Designation: D2330 – 02

Standard Test Method for Methylene Blue Active Substances[1]

This standard is issued under the fixed designation D2330; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

This standard has been approved for use by agencies of the Department of Defense.

1. Scope

1.1 This test method covers the determination of compounds that react with methylene blue under the conditions specified in the test procedure. They are referred to as methylene blue active substances (MBAS), and are calculated and reported in terms of the reference material, linear alkyl benzene sulfonate, LAS.

1.2 This test method is applicable for determining MBAS in water and wastewater. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

1.3 This test method is a simple, rapid, control procedure suitable for monitoring the effectiveness of a biodegradation or other linear alkyl benzene sulfonate (LAS) removal process. For greater specificity and interference removal, the pretreatment procedure in Annex A1 should be used. Data derived without the pretreatment procedure should be interpreted with care. This test method is applicable in the range from 0.03 to 1.5 mg/L for a 100-mL sample.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For a specific hazard statement, see 8.3.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D459 Terminology Relating to Soaps and Other Detergents
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D3370 Practices for Sampling Water from Closed Conduits
D3856 Guide for Good Laboratory Practices in Laboratories Engaged in Sampling and Analysis of Water
D4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data[3]
D5788 Guide for Spiking Organics into Aqueous Samples
D5789 Practice for Writing Quality Control Specifications for Standard Test Methods for Organic Constituents[3]
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
E60 Practice for Analysis of Metals, Ores, and Related Materials by Molecular Absorption Spectrometry
E131 Terminology Relating to Molecular Spectroscopy
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D1129 and E131.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *alkyl benzene sulfonate (ABS)*[4]—the generic name applied to the neutralized product resulting from the sulfonation of a branched-chain alkylated benzene. See also Terminology D459.

3.2.2 *linear alkyl benzene sulfonate (LAS)*[4]—a form of alkyl benzene sulfonate (ABS) in which the alkyl group is linear rather than a branched chain. See also Terminology D459.

4. Summary of Test Method [5]

4.1 This test method is based upon the formation of a blue-colored chloroform extractable ion pair by the reaction of cationic methylene blue and an anionic surfactant (including LAS, other sulfonates, and sulfate esters).

4.2 The sample is mixed with an acidified, aqueous solution of methylene blue. Any resulting hydrophobic ion pair which may be formed is extracted successfully with chloroform. The combined chloroform extracts are washed with an acid solution to remove the less hydrophobic ion pairs (having low partition coefficients) that can be formed by potentially interfering

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

Current edition approved July 10, 2002. Published August 2002. Originally published as D2330 – 65 T. Last previous edition D2330 – 88 (1995)$^{\varepsilon 1}$. DOI: 10.1520/D2330-02.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

[4] For a more complete discussion of terms relating to synthetic detergents and their significance, refer to "Syndets and Waste Disposal" by McKinney, R. E., *Sewage and Industrial Wastes*, Vol 29, Part 6, June 1957, pp. 654–666.

[5] Adopted from "Surfactants (Anionic) Methylene Blue Methods," *Standard Methods for the Examination of Water and Waste Water*, Twelfth Ed., 1965.

substances. The chloroform layer retains the highly hydrophobic methylene blue-LAS ion pairs.

4.3 The intensity of the blue color remaining in the chloroform extract is measured photometrically at the wavelength of maximum absorption near 650 nm. This intensity is related to the concentration of LAS by means of a calibration curve or chart.

5. Significance and Use

5.1 The widespread use and discharge of detergents into surface waters can result in a lowering of its aesthetic quality by foam formation and by causing toxicity to aquatic wildlife. This test method is capable of detecting small concentrations of detergents as MBAS so that they can be controlled to prevent such problems.

5.2 Biodegradable linear alkyl benzene sulfonates (LAS) have replaced the branched-chain alkyl benzene sulfonates (ABS) in detergent formulations, which were more resistant to biodegradation. Differentiation between linear and branched-chain alkyl benzene sulfonates, as well as differentiation of the various positional isomers of either type, is not possible by this test method. While the methylene blue method may be employed to monitor studies designed to measure biodegradability, it cannot be used to predict this quality.

6. Interferences

6.1 Any organic or inorganic compound that will form a chloroform extractible ion pair will interfere by producing high results, unless the ion pair formed is eliminated by the treatment described in 4.1. These positive interferences include organic sulfonates, carboxylates, phosphates, and phenols, as well as inorganic cyanates, chlorides, nitrates, and thiocyanates.

6.2 Any compound effectively competing with methylene blue to form a LAS ion pair will give negative results. This negative interference is demonstrated by some amines and has analytical significance in the case of quaternary ammonium compounds.

