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5540 SURFACTANTS*

5540 A. Introduction

1. Occurrence and Significance

Surfactants enter waters and wastewaters 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.

*Approved by Standard Methods Committee, 1988.

The surfactant hydrophobic group generally is a hydrocarbon radical (R) containing about 10 to 20 carbon atoms. The hydrophilic groups are 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 anionic surfactant ion is negatively charged, e.g., $(RSO_3)^-Na^+$, and a cationic one is positively charged, e.g., $(RMe_3N)^+Cl^-$. Nonionizing (nonionic) surfactants commonly contain a polyoxyethylene hydrophilic group ($ROCH_2CH_2OCH_2CH_2.....OCH_2CH_2OH$, often abbreviated RE_n, where n is the average number of $-OCH_2CH_2-$ units in the hydrophilic group). Hybrids of these types exist also.

In the United States, ionic surfactants amount to about two thirds of the total surfactants used and nonionics to about one third. Cationic surfactants amount to less than one tenth of the ionics and are used generally for disinfecting, fabric softening, and various cosmetic purposes rather than for their detersive properties. 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. Primary sludge concentrations range from 1 to 20 mg adsorbed anionic surfactant per gram dry weight.[1] In environmental waters the surfactant concentration generally is below 0.1 mg/L except in the vicinity of an outfall or other point source of entry.[2]

2. Analytical Precautions

Because of inherent properties of surfactants, special analytical precautions are necessary. 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. Adsorption of surfactant from aqueous solutions onto the walls of containers, when concentrations below about 1 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., $0.03N$ KH_2PO_4).[3]

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5540 B. Surfactant Separation by Sublation

1. General Discussion

a. Principle: The sublation process isolates the surfactant, regardless of type, from dilute aqueous solution, and yields a dried residue relatively free of nonsurfactant substances. It is accomplished by bubbling a stream of nitrogen up through a column containing the sample and an overlying layer of ethyl acetate. The surfactant is adsorbed at the water-gas interfaces of the bubbles and is carried into the ethyl acetate layer. The bubbles escape into the atmosphere leaving behind the surfactant dissolved in ethyl acetate. The solvent is separated, dehydrated, and evaporated, leaving the surfactant as a residue suitable for analysis. This procedure is the same as that used by the Organization for Economic Co-operation and Development (OECD),[1] following the development by Wickbold.[2,3]

b. Interferences: The sublation method is specific for surfactants, because any substance preferentially adsorbed at the water-gas interface is by definition a surfactant. Although nonsurfactant substances largely are rejected in this separation process, some amounts will be carried over mechanically into the ethyl acetate.

c. Limitations: The sublation process separates only dissolved surfactants. If particulate matter is present it holds back an equilibrium amount of adsorbed surfactant. As sublation removes the initially dissolved surfactant, the particulates tend to reequilibrate and their adsorbed surfactants redissolve. Thus, continued sublation eventually should remove substantially all adsorbed surfactant. However, if the particulates adsorb the surfactant tightly, as sewage particulates usually do, complete removal may take a very long time. The procedure given herein calls for preliminary filtration and measures only dissolved surfactant. Determine adsorbed surfactant content by analyzing particulates removed by filtration; no standard method is available now.

d. Operating conditions: Make successive 5-min sublations from 1 L of sample containing 5 g $NaHCO_3$ and 100 g NaCl. Under the conditions specified, extensive transfer of surfactant occurs in the first sublation and is substantially complete in the second.[2-4]

e. Quantitation: Quantitate the surfactant residue by the procedures in 5540C or D. Direct weighing of the residue is not useful because the weight of surfactant isolated generally is too low, less than a milligram, and varied amounts of mechanically entrained nonsurfactants may be present. The procedure is applicable to water and wastewater samples.

2. Apparatus

a. Sublator: A glass column with dimensions as shown in Figure 5540:1. For the sintered glass disk use a coarse-porosity frit (designation "c"–nominal maximum pore diam 40 to 60 μm as measured by ASTM E-128) of the same diameter as the column internal diameter. Volume between disk and upper stopcock should be approximately 1 L.

b. Gas washing bottle, as indicated in Figure 5540:1, working volume 100 mL or more.

c. Separatory funnel, working volume 250 mL, preferably with inert TFE stopcock.

d. Filtration equipment, suitable for 1-L samples, using medium-porosity qualitative-grade filter paper.

e. Gas flowmeter, for measuring flows up to 1 L/min.

