COLORIMETRIC DETERMINATION OF SULPHURIC ACID.

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In the benzidine method of volumetric determination of sulphuric acid, we precipitate the acid as benzidine sulphate by means of benzidine hydrochloride and titrate the precipitate directly by alkali. We may also determine the acid by the estimation of benzidine in the precipitate⁽¹⁾; similarly, when the amount of benzidine hydrochloride added is known, we can reckon the acid by estimating the residual benzidine in solution. Moreover, if benzidine can be estimated colorimetrically, the estimation of the acid may be simplified and be useful for some purposes.

A search for the reagent which will give a quantitative colouration with benzidine, has shown that furfurol is the most convenient and handy one among a good deal of varieties of chemicals for that purpose. The conditions and properties of this colouration were as follows :—

1. In the presence of small amounts of HCl and ethyl alcohol, aqueous solution of bezidine hydrochloride forms a canary yellow solution with furfurol at room temperature. The colour hardly changes its intensity on standing.

2. When the equal amounts of furfurol in large excess are added to the increasing amounts of benzidine, the depths of colouration produced are proportional to the amounts of benzidine.

Thus, 5 c.c. of benzidine hydrochloride solutions of the concentrations of 6 : 5 : 4 : 3 (6 = 0.006 mg. mol. per 5 c. c.) are mixed with 0.2 mg. mol. of furfurol. The relative depths of colouration are measured, comparing the neighbouring two liquids in a colorimeter.⁽²⁾ The depths of colouration are found to be proportional to the concentration of benzidine hydrochloride (Tab. 1). This holds also in higher and lower concentrations of benzidine.

TABLE 1.

Concentration of benzidine solutions	6 : 5 : 4 : 3		
Ratio of concentrations	1.20	1.25	1.33
Ratio of depths of colouration observed	1.19	1.25	1.33

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(1) Raiziss and Dubin, *J. Biol. Chem.*, **18** (1914), 297; Pezzi, *Giorn. chim. ind. applicata.*, **3**, 10 (*Chem. Zentr.*, 1921, II, 715).

(2) Colorimeter-nephelometer by Baudouin and Benard, Pellin et Cie., Paris, was used.

3. When the increasing amounts of furfurol are added to the equal amounts of benzidine hydrochloride, the colourations produced are not constant, but increase with the amount of furfurol as shown in Fig. 1.

4. Increase of acidity of the mixture somewhat deepens the colouration.

The above results show that when benzidine hydrochloride is submitted to colorimetry, the equal amounts of furfurol (excess to benzidine) must be added to the comparing solutions of the same acidity.

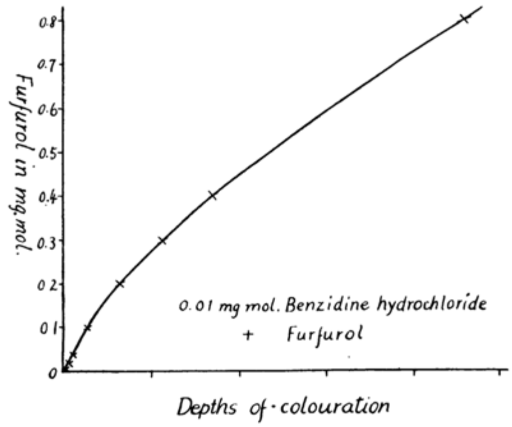

Fig. 1.

Ordinary Method.

Pure ammonium sulphate solution of the concentration of 4–5 millimols is prepared for standard solution. The concentration of the test solution should be nearly equal to this. To 5 c. c. of both solutions taken in large test-tubes (Fig. 2, B) add 2 drops of brom-phenol-blue (0.04%), and N-HCl until the indicator turns perfectly yellow, and then 5 c.c. of water respectively. Two c.c. of $M/20$ benzidine hydrochloride are next added, drop by drop, to each tube, followed by 5 c.c. of 90% acetone to complete the precipitation. After standing 10 minutes, the solutions are filtered by a filtering apparatus.

