

## MEASUREMENT OF DISSOLVED OXYGEN IN SWAMP WATERS. FURTHER MODIFICATION OF THE WINKLER METHOD

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### INTRODUCTION

There have been several modifications of the original Winkler method for the estimation of dissolved oxygen (Winkler, 1888) designed to overcome the sources of error due to the presence of oxidizing and reducing substances in natural waters and industrial wastes. In the course of work on tropical swamps we have been interested in the amount of oxygen in water which not only may contain large quantities of organic matter, ferrous or ferric iron, nitrites and other disturbing substances, but may also be deeply coloured or charged with sediment to give anything from a slightly turbid to a densely muddy liquid.

For such water none of the modifications so far described is satisfactory. It does not in fact appear that any independent check has been made on the absolute accuracy of any of these modifications, though the reliability of the original method has been proved by Wheatland & Smith (1955) by comparison with gasometric analysis of distilled water and sea water. An attempt is made to assess the absolute accuracy of the method described in this paper.

### IODINE-DIFFERENCE METHOD

The various modifications designed to overcome the difficulties mentioned above have been reviewed by Ohle (1953). Since the Winkler method involves the final estimation of an equivalent amount of iodine, the most satisfactory as well as the most rapid is the 'sample-blank' or 'iodine-difference' method of which an improved version was developed by Ohle. This involves two original identical samples to each of which the same measured quantity of iodine is first added, and the alteration in the amount of free iodine by the disturbing substances is therefore the same in both. One sample (A) is then subjected to the Winkler procedure, and the same reagents are added in the wrong order to the other (B or 'blank'), which, unlike A, then contains no additional iodine equivalent to the dissolved oxygen. The difference between the titres (A-B) is equivalent to the amount of dissolved oxygen.

An earlier variant of this method involved adding the iodine to the alkaline iodide solution which, with manganous sulphate, comprises the Winkler reagents (Adams, Barnett & Keller, 1943). Ohle (1953) objects to this procedure because it is difficult

to add exactly the same volume of an extremely alkaline solution to the two samples. The method described here avoids this difficulty in another way.

Ohle discovered an important source of error in dealing with what he called 'ein stark verschmutztes Wasser' from ponds. He found that the amount of free iodine left in the blank (B) became progressively less the longer the interval between adding the Winkler reagents and the final acidification. Since this did not apply to A, the apparent amount of oxygen became greater the longer this interval. This did not occur with clean water and he interpreted it as due to colloidal and particulate organic matter which was thrown down by the manganous hydroxide precipitate in A, but not in B where no precipitate is formed. He suggested that in the very alkaline conditions at this stage, when the added iodine is present as the very reactive hypoiodite, the suspended colloidal matter in B would be highly reducing and react with it. To obviate this error he advocated addition of acid immediately after the prescribed 10 sec. of shaking with the Winkler reagents. There was, however, no evidence that, during the minimum time needed to ensure completion of the Winkler reaction, there had not already been some reduction of iodine in B. This can only be decided by an independent estimate of the oxygen content, and it will be seen from the following that this error is not in fact overcome, even with moderately polluted water, by reducing the time taken over the operations to a minimum.

#### MODIFIED IODINE-DIFFERENCE METHOD

The procedure devised to eliminate the above error involves a preliminary clarification by aluminium hydroxide (Ellis, Westfall & Ellis 1948, p. 6) of a single sample of about 200 ml. from which the clear and decolorized supernatant is siphoned into two 35 ml. bottles, which are the A and B samples for the iodine-difference method. The added iodine is incorporated with the potassium hydroxide which produces the precipitate of aluminium hydroxide in the original sample, thus avoiding the difficult operation of adding exactly equal volumes of iodine solution to A and B. Clarification both removes colloidal organic matter and makes possible oxygen estimations in densely muddy water. A single original sample is preferable to two, because the identity of A and B in content of reducing substances is thereby assured.

The rest of the procedure is similar to that of Ohle (1953) except that, instead of altering the order of adding the reagents, saturated sodium chloride is substituted for manganous sulphate in the blank (B), and phosphoric acid is used in both because of the large amount of iron in swamp water.

The final titration with sodium thiosulphate was done with an Agla syringe micro-burette as in the micro-method of Fox & Wingfield (1938). Up to five 5 ml. aliquots can be taken from each sample.

#### REAGENTS

- (1) Potassium aluminium sulphate. Saturated solution (about 11 %) in saturated NaCl.
- (2) Iodine, 4 g.; KI, 100 g.; water, 100 ml.

