Washington & Lee University
Lexington Virginia
June 1923

Thesis

Fluorometry: H-ion. Influnceof pH and certain Neutral Salts upon the fluorescence of Sulfonic Acids.

by
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Presented to the Departmen of Chemistry of Washington and Lee University, as a partial fulfillment of the requirements for the degree of Bachelor of Arts.

FLUOREMETRY: INFLUENCE OF pH AND CERTAIN NEUTRAL SALTS UPON THE FLUORESCENCE OF SULFONIC ACIDS.

The method for the quantitative determination of substances in solution by comparative fluorescence, as suggested by L.J.Desha. # Journal of American Chemical Society, Vol. XVII Number 7, July, 1920, seems to warrant a further study of the conditions affecting such a determination. The work as carried out in this laboratory has been the study in the field of different salts, (1) the effect of dilution on the intensity of fluorescence, (2) the effect of H-ion concentration on the intensity of different salts, (3) the effect of neutral salts.

Experimental

Arrangement of Apparatus.

The apparatus used and its arrangement were the same as described by L.J.Desha (loc.cet.) except that the Kober nephelometer was equipped with the Klett Top Reader.

Compounds used were C.P. products of Eastman Kodak Company which were not further purified by recrystalization. Except p-phentidine sulfonic acid (technical grade), and the K Salt of Resorcin sulfonate, and K Salt of Hydroquinone sulfonate, prepared in the laboratory by T.R.Ralston according to the method described by Kauffman

Preparation of Solutions

Table I.

No.	Name of Compound	M.W.
1.	Na 1 naphthol 4 sulfonate.	230.166
2.	Beta naphthalene sulfonic acid.	208.17
3.	Sulfo-Salicyclic acid.	218.108
4.	Na-m-dimethyl amino benzene sulfonate.	223.188
5.	Na-m-diethyl amino benzene sulfonate.	251.176
6.	Phenyl p-toluene sulfonate.	248.216
7.	Tetrabrom eosine.	648.84
8.	P-phentidine sulfonic acid.	218.208
9.	Na Chloro Benzene (3-5 disulfonate.)	316.694
10.	Na 1 naphthol 2 sulfonate.	230.16
11.	Nitroso R Salt.	361.110
12.	Iodeosine.	835.84
13.	(Ralston's) K Salt of Resorcin sulfon.	228.176
14.	(Ralston's) K Salt of Hydroquinone sul.	228.175
15.	Fluorescein.	332.20

0.01 Molar solutions were made of each of the above salts. For the purpose of comparing the effects of changes in pH, there was prepared a series of buffer solutions consisting of phosphates and borates, mixtures recommended by Clark (The Determination of Hydrogen Ions, Page 76; 1920.). Carbonate free M/5 NaOH was prepared as follows: A Metallic sodium was cut and weighed under benzene, then dropped thru a reflux condenser into distilled water covered with a layer of ether. After the Na had been dissolved, this was diluted to the desired strength, and titrated against a standard acid.

Each of the below solutions were made up to 180 cc (so that for tests 9 cc may be diluted with 1 cc of fluorescent solution and still give designated pH).

Table II.

pH	M/5 KH_2PO_4	M/5 NaOH	Diluted To
5.8	50 cc	3.72 cc	180 cc
6.6	50 cc	17.80 cc	180 cc
6.8	50 cc	23.65 cc	180 cc
7.0	50 cc	29.63 cc	180 cc
7.2	50 cc	35.00 cc	180 cc
7.4	50 cc	39.50 cc	180 cc
8.0	50 cc	46.80 cc	180 cc

Table III.

pH	M/5 Boric Acid	M/5 KCl NaOH	Diluted To
8.0	50 cc	3.97 cc	180 cc
8.6	50 cc	12.00 cc	180 cc
9.6	50 cc	36.85 cc	180 cc

Table IV.

pH	M/5 Boric Acid	M/5 Na OH	Diluted To
8.0	50 cc	3.97 cc	180 cc
8.6	50 cc	12.00 cc	180 cc
9.6	50 cc	36.85 cc	180 cc

Qualitative Effects of Dilution
On Fluorescence.

