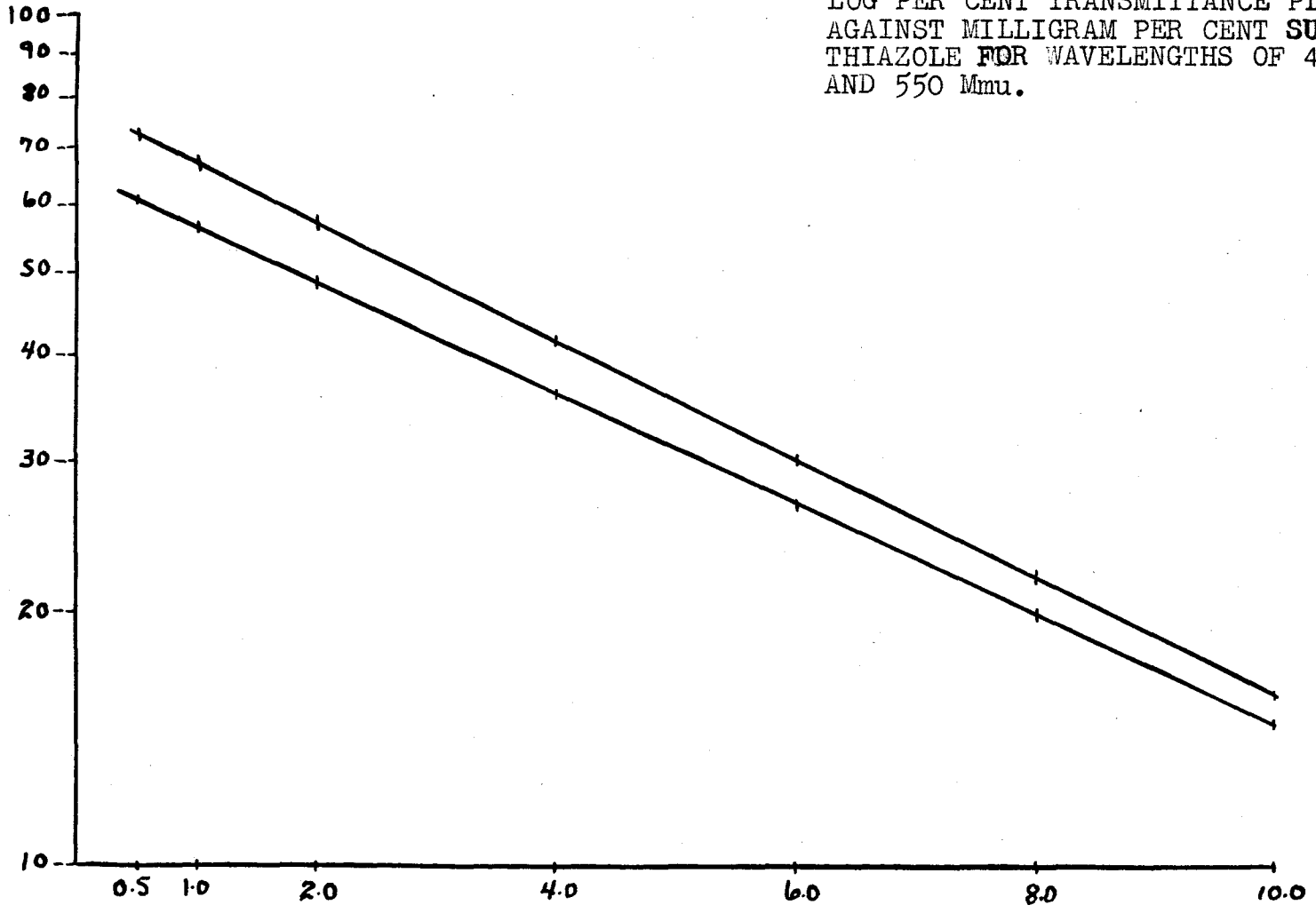
GRAPH III

LOG PER CENT TRANSMITTANCE PLOTTED AGAINST MILLIGRAM PER CENT SULFANILAMIDE FOR A WAVELENGTH OF 480 Mmu.



GRAPH IV

LOG PER CENT TRANSMITTANCE PLOTTED AGAINST MILLIGRAM PER CENT SULFATHIAZOLE FOR WAVELENGTHS OF 480 Mmu. AND 550 Mmu.



APPROVAL SHEET

The thesis submitted by Paul William Brna has been read and approved by three members of the Department of Chemistry.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical assuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 25, 1944
Date

G. M. Schmeing
Signature of Adviser