XXX. ON THE ESTIMATION OF β -HYDROXY-BUTYRIC ACID.

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The experiments described in this paper were undertaken in order to test those methods for the estimation of β -hydroxybutyric acid which are applicable to mixtures of its two optical isomers. Two such methods are available, dependent upon the conversion of the acid into crotonic acid, and into acetone, respectively.

The production of crotonic acid has been applied as a method of estimation by Darmstädter [1903], Ryffel [1905], and Pribram [1912]. Darmstädter's method has been shown by Shindo [1907] to be very inaccurate. Pribram obtained 99 % of the theoretical amount of crotonic acid in as little as 350 cc. of distillate. In the present investigation a large number of estimations were made by Pribram's method, and the yield was not more than 96 % in from 700 to 1200 cc. of distillate. The form of the curve obtained is very similar to that given below for the formation of acetone from β -hydroxybutyric acid; in the later stages of the distillation the crotonic acid comes over more and more slowly. In Ryffel's method this difficulty was avoided by calculating the ultimate result of the distillation from the rate of evolution observed in fractions of the first 400 cc. of distillate.

The conversion of β -hydroxybutyric acid into acetone by oxidation with sulphuric acid and potassium bichromate was applied as a method of estimation by Shaffer [1908]; the acetone produced was estimated by means of iodine and thiosulphate in the ordinary way. It seems preferable to employ the method of Scott-Wilson [1911] for determining the amount of acetone, since the reagent introduced by him is less likely than is iodine to be affected by the presence of other substances. This combination of the methods of Shaffer and Scott-Wilson has been used in the investigation of a series of cases of acidosis [Kennaway, Pembrey, and Poulton, 1913].

Results obtained with the Scott-Wilson method.

The acetone is precipitated by a reagent containing mercuric cyanide, silver nitrate, and caustic soda; the amount of mercury in the precipitate is estimated by titration with potassium sulphocyanide. On the assumption that one molecule of acetone combines with five atoms of mercury, Scott-Wilson obtained results which were about 3 % too low. The following experiments were carried out with acetone from the bisulphite compound (Kahlbaum).

TABLE I.

			Mg. acetone in 10 cc.
I.	(1)	0.0990 gm. acetone weighed and diluted to 1 litre ...	0.990
	(2)	100 cc. of above solution estimated by iodine method ...	(1) 0.981
			(2) 0.981
	(3)	10 cc. estimated by Scott-Wilson method. Mean of ten estimations (max. 0.980, min. 0.960) ...	0.9718
	(4)	5 cc. estimated by Scott-Wilson method. Mean of ten estimations (max. 0.986, min. 0.963)... ...	0.9707
II.	(1)	0.0747 gm. acetone weighed and diluted to 1 litre ...	0.747
	(2)	100 cc. of above solution estimated by iodine method ...	(1) 0.722
			(2) 0.722
	(3)	10 cc. of above solution estimated by Scott-Wilson method ...	(1) 0.719
			(2) 0.713
III.	Acetone solution of unknown strength:		
	(1)	100 cc. estimated by iodine method ...	0.8180
	(2)	10 cc. estimated by Scott-Wilson method ...	0.8177

In Scott-Wilson's experiments, the precipitation of acetone by the mercuric cyanide reagent was found to be complete in 10 minutes if the reagent were added in the proportion of not less than 25 cc. for 1 mg. of acetone. These estimations were made with a $\frac{N}{1000}$ acetone solution (i.e. 0.58 mg. in 10 cc.). Since the concentration of acetone in distillates from urine is often much lower than this, the question of the relation between this concentration and the rate of precipitation requires some further testing.

TABLE II.

Precipitation of acetone in different concentrations with the same time of precipitation.

Portions of 10 cc. of the acetone solution given under (II) above were precipitated with Scott-Wilson reagent, the concentration being in some cases lowered by addition of water, as shown in the third column below. The precipitation was in all cases allowed to proceed overnight.