1 cc of the 0.01 Molar solutions listed in Table No.1 were added to 8 cc of distilled H_2O and 1 cc of N/10 NaOH. A qualitative determination was made of their intensity and color of fluorescence. They are arranged below in the order of their intensity.

Table V.

Strength 0.001 M, color and intensity of fluorescence.

15	1	10	7	8*	11	5	12	9	4	13	14	2	6	3
G/Y	D/B	L/B	O	D/B	D/G	B	D/O	G	L/V	VL/G	VL/B	Slight		

Strength 0.0001 M.

L cc of 0.001 M solution, 8 cc of distilled water and 1 cc of N/10 NaOH.

15	1	10	7	8	11	12	5	9	13	14	2	6	3
D/G	D/B	L/B	Y	D/B	L/G	L/O			Slight.				

Strength 0.00001 M.

1 cc of 0.0001 M solution, 8 cc of H_2O and 1 cc of N/10 NaOH.

15	1	10	7	11	12
D/G	B	L/B	L/Y	B	P/Y

The following abbreviations for colors are used in the above tables:-

G/Y = Greenish Yellow; D/B = Dark Blue; L/B = Light Blue

O = Orange; D/G = Dark Green; B = Blue; D/O = Dark Orange.

G = Green; L/V = Light Violet; VL/G = Very light Green.

VL/B = Very light Blue; P/Y = Pale Yellow.

* Solution on Standing loses its intensity.

Qualitative Examination of Effects of Change in pH.

A qualitative examination of effect of change in pH was made by taking equal amounts of the buffer solution pH 5.8 + pH 8.0, and adding equal amount of fluorescence solution and noting the effect as below.

Table VI

Solutions As Table I. Number	pH 5.8	pH 8.0
1	Slight fluorescence.	Very Bright
10	Slight fluorescence.	Very Bright
11	Same as at pH 8	Same as at pH 5.8
5	Same as at pH 8	Same as at pH 5.8
7	Same as at pH 8	Same as at pH 5.8
15	Same as at pH 8	Same as at pH 5.8
12	Same as at pH 8	Same as at pH 5.8
13	Slight fluorescence.	Brighter.
14	Slight fluorescence.	Brighter.

With Table Number VI as basis, I made up for quantitative comparison the following solutions as given in Table Number VII. These buffers were made according to Clark's dilution to 180 instead of 200 cc so that 9 cc may be diluted with 1 cc of fluorescent solution and get designated pH.

Table VII.

pH	M/5 KH_2PO_4 NaOH diluted to 180 cc.	0.00001 M Na 1 naphthol 4 sulfonate.
5.8	45 cc	5 cc
6.6.	45 cc	5 cc
6.8	45 cc	5 cc
7.0	45 cc	5 cc
7.2	45 cc	5 cc
7.4	45 cc	5 cc
8.0	45 cc	5 cc

pH	M/5 Boric Acid KCl-NaOH diluted to 180 cc	0.00001 M Na 1 Naphthol 4 sulfonate.
8.0	45 cc	5 cc
8.6	45 cc	5 cc
9.6	45 cc	5 cc

pH	M/5 Boric Acid-NaOH diluted to 180 cc.	0.00001 M Na 1 naphthol 4 sulfonate.
8.0	45 cc	5 cc
8.6	45 cc	5 cc
9.6	45 cc	5 cc

The comparative fluorescence of the above solutions was then determined by the method of matching the intensities of columns of different heights as described by L.J.Desha(loc. cet.).

The Intensity In The Phosphate Mixture of pH 7.0 (Standard)

For convenience in comparison, and in the plotting of a curve to show the relation between intensity and pH value, the intensity due to pH 7.0 was called 100 and others calculated to this.

Results of Measurement.