- (3) 19% KOH in saturated NaCl } equal parts by volume.  
 Reagent (2)
- (4)  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 50 g.; water to 100 ml.  
 (5) KI, 15 g.; KOH, 70 g.; water to 100 ml.  
 (6) NaCl saturated solution.  
 (7) Orthophosphoric acid.  
 (8)  $\text{KIO}_3$  standard solution, e.g. 0.465 g./l.  
 (9) Standard iodine solution: (8) 2.00 ml., (7) 1 ml., KI crystals about 1 g., water to 100 ml.  
 (10)  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , 6 g.; NaOH 20%, 1 ml.; isobutyl alcohol, 10 ml.; water to 1 l.  
 (11) Sodium starch glycollate 5% in water (stock) diluted five times.

Reagents (1)–(6) are made up in freshly boiled distilled water to avoid super-saturation with air.

In (3) the iodine is in the form of hypoiodite leaving just sufficient free potassium hydroxide to precipitate aluminium hydroxide when added with (1) without raising the pH to a level likely to hasten the absorption of dissolved oxygen by the organic matter. It was shown in fact that the pH of a sample of swamp water, originally 6.5, was raised to 7.6 by this procedure, and that the copepod Crustacea in this sample remained alive above the precipitate for more than 5 hr.

#### PROCEDURE

The clarifying and Winkler reagents are injected with hypodermic syringes.

Operations (a)–(d) must be done in the field immediately after sampling.

(a) Samples are collected in rather tall 200 ml. reagent bottles. To each are added: reagent (1), 1.5 ml.; reagent (3), 0.6 ml.

The samples are stoppered, shaken vigorously and stood under water for the precipitate to settle. This takes 10–20 min. according to the nature of the suspended matter.

(b) With the simple device shown in Fig. 1 the clear supernatant is siphoned into the 35 ml. bottle A from which it passes into B. When B is full A has been sufficiently flushed and there is still enough water above the inlet of the siphon tube in the original sample to avoid contamination from the air above. This was confirmed by sampling deoxygenated water. A and B are stoppered and immediately treated with the Winkler reagents.

(c) To A are added: reagent (4), 0.17 ml.; reagent (5), 0.17 ml. The bottle is immediately stoppered and shaken for 10 sec. After standing until the precipitate has fallen from the neck of the bottle (10–20 sec.) 0.3 ml. of acid (7) is added and the bottle is restoppered and shaken until the precipitate is dissolved.

(d) To B are added: reagent (6), 0.17 ml.; reagent (5), 0.17 ml. This is then treated exactly as A, though no precipitate is formed.

A and B, after acidification, can be kept sealed and in the dark for at least 24 hr. without change in the iodine content.

(e) 5 ml. aliquots are titrated against thiosulphate (10) in an Agla syringe micro-burette after adding three drops of indicator (11). The thiosulphate is standardized against 5 ml. of the standard iodine solution (9).

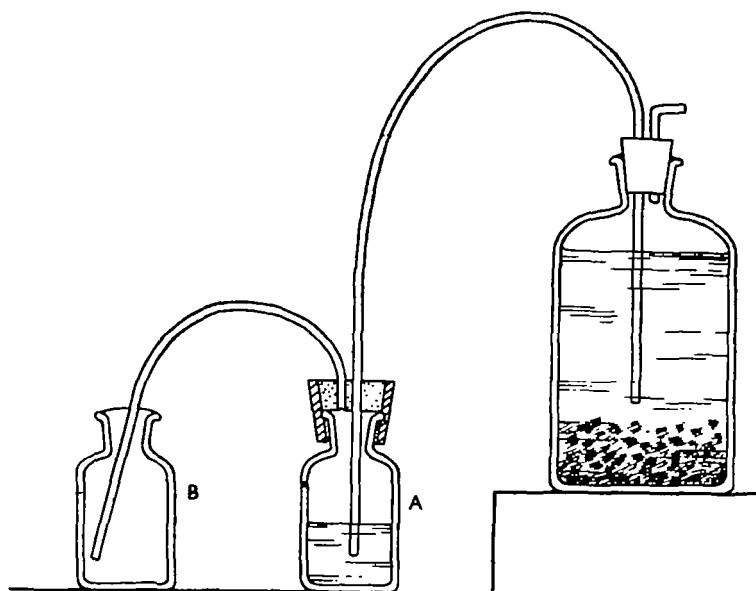


Fig. 1. The method for siphoning the A and B samples (each 35 ml.) from the supernatant of the original sample (200 ml.) after clarification. The double bung on bottle A ensures that this is flushed to the top so that it can be stoppered without including air bubbles. The Polythene tube passes loosely through the bung in the original sample bottle so that the depth can be adjusted according to the amount of precipitate.