Acetone solution, cc.	Scott-Wilson reagent, cc.	Total volume, cc.	Result of estimation	
			Cc. KCNS used	Mg. acetone
10	25	35	12.40	0.719
"	25	35	12.30	0.713
"	25	35	12.20	0.707
"	20	30	12.35	0.716
"	20	30	12.30	0.713
"	20	100	12.20	0.707
"	20	100	12.05	0.699
"	20	200	12.10	0.702
"	20	200	11.90	0.690
"	20	300	11.40	0.661
"	20	300	10.90	0.632
"	20	300	10.70	0.620

These results show that the precipitation can be rendered incomplete and irregular by sufficient dilution. The rate of precipitation was tested as follows:

TABLE III.

Precipitation of acetone at the same concentration with different times of precipitation.

Mixtures of Acetone solution		...	10 cc.
Scott-Wilson reagent		...	20 "
Water		...	70 "
Hours allowed for precipitation		Result of estimation	
		Cc. KCNS used	Mg. acetone
$\frac{1}{2}$	{	12.00	0.696
		12.00	
1	{	12.30	0.703
		11.95	
$1\frac{1}{2}$	{	11.90	0.690
		11.90	
2		12.00	0.696
$2\frac{1}{2}$		12.00	0.696
5	{	12.10	0.7047
		12.20	
		12.30	
18	{	12.20	0.7064
		12.05	

The differences observed in this series are almost within experimental error. A full investigation of the relations between different concentrations of acetone and of mercuric cyanide, and the rate of precipitation, would obviously be very laborious. In practice, this question does not give much trouble. With a little experience, it is easy to judge from the rate at which the precipitate develops whether an excessive amount of acetone has been added to the amount of reagent taken. In any case of doubt, one can (1) add more reagent to one of two duplicates; (2) keep one duplicate for a longer time before filtration; or (3) keep the filtrate to see whether any further precipitate develops in it. It is well to obtain distillates which contain more than 0.7 mg. acetone in 100 cc. (see Table II), to aim at using 30 cc. reagent for 1 mg. acetone, and to leave the mixtures when possible overnight before filtration.

Except where it is otherwise stated, all the acetone estimations given in this paper were made by the Scott-Wilson method.

Results obtained with Shaffer's method.

(a) The estimation of β -hydroxybutyric acid.

Shaffer [1908] obtained approximately correct yields (98.8 to 103.3%), using a preparation of β -hydroxybutyric acid the purity of which was not tested in a satisfactory manner. Cooke and Gorslin [1911] showed that they could obtain concordant duplicates by this method, but did not attempt to establish its accuracy. Mondschein [1912] obtained an average yield of 97.8%, but he gives no particulars of the preparation of the acid used in his experiments.

The preparation used in the estimations given below was made from the impure inactive acid (Kahlbaum). The liquid was diluted with water, filtered, and extracted with ether; the extract was distilled in vacuo, the residue neutralised with caustic soda, and the salt recrystallised five times from alcohol. It gave the following result on combustion¹:

0.1600 g.; 0.0806 g. H₂O; 0.2240 g. CO₂.

	C %	H %
Found	38.20	5.60
Calculated for C ₄ H ₇ O ₃ Na	38.09	5.55

When weighed amounts of this salt were treated by Shaffer's method,

¹ I am indebted to Mr V. Steele for carrying out this analysis.

the yield of acetone obtained was from 90 to 96.5 % of the theoretical amount. The concentration of acetone in successive portions of distillate becomes less and less until the amount, if any be present, is too small for estimation. (Table IV and Fig. 1.)

Fig. 1. Formation of acetone from β -hydroxybutyric acid.

Abscissae represent cc. distillate.

Ordinates represent mg. acetone.

Horizontal lines show the theoretical amounts of acetone.

