

# STANDARD OPERATING PROCEDURE For SM 5540 C

## The Determination of MBAS Anionic Surfactants as MBAS

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SOP #: SM 5540 C

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# MassDEP

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## LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	September 2000	None	
0	September 2000	Replaced old DEP Logo with state seal + MassDEP (December 2006)	Title page & header



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## SM METHOD 5540 C Approved for NPDES

TITLE: Anionic Surfactants as MBAS  
ANALYTE: Methylene Blue Active Substances  
INSTRUMENTATION: Spectrophotometer

### 1.0 SCOPE AND APPLICATION

- 1.1 Surfactants enter waters and waste waters mainly by discharge of aqueous wastes from household and industrial laundering and other cleansing operations. A surfactant combines in a single molecule a strongly hydrophobic group with a strongly hydrophilic one. Such molecules tend to congregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids, and particles, thus imparting properties such as foaming, emulsification, and particle suspension.
- 1.2 The surfactant hydrophobic group generally is a hydrocarbon radical (R) containing about 10 to 20 carbon atoms. The hydrophilic group is of two types, those that ionize in water and those that do not. Ionic surfactants are subdivided into two categories, differentiated by the charge. An ionic surfactant ion is negatively charged, e.g.  $(\text{RSO}_3)^- \text{Na}^+$ , and a cationic one is positively charged, e.g.,  $(\text{RMe}_3\text{N})^+ \text{Cl}^-$ . Nonionizing surfactants commonly contain a polyoxyethylene hydrophilic group  $(\text{ROCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH})$ , often abbreviated  $\text{RE}_n$ , where n is the average number of  $-\text{OCH}_2\text{CH}_2-$  units in the hydrophilic group). Hybrids of these types exist also.
- 1.3 In the U.S., ionic surfactants amount to about 2/3 of the total surfactants used and non-ionics to about 1/3. Cationic surfactants amount to less than 1/10 of the ionics and are used generally for disinfecting, fabric softening, and various cosmetic purposes rather than for their deterative properties.
- 1.4 At current detergent and water usage levels the surfactant content of raw domestic wastewater is in the range of about 1 to 20 mg/L. Most domestic wastewater surfactants are dissolved in equilibrium with proportional amounts adsorbed on particulates. In environmental waters the surfactant concentration generally is below 0.1 mg/L except in the vicinity of an out fall or other point source of entry.

### 2.0 SUMMARY OF METHOD

- 2.1 Methylene blue active substances (MBAS) bring about the transfer of methylene blue, a cationic dye, from an aqueous solution into an immiscible organic liquid upon equilibration. This occurs through ion pair formation by the MBAS anion and the methylene blue cation. The intensity of the resulting blue color in the organic phase is a measure of MBAS. Anionic surfactants are among the most prominent of many substances, natural and synthetic, showing methylene blue activity.



- 2.2 This method is relatively simple and precise. It comprises three successive extractions from acid aqueous medium containing excess methylene blue into chloroform ( $\text{CHCl}_3$ ) followed by an aqueous backwash and measurement of the blue color in the chloroform by spectrophotometry at 652 nm. The method is applicable at MBAS concentrations down to about 0.025 mg/L.
- 2.3 Soaps do not respond in the MBAS method. Those used in or as detergents are alkali salts of  $\text{C}_{10-20}$  fatty acids  $[\text{RCO}_2]^- \text{Na}^+$  and though anionic in nature they are so weakly ionized that an extractable ion pair is not formed under the conditions of the test. Non-soap anionic surfactants commonly used in detergent formulations are strongly responsive. These include principally surfactants of the sulfonate type  $[\text{RSO}_3]^- \text{Na}^+$ , the sulfate ester type  $[\text{ROSO}_3]^- \text{Na}^+$ , and sulfated non-ionics  $[\text{RE}_n\text{OSO}_3]^- \text{Na}^+$ . They are recovered almost completely by a single  $\text{CHCl}_3$  extraction.
- 2.4 Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant and is used to standardize the MBAS method. LAS is not a single compound, but may comprise any or all of 26 isomers and homo-logs with structure  $[\text{RC}_6\text{H}_4\text{SO}_3]^- \text{Na}^+$ , where R is a linear secondary alkyl group ranging from 10 to 14 carbon atoms in length. The manufacturing process defines the mixture, which may be modified further by the wastewater treatment process.
- 2.5 Sulfonate and sulfate type surfactants respond together in MBAS analysis, but they can be differentiated by other means. The sulfate type decomposes upon acid hydrolysis; the resulting decrease in MBAS corresponds to the original sulfate surfactant content while the MBAS remaining corresponds to the sulfonate surfactants.
- 2.6 Alkylbenzene sulfonate can be identified and quantified by IR spectrometry after purification. LAS can be distinguished from other alkylbenzene sulfonate surfactants by IR methods. LAS can be identified unequivocally and its detailed isomer homo-log composition determined by desulfonation gas chromatography.