Table VIII

Fluoremetric readings of Na 1 naphthol 4 sulfonate 0.00001M from pH 5.8 to pH 8.0 Phosphates.

pH	Compared To pH	Rdg. of Standard.	Rdg. of Unknown	Intensity taking that due to pH 7.0 as 100.
5.8	7.0	20.00	30.00	20/30 x 100 = 66.7
6.6	7.0	20.00	25.50	20/25.50 x 100 = 78.5
6.8	7.0	20.00	24.47	20/24.47 x 100 = 81.7
7.0	7.0	20.00	20.00	20/20 x 100 = 100.0
7.2	7.0	20.00	16.40	20/16.40 x 100 = 122.0
7.4	7.0	20.00	15.47	20/15.47 x 100 = 129.2
8.0	7.0	20.00	7.50	20/ 7.50 x 100 = 267.0

Then to get a higher pH than 8.0, Boric Acid KCl was used. pH 8.0 KH_2PO_4 as standard compared Boric Acid KCl pH 8.0 and found that there was a marked difference in the intensity, of pH 8.0 Boric Acid KCl and pH 8.0 of KH_2PO_4.

Results of Measurement.

Table IX.

pH 8.0 KH_2PO_4 Standard.

pH	Compared To pH.	Rdg. of Standard.	Rdg. of Unknown.	Intensity taking that due to pH 7.0 as 100.
8.0	8.0	20.00	35.00	20/35.00 x 100 = 57.2
8.6	8.0	20.00	31.30	20/31.30 x 100 = 63.9
9.6	8.0	20.00	30.00	20/30.00 x 100 = 66.6

Then to find the cause of the decrease in intensity when using Boric Acid free from KCl and compared pH 8.0 Boric Acid without KCl with pH 8.0 of KH_2PO_4. These checked within 0.04 %. Then compare pH 8.0 of Boric Acid KCl and there was the same effect as with KH_2PO_4.

Result of Measurement.

Boric Acid KCl and Boric Acid free from KCl and Na 1 naphthol 4 sulfonate 0.00001M.

Table X.

Boric Acid Free From KCl.		Boric Acid KCl.		
pH	Rdg.of Stan.	pH	Rdg.Unknown.	Intensity taking that due to pH 7.0 as 100.
8.0	20.00	8.0	35.00	35/ 267
8.0	20.00	8.6	31.30	35/31.30 x 267=298
8.0	20.00	9.6	30.00	35/30.00 x 267=309

Na 1 naphthol 2 sulfonate same results as Na 1 naphthol 4 sulfonate.

The decrease in intensity due to KCl was determined to be about 44.5 %.

Result of Measurement.

Table XI.

Fluoremetric readings of Ralston's Salt of Hydroquinone Sulfonate 0.0001M from pH 5.8 to pH 8.0 Phosphates.

pH	Compared To pH.	Rdg. of Standard.	Rdg. of Unknown.	Intensity taking that due to pH 7.0 as 100.
5.8	7.0	20.00	27.00	20/27.00 x 100= 74.1
6.6	7.0	20.00	23.00	20/23.00 x 100= 87.0
6.8	7.0	20.00	21.00	20/21.00 x 100= 95.2
7.0	7.0	20.00	20.00	20/20.00 x 100=100.0
7.2	7.0	20.00	17.40	20/17.40 x 100=115.0
7.4	7.0	20.00	15.40	20/15.40 x 100=128.1
8.0	7.0	20.00	8.50	20/ 8.50 x 100=235.0

Boric Acid KCl had same effect on K Salt of Hydroquinone (Ralston's) as on Na 1 naphthol 4 sulfonate Boric Acid KCl compared to KH_2PO_4 pH 8.0.

pH	Compared to pH.	Rdg. of Standard.	Rdg. of Unknown.	Intensity taking that due to pH 7.0 as 100.
8.0	8.0	20.00	35.50	20/35.50 x 100= 56.4
8.6	8.0	20.00	31.00	20/31.00 x 100= 64.5
9.6	8.0	20.00	29.00	20/29.20 x 100= 69.0

Compound Boric Acid KCl with Boric Acid free from KCl pH 8.0.

