1 L/min. If a lower flow rate is necessary to avoid phase intermixing, prolong sublation time proportionally. If the volume of the upper phase has decreased by more than about 20%, repeat the operation on a new sample but avoid excessive intermixing at the interface. Draw off entire ethyl acetate layer through upper stopcock into the separatory funnel; return any transferred water layer to the sublator. Filter ethyl acetate layer into a 250-mL beaker through a dry, medium-porosity, qualitative filter paper (prewashed with ethyl acetate to remove any adventitious surfactant) to remove any remaining aqueous phase.

Repeat process of preceding paragraph with a second 100-mL layer of ethyl acetate, using the same separatory funnel and filter, and finally rinse sublator wall with another 20 mL, all into the original beaker.

Evaporate ethyl acetate from the beaker on a steam bath in a hood, blowing a gentle stream of nitrogen or air over the liquid surface to speed evaporation and to minimize active boiling. Evaporate the first 100 mL during the second sublation to avoid overfilling the beaker. To avoid possible solute volatilization, discontinue heating after removing the ethyl acetate. The sublated surfactant remains in the beaker as a film of residue.

Draw off aqueous layer in the sublator and discard, using the stopcock just above the sintered disk to minimize disk fouling.

5. Precision and Bias

Estimates of the efficiency of surfactant transfer and recovery in the sublation process include the uncertainties of the analytical methods used in quantitating the surfactant. At present the analytical methods are semiquantitative for surfactant at levels below 1 mg/L in environmental samples.

With various known surfactants at 0.2 to 2 mg/L and appropriate analytical methods, over 90% of added surfactant was recovered in one 5-min sublation from 10% NaCl. Without NaCl, recovery of nonionics was over 90% but recovery of anionics and cationics was only 2 to 25%.[4]

Five laboratories studied the recovery of five anionic surfactant types from concentrations of 0.05, 0.2, 1.0, and 5.0 mg/L in aqueous solutions.[5] The amount in each solution was determined directly by methylene blue analysis and compared with the amount recovered in the sublation process, also analyzed by methylene blue. The overall average recovery was 95.9% with a standard deviation of ±7.4 (n = 100). The extreme individual values for recovery were 65% and 115% and the other 98 values ranged from 75% to 109%. Recovery did not depend on surfactant concentration (average recoveries ranging from 94.7% at 5.0 mg/L to 96.8% at 1.0 mg/L) nor on the surfactant type (average recoveries ranging from 94.7% to 96.6%). Average recoveries at the five laboratories ranged from 90.0% to 98.0%.

TABLE 5540:I. SURFACTANT RECOVERY BY SUBLATION

Variable	MBAS	CTAS
Sample volume, mL	200–300	500
Concentration without sublation, mg/L	2.2–4.7	—
Concentration found in sublate,* mg/L	1.8–4.4	0.3–0.6
Recovery in sublate, %	87 ± 16†	—
Amount in second sublate,‡ mg	0.02 ± 0.02†	0.08 ± 0.01†
Amount added, mg	0.05–0.10§	0.50–0.67‖
Recovery in sublation,# %	94 ± 17†	92 ± 6†

* Two 5-min sublations.
† Average ± SD (n = 8).
‡ Two more 5-min sublations.
§ Reference LAS.
‖ Linear alcohol ethoxylate $C_{12-18}E_{11}$.
Fifth and sixth 5-min sublations.

Application of the sublation method in three laboratories to eight different samples of raw wastewater in duplicate gave the results shown in Table 5540:I. Methylene blue active substances (MBAS) recovery in double sublation averaged 87 ± 16% of that determined directly on the filtered wastewater; these results would have been influenced by any nonsurfactant MBAS that might have been present. Repeating double sublation on the spent aqueous phase yielded another 0.02 mg MBAS and another 0.08 mg cobalt thiocyanate active substances (CTAS). Adding 0.05 to 0.10 mg of known linear alkylbenzene sulfonate (LAS) or 0.50 to 0.67 mg of known linear alcohol-based $C_{12-18}E_{11}$ to the same sublator contents and again running double sublation resulted in over 90% recovery of the amount added.

