



accu-Test™ Micro Chemical Oxygen Demand System

The Micro-COD Test Method - Accu-Test™ Mercury-Free Vials, Spectrophotometric Method

Scope and Application

- 1.1 The Chemical Oxygen Demand (COD) test determines the quantity of oxygen required for oxidation of reduced species including organic matter in a water sample using a specific oxidizing agent, temperature and time of reaction. This bulletin covers the use of reagents prepared without mercuric salts. Their use is limited to the analysis of samples containing low amounts of chloride ion. Use of these reagents is **NOT** approved for NPDES reporting purposes.
- 1.2 Bioscience, Inc. offers prepackaged mercury-free reagents for use in determining COD in the following ranges:
 - Low Range COD Vials 5-150 mg/L COD
 - Standard Range COD Vials 20-900 mg/L CODThese products are for use with the COD method described here. Samples with higher COD can be analyzed after dilution. When the chloride concentration of a sample exceeds 10% of the expected COD of the sample (please refer to the discussion in Section 4.3), the sample may be analyzed after pretreatment with mercuric sulfate or dilution.

Summary of Method

- 2.1 Organic and inorganic compounds are oxidized in a sealed 10 ml borosilicate glass vessel (expendable tube) which contains premixed COD reagents. Chlorides may be complexed by pretreatment with mercuric sulfate, if necessary. The reagents and catalysts used are in the same proportions as those used in the Standard Titrimetric Methods^{3,4,5} with the exception of the normality of the dichromate oxidizing agent and the deletion of mercuric sulfate. The normality of the dichromate is that specified by Jirka and Carter⁶ and by EPA Method 410.4². For samples in the range of 20-900 mg/L (Standard Range), the COD is determined using a spectrophotometer at 600 nm by measuring the concentration of the produced Cr (III) ion. This wavelength is that specified by Method 410.4 and Jirka and Carter. For samples in the range of 5-150 mg/L, the COD can be determined using a spectrophotometer at 440 nm by measuring the decrease in concentration of the Cr (VI) ion as specified by Jirka and Carter. The path length of the tubes is 1.40 cm.

Sampling and Preservation

- 3.1 Care must be taken to store COD vials in light-proof containers until use. The guaranteed shelf life of these products is one year if stored in the dark and at room temperature.

WARNING: Do not expose stored vials to temperatures below 4 degrees Celsius or above 45 degrees Celsius.

- 3.2 Samples should be collected in glass bottles. Plastic containers may be used if the containers are known to contain no contaminants which would interfere with the test.
- 3.3 Samples containing active microorganisms should be analyzed as soon after collection as possible. Samples containing materials which have settled to the bottom should be well-mixed (preferably homogenized) to permit analysis of representative aliquots.
- 3.4 Samples which cannot be run immediately (within 5 hours) after collection should be preserved with a sufficient concentration of H₂SO₄ to lower the pH to below 2 and be refrigerated at 4 degrees Celsius until analysis can be performed.

Interferences

- 4.1 Any contamination of the sample with foreign organic matter will cause an error in the analysis.
 - 4.1.1 Water used for dilution and blanks should be as low in organic material as possible. Distilled or type II deionized water is recommended. All water used in any set of analyses should preferably be from the same batch or container.
 - 4.1.2 Any glassware used in sample preparation should be proven to be as free of organics as possible. Rinse with 20% H₂SO₄ and dry at 150°C.
- 4.2 Volatile materials may be lost if the sample is mixed with reagents before sealing the vessel. To avoid this possibility, samples should be carefully added to vials down the side of the vessel to effect the layering of samples over the denser reagents until after the vial is sealed.
- 4.3 Chlorides are among the reduced species oxidized by dichromate and will cause erroneously high COD values if not complexed. Samples with chloride concentrations greater than 10% of the sample COD may cause significant inaccuracies of the COD results. Sufficient mercuric sulfate can be added to the reagents (or the sample can be pretreated as described in Section 7.2) to complex chloride before the chloride reacts with the dichromate.
- 4.4 Spectrophotometric interferences in the sample can cause erroneously high COD values. Although samples with such interfering species are not typically encountered, those which might contain such species should be checked against the titration method⁹ to establish comparability.
- 4.5 Spectrophotometric interference in the form of light attenuation can result from smudged or scratched vessels. If a suitable "window" area for light transmission does not exist on the vessel, the sample must be transferred to a cell or to another vessel for reading. When transfer is necessary, care must be taken not to resuspend or transfer precipitated salts which may be present. All samples and standards must be measured with comparable cells or reagent vials.
- 4.6 Spectrophotometers with narrow transmission bandwidths (typically the more

expensive instruments) may accentuate imperfections in the optical quality of the glass vessel resulting in loss of precision of replicates. Spectrophotometers with bandwidths of 20 nm or more are recommended.