#### CALCULATION

Oxygen in sample =  $\frac{1}{2}KF(A-B)$  mg./l.

$K$  = factor converting micrometer units to mm.<sup>3</sup>. This is approximately 20, but should be determined accurately by weighing mercury delivered from the burette over the range used.

$F$  = the oxygen equivalent of the standardized thiosulphate, in g. O<sub>2</sub>/l.

$A$  and  $B$  = volume in micrometer units of thiosulphate equivalent to 5 ml. of  $A$  and  $B$ , respectively.

#### CORRECTION FOR OXYGEN IN REAGENTS

Some users of the Winkler method (Ohle, 1953) have made a correction for the oxygen added in the reagents, including the acid. It was, however, shown by Adams *et al.* (1943) that the oxygen in the acid does not react with the manganous hydroxide and that a correction for the oxygen in the Winkler reagents would amount to no more than 0.01 mg. O<sub>2</sub>/l. A more direct proof of the insignificance of this correction was given by the comparison by Wheatland & Smith (1955) of gasometric with

Winkler estimations of dissolved oxygen in distilled water and sea water. The mean difference between the results from the two methods was 0.01 mg.  $O_2$ /l., the gasometric being the higher. No correction is therefore introduced here, but the clarifying reagents (1) and (3) are made up in a saturated solution of NaCl to reduce to the minimum the oxygen dissolved in them.

#### END-POINT OF IODOMETRIC TITRATION

An improved end-point was obtained by using a magnetic stirrer made of a piece of iron wire about 5 mm. long sealed in a glass tube. The rotation of this at the bottom of the titration tube, by a rotating magnet below, caused a vortex down the centre of the liquid. The tip of the microburette (hypodermic needle) was lowered by a screw-stand into the top of the vortex. On nearing the end-point the vortex could be distinguished as a column of fluid, clear compared with the surrounding faint bluish tint, just after a small amount of thiosulphate was injected. The end-point was reached when the injection of another division of the micrometer head (0.2 mm.<sup>3</sup>) gave no colour differentiation of the vortex column. The titration tube was illuminated from behind through frosted glass. In the field stirring was done by mouth-blowing through a fine Polythene tube.

#### SPECTROPHOTOMETRIC ESTIMATION OF IODINE

After the work described in this paper had been completed it was discovered that the final estimation of iodine could be done, as accurately as by titration and vastly more quickly, by direct measurement of optical density in a Unicam spectrophotometer (S.P. 600). Preliminary trials with a series of iodine solutions within the required range, in quartz optical cells of 1 cm. depth, showed that 420 m $\mu$  was a suitable wave-length, giving a steep curve of optical density against iodine concentration. The instrument was then calibrated by measuring the optical density at 420 m $\mu$  of a series of standard iodine solutions made up in water containing acidified Winkler reagents, the same reagents without free iodine being used as blank. Two curves were drawn (Fig. 2*a*, *b*) relating optical density to the oxygen equivalent of the iodine solution, one over the range 0–8 mg.  $O_2$ /l., the other on a larger scale over the range 0–1 mg.  $O_2$ /l., the latter covering most of the values normally obtained from swamp waters in the field. The relation is clearly linear.

Table 1 compares spectrophotometric with titrimetric estimations, each sample being finally estimated by both methods. It will be shown later that the present method, ending with titration, cannot be more accurate than  $\pm 0.2$  mg.  $O_2$ /l. The agreement between the two is therefore satisfactory. The negative figures (i.e.  $B > A$ ) obtained from stagnant swamp water are discussed below (p. 563).

The spectrophotometric estimation is particularly suited to the iodine-difference method because the sample containing the lesser amount of iodine (B) can be used as the blank and the difference is read directly.

To titrate in duplicate 5 ml. aliquots from one pair of A and B samples requires about 15 min. In 5 min. it is possible to estimate photometrically the difference in

optical density between four pairs of samples and to read off the equivalent oxygen concentrations from the calibration curve.