6. References

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5540 C. Anionic Surfactants as MBAS

1. General Discussion

a. Definition and principle: 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. The MBAS method is useful for estimating the anionic surfactant content of waters and wastewaters, but the possible presence of other types of MBAS always must be kept in mind.

This method is relatively simple and precise. It comprises three successive extractions from acid aqueous medium containing excess methylene blue into chloroform ($CHCl_3$), followed by an aqueous backwash and measurement of the blue color in the $CHCl_3$ by spectrophotometry at 652 nm. The method is applicable at MBAS concentrations down to about 0.025 mg/L.

b. Anionic surfactant responses: Soaps do not respond in the MBAS method. Those used in or as detergents are alkali salts of C_{10-20} fatty acids $[RCO_2]^-Na^+$, and though anionic in nature they are so weakly ionized that an extractable ion pair is not formed under the conditions of the test. Nonsoap anionic surfactants commonly used in detergent formulations are strongly responsive. These include principally surfactants of the sulfonate type $[RSO_3]^-Na^+$, the sulfate ester type $[ROSO_3]^-Na^+$, and sulfated nonionics $[RE_nOSO_3]^-Na^+$. They are recovered almost completely by a single $CHCl_3$ extraction.

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 homologs with structure $[R'C_6H_4SO_3]^-Na^+$, 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.

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. Alkylbenzene sulfonate can be identified and quantified by infrared spectrometry after purification.[1] LAS can be distinguished from other alkylbenzene sulfonate surfactants by infrared methods.[2] LAS can be identified unequivocally and its detailed isomer-homolog composition determined by desulfonation-gas chromatography.[3]

c. Interferences: Positive interferences result from all other MBAS species present; if a direct determination of any individual MBAS species, such as LAS, is sought, all others interfere. Substances such as organic sulfonates, sulfates, carboxylates and phenols, and inorganic thiocyanates, cyanates, nitrates, and chlorides also may transfer more or less methylene blue into the chloroform phase. The poorer the extractability of their ion pairs, the more effective is the aqueous backwash step in removing these positive interferences; interference from chloride is eliminated almost entirely and from nitrate largely so by the backwash. Because of the varied extractability of nonsurfactant MBAS, deviations in $CHCl_3$ ratio and backwashing procedure may lead to significant differences in the total MBAS observed, although the recovery of sulfonate- and sulfate-type surfactants will be substantially complete in all cases.

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. Although some of the adsorbed MBAS may be desorbed and paired during the $CHCl_3$ extractions, recovery may be incomplete and variable.

Minimize interferences by nonsurfactant materials by sublation if necessary (5540B). Other countermeasures are nonstandard. Remove interfering cationic surfactants and other cationic materials by using a cation-exchange resin under suitable conditions.[3] Handle adsorption of MBAS by particulates preferably by filtering and analyzing the insolubles. With or without filtration, adsorbed MBAS can be desorbed by acid hydrolysis; however, MBAS originating in any sulfate ester-type surfactant present is destroyed simultaneously.[1] Sulfides, often present in raw or primary treated wastewater, may react with methylene blue to form a colorless reduction product, making the analysis impossible. Eliminate this interference by prior oxidation with hydrogen peroxide.

d. Molecular weight: 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 $CHCl_3$ layer, although the amounts by weight may differ significantly. If results are to be expressed by weight, as generally is desirable, 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, report results in terms of a suitable standard calibration curve, for example "0.65 mg MBAS/L (calculated as LAS, mol wt 318)."

e. Minimum detectable quantity: About 10 μg MBAS (calculated as LAS).

f. Application: The MBAS method has been applied successfully to drinking water samples. In wastewater, industrial wastes, and sludge, numerous materials normally present can interfere seriously if direct determination of MBAS is attempted. Most nonsurfactant aqueous-phase interferences can be removed by sublation. The method is linear over an approximate range of 10 to 200 μg of MBAS standard. This may vary somewhat, depending on source of standard material.

g. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer,* for use at 652 nm, providing a light path of 1 cm or longer.

2) *Filter photometer,* providing a light path of 1 cm or longer and equipped with a red color filter exhibiting maximum transmittance near 652 nm.

b. Separatory funnels: 500-mL, preferably with inert TFE stopcocks and stoppers.