Various attempts were made to increase the yield by altering the manner in which the oxidation was carried out. If the concentration of sulphuric acid be increased to six volumes per cent., which is twice that recommended by Shaffer, the reaction comes to an end sooner, but the total yield is not increased (see Expts. VII and VIII, Table IV). Slow addition of the bichromate produces a somewhat higher result than the more rapid addition of the same amount (Fig. 2). Theoretically, 1 part of acetone should be produced by 1.7 parts of bichromate. Large excess of bichromate certainly diminishes the yield, as Shaffer stated; on the other hand, the theoretical

amount of bichromate produces only about $\frac{2}{3}$ of the theoretical yield of acetone (see Expt. VI, Table IV; and Fig. 2). Between these two extremes of excess and defect, there is a wide range over which the amount of bichromate seems to have no regular influence on the result. This is shown in Table V, in which the yields are arranged in descending order.

TABLE IV.

Sodium β -hydroxybutyrate distilled with 3 volumes % sulphuric acid, and potassium bichromate.

Expt.	Distillate, cc.	Acetone found, mg.	Acetone calculated, mg.	Yield %	Potassium bichromate, mg.	
					used	calculated
I	500	18.94				
	300	1.63				
	300	0.26				
	1100	20.83	22.69	91.9	250	40
II	800	81.78				
	500	trace				
	1300	81.78	91.2	90	700	155
III	800	28.10				
	200	0.17				
	1000	28.27	29.64	95.4	600	50
IV	800	29.69				
	200	1.95				
	800	1.22				
	1800	32.86	34.47	95.3	440	59
V	800	23.74				
	300	0.49				
	1100	24.23	25.77	94	400	44
VI	800	39.45			100	
[Effect of theoretical amount of bichromate]	800	16.54			150	
	300	0.72				
	1900	56.71	60.39	93.9	250	103
VII	800	26.216				
[6 vols. % sulphuric acid]	800	Nil				
	1600	26.216	27.93	93.9	300	48
VIII	800	12.934				
[6 vols. % sulphuric acid]	800	Nil				
	1600	12.934	13.96	92.6	200	24

Fig. 2. Effect of amount of bichromate on the yield of acetone from β -hydroxybutyric acid. Abscissae represent cc. distillate. Ordinates represent mg. acetone or cg. bichromate. Continuous lines represent acetone; the horizontal lines show the theoretical yield. Dotted lines represent bichromate. Curve I. Effect of theoretical amount of bichromate (horizontal dotted line). Curves II and III. Duplicate determinations on urine with added sodium β -hydroxybutyrate. Slow (II) and rapid (III) addition of bichromate.

TABLE V.

Effect of amount of bichromate upon the yield of acetone from sodium β -hydroxybutyrate.

Quotient		Yield % ₀
Bichromate ^e used		
Bichromate calculated		
12		95.4
7		95.3
9		94.0
2.5		93.9
6		93.9
8		92.6
6.5		92.1
6.25		91.9
4.6		90.0
Mean	6.6	93.2

When the sodium salt is added to urine, and the urine then treated in the ordinary way by Shaffer's method, the yield is as a rule somewhat higher than that given by a pure solution (Table VI); probably the increase is due to concentration of the fluid brought about by the bulky precipitate of lead compounds¹.

TABLE VI.

Sodium β -hydroxybutyrate added to urine and treated by Shaffer's method.

Expt.	Distillate, cc.	Acetone found, mg.	Acetone calculated, mg.	Yield %
I	800	23.90		
	300	0.08		
	1100	23.98	25.69	93.4
II	800	28.72		
	300	0.44		
	1100	29.16	31.65	92.1
III	800	16.07		
	400	1.30		
	400	0.17		
	1600	17.54	18.52	94.6
IV	800	16.80		
	800	0.30		
	1600	17.10	18.19	94
V	800	16.88		
	200	0.44		
	500	0.23		
	1500	17.55	18.19	96.5
VI	800	15.72		
	700	1.71		
	600	trace		
	2100	17.43	18.19	95.8
VII	800	16.72		
	600	0.62		
	1400	17.34	18.19	95.3

It is then easy to show that Shaffer's method does not give a quantitative yield of acetone, but it is of course much more difficult to find out whether the acetone obtained from urine by oxidation is derived wholly from β -hydroxybutyric acid, and there seems to be no satisfactory way of doing this. However, the following experiment was made to investigate this question. A diabetic urine containing 3.4% sugar, 0.228% aceto-acetic acid, and

¹ In these and all other cases where substances were added to urine, the amount yielded by the urine alone was of course subtracted from the result.

