

Benzoquinone picrate	120	80 44	82 65	86 82	— —	Ammoniacal AgNO ₃ Fluorescence
Benzoquinone- β -Naphthol	156	80 86	82 85	86 95	— —	Ammoniacal AgNO ₃ Pauly reagent
Benzoquinone- Phenol	71	81 95	81 100	— —	— —	Ammoniacal AgNO ₃ Pauly reagent
Benzoquinone- Resorcinol	>300	80	82	86	—	Ammoniacal AgNO ₃ Pauly reagent
Benzoquinone- Hydroquinone	171	79	82	88	—	Ammoniacal AgNO ₃

* Solvent—A : BuOH:H₂O (saturated)

B : BuOH:AcOH:H₂O (4:1:2)

C : EtOH:30% Urea aq. (3:1)

D : BuOH:10% NH₄OH aq. (4:3)

Quinone, resorcinol, and hydroquinone gave identical R_f values by the solvents used in the present experiments that it was impossible to make any conclusive judgement but since other quinhydrone-type had clearly been isolated, these must have been separated too.

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Kiyoshi Yoshimura and Minoru Morita : Colorimetric Determination of Homosulfamine.*

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It has previously been reported that homosulfamine produced a yellow color with *p*-dimethylaminobenzaldehyde in diluted hydrochloric acid¹⁾ and an orange yellow color with sodium 1,2-naphthoquinone-4-sulfonate in alkaline solution.²⁾ Momose and Yasumura³⁾ utilized the latter for the colorimetric determination of homosulfamine and established a simple colorimetric method which is described in the J. P. VI. However, the color is not stable and it is reported that the error is about 10%.

Recently Perez and Poirier³⁾ reported on the colorimetric determination of primary aliphatic amines such as methylamine, ethylamine, and propylamine, using diazotized *p*-nitraniline. According to Beyer,⁴⁾ this coloration (red) is due to the existence of quinoid derivatives. In view of the fact that homosulfamine is one of the aliphatic amines, it was reacted with the above-mentioned diazonium reagent and it produced a red color having the absorption maximum at 520 m μ as shown in Fig. 1. Experiments were carried out to determine very small amounts of this drug by a colorimetric method in which the color is more stable. On the other hand, the color intensity was greatly influenced by various factors such as the amount of diazonium reagent, reaction temperature, length of reaction period, time elapsed after addition of all reagents, and pH during reaction, as shown in Figs. 2~5 and Table I.

TABLE I.

pH	6.3	7.0	7.7	7.85	8.0
Absorbancy	0.163	0.421	0.638	0.660	0.729

As pH increases, blank soln. becomes somewhat red.

* Paper read at the Kinki Local Meeting of the Pharmaceutical Society of Japan, Oct. 16, 1954.

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1) W. Hoffmann, G. Willkens : Pharm. Ztg., **83**, 65~75.

2) T. Momose, *et al.* : J. Pharm. Soc. Japan, **70**, 672(1950).

3) M. Perez, P. Poirier : Bull. soc. chim. France, **1953**, 754.

4) B. H. Beyer : J. Am. Chem. Soc., **64**, 1318(1942).

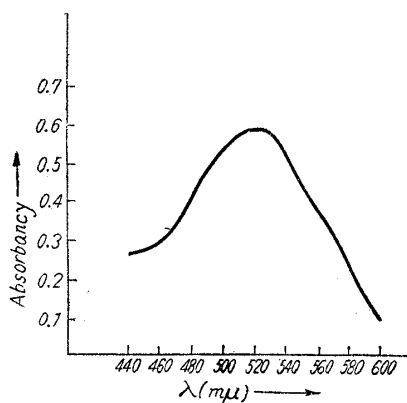


Fig. 1 Absorption Curve by Beckman Model B Spectrophotometer (20 γ /cc Soln.)

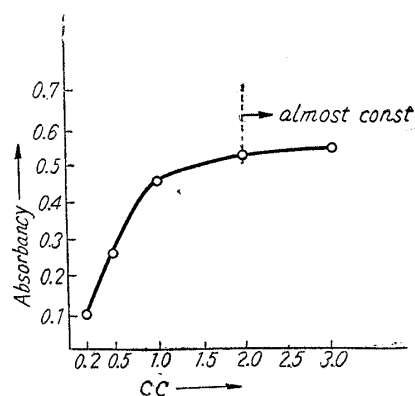


Fig. 2 Influence of Amt. of Diazonium Reagent on Intensity of Color (20 γ /cc. Soln.)

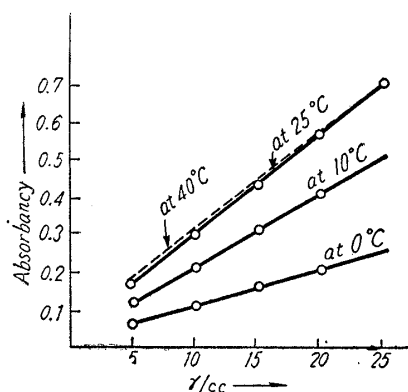


Fig. 3 Influence of Temp. on Intensity of Color

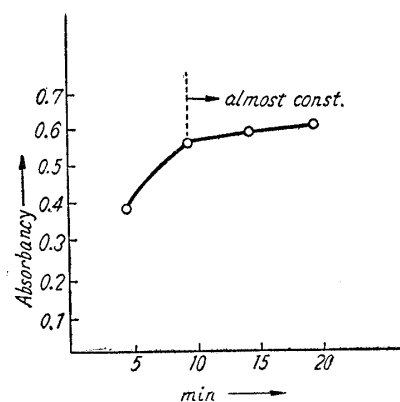


Fig. 4 Variation of Intensity of Color with Length of Reaction Period (20 γ /cc. Soln.)