3. Reagents

a. Stock LAS solution: Weigh an amount of the reference material* equal to 1.00 g LAS on a 100% active basis. Dissolve

* For sources of suitable reference material, contact *Standard Methods* technical information manager at www.standardmethods.org/contact/.

in water and dilute to 1000 mL; 1.00 mL = 1.00 mg LAS. Store in a refrigerator to minimize biodegradation. If necessary, prepare weekly.

b. Standard LAS solution: Dilute 10.00 mL stock LAS solution to 1000 mL with water; 1.00 mL = 10.0 μg LAS. Prepare daily.

c. Phenolphthalein indicator solution, alcoholic.

d. Sodium hydroxide (NaOH), 1*N*.

e. Sulfuric acid (H_2SO_4), 1*N* and 6*N*.

f. Chloroform ($CHCl_3$): **CAUTION: Chloroform is toxic and a suspected carcinogen. Take appropriate precautions against inhalation and skin exposure.**

g. Methylene blue reagent: Dissolve 100 mg methylene blue† in 100 mL water. Transfer 30 mL to a 1000-mL flask. Add 500 mL water, 41 mL 6*N* H_2SO_4, and 50 g sodium phosphate, mono- basic, monohydrate, $NaH_2PO_4 \cdot H_2O$. Shake until dissolved. Dilute to 1000 mL.

h. Wash solution: Add 41 mL 6*N* H_2SO_4 to 500 mL water in a 1000-mL flask. Add 50 g $NaH_2PO_4 \cdot H_2O$ and shake until dissolved. Dilute to 1000 mL.

i. Methanol (CH_3OH). **CAUTION: Methanol vapors are flammable and toxic; take appropriate precautions.**

j. Hydrogen peroxide (H_2O_2), 30%.

k. Glass wool: Pre-extract with $CHCl_3$ to remove interferences.

l. Water, reagent-grade, MBAS-free. Use for making all reagents and dilutions.

4. Procedure

a. Preparation of calibration curve: Prepare an initial calibration curve consisting of at least five standards covering the referenced (5540C.1*f*) or desired concentration range. Provided that linearity is demonstrated over the range of interest (r = 0.995 or better) run daily check standards at the reporting limit and a concentration above the expected samples' concentration. Check standard results should be within 25% of original value at the reporting limit and 10% of original value for all others. Otherwise, prepare a new calibration curve.

Prepare a series of separatory funnels for a reagent blank and selected standards. Pipet portions of standard LAS solution (5540C.3*b*) into funnels. Add sufficient water to make the total volume 100 mL in each separatory funnel. Treat each standard as described in ¶s *d* and *e* below, and plot a calibration curve of absorbance vs. micrograms LAS taken, specifying the molecular weight of the LAS used.

b. Sample size: For direct analysis of waters and wastewaters, select sample volume on the basis of expected MBAS concentration:

Expected MBAS Concentration *mg/L*	Sample Taken *mL*
0.025–0.080	400
0.08–0.40	250
0.4–2.0	100

† Eastman No. P573, or equivalent.

If expected MBAS concentration is above 2 mg/L, dilute sample containing 40 to 200 μg MBAS to 100 mL with water.

For analysis of samples purified by sublation, dissolve sublate residue (5540B.4*e*) in 10 to 20 mL methanol, quantitatively transfer the entire amount (or a suitable portion if more than 200 μg MBAS is expected) to 25 to 50 mL water, evaporate without boiling until methanol is gone, adding water as necessary to avoid going to dryness, and dilute to about 100 mL with water.

c. Peroxide treatment: If necessary to avoid decolorization of methylene blue by sulfides, add a few drops of 30% H_2O_2.

d. Ion pairing and extraction:

1) Add sample to a separatory funnel. Make alkaline by dropwise addition of 1*N* NaOH, using phenolphthalein indicator. Discharge pink color by dropwise addition of 1*N* H_2SO_4.