0.925 % β -hydroxybutyric acid, was acidified and extracted with ether until the extract was optically inactive. The second distillate from the residue when treated by Shaffer's method gave with Scott-Wilson's reagent a barely perceptible trace of precipitate, such as might well have been due to incomplete extraction of the β -hydroxybutyric acid. This experiment of course shows merely that the ether-insoluble substances did not yield acetone.

After nearly all the estimations given in this paper had been made¹, Shaffer and Marriot [1913] published some very similar results on the formation of acetone from β -hydroxybutyric acid. They obtained from 90 to 94 % of the theoretical amount; by very slow oxidation this was increased to from 95 to 97 %.

(b) *The estimation of aceto-acetic acid.*

The accuracy of Shaffer's method as a means of estimating aceto-acetic acid, which is greatly preponderant over acetone in urine [Arnold, 1900], appears never to have been tested. The estimations made with this substance were not altogether satisfactory, as difficulty was experienced in establishing a constant boiling point for the ester employed. However, the following experiments were carried out.

3.2525 gram aceto-acetic ester was saponified in the cold with the calculated amount of N caustic soda (25 cc.), and diluted to 1 litre (Solution I).

	Acetone mg.		Yield %
	Found by Scott-Wilson estimation	Calculated from weight of ester	
10 cc. of above solution distilled by Shaffer's method	14.15	14.50	97.6
25 cc. " " " " "	35.96	36.25	99.0

This solution gave low results when added to urine. Probably some ester was still present, which was decomposed to acetic acid by the strong ammonia used in Shaffer's method. The solution was therefore shaken out five times with ether to remove ester; it had then taken up acetone from the ether, but the results obtained before and after addition to urine were compared. (Solution II.)

Solution II. 25 cc. distilled gave 54.37 mg. acetone.
 25 cc. added to urine gave 53.12 mg. acetone=97.7 %.

Another preparation (III) gave the following results:

50 cc. distilled gave 10.526 mg. acetone.
 50 cc. added to urine gave 10.522 mg. acetone.

¹ A preliminary communication was given to the Physiological Society, Nov. 15th, 1913. [Kennaway, 1913.]

The recovery of acetone from urine was tested as follows:

10 cc. acetone solution precipitated with Scott-Wilson's reagent gave 0.597 mg.
 100 cc. added to urine gave 6.037 mg. = 101 %.

Analyses of mixtures of sodium aceto-acetate and sodium β -hydroxybutyrate.

	Acetone, mg.		Yield %
	Calculated	Found	
1. Aceto-acetic acid (I)	36.25	35.23	97.2
β -hydroxybutyric acid	31.94	29.41	92.1
2. Aceto-acetic acid (I)	36.25	35.23	97.2
β -hydroxybutyric acid	25.76	24.23	94.0
3. Added to urine			
Aceto-acetic acid (III)	6.737	6.826	101.0
β -hydroxybutyric acid	28.50	26.34	92.1

A combination of the Shaffer and Scott-Wilson methods.

This may be illustrated by stating in detail the quantities used in a particular estimation. The method is taken from the papers of Shaffer [1908], Cooke and Gorslin [1911], and Scott-Wilson [1911], with various modifications which are mentioned below.

