posure to SΦU: stripping of the gill tissue in the fish which died, little change in the fish which survived.

The fish exposed to the continuous activated sludge effluents (Table II) showed a similar pattern. Those from the control run fed no LAS exhibited a slight roughening of the gill tissue as compared with control fish in standard water. The survivors from Runs A–D showed a further slight loss of smoothness and some indication of isolated points of damage. The 11-day exposure received by the three fish in Run E resulted in a further incremental change in microscopic appearance, but with damage less severe than illustrated in Figure 3 b. Macroscopic appearance and behavior still could not be distinguished from the controls. The one casualty, in Run B, showed complete loss of the mucous layer and soft tissue of the gill structure, similar to those which had died in the TL_m determinations.

In the static tests using unacclimated sludge effluent, no difference in the microscopic appearance could be detected between the control fish (Table IV, Run 1) and those exposed to 1 mg/liter of C_{14} LAS (Run 2), which was significantly higher than the standard TL_m value. Each group showed a very slight loss of smoothness in texture of the gill tissue, while those which died (Table IV, Runs 3 and 4) suffered near complete loss of color and soft tissues.

Thus in all cases the attack on the gill tissue appears to be related not only to the LAS concn, but also to the sensitivity of the individual fish. The susceptible individual suffers rapid loss of gill mucosa with resultant death, while the resistant individual survives under the same conditions with little if any detectable damage. Such differences in physiological response between individuals of any species are not uncommon.

Schmid and Mann have reported that sodium dodecylbenzene sulfonate attacks the mucous cells on the top of the gill lamina of trout starting at a concn of 5 mg/liter (27). Cairns and Scheier noted similar effects on pumpkinseed and bluegill sunfish after several months' exposure to 3.1 mg/liter of tetrapropylene ABS, ca. one-fourth to one-third of the TL_m concn (28). The present work shows that LAS likewise attacks the gill mucous membranes at concn near or above the TL_m value, but with much less damage to the fish that survive than to those that die at the same concn, and that no damage at all is observed in four days at concn below two-thirds of the TL_m.

ACKNOWLEDGMENTS

Design of the activated sludge units by W. M. Davis; development of operational techniques by R. J. Day; and development of operational techniques and supervision of the daily operation of the activated sludge units by J. C. Gromada.

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[Received June 17, 1964—Accepted September 11, 1964]

The Determination of Polyoxyethylene Nonionic Surfactants in Water at the Parts per Million Level

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Abstract

A method has been developed to determine the concn of polyoxyethylene nonionic surfactant (PNS) in the parts/million concn range in a water-bacteria medium. The method has successfully determined the concn of PNS during the course of biodegradation studies using either activated sludge or river water as the bacterial source. The nonionic surfactant was removed from the water solution by continuous ether extraction. Detection and measurement of the PNS was accomplished using cobalt thiocyanate and measuring the absorbance of the blue cobalt-PNS complex at 620 mμ in a five-cm absorption cell.

Optimum extraction conditions required a neutral pH and a low ionic strength. The colorimetric step required that each molecule of PNS contain at least six ethylene oxide units for color development, and since the absorbance varies with the length of the polyoxyethylene chain, the method must be standardized using the particular compound under investigation.

Introduction

AN IMPORTANT MEMBER of the detergent family is the nonionic surfactant prepared by adding ethylene oxide to an alcohol, amine or alkylphenol starter. This variety of detergent is known as a polyoxyethylene nonionic surfactant which henceforth will be designated as nonionic surfactant or PNS. Bifunctional solubility is built into the molecule as is evidenced by the organic soluble, hydrophobic nature of the

alkyl function compared with the water soluble, hydrophilic character of the polyoxyethlene chain which is at least six moles of ethylene oxide in length. These PNS find extensive usage as soaps and detergents and they also have application as chemical reagents.

Most surface tension reducing agents, surfactants, are capable of causing foam when they are present in minute amt in water. For example, PNS will cause foaming in water when their concn is five parts/million or lower. Detergent foaming after passing through waste water treatment facilities has been verified to be caused by unbiodegradable "hard detergents." Analytical testing procedures are available for the measurement of parts/million cationic and anionic surfactants in water (1); however, a method for the determination of PNS in the parts/million range in water in a biological environment was previously non-existent.

We are actively engaged in the evaluation of synthetic surfactants prepared by reacting ethylene oxide with suitable alkylphenol or alcohol starters to form PNS. Biological degradation studies of these PNS required that a chemical method be developed for their determination in the parts/million range in water. PNS have high boiling points which prevent their analysis by gas chromatography or mass spectrography and, in addition, the chemically inert nonionic nature of these surfactants has restricted their chemical reactions to a limited number of precipitation (2,3,4) or colorimetric reactions (5,6).