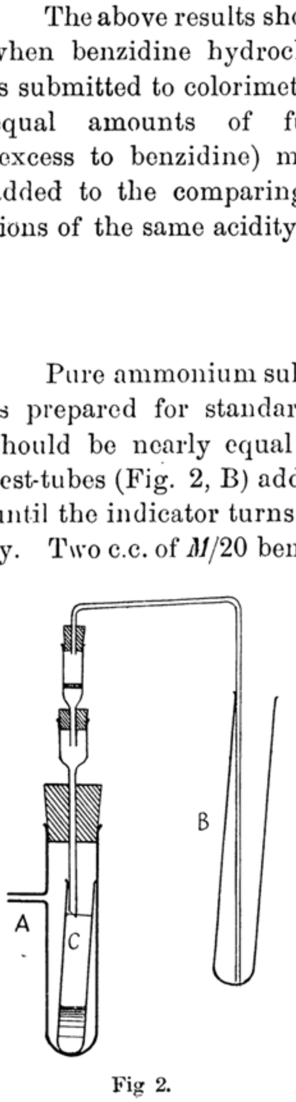

Fig 2.

As shown in Fig. 2, the filtering apparatus is like that of Pregl's in the determination of halogens, provided that the "glass filter" (Pattern 12 G 2/<7, Schott & Gen, Jena) is preferably used in the place of "Filterröhrchen". By a gentle suction from A, the precipitates are all brought into the filter, where it is washed 3 times by 2 c.c. of 90% acetone and finally once by 3 c.c. of it. When all acetone has gone through the filter, it is sucked strongly for a few minutes to dry up any wash liquid left in the filter. The suction is then stopped, the filtrate in A is poured out and in that place a clean test-tube with a mark at 20 c.c. (Fig. 2, C) is settled. Fifteen c.c. of a mixture of absolute alcohol and *N*/10 NaOH (1 : 1 by volume) are taken into the tube formerly used for the precipitation and are shaken to dissolve any benzidine sulphate adhered to the wall of the vessel. Then, the solution is sucked into the filter, which dissolves the precipitate and gathers into the test-tube.

This operation is performed twice with both solutions at a single apparatus or at the same time by a pair of apparatuses, which may save time. The two solutions collected under the filter are then taken out, added by 10 drops of 10% $BaCl_2$, 1 drop of phenolphthalein and with gentle agitation *N*-HCl drop by drop, until the alkaline reaction fades, and 10–15 drops furthermore. Fill the tubes up to the mark, mix well and pipette out 5 c.c. from each solution into a pair of clean test-tubes. To them add 2 c.c. of 1% furfurol aq. and put the resulting coloured liquids to a colorimeter. It takes about 2 hours for the operation. The solution before adding furfurol can be preserved unchanged for days. It is advisable to use sky-blue glasses on the eye-piece of a colorimeter; they facilitate the comparison well.

For the furfurol solution, freshly distilled furfurol should be used. The pale yellow colour of the solution does no harm on the comparison. Also commercial furfurol can be used, when it is decolourized by animal charcoal in aqueous solution. In this case, the concentration of furfurol is somewhat lowered.

In comparing two ammonium sulphate solutions (0.050 and 0.025 mg. mol. per 5 c.c.) by this method, the result obtained was 2 : 0.99, showing that the sulphate was quantitatively derived into coloured substances.

Application to urine analysis. The sulphates in urine are determined after the accompanying phosphate is removed by the method of Fiske(1) as follows. Into a 100 c.c. volumetric flask sufficient urine to contain 40–50 mg. H_2SO_4 in the form of inorganic sulphate (ordinarily, an amount of urine corresponding to a 30-minute period will be suitable) is introduced and diluted to about 50 c.c. with water. A drop of phenolphthalein solution is added, followed by ammonia water until the contents become faintly pink. Now add about 1.5 gr. of finely powdered basic magnesium carbonate, make up to the mark, shake for a minute and let it stand for 30 minutes. In case of foaming, a drop of caprylic alcohol facilitates to make up to the mark exactly. In accurate work, the volume of carbonate should be taken into calculation (about 0.8 c.c.). The mixture is filtered through a dry paper into a dry flask. This filtrate is used for the following two determinations.

Inorganic sulphate. Five c.c. of the filtrate are directly proceeded as above.

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(1) *J. Biol. Chem.*, 47 (1921), 62.