6.3 An evaluation of the effect of various potential interferences is summarized in Table 1. The listed compounds, in the concentrations indicated, were added to solutions containing 1 mg/L LAS.

6.4 When interferences are present, the pretreatment procedure described in Annex A1 should be used. Table 2 shows the interferences that can be present even though the pretreatment was used.

6.5 When a concentrated acid chromate cleaning solution is used to clean glassware, including separatory funnels, between samples, care must be taken to completely flush all of the acid chromate cleaning solution from all surfaces and, in particular, from the space between the barrel and plug of the separatory funnel stopcock. Failure to remove the acid can result in an error in results.

6.5.1 Never use a detergent to clean any glassware used in this test method as a detergent is difficult to remove from surfaces. Any residual detergent could cause a high result.

TABLE 1 Evaluation of Potential Interferences in the Methylene Blue Method

Added to 1.0 mg/L LAS Solution	Concentration, mg/L	Indicated LAS, mg/L
Acetic acid	100	1.0
Ammonium diethylphosphorodithioate	20	1.1
Benzene sulfonic acid	100	1.3
Cholesterol	100	1.0
2,4-dichlorophenol	100	1.0
Diethanolamine	1000	1.0
Disodium phenylphosphate	10	1.0
Isopropylamine	14	1.0
Leucine	10	1.0
N-1-(naphthylethylenediamine) hydrochloride	100	0.9
Nonyl phenol + 9 EtO	100	1.0
Phenol	100	1.0
Picric acid	5	4.6
Potassium chloride	100	1.0
Potassium cyanate	100	1.0
Potassium nitrate	100	1.0
Potassium thiocyanate	2	1.0
Potassium thiocyanate	100	4.1
Proteins (Knox gelatine)	100	0.9
Sodium dodecyl sulfate	10	14.6
Sodium dodecane sulfonate	5	5.0
Sodium naphthalene sulfonate	5	5.1
Sodium stearate	100	1.0

TABLE 2 Evaluation of Potential Interferences in the Methylene Blue Method with Pretreatment Described in Annex A1

Added to 1.0 mg/L LAS Solution	Concentration, mg/L	Indicated LAS, mg/L
Sodium dodecane sulfonate	5	3.7
Sodium benzene sulfonate	100	1.2
Sodium dodecyl sulfate	10	0.9
Potassium thiocyanate	100	1.0
Picric acid	10	1.0

7. Apparatus

7.1 *Filter Photometer or Spectrophotometer*, suitable for measurement at a wavelength in the region near 650 nm and equipped with 50-mm and 10-mm light path absorption cells.

NOTE 1—Photometers and photometric practices prescribed in this test method shall conform to Practice E60. Spectrophotometers shall conform to Practice E275.

7.2 *Separatory Funnels*, 250-mL size, Squibb-type, glass-stoppered, preferably with TFE-fluorocarbon stopcocks.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

[6] *Reagent Chemicals, American Chemical Society Specifications,* American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D1193, Type II.

8.3 *Chloroform* ($CHCl_3$). (**Warning—**Chloroform ($CHCl_3$) is toxic and is suspected of being a possible carcinogen: avoid ingestion, inhalation, or absorption through the skin. Use a well-ventilated fume hood to carry off chloroform vapors during analysis.)

8.4 *Linear Alkyl Benzene Sulfonate Solution, Stock (1.0 mL = 1.0 mg LAS)*—Weigh the amount of reference material[7] necessary to provide the equivalent of 1.000 g of LAS on a 100 % active basis. Dissolve in water and dilute to 1 L, mixing gently to prevent foam formation. Record the molecular weight of the LAS reference material as supplied. The stock solution may be stored at 4°C in the dark for 12 months in a well-stoppered flask without deterioration.[8]

8.5 *Linear Alkyl Benzene Sulfonate Standard Solution (1.0 mL = 0.01 mg LAS)*—Dilute 10.0 mL of the foam-free stock solution (8.4) to 1 L with water that has been previously adjusted to pH 2 with sulfuric acid and mix. The standard solution may be stored at 4°C in the dark for at least 12 months in a well-stoppered flask without deterioration.[8]

8.6 *Methylene Blue Solution (30 mg/L)*—Dissolve 0.1 g of methylene blue chloride in 100 mL of water. Transfer 30 mL of this solution to a 1-L volumetric flask and add 500 mL of water. Add carefully 50 mL of 14 % sulfuric acid stock solution (8.10) and 50 g of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$). Shake until solution is complete and then dilute to 1 L with water and mix.