A review of the PNS analysis procedures indicated that in order to determine PNS in the parts/million range in water, a method for the removal of the PNS from a water-bacteria solution accompanied by concn of the PNS would be desirable. Removal of the PNS from water accompanied by simultaneous concn of the surfactant was accomplished by continuous ether extraction of the aqueous solution followed by color development of the extracted PNS with cobalt thiocyanate.

Experimental

In order to be able to determine the PNS at a concn of 0.1 mg/100 ml water, it was desirable to conc the surfactant by extraction from water using a continuous ether extraction assembly similar to that constructed by Knapp (7). After a suitable extraction time, the collected ether overflow was evaporated to dryness and a measured volume of cobalt thiocyanate was added. The blue PNS-cobalt complex was extracted into chloroform and the absorbance of the solution was measured at 620 mμ. The concn of the PNS was determined using previously prepared calibration curves.

Apparatus

The extraction apparatus is shown in Figure 1. A separatory funnel with a Teflon stopcock was used as a receiver to reduce the number of transfers to a minimum.

Reagents

1) Ammonium cobalt thiocyanate: prepared by dissolving 15 g $Co(NO_3)_2 \cdot 6H_2O$ and 100 g NH_4SCN in 500 ml distilled water.
2) Ethyl ether, anhydrous reagent grade.
3) Chloroform, spectral grade.
4) Potassium chloride, reagent grade.

Procedure

One gram KCl is added to the extraction column followed by 100 ml test sample. The column is swirled to promote solution of the KCl. The fritted ether dispersion tube is inserted into the column and the condenser is attached. Fifty ml ethyl ether is added to the separatory funnel and reflux of the ether is obtained by heating the funnel in a suitable manner, e.g. heating tape or heating mantle.

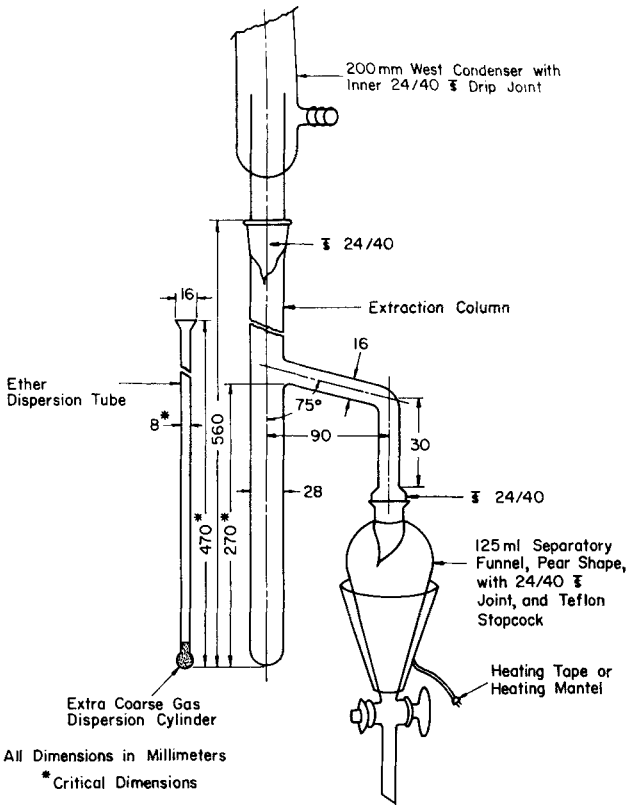

FIG. 1. Continuous ether apparatus for the determination of PNS in water.

The ether boil-up rate is adjusted so that the height of the ether in the dispersion tube is 17 cm above the ether overflow level in the extraction column. This boil-up rate will produce a stream of fine bubbles of ether at the glass frit which will rise up through the water layer. Extraction is continued at this rate for three hr, after which time the funnel heating device is disconnected and the apparatus is permitted to cool. After the apparatus has cooled, the separatory funnel is removed and the ether is boiled to dryness by submersion of the separatory funnel into a beaker of hot water.

The separatory funnel is removed from the water, the exterior is rinsed with acetone (including inside of the tip), and dried. Five ml ammonium cobalt thiocyanate is added to the separatory funnel and the contents are swirled for one min. Five ml spectral grade chloroform is added to the cobalt thiocyanate solution and the contents are shaken or swirled for one min. The layers are permitted to separate and the chloroform layer is drained into a 15-ml graduated centrifuge tube. The chloroform extraction is repeated with a second 5-ml portion and finally with a 6-ml portion of chloroform. A total of 15 ml chloroform extract is collected/sample in a single centrifuge tube. Precise chloroform-aqueous phase separations are not necessary, but it is necessary that 15 of the 16 ml chloroform are collected. Centrifugation of the chloroform extract can be avoided by carefully collecting the chloroform extract without carrying water over into the centrifuge tube.