Direct spectrophotometric estimation of iodine was used by Littmann & Benoliel (1953) for continuous recording of oxidants in the atmosphere, for which filters were used giving wave-lengths in the near ultra-violet (320–380 m $\mu$ ). Ovenston & Watson (1954) used a Unicam spectrophotometer S.P. 500 to estimate small

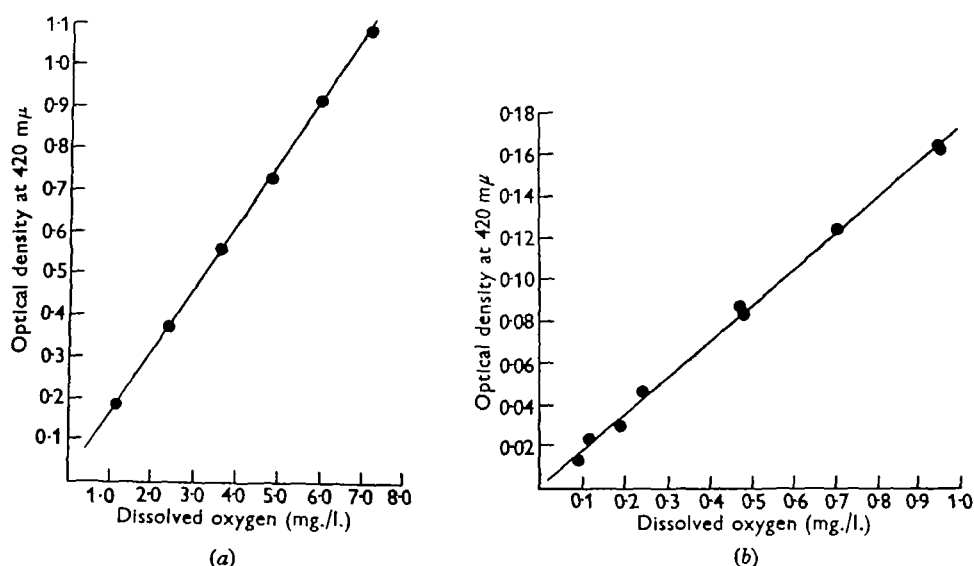


Fig. 2. Curves showing the relation between the concentration of free iodine (represented as the equivalent concentration of dissolved oxygen) and the optical density of the solution measured with a Unicam Spectrophotometer S.P. 600 at a wave-length of 420 m $\mu$  in quartz optical cells of 1 cm. depth, in the range (a) 0–8.0, and (b) 0–1.0 mg. O<sub>2</sub>/l.

Table 1

Sample	Type of Winkler	Oxygen (mg./l.)		
		Spectro-photometer	Titrimetric	Difference
Boiled distilled water	Unmodified	1.02	1.03	–0.01
		1.85	1.88	–0.03
Tap water	Unmodified	7.20	7.11	+0.09
		2.22	2.09	+0.13
		6.78	6.82	–0.03
		4.81	4.64	+0.17
		6.90	6.74	+0.16
		1.58	1.59	–0.01
Stored swamp water	Present iodine difference	8.40	8.43	–0.03
		8.14	8.31	–0.17
		2.92	2.85	+0.07
		2.85	2.80	+0.05
Stagnant swamp water	Present iodine difference	–0.28	–0.20	–0.08
		–0.19	–0.13	–0.06
		–0.14	–0.01	–0.13
		–0.01	–0.08	–0.07

amounts of oxygen in boiler water by the Winkler method, the optical density of the iodine solution being estimated at  $353\text{ m}\mu$ . Briggs, Knowles & Scragg (1954) developed a continuous dissolved oxygen recorder in which iodine from the Winkler reaction was recorded spectrophotometrically at a wave-length of  $430\text{ m}\mu$  given by a filter. This is close to the wave-length used in the present method ( $420\text{ m}\mu$ ). They also found that the normal unavoidable variation in the amount of potassium iodide added during the Winkler procedure had no significant effect on the amount of light absorbed by the iodine solution.

#### ACCURACY OF MODIFIED IODINE-DIFFERENCE METHOD

In the highly polluted water of tropical swamps there is an unstable equilibrium between the reducing processes below and the penetration of oxygen from the air above. This results in a very steep oxygen gradient from the surface film downwards. A 200 ml. sample cannot therefore be drawn from a region of uniform oxygen content, and great sensitivity in the estimation would have no value. It is anyhow not to be expected, in view of the instability and of the great variety of reactive

Table 2

Swamp water previously aerated for 48 hr. at  $30.0^\circ\text{C}$ ., and aeration continued throughout. A 200 ml. sample siphoned off daily together with a 35 ml. sample (figure in brackets) the latter analysed by the unmodified Winkler method. Barometric pressure 660 mm. mercury.