25 cc. of diabetic urine giving a moderately strong ferric chloride reaction was placed in a 250 cc. flask, diluted with water, and mixed with 50 cc. basic lead acetate solution¹ and 10 cc. strong ammonia. A drop of dilute sulphuric acid in the supernatant fluid showed that excess of lead was present. The mixture was made up to 250 cc. 100 cc. of the filtrate was made faintly acid with 9 cc. 20 vol. % sulphuric acid, and made up to 250 cc. 100 cc. of this solution was placed in the distilling flask with 400 cc. water, 15 cc. concentrated sulphuric acid and talc, and 300 cc. distilled off at constant volume. The distillate was made up to 500 cc., and two portions of 50 cc. precipitated with 20 cc. Scott-Wilson reagent.

800 cc. was then distilled off at constant volume with addition of 400 cc. 0.2 % potassium bichromate and water; the distillate was made up to 1 litre, and two portions of 50 cc. precipitated with 20 cc. Scott-Wilson reagent.

300 cc. was then distilled off at constant volume with addition of water,

¹ The U.S.P. solution used by Shaffer is prepared as follows: 180 g. lead acetate is dissolved in 700 cc. water; the solution is boiled for half an hour, while 110 g. of litharge is added in small portions. The solution is made up to 1 litre. The amount required is in most cases from one-half to twice the volume of urine.

and made up to 500 cc.; 100 cc. of this with 15 cc. reagent gave no precipitate on standing overnight.

The Scott-Wilson titrations gave the following results:

- (1) Aceto-acetic acid + acetone.

$$\left. \begin{array}{l} \text{KCNS } 9.45 \text{ cc.} \\ 9.45 \text{ cc.} \end{array} \right\} = 0.548 \text{ mg. acetone in } \frac{1}{10} \times \frac{1}{2} \times \frac{1}{2} \times 25 = 0.4 \text{ cc. urine.}$$

- (2) β -hydroxybutyric acid.

$$\left. \begin{array}{l} \text{KCNS } 10.70 \text{ cc.} \\ 10.75 \text{ cc.} \end{array} \right\} = 0.622 \text{ mg. acetone in } \frac{1}{10} \times \frac{1}{2} \times \frac{1}{2} \times 25 = 0.2 \text{ cc. urine.}$$

The estimation of such small quantities does not involve any considerable error. If the last two titration figures given above had been 10.5 cc. and 10.9 cc., and the day's volume of urine had been 7000 cc., the total amount of β -hydroxybutyric acid reckoned separately from the two titrations would have been 38.3 or 39.8 grams. The titrations as a rule do not differ by more than 0.2 cc.

The reasons for taking as much as 25 cc. of urine when the ultimate estimation is carried out upon the equivalent of 0.2 or 0.4 cc. are (1) the errors of the series of measurements are lessened by working with large volumes; (2) it is important that the concentration of acetone in the distillate should not be too low (see Table II)¹; (3) with some urines, but not with all, the conversion of a larger quantity of β -hydroxybutyric acid into acetone takes place more rapidly and completely than that of a smaller quantity (Fig. 3).

It is most convenient to have from 0.5 to 1 mg. of acetone in 25 to 50 cc. of distillate. 1 mg. of acetone requires 17.3 cc. N/100 KCNS. The amount of acetone bodies in a urine can be judged roughly by colour tests. If Roth's reaction gives a strong colour within a few seconds, probably more than 0.25 % aceto-acetic acid is present; the reaction is still given by a concentration of 0.0005 % or less [Hurtley, 1913]. The ferric chloride reaction is not obvious unless more than 0.07 % aceto-acetic acid is present. In order to form some idea of the amount of β -hydroxybutyric acid, one may remember that in well-marked cases of acetonuria this acid forms from 60 to 80 % of the total acetone bodies [Neubauer, 1910]; if this percentage were 66, the concentrations of acetone in the two distillates obtained by the method given above would be equal.

The urine is measured into the flask first, and diluted before adding the basic lead acetate; there is then less frothing. The froth can be

¹ Direct distillation into the reagent, as practised by Scott-Wilson with normal urine, would not be practicable in pathological cases.

dispersed, when brought up into the neck of the flask, by rubbing with a glass rod and piece of filter-paper¹.