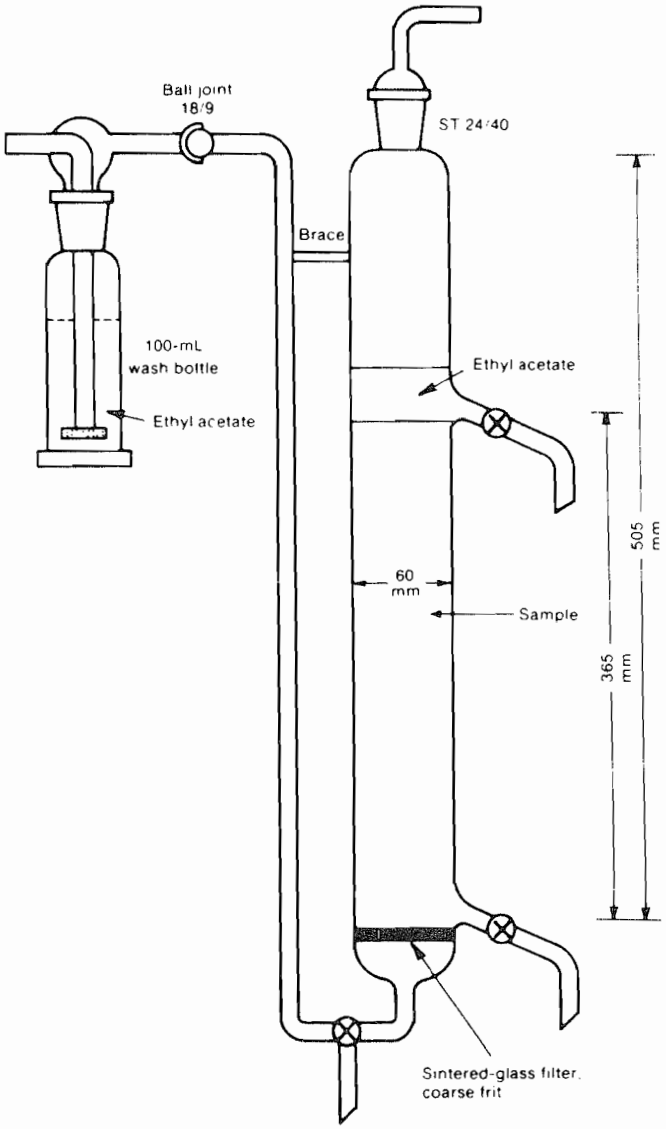

Figure 5540:1. Sublation apparatus.[1] See Section 5540B.2*a* and *b* and 4*c*. Bottom stopcock: TFE plug, 4-mm bore; side stopcocks: TFE plug, 2-mm bore.

3. Reagents

a. Nitrogen, standard commercial grade.

b. Ethyl acetate: CAUTION: *Ethyl acetate is flammable and its vapors can form explosive mixtures with air.*

c. Sodium bicarbonate, $NaHCO_3$.

d. Sodium chloride, NaCl.

e. Water, surfactant-free.

4. Procedure

a. Sample size: Select a sample to contain not more than 1 to 2 mg surfactant.[4] For most waters the sample volume will be about 1 L; for wastewater use a smaller volume.

b. Filtration: Filter sample through medium-porosity qualitative filter paper. Wash filter paper by discarding the first few hundred milliliters of filtrate.

c. Assembly: Refer to Figure 5540:1.

Connect nitrogen cylinder through flowmeter to inlet of gas washing bottle. Connect gas outlet at top of sublator to a gas scrubber or other means for disposing of ethyl acetate vapor (e.g., vent to a hood or directly outdoors). In the absence of a flowmeter, ensure proper gas flow rate by measuring volume of gas leaving the sublator, with a water-displacement system.

d. Charging: Fill gas washing bottle about two-thirds full with ethyl acetate. Rinse sublation column with ethyl acetate and discard rinse. Place measured filtered sample in sublator and add 5 g $NaHCO_3$, 100 g NaCl, and sufficient water to bring the level up to or slightly above the upper stopcock (about 1 L total volume). If sample volume permits, add salts as a solution in 400 mL water or dissolve them in the sample and quantitatively transfer to the sublator. Add 100 mL ethyl acetate by running it carefully down the wall of the sublator to form a layer on top of the sample.

e. Sublation: Start the nitrogen flow, increasing the rate carefully to 1 L/min initially but do not exceed a rate at which the liquid phases begin vigorous intermixing at their interface. Avoid overly vigorous intermixing, which will lead to back-extraction of the surfactant into the aqueous phase and to dissolution of ethyl acetate. Continue sublation for 5 min at 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.

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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. Sub-

stances 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 (Section 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.

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 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, monobasic, 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 a series of 10 separatory funnels with 0, 1.00, 3.00, 5.00, 7.00, 9.00, 11.00, 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. Treat each standard as described in ¶s 4*d* and *e* following, 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

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

* A suitable reference material can be obtained from U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio 45268.