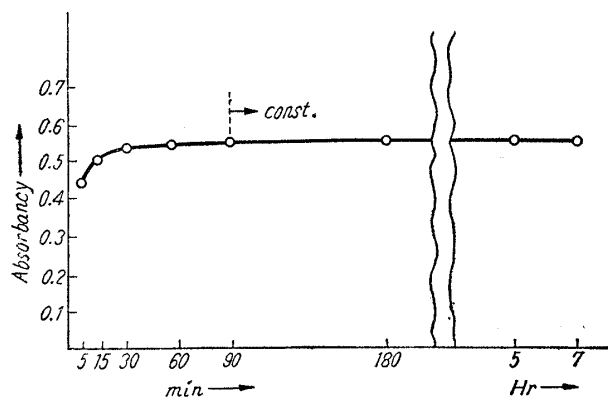


Fig. 5 Variation of Intensity of Color with Time Elapsed (20 γ /cc.)

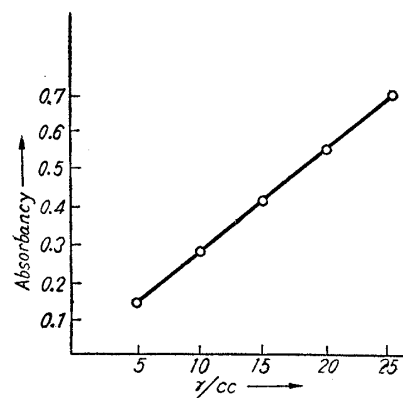


Fig. 6 Calibration Curve

Experimental

Reagent—(1) Diazonium reagent: 100 mg. of *p*-nitraniline which is recrystallized from water is weighed into a 100-cc. amber-colored volumetric flask, add 2 cc. of 2*N* HCl and heat on a water bath to dissolve the base. After cooling to room temperature, add about 15 cc. of distilled water and cool to about 8°. Then add 2.8 cc. of 2% NaNO₂ solution all at once and shake well in the thermostat at about 8°. Then add distilled water to the mark and filter through filter paper into another amber-colored bottle. This reagent may be used even 24 hrs. after its preparation if kept in amber-colored bottle at 0°.

(2) EtOH: First grade EtOH redistilled.

(3) Alkaline solution: 10% NaOH solution.

(4) Buffer solution: 0.25M Na_2HPO_4 solution adjusted to pH 7.5 by addition of 0.25M KH_2PO_4 solution.

(5) Standard material: Homosulfamine which corresponds to all requirements of J.P. VI was recrystallized from 50% EtOH and dried at 110° to const. weight, m.p. 263~264°. *Anal.* Calcd. for $\text{C}_7\text{H}_{11}\text{O}_2\text{N}_2\text{ClS}$: N, 12.58. Found (Kjeldahl): N, 12.55.

Preparation of Calibration Curve: Solution containing from 5 to 25 γ per cc. was prepared from dried anhyd. homosulfamine, 5 cc. portions of each solution were pipetted into five amber-colored test tubes, and color development was made as described in the procedure beginning with "add 5 cc. of buffer solution....". Calibration curve was made by plotting absorbancy against concentration of homosulfamine on section paper (Fig. 6). The apparatus used was an AKA photoelectric colorimeter. The filter was S-53. The cells were 1.000 cm.

Procedure—Pipet 5 cc. of solution containing about 15 γ per cc. into an amber-colored test tube, add 5 cc. of buffer solution and place in a thermostat keeping at 25° for 15 mins. Then add 2 cc. of 10% NaOH and 2 cc. of EtOH successively, and shake well. After 1.5 hrs., measure the absorbancy with S-53 filter (AKA photoelectric colorimeter) or at 520 $\text{m}\mu$ (Beckman Model B spectrophotometer) using a blank solution of the reagents which has been treated in the same way as the sample and determine the homosulfamine content by referring to the calibration curve.

Discussion

This method is directly applicable to such preparations as injection and eye water containing boric acid and zinc sulfate. Phenols, other sulfa drugs, aromatic amines, and aliphatic amines give red colors. Ephedrine hydrochloride does not produce a red color but reacts with diazonium reagent. Table II gives the minimum amount of several interfering materials. We carried out experiments 19 times using the solution containing 15 γ per cc. for the purpose of finding the error due to the color developing procedure, the results of which are shown in Table III. From these data, standard deviation is 0.000785 and the error is 1.81%.

TABLE II.

Interfering materials tested	Amount in γ /cc. that will not interfere
Taurine	0.3
Sulfamine	1
Sulfamerazine	1
Sulfadiazine	1
Ephedrine Hydrochloride	1

TABLE III.

0.449	0.439	0.419	0.440	0.432
0.435	0.432	0.434	0.421	0.440
0.434	0.433	0.434	0.435	0.439
0.420	0.445	0.429	0.438	
\bar{x} 0.434, xf 18, S. D. 0.000785				
Error 1.81%				

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