Apparatus

- 5.1 Premixed COD reagents in screw-cap glass tubes.

COD Twist-Tubes, Standard Range (20-900 mg/L COD) .. Part #185-252
COD Twist-Tubes, Low Range (5-150 mg/L COD)Part #185-240
- 5.2 COD reactor capable of maintaining 150 ± 2 degrees Celsius.

COD Reactor (15 Tubes). Part #163-486 (110V), Part #163-686 (220V)
COD Reactor (30 Tubes). Part #163-466 (110V), Part #163-666 (220V)
- 5.3 Spectrophotometer with 16 mm sample holder suitable for measurements at 600 nm and 440 nm.

Adaptor, Coleman (16 mm) Part #163-501
Adaptor, Hach DR2 (16 mm) Part #163-519
Adaptor, Spectronic 20 (16 mm). Part #163-494
- 5.4 (Optional) Bioscience COD Data Management Software System . . . Part #200-100
- 5.5 Chloride Test Kit. Part #185-900, Refill Kit.Part #185-905

Reagents

- 6.1 Premixed reagents in the vials are specified in Section 5. These vessels contain all reagents and catalyst needed for the COD test, except those required for chloride pretreatment.
- 6.2 Sulfuric acid (95% H_2SO_4), for chloride pretreatment.
- 6.3 Mercuric sulfate ($HgSO_4$), for chloride pretreatment (if desired).
- 6.4 Distilled water. Precautions should be taken to insure that water used in the test be low in organic matter, preferably containing less than 0.3 ppm organic carbon.
- 6.5 Distilled or deionized type II water should be used in the preparation of all standard solutions.
- 6.6 Potassium acid phthalate (KHP): $KOCOC_6H_4COOH$ ACS primary standard grade.
 - 6.6.1 KHP stock solution: To obtain a 10 g/L COD stock solution, add 8.5034 g of potassium acid phthalate (KHP pre-dried to a constant weight at 110 degrees Celsius) to a 1000 ml volumetric flask and dilute to 1000 ml using deionized water.
 - 6.6.2 COD working standards: A series of standard solutions that cover the expected range of sample concentrations is prepared by diluting appropriate volumes of the KHP stock solution. A procedural blank

(with distilled water) should be prepared with the other standards to zero the spectrophotometer

For Standard Range reagent, prepare standards in the range of 50 to 900 mg/l (e.g. 5.00 ml of KHP stock solution diluted to 1.000 l produces a 50 mg/l working standard.

For Low Range reagent, prepare standard in either the 5 to 50 mg/l range (in conjunction with standard range to cover 5 to 900 mg/l range) or 10 to 150 mg/l range (e.g. dilute 10.0 ml KHP stock solution to 100.0 ml to produce a 1000 mg/l standard. Dilute portions of this standard to obtain the required dilute standards. 5.00 ml of 1000 mg/l standard diluted to 1.000 l equals 5 mg/l working standard.

Procedure

- 7.1 Preheat the reactor (heater block) to 150 degrees Celsius.
- 7.2 This section describes procedures for pretreatment of chlorides. If pretreatment is not necessary, skip to Section 7.3.
 - 7.2.1 Place 30 ml of the sample into an Erlenmeyer flask.
 - 7.2.2 Add 10 mg HgSO₄ for every milligram of Cl⁻ present in the sample.
 - 7.2.3 Carefully add 5 ml of 95% H₂SO₄ with stirring to the contents of the flask and mix thoroughly.
- 7.3 Remove the cap from a COD vial.

CAUTION: Use of safety glasses is recommended.
- 7.4 Carefully add 2.5 ml of sample (or standard solution) down the side of the vial such that it forms a layer on top of the reagents.

NOTE: Samples containing a significant amount of suspended solids should be thoroughly homogenized and milled in a blender or similar device before adding the sample to the vial.
- 7.5 Carefully replace the cap and tighten cautiously.

NOTE: Caps and seals can be broken by over-tightening.
- 7.6 Thoroughly mix the contents of the sealed vial by shaking.

CAUTION: The vial will get very hot during mixing. Samples should be mixed either in racks or with use of insulated gloves. Appropriate clothing and eye protection should be worn.
- 7.7 Process standards and blanks exactly as the samples.
- 7.8 Place the vial in the reactor maintaining 150 ±2 degrees Celsius for 2 hours. The 2-hour oxidation period is standard practice²⁻⁷ and is sufficient for complete oxidation of most compounds. (See Reference 5 for relative digestion efficiency of pure compounds).