† Eastman No. P573 or equivalent.

residue (Section 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 (¶ 4*a*) read micrograms of apparent LAS (mol wt ___) corresponding to the measured absorbance.

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

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]

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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.

c. Reference nonionic surfactant: Until it is practical to determine the nature and molecular composition of an unknown mixed CTAS, and to calculate or determine the CTAS responses of its component species, exact quantitation of uncharacterized CTAS in a sample in terms of weight is not possible. Instead, express the analytical result in terms of some arbitrarily chosen reference nonionic surfactant, i.e., as the weight of the reference that gives the same amount of CTAS response. The reference is the nonionic surfactant $C_{12-18}E_{11}$, derived from a mixture of linear primary alcohols ranging from 12 to 18 carbon atoms in chain length by reaction with ethylene oxide in a molar ratio of 1:11. $C_{12-18}E_{11}$ is reasonably representative of nonionic surfactants in commercial use; its CTAS response is about 0.21 absorbance units/mg.

If the identity of the nonionic surfactant in the sample is known, use that same material in preparing the calibration curve.

d. Interferences: Both anionic and cationic surfactants may show positive CTAS response[1,4] but both are removed in the ion-exchange step. Sublation removes nonsurfactant interferences. Physical interferences occur if some of the CTAS is adsorbed on particulate matter. Avoid such interference by filtering out the particulates for the sublation step; this will measure only dissolved CTAS.

e. Minimum detectable quantity: About 0.1 mg CTAS, calculated as $C_{12-18}E_{11}$, which corresponds to 0.1 mg/L in a 1-L sample.

f. Application: The method is suitable for determining dissolved nonionic surfactants of the ethoxylate type in most aqueous systems.

2. Apparatus

a. Sublation apparatus: See Section 5540B.2.

b. Ion-exchange column, glass, about 1- × 30-cm. Slurry anion-exchange resin in methanol and pour into column to give a bed about 10 cm deep. Insert plug of glass wool and then add a 10-cm bed of cation-exchange resin on top in the same manner. One column may be used for treating up to six sublated samples before repacking.

c. Spectrophotometer and 2.0-cm stoppered cells, suitable for measuring absorbance at 620 nm.

d. Separatory funnels, 125-mL, preferably with TFE stopcock and stopper.

e. Extraction flasks, Soxhlet type, 150-mL.

3. Reagents

a. Sublation reagents: See Section 5540B.3.

b. Anion-exchange resin, polystyrene-quaternary ammonium-type,* 50- to 100-mesh, hydroxide form. To convert chloride form to hydroxide, elute with 20 bed volumes of 1*N* NaOH and wash with methanol until free alkali is displaced.

c. Cation-exchange resin, polystyrene-sulfonate type,† 50- to 100-mesh, hydrogen form.

d. Cobaltothiocyanate reagent: Dissolve 30 g $Co(NO_3)_2 \cdot 6H_2O$ and 200 g NH_4SCN in water and dilute to 1 L. This reagent is stable for at least 1 month at room temperature.

e. Reference nonionic surfactant, $C_{12-18}E_{11}$: Reaction product of C_{12-18} linear primary alcohol with ethylene oxide in 1:11 molar ratio.‡

f. Reference nonionic surfactant stock solution, methanolic, approximately 2 mg nonionic/mL methanol: Quantitatively transfer entire contents (approximately 1 g nonionic) from preweighed ampule into 500-mL volumetric flask, thoroughly rinse ampule with methanol, make up to volume with methanol, and reweigh dried ampule. Calculate concentration in milligrams per milliter as in ¶ 5*a*. Because of possible phase separation, use all material in the ampule.

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* Bio-Rad, AGI-X2, or equivalent.

† Bio-Rad AG 50W-X8, or equivalent.

‡ This material can be obtained from U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio 45268.

g. Reference nonionic surfactant standard solution, methanolic, approximately 0.1 mg nonionic/mL methanol: Dilute 10.00 mL stock solution to 200 mL with methanol. Exact concentration is 1/20 that of the stock solution.

h. Sodium hydroxide, NaOH: 1*N*.

i. Glass wool: Pre-extract with chloroform or methylene chloride.

j. Methanol, CH_3OH: CAUTION: *Methanol vapors are flammable and toxic; take appropriate precautions.*

k. Methylene chloride, CH_2Cl_2: CAUTION: *Methylene chloride vapors are toxic; take adequate precautions.*

l. Water: Use distilled or deionized, CTAS-free water for making reagents and dilutions.

