SIMPLIFIED METHOD FOR MEASURING DISSOLVED OXYGEN IN STREAMS*

CHARLOTTE E. WILCOX
Illinois State Normal University, Normal

From the point of view of the biologist, no one factor has more significance than the dissolved oxygen content of the body of water in which aquatic life is being studied. The dissolved oxygen content of such bodies of water is also a factor, indicative of the sanitary condition, to be taken into consideration by health authorities and directors of outdoor activities. The regular Winkler Method and other methods as given in *Standard Methods for the Examination of Water and Sewage* are not suitable for field work and involve laboratory resources and techniques not available to those most interested in stream conditions.

A simple colorimetric method developed by Dr. Issacs and modified by Dr. Gilcreas as reported in the *Sewage Works Journal* in May of 1935 looked particularly promising. It seemed simple, on the face of it, for it took only small variations in the amount of oxygen to change the color of the solution, and these colors accordingly would be easy to match. Dr. Issacs' method employs the use of amidol di-aminophenal hydrochloride.

Samples were taken and tested according to the method and an attempt was made to match them against the standards, as proposed by Issacs and Gilcreas, but they were not of the shade of color and could not be accurately matched. The yellow color appearing in the tests, which caused them to add potassium dichromate to the cobalt chloride to get the necessary shade of color, was totally lacking in the tests made by the writer. This necessitated finding out what was wrong with the tests of this experimental study or the tests made by Issacs and Gilcreas. In testing distilled water for oxygen, an absence of oxygen was expected. It was a surprise to find a yellow color developing which gave the first clue to the origin of the color which Issacs and Gilcreas noted. Since it was known that the distilled water in the laboratory contained a trace of nitrites it occurred to the writer that the yellow of Issacs and Gilcreas's tests arose as impurities in their distilled water, in their chemicals, or in the waters tested. To demonstrate this, nitrogen-free water was used, and it was discovered that by adding traces of sodium nitrite, the yellow color showed up immediately, while amounts of more than two parts per million gave an immediate wine-red color, a condition known to Issacs and Gilcreas.

Consequently by using chemically pure distilled water and analyzed chemicals of reagent grade, a set of standards was made, omitting the yellow-forming potassium dichromate, which would check with our test solutions.

These standards were made by dissolving 238 grams of cobalt chloride in 100 cc. of concentrated hydrochloric acid. Enough water was added to make one liter and when this solution was diluted one half it was seen to match the fourteen parts

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per million (p.p.m.) of Standard II which Gilcreas developed in his set of standards. The comparison of the two was made by screening out the yellow of Gilcreas' Standard II. Then twenty cc. of this 14 p.p.m. stock solution was taken as the constant amount of the cobalt chloride solution to be diluted in the making of other standards, expressed in parts per million to be made. Standards were made up for ½ p.p.m. to 14 p.p.m.

The formula used in making these dilutions was:

$$\text{p.p.m.} = 14\,\frac{\text{Orig. vol. of standard}}{\text{Final vol.}}$$

$$\text{p.p.m.} = 14\,\frac{\text{Orig. vol.}}{X}$$

Key to formula:

p.p.m. = the desired p.p.m. for the standard

14 = the p.p.m. of the solution of cobalt chloride to be diluted

Original volume = a matter of choice and here 20 cc. was used for each standard of the set

X = the final or total volume of the cobalt chloride solution plus the distilled water added

Thus in finding the dilution for the ½ p.p.m. standard the formula becomes:

$$0.5 = 14 \text{ times } \frac{20}{X}$$

$$0.5X = 14 \text{ times } 20$$

$$0.5X = 280$$

X = 560 cc. of water and original solution. Thus 20 cc. of the original solution plus 540 cc. of distilled water will make the total volume of 560 cc. of solution. When this solution is poured into the standard bottle the pink of this solution represents ½ p.p.m., and a sample containing amidol or amidol and citrate if matching this pink can be said to contain ½ p.p.m. of dissolved oxygen.

These color standards were carried into the field and found very convenient for practical use. They were kept in a dark place and no color change was noticed during the period of months they were used.

In making the actual oxygen tests quantities of amidol proportional to those of Issacs were used. Amidol weighing 0.176 grams was put into the 32.5 cc. square glass-stoppered color-standard bottle. Then the potassium citrate solution was made by dissolving 72 grams of citrate in distilled water made up to 100 cc. This citrate solution was taken into the field in a bottle and when the test for dissolved oxygen was made 0.68 cc. of it was pipetted into the bottle containing the amidol and water sample, after which the bottle was stoppered so all the air was excluded. After one half hour this solution was matched against cobalt standard described instead of the Gilcreas and Issacs standards for reasons previously explained.

As mentioned, our first tests did not reveal any interference from small amounts of nitrites, but tests taken when the sewage treatment plant was operating improperly did show such interference. Consequently where interference is indicated, two methods were developed by which the oxygen test could be compared to the standards developed using cobalt chloride. The methods are based on the discovery that nitrites give an immediate color reaction with amidol whereas dissolved oxygen gives a delayed color reaction. One method was developed by which oxygen tests with traces of nitrite present could be tested and the other method for samples containing more than 2 p.p.m. nitrites.

PROCEDURE WITH TRACES OF NITRITE PRESENT

Equipment:

1. Put proper amount of amidol into color-standard bottle I.

2. Put proper amount of amidol into color-standard bottle II.
3. One empty color-standard bottle III.
4. One bottle IV for collecting water sample.

Making the test:

1. Put sample of water to be tested into amidol color-standard bottle I. Add citrate solution and stopper tightly.
2. Collect sample of water tested in water-sample bottle IV.
3. In one half hour fill empty color-standard bottle III with water from water sample bottle IV.
4. At the same time fill amidol color-standard bottle II (no citrate) with water from sample bottle IV, tightly stopper. (Step four is done at the same time as step three.)
5. Immediately match amidol color-standard bottle I (set up one half hour previously) with the cobalt chloride standards. Eliminate the error caused by the yellow color of traces of nitrites by placing amidol color-standard bottle II (showing yellow color produced by traces of nitrites only) in front of the cobalt chloride color-standard bottle being matched; and by placing the color-standard bottle III containing nothing but test water in front of amidol color-standard bottle I. A block comparator may be used for convenience. This modified procedure effectively eliminates errors introduced by the yellow color from traces of nitrites, which gave Issacs and Gilcreas so much concern.

PROCEDURE WITH NITRITES OVER 2 P.P.M.

When more than 2 p.p.m. of nitrites are present the wine-red color which develops immediately is matched against the standards and this reading subtracted from the combined wine-red color of nitrite and dissolved oxygen developing one half hour later.

Under our field conditions, over 2 p.p.m. of nitrites did not occur and there was no necessity for using the second procedure just presented, but it has been given in case such amount might by rare chance be found elsewhere. Both procedures were tested on samples in the laboratory and the procedure involving traces of nitrites was used successfully in the field.

The factors which interfere with the accuracy of this method of testing dissolved oxygen are so few, and the amounts needed to interfere so high, and the elimination of these interfering factors so easy, that the method, in this respect, is well ahead of the standard but lengthy and complex Winkler procedures.