### 3.0 DEFINITIONS

- 3.1 LAS: Linear Alkylbenzene Sulfonate  $[\text{R-C}_6\text{H}_4\text{SO}_3]^- \text{Na}^+$
- 3.2 Sulfonate:  $[\text{R-SO}_3]^- \text{Na}^+$
- 3.3 Sulfate ester:  $[\text{R-O-SO}_3]^- \text{Na}^+$
- 3.4 Sulfated nonionic:  $[\text{RE}_n\text{OSO}_3]^- \text{Na}^+$
- 3.5 MBAS: methylene blue active substance; all of the above are mbas
- 3.6 Cationic surfactant:  $[\text{RMe}_3\text{N}]^+ \text{Cl}^-$
- 3.7 Nonionizing surfactant:  $(\text{ROCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OH})$



## **4.0 INTERFERENCES**

- 4.1 Positive interference results from all other MBAS species present. Substances such as organic sulfonates, sulfates, carboxylates, and phenols, and inorganic thiocyanates and cyanates may transfer methylene blue into the chloroform phase.
- 4.2 Negative interferences can result from the presence of cationic surfactants and other cationic materials, such as amines, because they compete with the methylene blue in the formation of ion pairs. Particulate matter may give negative interference through adsorption of MBAS.
- 4.3 Sulfides, often present in raw or primary treated wastewater, may react with methylene blue to form a colorless reduction product. Eliminate this interference by prior oxidation with hydrogen peroxide.

## **5.0 SAFETY**

- 5.1 Chloroform is a suspected carcinogen and all extractions must be performed under a hood. Gloves and safety glasses must be worn when conducting the extraction.

## **6.0 EQUIPMENT AND SUPPLIES**

- 6.1 Spectrophotometer: for use at 652 nm, providing a light path of 1 cm.
- 6.2 Separatory funnels: 250 or 500 ml, preferably with inert TFE stopcocks and stoppers.

## **7.0 REAGENTS AND SUPPLIES**

- 7.1 Stock LAS solution: Weigh an amount of the reference material equal to 1.00 g LAS on a 100% active basis. Dissolve in reagent water and dilute to 1000 mL, 1.00 mL = 1.00 mg LAS. Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly.
- 7.2 Standard LAS solution: Dilute 10.00-mL stock LAS solution to 1000-mL with water; 1.00-mL = 10.0 µg LAS. Prepare daily.
- 7.3 Sodium Hydroxide, NaOH, 1N
- 7.4 Sulfuric acid, H<sub>2</sub>SO<sub>4</sub>, 1N and 6N
- 7.5 Chloroform, CHCl<sub>3</sub>: Caution: Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure.
- 7.6 Methylene blue reagent: Dissolve 100mg methylene blue in 100-mL water. Transfer 30-mL to a 1000-mL flask. Add 500-mL water, 41-mL of 6N H<sub>2</sub>SO<sub>4</sub>, and 50 g sodium phosphate, monobasic, monohydrate, NaH<sub>2</sub>PO<sub>4</sub>(H<sub>2</sub>O). Shake until dissolved. Dilute to 1000-mL.
- 7.7 Wash solution: Add 41 mL 6N H<sub>2</sub>SO<sub>4</sub> to 500-mL reagent water in a 1000-mL flask. Add 50 g NaH<sub>2</sub>PO<sub>4</sub>(H<sub>2</sub>O) and shake until dissolved. Dilute to 1000-mL.



7.8 Glass wool: Pre-extract with  $\text{CHCl}_3$  to remove interferences.

7.9 Hydrogen peroxide:  $\text{H}_2\text{O}_2$ , 30%.

## 8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGEE

8.1 Avoid foam formation because the surfactant concentration is higher in the foam phase than in the associated bulk aqueous phase and the latter may be significantly depleted. If foam is formed let it subside by standing, or collapse it by other appropriate means, and remix the liquid phase before sampling.

8.2 Adsorption of surfactant from aqueous solutions onto the walls of container, when concentrations below about 1.0 mg/L are present may seriously deplete the bulk aqueous phase. Minimize adsorption errors if necessary by rinsing container with sample and for anionic surfactants by adding alkali phosphate (e.g., 0.03N  $\text{KH}_2\text{PO}_4$ ).

8.3 Samples should be stored at 4°C and analyzed within 48 hours.

## 9.0 QUALITY CONTROL

9.1 An LRB and at least one LFB is analyzed with every batch.

9.2 Duplicates and LFM's are analyzed at a frequency of 10 per cent.

9.3 A QCS is analyzed quarterly.

## 10.0 CALIBRATION AND STANDARDIZATION

10.1 Spectrophotometer procedure

10.1.1 Turn on and let warm up for 20 minutes.

10.1.2 When main menu appears, press 1 to put in abs/%T/conc. mode.

10.1.3 Press 'Go to WL" key and type 652 which is the correct wavelength.

10.1.4 Press the ENTER key.

10.1.5 Put cal blank std in cell and place in first cell holder. Press auto-zero key to zero the instrument with blank.

10.1.6 Place stds and samples in cell and record absorbance directly from screen.

## 11.0 PROCEDURE

11.1 Preparation of calibration curve: Prepare a series of 8 separatory funnels with 0, 1.00, 3.00, 5.00, 9.00, 13.00, 15.00, and 20.00-mL standard LAS solution. Add sufficient water to make the total volume 100-mL in each separatory funnel. Follow the procedure give below. Plot a calibration curve of absorbance vs. micrograms LAS taken, specifying the molecular weight of the LAS used.