8.7 *Phenolphthalein Indicator Solution (5.0 g/L)*—Dissolve 0.5 g of phenolphthalein in 50 mL of 95 % ethyl alcohol and dilute to 100 mL with water and mix.

NOTE 2—Specially denatured ethyl alcohol conforming to Formula No. 3A or 30 of the U. S. Bureau of Alcohol, Tobacco, and Firearms may be substituted for 95 % ethyl alcohol.

8.8 *Phosphate Wash Solution*—Dissolve 50 g of sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) in 500 mL of water in a 1-L volumetric flask. Add carefully 50 mL of 14 % sulfuric acid stock solution (8.10) and dilute to volume with water and mix. The solution has a pH of approximately 1.8.

8.9 *Sodium Hydroxide Solution (10 g/L)*—Dissolve 10 g of sodium hydroxide (NaOH) in water, dilute to 1 L and mix.

8.10 *Sulfuric Acid Stock Solution (14 % volume per volume)*—Add carefully 140 mL of concentrated sulfuric acid (H_2SO_4, sp gr 1.84) to 700 mL of cold (0 to 5°C) water with good stirring, dilute to 1 L with water and mix.

8.11 *Sulfuric Acid Solution, Dilute (0.7 % volume per volume)*—Dilute carefully 50 mL of 14 % sulfuric acid stock solution (8.10) to 1 L with water and mix.

9. Sampling

9.1 Collect the sample in accordance with Practices D3370.

9.2 Samples may be preserved against biological oxidation by adding concentrated sulfuric acid (H_2SO_4) to adjust the sample to pH 2 or less and storing at 4°C. Analyze the preserved sample as soon as possible, or within one week after collection. Data on decomposition are not available.

9.3 Rinse the sample container and cap well to free them of detergent if they have been used previously and cleaned prior to recycling.

10. Preparation of Apparatus

10.1 *Glassware Conditioning*:

10.1.1 All glassware used for the determination of LAS should be free of scratches and etch marks because of the tendency of surface-active materials to adsorb on this type of surface. All volumetric flasks and photometer cells, projected for use in LAS determinations, should, as instructed herein, be preconditioned as follows: Obtain the chloroform extract from 12.0 mL of the standard LAS solution as described in 11.4. Transfer sequentially to each of the volumetric flasks and photometer cells and permit a minimum contact time, in each case, of 5 min. Rinse thoroughly with chloroform and drain (**Warning**—see 8.3).

11. Calibration

11.1 Prepare a series of standards by adding the standard solution (8.5) from a 25-mL buret to a series of 250-mL separatory funnels (see 6.5) and dilute the standards to 100 mL volume with water, yielding solutions as follows:

Standard, mL (1.0 mL = 0.01 mg LAS)	LAS, mg (per 100 mL of extract)
0.00	0.00
1.00	0.01
3.00	0.03
5.00	0.05
7.00	0.07
9.00	0.09
12.00	0.12

NOTE 3—If desired, additional standards in the range from 0.00 to 0.12 mg of LAS may be prepared for the calibration series.

11.2 Add 3 drops of phenolphthalein solution (8.7) and just enough sodium hydroxide solution (8.9) to produce a pink color. Add dilute sulfuric acid solution (8.11), in small increments until the pink color is barely discharged.

11.3 Add 25 mL of methylene blue solution (8.6) and mix. Add 25 mL of chloroform (**Warning**—see 8.3) and mix thoroughly for 30 s with shaking. Vent carefully, permit the phases to separate and then drain the chloroform layer into a second 250-mL separatory funnel (see 6.5). Leave any emulsion layer in the first separatory funnel. Repeat the extraction, serially, with two additional 25-mL portions of chloroform.

NOTE 4—Vent the separatory funnel through the stopcock with the funnel tip directed away from the face to avoid contact with any sample spray (**Warning**—see 8.3).

11.4 Add 50 mL of phosphate wash solution (8.8) to the combined chloroform extracts in the second separatory funnel and shake vigorously for 30 s (see Note 4). Hold the separatory

[7] Linear alkyl benzene sulfonate reference material may be obtained from the United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

[8] Data supporting the precision and bias statements and the stability of the LAS Stock and Standard Solutions are available from ASTM Headquarters. Request RR:D19-169.

funnel in a vertical position and swirl the contents. Permit settling for 1 min. Filter the chloroform layer through a glass wool plug into a conditioned (see 10.1) 100-mL volumetric flask. Add 20 mL of chloroform to the second separatory funnel and repeat the shaking, swirling, and settling steps (see Note 4). Combine the chloroform layer through the glass wool into the volumetric flask. Add additional chloroform as needed to bring the flask to 100-mL volume and mix thoroughly.