Fig. 3. Conversion of different amounts of β -hydroxybutyric acid into acetone.

Abscissae represent cc. distillate.

Ordinates represent mg. acetone.

I. Estimation on equivalent of 12 cc. urine.

II. " " " 4 "

III. " " " 2 "

In the right-hand figure, the results given in curves I and III are calculated for 4 cc. urine, to facilitate comparison.

Estimation on equivalent of—12 cc. urine		12 cc.	4 cc.	2 cc.
1st distillation, 500 cc.	55.98	55.97	17.57	8.062
2nd " 300 "	Nil	Nil	1.14	0.823
3rd " 300 "			Nil	0.14
4th " 300 "				Nil
Mg. acetone, total	55.98	55.97	18.71	9.025
	$\div 3 = 18.66$			$\times 2 = 18.05$

The apparatus which has been used in these estimations is shown in Fig. 4. The distillate from the first flask is passed through a second flask, as in Scott-Wilson's method, containing about 30 cc. 40 % caustic soda and an equal volume of water, with a little talc; this is kept at more or less constant volume by a small flame, and towards the close of the distillation

¹ A drop of ether is of course highly effectual, but must on no account be used, as ordinary ether, and even that sold as "distilled over sodium," contains quite enough acetone to affect a Scott-Wilson estimation.

is boiled down to a small volume. This flask must rest on a sand-bath, as it is apt to crack on wire gauze. The distillate is received into a 500 cc. or 1000 cc. measuring flask; the condenser and delivery-tube should be in one piece, as no rubber junction at this point is satisfactory. The measuring flask is connected with a water trap in a 50 cc. cylinder; it is well to let the water suck back here soon after the start of the distillation, to make sure that the apparatus is tight. In carrying out the oxidation, it is generally convenient to keep the funnel filled with 0.2 % bichromate, and if necessary to add water or a few cc. of 5 % bichromate.

In the estimation of β -hydroxybutyric acid, a second distillation is necessary to show whether the formation of acetone is completed. The result is in many cases nil, or an amount too small to estimate; in other cases it may be as much as 10 % of the whole. The obvious method of avoiding this lengthy distillation and the large volume of distillate is to carry out the oxidation with a reflux condenser and bulbs, and then distil off the acetone formed. The results obtained thus were always too low, this being due probably to loss of uncondensed acetone when changing to the distillation apparatus. In estimations on urine, the formation of acetone is as a rule completed more rapidly than when the pure acid is used.

The second distillation with hydrogen peroxide described by Shaffer was not found to make any considerable difference in those cases where it was tested (Table VII). When a somewhat lower result is obtained from the

TABLE VII.

Comparison of results with Iodine and Scott-Wilson methods.

Milligrams acetone.

From aceto-acetic acid + acetone			From β -hydroxybutyric acid			
			Before H ₂ O ₂		After H ₂ O ₂	
	Iodine method	Scott-Wilson method	Iodine method	Scott-Wilson method	Iodine method	Scott-Wilson method
Urine 1	15.46	15.48	41.82	41.90	42.30	41.83
„ 2	16.19	15.98	39.40	38.64	38.68	38.78
„ 3	14.75	14.93	52.22	51.11	50.76	50.60
„ 4	15.59	15.83	50.76	50.60	49.05	49.45
„ 5	5.560	5.568	17.88	17.57	17.52	17.40
„ 6	43.51	41.76	111.19	106.15		
„ 7	6.16	6.32	21.75	21.97		
„ 8	9.91	9.12	49.31	46.40		
„ 9	4.83	5.18	24.65	24.48		
Blood 1	11.12	11.34	24.17	20.88		
„ 2	12.33	12.29	28.04	24.52		

second distillation, the diminution may be due to loss of acetone rather than to removal of other substances; possibly these are eliminated by the method of distillation through caustic soda adopted in these experiments (Fig. 4). When blood is treated in the same way as urine, besides acetone, other substances which combine with iodine are undoubtedly formed (Table VII).