Boric Acid free from KCl.		Boric Acid KCl		Intensity taking that due to pH 7.0 as 100.
pH	Rdg. Standard	pH	Rdg. Unknown.	
8.0	20.00	8.0	35.50	235
8.0	20.00	8.6	31.00	35.5/31.00 x 100=265
8.0	20.00	9.6	29.00	35.5/29.00 x 100=280

Results of Measurement.

Table XII.

Fluoremetric reading of (Ralston's) K Salt of Resorcin sulfonate 0.0001 M from pH 5.8 to pH 8.0.

pH	Compared to pH.	Rdg. of Standard.	Rdg. of Unknown.	Intensity taking that due to pH 7.0 as 100.
5.8	7.0	20.00	30.00	20/30.00 x 100 = 66.7
6.6	7.0	20.00	24.50	20/24.50 x 100 = 85.2
6.8	7.0	20.00	21.00	20/21.00 x 100 = 95.2
7.0	7.0	20.00	20.00	20/20.00 x 100 = 100.0
7.2	7.0	20.00	19.00	20/19.00 x 100 = 105.2
7.4	7.0	20.00	17.00	20/17.00 x 100 = 117.6
8.0	7.0	20.00	8.90	20/ 8.90 x 100 = 224.7

Boric Acid KCl had the same effect on K Salt of Resorcin Sulfonate as on the others salts above.

Boric Acid compound with KH_2PO_4 pH 8.0

pH	Compared to pH.	Rdg. of Standard.	Rdg. of Unknown.	Intensity taking that due to pH 7.0 as 100.
8.0	8.0	20.00	37.00	20/37.00 x 100 = 54.0
8.6	8.0	20.00	31.50	20/31.50 x 100 = 63.5
9.6	8.0	20.00	30.00	20/30.00 x 100 = 66.6

Compared Boric Acid KCl with Boric Acid free from KCl, pH 8.

Boric Acid Free from KCl.		Boric Acid KCl.		Intensity taking that as due to pH 7.0 as 100.
pH	Rdg.Stand.	pH	Rdg.Un.	
8.0	20.00	8.0	37.00	37.00/37.00 x 224.7 = 224.7
8.0	20.00	8.6	31.50	37.00/31.50 x 224.7 = 263.8
8.0	20.00	9.6	30.00	37.00/30.00 x 224.7 = 277.1

Discussion of Results.

The results from Table V. show that, for the best results in the fluoremetric study, Na 1 naphthol 4 sulfonate and Na 1 naphthol 2 sulfonate should be diluted to 0.00001M and (Ralston) K Salt of Hydroquinone sulfonate and K Salt of Resorcin sulfonate should be diluted to 0.0001M.

The results given in Table VIII and the curve of Figure 1 taken from these results, show that there is an increase in the intensity of fluorescence of Na 1 naphthol 4 sulfonate and Na 1 naphthol 2 sulfonate as the pH increases. This increase is seen to be very gradual up to pH 7.2. From pH 7.2 to pH 8.6 the increse is seen to be very rapid and regular. Beyond pH 8.6 the increase is very slight, becoming almost nothing as the pH still further increased.

The results of Tables VIII, IX, X, XI, and XII show that the intensity of fluorescence of Na 1 naphthol 4 sulfonate, Na 1 naphthol2sulfonate, and (Ralston's) K Salt of Hydroquinone sulfonate, and K Salt of Resorcin sulfonate is the same in the presence of phosphates as it is in the presence of borates. These tables also show that the presence of KCl in the borate has a dampening effect of 44.5 % on the intensity of fluorescence.