Total sulphate. Five c.c. of the filtrate are pipetted into a test-tube, to which 0.5 c.c. conc. HCl is added, and the tube is dipped into vigorously boiling water for 30 minutes. The hydrolysed brown liquid is then poured into a small dish with the aid of small amount of water. The contents of the dish are evaporated on a water bath to perfect dryness and laid heated 10 minutes longer. The residue is extracted with 10 c.c. of hot water in several times, transferred into a precipitation tube and treated as before, provided that there is no need of adding 5 c.c. of water before the precipitation with benzidine hydrochloride.

Results. Two samples of normal urine are taken and their contents of sulphates are determined both by gravimetric and colorimetric methods to compare results.

TABLE 2.

Urine No.	d_{15°	Cl present at the time of precipitation mg.	Inorganic sulphate SO_3. mg./c.c. urine		Total sulphate SO_3. mg./c.c. urine	
			gravimetric	colorimetric	gravimetric	colorimetric
I	1.024	6.4	1.614	1.61		
		221*			1.90	1.56
II	1.030	11.0	2.121	2.14		
		8.5			2.265	2.23

* HCl for hydrolysis was neutralized, not evaporated off.

As seen from the table, when there is a large amount of chloride present in the sample, it gives bad results. This is why in total sulphate estimation the hydrolysing HCl must be removed by evaporation.

Rapid Method.

When the testing solution is colourless, or can be made colourless, we can determine its sulphuric acid in estimating the residual benzidine in solution colorimetrically, after the precipitation of sulphuric acid by benzidine. In such a case, the estimation is very simple.

Five c.c. of the standard (0.01 mg. mol. $(NH_4)_2SO_4$ per 5 c.c.) and testing solutions are taken into a pair of centrifugal tubes, and each solution is acidified with 5 drops of *N*-HCl To them add 2 c.c. of benzidine hydrochloride solution (0.03 mg. mol. per 2 c.c., accurately) followed by 2 c.c. of 1% furfurol solution respectively. After careful mixing, the solutions are centrifuged and the supernatant clear liquids are put to colorimeter.

In the case when more concentrated sample can be obtained, the following recipe is advantageous: 5 c.c. of the standard (0.025 mg. mol. $(NH_4)_2SO_4$

per 5 c.c.) and testing solutions, 5 drops of N-HCl, 5 c.c. of benzidine hydrochloride (0.075 mg. mol. per 5 c.c.) and 5 c.c. of furfurol solution respectively.

Of course the above performance can be applied to any other proportion of reagents and sample, but always the benzidine reagent must not be added too excessively, for it brings the ratio of residual benzidine in two solutions near to 1, and it requires to observe the ratio of the two more accurately, which is not advantageous for the colorimetry. About 3 times excessive amount of benzidine reagent is suitable.

Preparation of solutions. *Standard solution.* 0.1320 Gr. (for the first recipe) or 0.3300 gr. (for the second) of pure ammonium sulphate is dissolved in water and made up to 500 c.c.

Benzidine solution. The solution of benzidine in dilute HCl is heated with small amount of animal charcoal and filtered. To the filtrate conc. HCl is added, until the white precipitate ceases to separate, which is soon collected by a centrifuge and dissolved in hot water. The solution is filtered hot into a pure NaOH solution and after the mixture has cooled, the precipitated benzidine is taken on a filter, washed by water, recrystallized from a large amount of water and dried in air oven. 1.380 Gr. of this purified benzidine is dissolved in water with 36 c.c. N-HCl and made up to 500 c.c. This solution is colourless and can be kept long without deterioration.

Calculation. Standard solution containing a mg. mol. sulphate and testing solution containing x mg. mol. sulphate in equal volume are mixed with b mg. mol. benzidine hydrochloride respectively. There remains $b-a$ and $b-x$ mg. mol. benzidine hydrochloride in solutions after the precipitation. Furfurol solution is added to each solution and the colouration that is proportional to residual benzidine is produced. Let the layers of these liquids giving the same intensity of colour be s and s_x. Then,

$$s\,(b-a) = s_x\,(b-x), \quad \text{or} \quad x = b-(b-a)\,\frac{s}{s_x}.$$

In the first instance of the above recipes, $x = 0.03 - 0.02\,\dfrac{s}{s_x}$.