Days after start	Oxygen found (mg./l.)	Theoretical oxygen (mg./l.)	Difference (mg./l.)
2	6.50 (5.81)	6.50	0.0
3	6.40 (6.01)	6.50	-0.10
4	6.30 (6.05)	6.50	-0.20
5	6.43 (6.00)	6.50	-0.07
6	6.37 (6.00)	6.50	-0.13
7	6.35 (5.75)	6.50	-0.15

substances which could be present in such waters, that oxygen could be estimated with great accuracy.

The accuracy of the method was tested on swamp water saturated with air (Table 2). About 3 l. of water were vigorously aerated through a diffuser for 48 hr. at constant temperature ( $30.0^\circ\text{C}$ ). During the next 6 days a 200 ml. sample was siphoned off daily and analysed. The theoretical amount of dissolved oxygen was calculated from the temperature and barometric pressure (660 mm. mercury) using the nomogram of Mortimer (1956). In addition a 35 ml. sample was taken daily and analysed by the unmodified Winkler method. The lower figures from the latter demonstrated the continued existence of reducing substances throughout the experiment.

The figures in Table 2 indicate that the sensitivity of the method for water of high oxygen content is about  $0.2\text{ mg. O}_2/\text{l.}$

It is obviously more difficult to assess the accuracy for natural waters containing very little or no oxygen, because they are likely to be in a very unstable condition and to contain much reducing matter. The problem was complicated by the discovery of 'negative' values with very polluted samples. Determinations on long-standing swamp pools, and on samples of swamp or pond water sealed in bottles for some days to exhaust the oxygen, gave a titre for B greater than that for A and thus a 'negative' oxygen figure equivalent to anything up to 0.5 mg.  $O_2$ /l. Whatever the cause of this phenomenon it was obviously necessary to be sure that 'negative' values are got only from samples containing no oxygen.

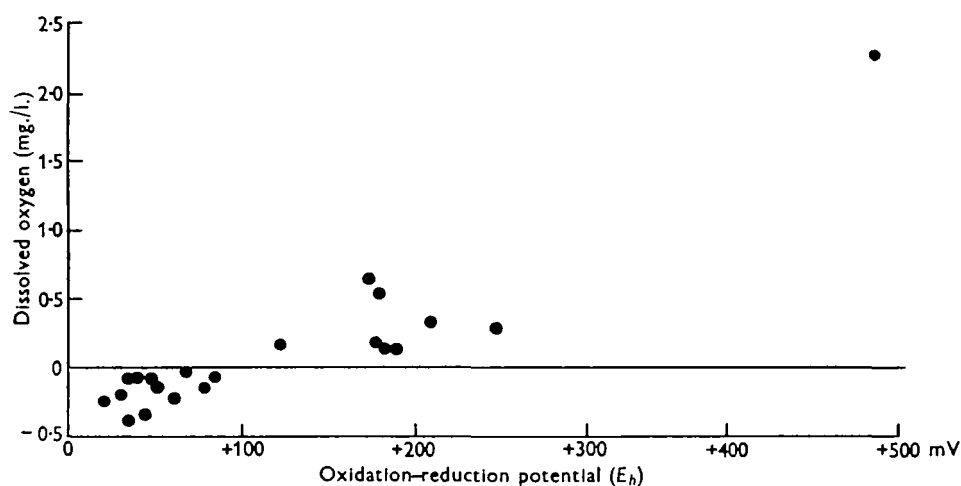


Fig. 3. The relation between estimated oxygen concentration and oxidation-reduction potential ( $E_h$ ) of swamp-water samples, containing little or no dissolved oxygen. The negative oxygen figures denote that sample B contained more iodine than A. The pH of the samples was 6.6–6.8.

To decide this point a large tank was set up containing swamp water together with mud and decomposing vegetable matter. From this a pair of 200 ml. samples were siphoned in series to ensure complete uniformity. One was analysed by the present method, in the other oxidation-reduction potential was measured by a bright platinum electrode. This was lowered through the neck of the bottle to near the bottom, the sample being connected to the saturated calomel reference electrode by an agar bridge. With samples giving potentials ( $E_h$ ) of less than +200 mV. the readings were reasonably steady after 5 min. Less reliance can be placed on the higher figures, but the latter are in fact of no interest in the present connexion. Readings were taken on a Cambridge potentiometer after 10 min. The pH of all samples was 6.6–6.8. Sampling was done over a period of a week. The water was siphoned from various depths and after varying degrees of stirring in order to get a wide range of oxygen concentrations.