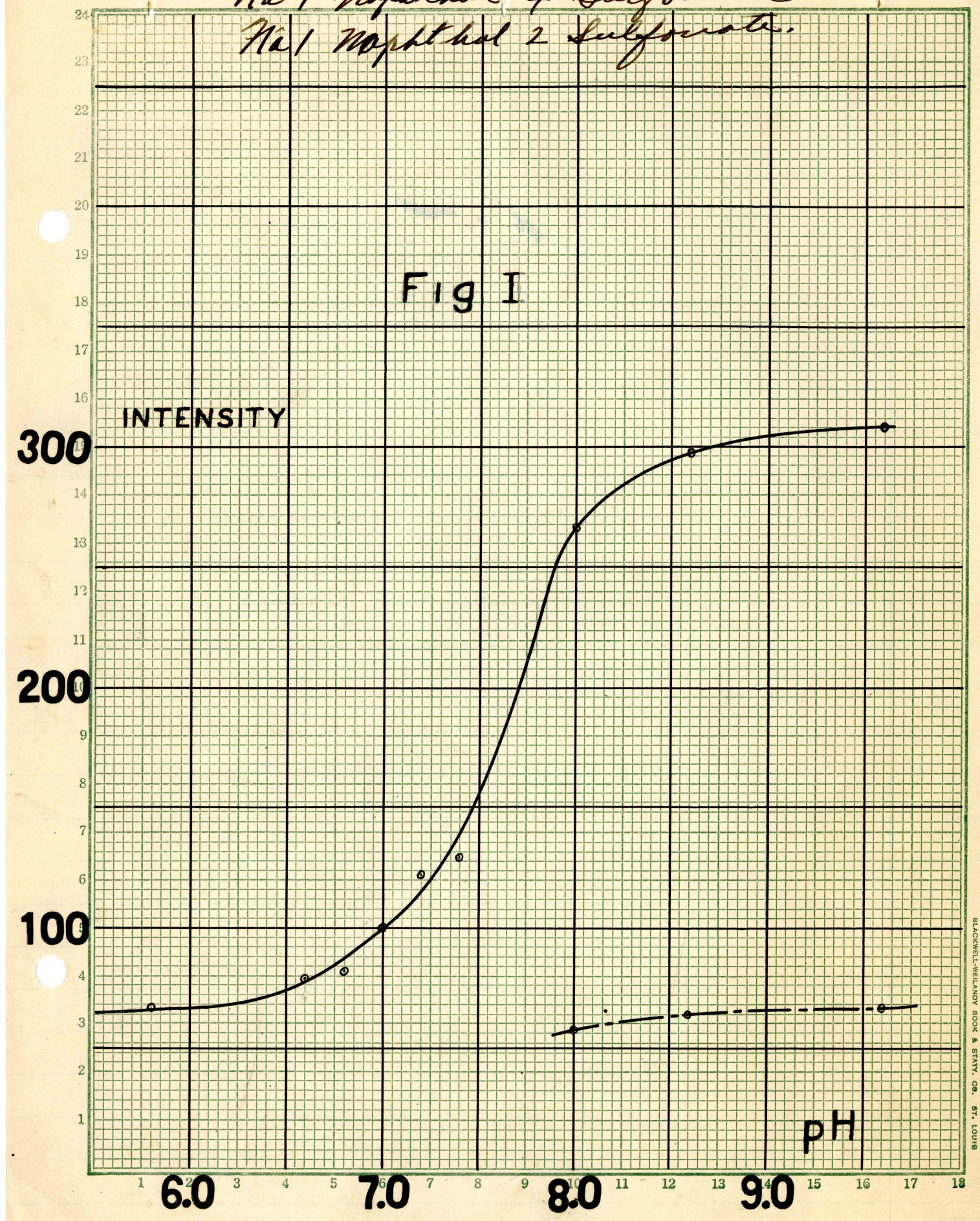
Summmary.