In the second instance, $x = 0.075 - 0.050\,\dfrac{s}{s_x}$.

This method gives high results in the presence of phosphate and low results in the presence of chloride (Tab. 3). It is necessary to remove phosphate when this is present over 5 times (in mol) excess to sulphate.

The effect of chloride can be partly decreased by the use of more concentrated sample and benzidine solutions. But by the use of a standard solution containing approximately equal amount of chloride, the most of the effect can be canceled. The method is, therefore, a convenient one in a routine work when the contents of chloride are approximately known.

TABLE 3.

SO_4 mg. mol.	Cl mg. mol.	PO_4 mg. mol.	SO_4 found mg. mol.
0.0125	0	0	0.0125
0.0125	0	0.125	0.0128
0.0125	0.375	0	0.0113

Application to urine analysis. This method can be applied on urine after it is decolourized by animal charcoal. The charcoal should be freed from sulphate, phosphate and especially from iron, as far as possible, by digesting with hydrochloric acid and subsequent washing with water. O. Warburg's " Silicat-zuckerkohle "(1) well serves for this purpose, as it is free from these substances.

For inorganic sulphate, 1 gr. or less of dried charcoal is added to 20 c.c. of urine solution (phosphate removed, see page 175), and after 20 minutes the solution is filtered through a fine filter paper into a dry flask. Five c.c. of this decolourized solution is proceeded as directed above.

In case of total sulphate, 10–20 c.c. of urine solution are hydrolysed in a large test-tube with its 1/10 volume of conc. HCl as directed in ordinary method. The residue is extracted with hot water, the extract is diluted to a definite volume (say, 20 c.c.), and is decolourized by a small amount (0.2–0.5 gr.) of animal charcoal. Five c.c. of this solution are proceeded as previously noted.

Standard solution. The injurious effect of chloride originally present in urine (see Urine III, Tab. 4) can be eliminated by the addition of sodium chloride to the standard solution. 0.1320 Gr. recrystallized ammonium sulphate and 1.2 gr. sodium chloride are dissolved in water and made up to 500 c.c. The solution serves as a standard for these estimations and gave following results.

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(1) *Biochem. Z.*, **145** (1924), 473.

TABLE 4.

Urine No.	d_{15°	Cl present at the time of precipitation. mg.	Inorganic sulphate SO_3. mg.		Total sulphate SO_3. mg.		Remarks	
			gravimetric	colorimetric	gravimetric	colorimetric	Standard solution used	Preservative added
III	1.016	7.58	0.816	0.77			$(NH_4)_2SO_4$ 0.01 mg. mol. per 5 c.c.	Toluol
		—			0.904	0.91		
V	1.024	6.73	2.130	2.10			$(NH_4)_2SO_4$ 0.0125 per 5 c.c.	”
		13.1			2.283	2.31	NaCl 0.20 per 5 c.c.	
VI	1.016	8.91	0.835	0.82			$(NH_4)_2SO_4$ 0.025 per 5 c.c.	Phenol
		17.8			0.927	0.93	NaCl 0.40 per 5 c.c.	
VII	1.028	9.70	2.228	2.23			”	—
		19.0			2.368	2.37		

Remarks.

The main parts of these methods lie in the precipitation of benzidine sulphate and colorimetry, and especially for the former performance; the following precautions are necessary: Sulphate is precipitated in slightly acidic solution, containing less than 5 times (in mol) of phosphate or 5 times of chloride (the effect of chloride is smaller in ordinary method than in rapid method). In the presence of other acid radicals which form difficultly soluble salts with benzidine or any oxidizing agent which attacks benzidine, the methods are not applicable.

Summary.

1. Sulphate is precipitated by benzidine hydrochloride and on resulting precipitate of filtrate the amount of benzidine is determined colorimetrically. From that value, the amount of sulphate is calculated.
2. "Rapid method" is given, which is simple in execution and does not take much time. The purification of benzidine for this method is also given.
3. The methods are applied on urine analyses and seem to be useful especially in clinical works.
4. In ordinary method 2–2.5 mg. H_2SO_4 per 10 c.c. can be determined; in rapid method 1–1.5 mg. per 5 c.c. is necessary.
5. When any hindering substances are absent, the accuracy of the results always lies within the range of accuracy of colorimetry.

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