4. Procedure

a. Purification by sublation: Proceed according to Section 5540B, using sample containing no more than 2 mg CTAS. (NOTE: For samples of known character containing no interfering materials, omit this step.)

b. Ion-exchange removal of anionic and cationic surfactants: Dissolve sublation residue in 5 to 10 mL methanol and transfer quantitatively to ion-exchange column. Elute with methanol at 1 drop/s into a clean, dry 150-mL extraction flask until about 125 mL is collected. Evaporate methanol on a steam bath aided by a gentle stream of clean, dry nitrogen or air, taking care to avoid loss by entrainment; remove from heat as soon as the methanol is completely evaporated. (NOTE: With samples of known character containing no anionic or cationic materials, omit step *b*.)

c. CTAS calibration curve: Into a series of 150-mL extraction flasks containing 10 to 20 mL methanol place 0.00, 5.00, 10.00, 20.00, and 30.00 mL reference nonionic surfactant standard solution and evaporate just to dryness. Continue as in ¶s 4*d* and *e*, below, and plot a calibration curve of absorbance against milligrams of reference nonionic taken, specifying its identity (e.g., $C_{12-18}E_{11}$ and lot number).

d. Cobalt complexing and extraction: Charge a 125-mL separatory funnel with 5 mL cobaltothiocyanate reagent. With precautions against excessive and variable evaporation of the methylene chloride, dissolve residue from ion-exchange operation, ¶ 4*b*, by adding 10.00 mL methylene chloride and swirling for a few seconds. Immediately transfer by pouring into the separatory funnel. *Do not rinse flask.* (NOTE: Because of the volatility of methylene chloride, rigidly standardize these operations with respect to handling and elapsed time; alternatively, evaporate the methanol in 200-mL erlenmeyer flasks to be stoppered with glass or TFE stoppers during dissolution. Transfer as directed here is incomplete, but in this case it will not introduce error because the loss of nonionics is exactly compensated for by the diminished volume of the organic layer in the extraction.) Shake separatory funnel vigorously for 60 s and let layers separate. Run lower layer into a 2.0-cm cell through a funnel containing a plug of pre-extracted glass wool and stopper. Be sure filtrate is absolutely clear. (NOTE: If desired, clarify by running the lower layer into a 12-mL centrifuge tube, stopper, spin at or above 1000 × *g* for 3 min, and transfer to the cell by a Pasteur pipet; use same procedure for both calibration and samples.)

e. Measurement: Determine absorbance at 620 nm against a blank of methylene chloride. (NOTE: If haze develops in the cell, warm slightly with a hot air gun or heat lamp to clarify.)

5. Calculations

a. Nonionic surfactant in reference nonionic stock solution ¶ 3*f*:

mg nonionic/mL methanol = mg reference sample/500 mL

b. Nonionic surfactant in sample: From the calibration curve read milligrams of reference nonionic corresponding to the measured absorbance:

mg CTAS/L = mg apparent nonionic/L sample

Report as "CTAS, calculated as nonionic surfactant $C_{12-18}E_{11}$."

6. Precision and Bias

Twenty-four samples of 6.22% w/v solution of reference nonionic surfactant $C_{12-18}E_{11}$ were analyzed in three laboratories by CTAS alone, without sublation or ion exchange. The overall relative standard deviation was about 3%. Results of the three laboratories individually were:

Laboratory	% w/w ± SD
A	6.08 ± 0.14 (n = 36)
B	6.56 ± 0.17 (n = 6)
C	6.25 ± 0.14 (n = 36)
Overall	6.20 ± 0.19 (n = 78)

Samples of raw wastewater were freed of surfactants by four successive sublations, then 0.50 or 0.67 mg reference nonionic surfactant $C_{12-18}E_{11}$ was added and carried through the entire sequence of sublation, ion exchange, and CTAS extraction. Recoveries averaged 92% with overall standard deviation around 6%:

Laboratory	% Recovery ± SD
A	87 ± 4 (n = 4)
B	97 ± 1 (n = 4)
Overall	92 ± 6 (n = 8)

The above data relate to the bias and precision of the method when applied to a known nonionic surfactant. When the nature of the nonionic surfactant is unknown, there is greater uncertainty. The response of the reference $C_{12-18}E_{11}$ is about 0.21 absorbance units/mg, while that of the eight nonionic types mentioned under ¶ 1*b* ranged from 0.20 to 0.36, and environmental nonionics might differ still more. If the nonionic surfactant in the sample has a response of 0.42, the result calculated in terms of milligrams $C_{12-18}E_{11}$ would be double the actual milligrams of the unknown nonionic.

7. References

1. SOAP AND DETERGENT ASSOCIATION ANALYTICAL SUBCOMMITTEE. 1977. Analytical methods for nonionic surfactants in laboratory biodegradation and environmental studies. *Environ. Sci. Technol.* 11:1167.
2. CRABB, N.T. & H.E. PERSINGER. 1964. The determination of polyoxyethylene nonionic surfactants in water at the parts per million level. *J. Amer. Oil Chem. Soc.* 41:752.

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8. Bibliography

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