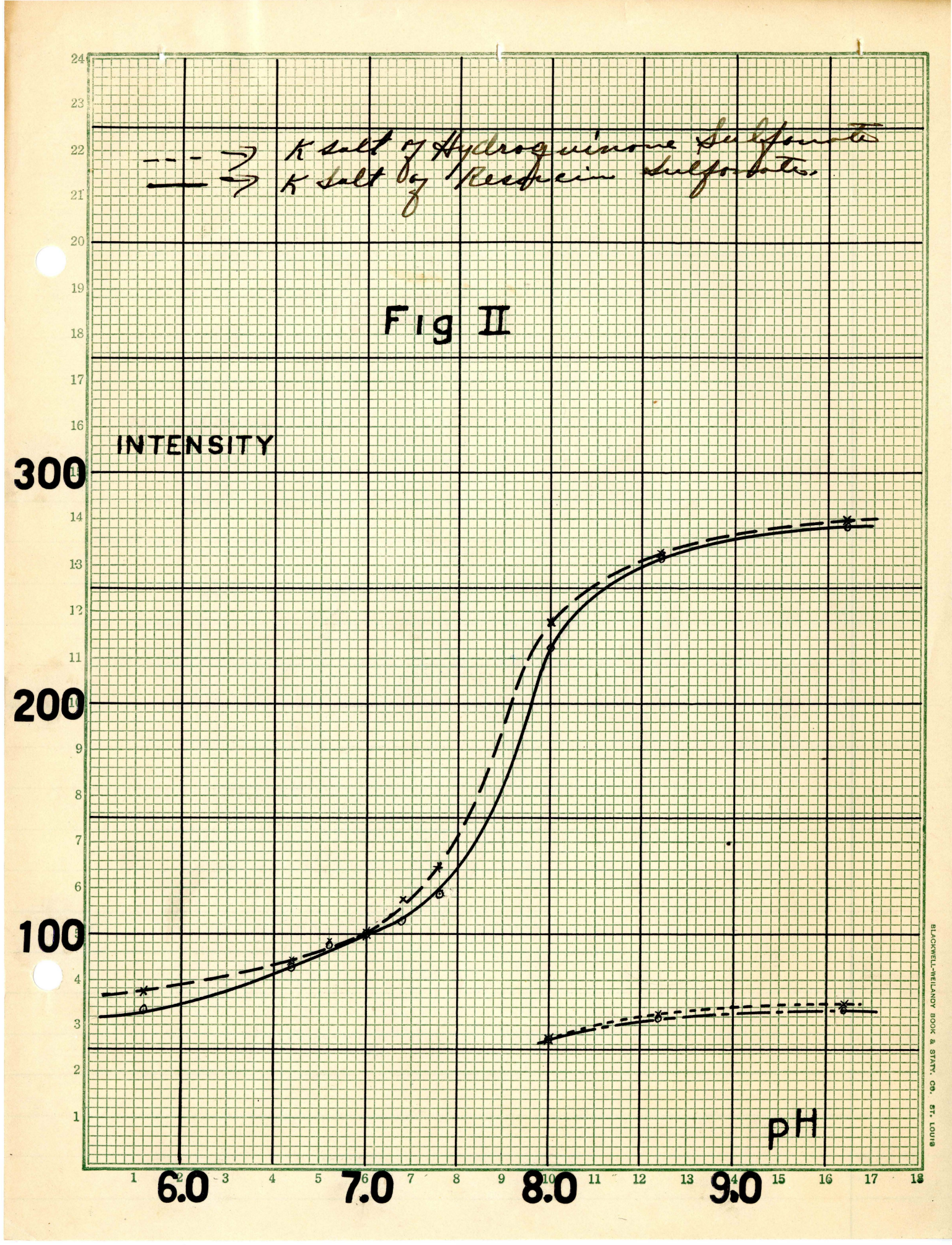
In conclusion, we may say that: (1) For fluoremetric study 0.00001M solutions of Na 1 naphthol 4 sulfonate, and Na 1 naphthol 2 sulfonate, and 0.0001M of (Ralston's) K Salt of Hydroquinone sulfonate, and K Salt of Resorcin sulfonate, should be used; (2) For these salts, the intensity of fluorescence increases gradually with decreased H-ion concentration up to a definite critical point---then, there is a rapid and regular increase up to a second critical point, after which the increase is very slight; (3) the intensity is the same in the presence of phosphates and borates, but KCl has a marked dampening effect.

The End.