11.5 Using a 50-mm (Note 5) light-path cell, at 650-nm wavelength, set the photometer to zero absorbance with the extract of the calibration blank.

NOTE 5—If a shorter 10-mm light path cell is employed, the volumes of standard LAS solution selected for the calibration should be proportionately increased.

11.6 Measure the absorbance of each of the extracts. Because of a tendency to fade slowly, the absorbance of the extracted methylene blue complex should be measured within 30 min after formation. Prepare a calibration curve by plotting photometer readings in absorbance against concentration of LAS in milligrams per 100 mL of extract on rectilinear graph paper, and record the molecular weight of the LAS reference material, as supplied,[6] on the graph.

NOTE 6—If the scale of the photometer reads in percent transmittance, plot the results on semilog paper, using the vertical log axis for transmittance and the horizontal linear axis for concentration in milligrams of LAS per 100 mL of extract.

NOTE 7—A separate calibration curve must be made for each photometer and each cell used. Each calibration curve must be checked periodically to ensure repeatability. If a subsequent calibration curve does not reproduce the previous curve, then recheck the curve again. Make sure that the LAS reference material as supplied has the same molecular weight as that which was used to produce the previous curve.

12. Procedure

12.1 Select a volume of sample consistent with the anticipated LAS content. If the LAS concentration is not expected to exceed 1 mg/L, use a 100-mL sample. For LAS in the 10 mg/L range, use a 10-mL sample diluted to 100 mL with water. The sensitivity of the method may be improved in the cases of relatively unpolluted waters by concentrating larger sample volumes to 100 mL by evaporation.

12.2 Process the sample or a set of samples, a quality control standard (see Annex A2), selected from near the midscale of the series used to prepare the calibration chart in 11.1, and a parallel procedure blank, using 100 mL of water, in 250-mL separatory funnels as outlined in 11.2 to 11.6.

NOTE 8—If an excessive amount of emulsion forms with a sample in 11.3 and it is evident that a substantial loss of MBAS will occur, then the analyst is advised to use well-known techniques in an attempt to break the emulsion. Several known techniques are (*1*) the brief local application of heat by a hot-water stream applied to the outside of the separatory funnel in the area of the emulsion layer and (*2*) filtering the emulsion through a wad of glass wool to remove particulate matter, etc. If the emulsion cannot be broken, then make a note recording the fact, or else, make another attempt at analysis by using a smaller sample size.

13. Calculations

13.1 Calculate and express as MBAS, the apparent concentration of linear alkyl benzene sulfonate as follows:

$$\text{MBAS, mg/L} = W \times 1000/S$$

where:

W = LAS in the sample from the calibration chart for the appropriate absorption cell, milligrams, and

S = sample volume selected in accordance with 12.1, millilitres.

13.2 Follow the procedures outlined in Annex A2 and Annex A3: to evaluate the quality control standard, to determine if the method is under control, and to accept or reject the results from that set of analyses.

14. Report

14.1 Include in the report the molecular weight (mw) of the LAS used to prepare the calibration curve in Section 11. Report results as:

MBAS (calculated as LAS, mw ___) = ___ mg/L

15. Precision and Bias [7]

15.1 Seven operators from seven laboratories determined three concentration levels of LAS on three days in reagent water and in selected water matrices.

15.2 The overall and single-operator precision of this test method within its designated range for reagent water and selected water matrices varies with the quantity tested in accordance with the values cited in Table 3 .

TABLE 3 Precision

Amount added, mg/L	Overall, S_t	Single-Operator, S_o
	Reagent Water	
0.23	±0.042	±0.021
0.78	±0.044	±0.028
1.28	±0.203	±0.091
	Matrix Water	
0.23	±0.045	±0.039
0.78	±0.131	±0.102
1.28	±0.063	±0.054

15.3 Recoveries of known amounts of LAS, calculated as MBAS, from reagent water (Types I, II, and III) and selected water matrices (drinking, natural, and treated waste) were as shown in Table 4 .

TABLE 4 Recovery

Amount Added, mg/L	Amount Found, mg/L	Bias, %	Statistically Significant, 95 % Confidence Level
		Reagent Water	
0.23	0.225	–2.2	no
0.78	0.756	–3.1	yes
1.28	1.223	–4.5	no
		Matrix Water	
0.23	0.224	–2.6	no
0.78	0.742	–4.9	no
1.28	1.037	–19.0	yes

15.4 These collaborative test data were obtained on reagent grade water, drinking, natural, and treated waste waters. It is the user's responsibility to ensure the validity of this test method for water of untested matrices.