- 11.2 Sample size: take 100-mL of sample if expected concentration is less than 2.0 mg/L. If higher, take appropriate dilution.
- 11.3 Peroxide treatment: If necessary to avoid decolorization of methylene blue by sulfides, add a few drops of 30% H<sub>2</sub>O<sub>2</sub>.
- 11.4 Add sample to a separatory funnel. Make alkaline by drop wise addition 1N NaOH, using phenolphthalein indicator. Discharge pink color by drop wise addition of 1N H<sub>2</sub>SO<sub>4</sub>.
- 11.5 Add 10 mL CHCl<sub>3</sub> and 25-mL methylene blue reagent. Rock funnel vigorously for 30 s and let phases separate. Excessive agitation may cause emulsion formation. (To break persistent emulsions add a small volume of isopropyl alcohol [ $<10$  mL]; add same volume to all standards). Before draining CHCl<sub>3</sub> layer, swirl gently and then let settle.
- 11.6 Draw off CHCl<sub>3</sub> layer into a second separatory funnel. Rinse delivery tube of first separatory funnel with a small amount of CHCl<sub>3</sub>. Repeat extraction two additional times using 10 mL CHCl<sub>3</sub> each time. If blue color in water phase becomes faint or disappears, discard and repeat using a smaller sample.
- 11.7 Combine all CHCl<sub>3</sub> extracts in the second separatory funnel. Add 50-mL wash solution and shake vigorously for 30 s. Let settle, swirl, and draw off CHCl<sub>3</sub> through a funnel containing a plug of glass wool into a 100-mL volumetric flask; filtrate must be clear. Extract wash solution twice with 10-mL CHCl<sub>3</sub> each and add to flask through the glass wool. Rinse glass wool and funnel with CHCl<sub>3</sub>. Collect, washing in volumetric flask, dilute to mark with CHCl<sub>3</sub>, and mix well.
- 11.8 Measurement: Determine absorbance at 652 nm against a blank of CHCl<sub>3</sub>.

## 12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Test results will appear to differ if expressed in terms of weight rather than in molar quantities. Equimolar amounts of two anionic surfactants with different molecular weights should give substantially equal colors in the CHCl<sub>3</sub> layers, although the amounts by weight may differ significantly. If results are to be expressed by weight, the average molecular weight of the surfactant measured must be known or a calibration curve made with that particular compound must be used. Because such detailed information generally is lacking, results are reported in terms of calibration curve made from LAS which has a molecular weight of 318.
- 12.2 Calculation: From the calibration curve read micrograms of apparent LAS (mol wt 318) corresponding to the measured absorbance.

mg MBAS/L =  $\frac{\mu\text{g apparent LAS}}{\text{mL original sample}}$

Report as "MBAS, calculated as LAS, molecular wt 318"

## 13.0 METHOD PERFORMANCE

- 13.1 MDL = 0.04 mg/L (7/7/98)



13.2 For an LFB of 0.35, the average recovery = 0.354 mg/L (101.1%).

Sd = 0.013 mg/L.

13.3 Duplicates: for the RPD, the average = 5.84

The standard deviation of the RPD = 6.2

## **14.0 POLLUTION PREVENTION**

14.1 The quantity of chemicals purchased should be based on expected usage during its shelf life.

14.2 Actual reagent preparation volumes should reflect anticipated usage during its shelf life.

14.2 All chloroform waste from extractions is collected in glass bottles and stored in hazardous waste room.

## **15.0 WASTE MANAGEMENT**

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations.

## **16.0 REFERENCES**

16.1 Standard Methods for the Examination of Water and Wastewater, 19th Edition, 1995.



## 17.0 TABLES AND VALIDATION DATA

**Table 1. Validation Data**

Sample # Value      Reported Value      True Value      Acceptance

**TABLE 2. Precision of MBAS in Reagent Water by Standard Method 5540 C**

Date	Based on Sample Numbers	No. of Samples (n)	Precision (RPD) <sup>b</sup>					
			Mean	SD <sup>a</sup>	Warning Limits (± 2 SD)		Control Limits (± 3 SD)	
					Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)

<sup>a</sup> SD = Standard deviation  
<sup>b</sup> RPD = Relative percent difference

**TABLE 3. Method Detection Limit (MDL) for MBAS Analysis in Reagent Water by Standard Method 5540 C**

Date of Study	No. of Samples Spiked (n)	Spiked Concentration (mg/L)	Accuracy (Mean % Recovery <sup>a</sup> )	Precision (SD <sup>b</sup> in mg/L)	MDL (mg/L)

<sup>a</sup> Recovery of spiked concentration  
<sup>b</sup> SD = standard deviation of mean concentration measured  
<sup>c</sup> Method Detection Level (MDL) is 0.02 mg/L F. WES' Reporting Detection Level (RDL) is 0.10 mg/L F.