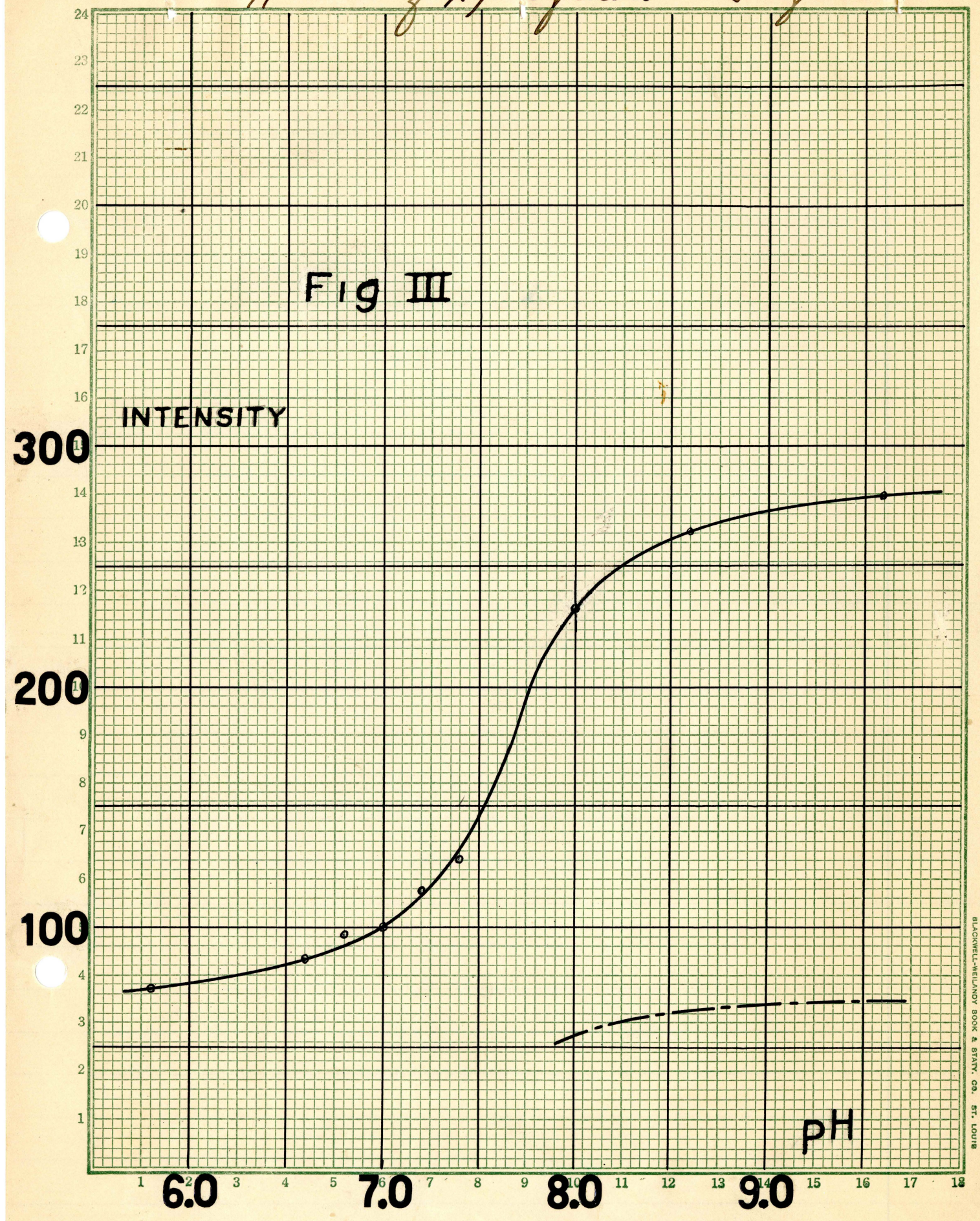
Na 1 Naphthol 4 Sulfonate

Na 1 Naphthol 2 Sulfonate.





K salt of Hydroquinone Sulfonate



K salt of Resorcin Sulfonate.