The relation between oxygen concentration and  $E_h$  is shown in Fig. 3. Negative oxygen figures were obtained only from samples having  $E_h$  less than +100 mV. The work of Pearsall & Mortimer (1939) on oxidation-reduction potentials in



natural waters and muds makes it clear that an  $E_h$  as low as +100 mV. can only be obtained in water devoid of dissolved oxygen.

Many attempts were made to discover the cause of this 'negative' phenomenon so that the procedure might be modified to avoid it. Complete failure in this, though unsatisfactory, does not appear in view of the above experiments (Fig. 3) to affect the reliability of the method. On the other hand, the 'positive' error in the standard iodine-difference method, discussed in the next section, is a serious disadvantage.

#### COMPARISON WITH STANDARD IODINE-DIFFERENCE METHOD

The preliminary clarification was introduced in order (a) to make possible estimations on very muddy waters, and (b) to avoid the error reported by Ohle (1953) in the standard iodine-difference method whereby, with polluted waters, the apparent oxygen-content increases with the time taken between adding the Winkler reagents and acidification, owing to progressive reduction of free iodine in B only. The experiments recorded in Table 3 show that the error cannot be avoided by reducing this time interval to the minimum possible.

Table 3

Samples	Oxygen (mg./l.)	
	Standard iodine-difference method	Present method
(a) From stagnant swamp pool, probably anaerobic. Sample pairs run off daily for 6 days	(i) 0.36	0.07
	(ii) 0.68	0.12
	(iii) 0.36	0.13
	(iv) 0.24	0.05
	(v) 0.86	Nil
	(vi) 0.80	Nil
(b) Hay infusion ( $E_7 = -214$ mV.)	0.46	-0.11
(c) Filtered swamp water aerated for 1 hr.	(i) 5.08	4.25
	(ii) 4.97	4.18
(d) Pond water, stirred	4.32	3.88

In order to ensure that identical samples were taken for comparison of the two methods, the water was stored in an aspirator bottle and the two 200 ml. samples were run off simultaneously through a Y-tube. The standard method was followed by adding free iodine to the original sample, the requisite quantity being made up in a volume of saturated sodium chloride equal to the volume of the clarifying reagents used in the present method. The subsequent procedure was identical for both methods. The acid was added *immediately* after shaking for 10 sec. with the Winkler reagents.

In all cases the figures from the standard method were in excess of those obtained by the present method. It is significant that the lower the oxygen content the greater was the proportionate difference between these two figures. But it should

be noted that there was a significant difference between the figures even when the water was not excessively polluted ((c) and (d)). We have reason to believe that figures obtained by the present method are correct to within 0.2 mg. O<sub>2</sub>/l. (see previous section). In the case of the extremely polluted hay infusion in which there was undoubtedly no dissolved oxygen ( $E_7 = -214$  mV.) and which was giving a negative figure by the present method (see previous section), the standard iodine-difference method gave 0.46 mg. O<sub>2</sub>/l.

#### SUMMARY

1. A modification of the iodine-difference Winkler method is described which involves a preliminary clarification of a single sample, from which two smaller samples are siphoned.
2. This makes possible estimations on extremely muddy and polluted water and avoids an error in the standard iodine-difference method which is very large with highly polluted waters.
3. The iodine is added with the clarifying reagents to the original sample, thus avoiding the necessity for quantitative addition of iodine to two separate samples, which is another potential source of error in the standard method.
4. Estimations on swamp water saturated with air under standard conditions indicate that the method is accurate to within 0.2 mg. O<sub>2</sub>/l.
5. With polluted water, having an oxidation-reduction potential ( $E_h$ ) of less than +100 mV., 'negative' figures for oxygen content are obtained. The cause of this phenomenon could not be discovered but, since this potential indicates anaerobic conditions, the method is not thereby affected.
6. An improved iodine-titration end-point is described involving the use of a magnetic stirrer.
7. Direct spectrophotometric estimation of iodine is shown to be as accurate as, and very much more rapid than titration, and particularly suited to the iodine-difference method.

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