Fig. 4.

Scott-Wilson showed that urine when decomposed by bacterial action yielded more acetone, or some substance reacting like acetone with his reagent. This change occurs slowly even when the urine is preserved with thymol in a cold room, and remains acid and apparently fresh (Table VIII); the β -hydroxybutyric acid is as a rule unaffected. Thymol when oxidised with bichromate and sulphuric acid yields some substance which is precipitated by Scott-Wilson's reagent, but comparative experiments show that the amounts

of thymol present in the filtered and diluted urine are not sufficient to affect the result. Chloroform cannot be used, as it causes reduction of the reagent, and the results are too high.

TABLE VIII.

			In 100 cc. urine	
			Aceto-acetic acid + acetone as acetone, grams	β -hydroxybutyric acid as acetone, grams
<i>Urine I.</i>	Estimated fresh.	...	0.187	0.812
	Kept with thymol at room temperature	...		
	6 months later (acid) ...	...	0.229	0.819
<i>Urine II.</i>	Estimated fresh.	...	0.062	0.1911
	Kept with thymol in cold room	...		
	5 months later (acid) ...	...	0.068	0.1907
<i>Urine III.</i>	Estimated fresh.	...	0.1305	0.300
	Kept with thymol in cold room	...		
	10 days later	...	0.1298	0.2994
	10 weeks later	...	0.138	0.311
	12 weeks later (acid) ...	...	0.137	0.311

Some notes on the Scott-Wilson method.

Scott-Wilson [1911] says that the mercuric cyanide reagent remains perfectly clear for months; in my experience it invariably develops a finely divided precipitate soon after preparation. This substance no doubt contains mercury or silver, or both, and must be removed. It is best to make up the reagent at least a week before it is required, and to filter through glass wool before use.

The constituents of the acid mixture are given by Scott-Wilson in "parts," but it is not stated whether these are by weight or by volume. The following mixture has been used in the present investigation: water 550 cc., concentrated sulphuric acid 27 cc., concentrated nitric acid 280 cc.

The precipitation can be carried out in beakers, as the precipitate is removed from these much more easily than from Erlenmeyer flasks. The precipitate after standing adheres very tenaciously to the vessel; it is best removed by rubbing with a glass rod and piece of filter paper; the paper is transferred on to the filter, and is used again in the same way to wipe out the funnel before the titration. If rubber tubing on a glass rod is used, the very fine precipitate gets inside the rubber however closely this be attached.

A Gooch crucible does not seem very suitable for the filtration. If an ordinary glass funnel be used with a porcelain disc, the final removal of the

precipitate is much easier, and the white particles are more visible on glass than on porcelain. The filter paper on the disc is covered with a suspension of talc in water, the talc being well sucked down before beginning the filtration. Such filters will stand the full force of the pump, in spite of the destructive action of the reagent upon filter paper. The precipitate and beaker should be washed five times after wiping the precipitate out of the beaker.

After washing, the material is lifted into an Erlenmeyer flask with a glass rod, and the funnel wiped with filter paper. Not more than 25 cc. of acid mixture should be used in washing down, as much dilution renders the end-point very vague. About 2 cc. of N/5 permanganate are added, and the mixture boiled till it is colourless; the sides of the flask are then washed down with a jet of acid mixture, about 1 cc. of permanganate is added, and the boiling repeated. No concordant results were obtained till this method of decomposing the precipitate was adopted.

The titration is carried out with N/100 KCNS, which can be standardised against silver nitrate. The first faint brown colour is the end-point; three more drops of sulphocyanide produce an obvious reddish-brown tint, and it is well always to obtain this second colour in order to be sure of the first.

SUMMARY.

The oxidation of β -hydroxybutyric acid with sulphuric acid and potassium bichromate in Shaffer's method of estimation gives from 90 to 96% of the theoretical amount of acetone.

A combination of Shaffer's method with Scott-Wilson's method for the estimation of acetone is described.

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