- 7.9 Mix vial contents by shaking and allow to cool. If rapid cooling is desired, the vials may be placed in a water bath. If crystals form during rapid cooling, discontinue and allow vials to cool slowly in the room air.
- 7.10 Carefully wipe the vials clean.
- 7.11 Allow any suspended precipitate to settle for 10 minutes.
- 7.12 Setting the spectrophotometer zero
 - 7.12.1 When using Standard Range vials, place a procedural (distilled water) blank vessel in the spectrophotometer set at 600 nm wavelength and re-zero the absorbance reading.
 - 7.12.2 When using Low Range COD vials, use the highest standard (e.g. 150 mg/L COD) to set the spectrophotometer to zero absorbance at 440 nm.
- 7.13 Read the absorbance of each standard and sample on the spectrophotometer.
- 7.14 As an alternative or for comparison to the spectrophotometric determination, the cap may be removed; the entire contents may then be quantitatively transferred from the vial and the COD determined by the titrimetric method³⁻⁵. A technical bulletin is available from Bioscience, Inc. describing the COD titration method.
- 7.15 If a separate chloride analysis is required for chloride correction, follow the analytical procedure included with the test kit (or other recognized analytical procedures).

Calculation

- 8.1 Prepare a graphic calibration curve by plotting the absorbance on the ordinate (Y) and the COD (mg/L) of standards on the abscissa (X). For the Standard Range Method, the best-fit curve will have a positive slope. For the Low Range Method, the best-fit curve will have a negative slope. (Note: The Bioscience COD Personal Computer Data Management System will automatically calibrate the spectrophotometer for you.)
- 8.2 The absorbance of samples can be converted graphically into measured COD (or automatically, using the COD Data Management System). Linear regression is the preferred data treatment and is automatically performed by the COD Data Management System. Alternatively, use a scientific calculator or computer program.
- 8.3 The measured COD must be multiplied by a dilution factor to correct for the dilution of the 30 ml sample if 5 ml of H₂SO₄ was added for complexing chlorides (per Section 7.2).
- 8.4 If a mathematical solution for the COD of samples is required, perform the following calculation:

$$\text{COD (mg/L)} = \frac{DA \times (C_2 - C_1)}{(A_2 - A_1)} + B$$

C₂ = The COD (mg/L) of any standard solution

C₁ = The COD (mg/L) of any standard lower than C₂

A₂ = The absorbance of the standard C₂

- A₁ = The absorbance of the standard C₁
- D = Dilution factor
 - = 1 if H₂SO₄ was not added to a 30 ml sample
 - = 35/30 = 1.167 if 5 ml H₂SO₄ was added to a 30 ml sample
- A = The absorbance of the sample
- B = Zero for Standard Range Method
 - = COD (mg/L) of highest standard for Low Range Method

Precision and Accuracy

- 9.1 After a thorough review and evaluation by the Environmental Protection Agency of the results of a comparability testing and other information submitted in accordance with Section 136.5, 40 CFR Part 136, the EPA determined the Standard Range micro-COD vial method to be a suitable alternate procedure to EPA Method 410.1¹.

Spectrophotometric determination of twist-cap vials conforms to Method 410.4. Precision of four replicates of each of 30 samples averaged $\pm 2.7\%$. The data submitted for this evaluation have been published by Messenger⁷. Reprints of this article are available from Bioscience Inc. upon request. Use of twist-cap closures and spectrophotometric determination are EPA accepted as equivalent to EPA 410.4.

- 9.2 ASTM has published a round-robin study using the micro-COD methods. Results of these tests indicate a standard deviation of 4 mg/l for the low range reagent using KHP standards (5 to 45 mg/l) in Type II water. In the presence of 1000 mg/l sodium chloride, the standard deviation was 8 mg/l. A significant positive bias was demonstrated for low standard concentrations (5 and 12 mg/l) in the presence of sodium chloride. The standard deviation ranged from 4.5 to 44 mg/l for the standard range reagent using KHP standards (27 to 750 mg/l) in Type II water. The relative standard deviation decreased from a high of 20 - 25% to less than 5% as the standard concentration increased. The standard deviation ranged from 7.8 to 104 mg/l in the presence of 1000 mg/l sodium chloride. A significant positive bias was noted at the lowest concentration tested (27 mg/l) and a significant negative bias was noted at the highest concentration (750 mg/l). Supporting data are available from ASTM Headquarters, 1916 Race Street, Philadelphia, PA 19103-1187. Request Document RR:D19-1044.

Bibliography

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