2) Add 10 mL $CHCl_3$ and 25 mL methylene blue reagent. Rock funnel vigorously for 30 s and let phases separate. Alternatively, place a magnetic stirring bar in the separatory funnel; lay funnel on its side on a magnetic mixer and adjust speed of stirring to produce a rocking motion. Excessive agitation may cause emulsion formation. To break persistent emulsions add a small volume of isopropyl alcohol (<10 mL); add same volume of isopropyl alcohol to all standards. Some samples require a longer period of phase separation than others. Before draining $CHCl_3$ layer, swirl gently, then let settle.

3) Draw off $CHCl_3$ layer into a second separatory funnel. Rinse delivery tube of first separatory funnel with a small amount of $CHCl_3$. Repeat extraction two additional times, using 10 mL $CHCl_3$ each time. If blue color in water phase becomes faint or disappears, discard and repeat, using a smaller sample.

4) Combine all $CHCl_3$ extracts in the second separatory funnel. Add 50 mL wash solution and shake vigorously for 30 s. Emulsions do not form at this stage. Let settle, swirl, and draw off $CHCl_3$ layer 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 $CHCl_3$ each and add to flask through the glass wool. Rinse glass wool and funnel with $CHCl_3$. Collect washings in volumetric flask, dilute to mark with $CHCl_3$, and mix well.

e. Measurement: Determine absorbance at 652 nm against a blank of $CHCl_3$.

5. Calculation

From the calibration curve (5540C.4*a*) read micrograms of apparent LAS (mol wt ___) corresponding to the measured absorbance.

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

Report as "MBAS, calculated as LAS, mol wt ___."

6. Precision and Bias

A synthetic sample containing 270 μg LAS/L in distilled water was analyzed in 110 laboratories with a relative standard deviation of 14.8% and a relative error of 10.6%.

A tap water sample to which was added 480 μg LAS/L was analyzed in 110 laboratories with a relative standard deviation of 9.9% and a relative error of 1.3%.

A river water sample with 2.94 mg LAS/L added was analyzed in 110 laboratories with a relative standard deviation of 9.1% and a relative error of 1.4%.[4]

7. References

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5540 D. Nonionic Surfactants as CTAS

1. General Discussion

a. Definition and principle: Cobalt thiocyanate active substances (CTAS) are those that react with aqueous cobalt thiocyanate solution to give a cobalt-containing product extractable into an organic liquid in which it can be measured. Nonionic surfactants exhibit such activity, as may other natural and synthetic materials; thus, estimation of nonionic surfactants as CTAS is possible only if substantial freedom from interfering CTAS species can be assured.

The method requires sublation to remove nonsurfactant interferences and ion exchange to remove cationic and anionic surfactants, partition of CTAS into methylene chloride from excess aqueous cobalt thiocyanate by a single extraction, and measurement of CTAS in the methylene chloride by spectrophotometry at 620 nm. Lower limit of detectability is around 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$. Beyond the sublation step the procedure is substantially identical to that of the Soap and Detergent Association (SDA).[1]

b. Nonionic surfactant responses: For pure individual molecular species the CTAS response is negligible up to about RE_5, where it increases sharply and continues to increase more gradually for longer polyether chains.[2,3] Fewer than about six oxygens in the molecule do not supply enough cumulative coordinate bond strength to hold the complex together. Commercial nonionic surfactants generally range from about RE_7 to RE_{15}; however, each such product, because of synthesis process constraints, is actually a mixture of many individual species ranging from perhaps RE_0 to RE_{2n} in a Poisson distribution averaging RE_n.

The hydrophobes used for nonionic surfactants in the U.S. household detergent industry are mainly linear primary and linear secondary alcohols with chain lengths ranging from about 12 to about 18 carbon atoms. Nonionics used in industrial operations include some based on branched octyl- and nonylphenols. These products give strong CTAS responses that may differ from each other, on a weight basis, by as much as a factor of 2. Specifically, eight such products showed responses from 0.20 to 0.36 absorbance units/mg by the SDA procedure.[1]

As with anionic surfactants measured as MBAS, the nonionic surfactants found in water and wastewater might have CTAS responses at least as varied as their commercial precursors because the proportions of the individual molecular species will have been changed by biochemical and physicochemical removal at varied rates, and further because their original molecular structures may have been changed by biodegradation processes.