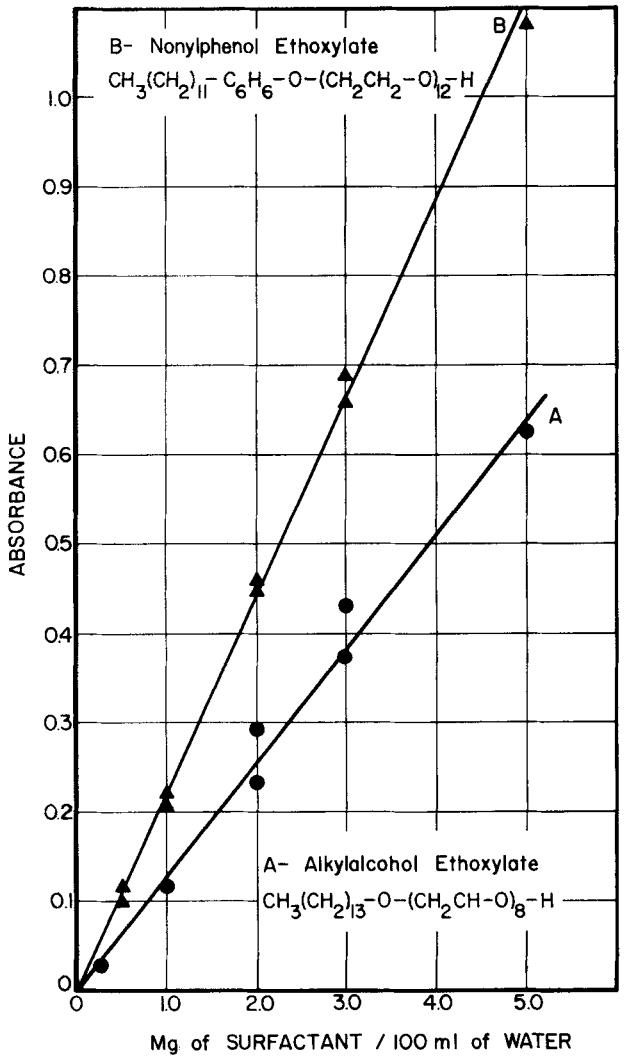

FIG. 2. Calibration curves for nonylphenol and alkylalcohol ethoxylates, absorbance vs. mg/100 ml water.

The collected chloroform extract is transferred to a 5-cm absorption cell and the absorbance is measured at 620 mμ vs. a blank, 100-ml aqueous solvent carried through the complete procedure. The concn of the PNS is obtained from a previously prepared calibration curve.

Calibration

The calibration curve is obtained by accurately weighing one g PNS into a 1000-ml volumetric flask and diluting to the mark with distilled water. This solution contains 1000 ppm PNS. Aliquots (2,5,10,15 and 20 ml) are removed and added to separate 1000-ml volumetric flasks which are filled to the mark with water. These solutions contain 2,5,10,15 and 20 parts/million, respectively. Duplicate analyses of each standard solution according to the above procedure will yield absorbance measurements for these concn. A calibration curve is obtained by plotting these absorbance values vs. parts/million PNS.

Discussion

An important quality of modern surfactants is the degree to which the surfactant will degrade biologically in waste water treatment facilities. The magnitude of the biodegradation of synthetic PNS has been examined using activated sludge and river water by the water treatment control group at Union Carbide's Technical Center located in South Charleston, W. Va. The colorimetric cobalt procedure has been used successfully to follow the degradation of this type of surfactant in both of these media.

TABLE I
Recovery of a 12-Mole Ethoxylate of Dodecyl Phenol

Concentration (ppm)	Absorbance, 620 mμ	Deviation from avg	Percentage of avg deviation
5	0.120	+0.007	
	0.100	−0.013	
	0.120	+0.007	
	Avg 0.113	0.009	±8.2
10	0.186	−0.023	
	0.211	+0.002	
	0.221	+0.012	
	Avg 0.209	0.037	±5.7
20	0.400	−0.037	
	0.450	+0.013	
	0.462	+0.025	
	Avg 0.437	0.025	±5.7

Analysis of the ether extraction step showed that the extraction reached a max (determined by the absorbance of the cobalt-PNS complex) after a three-hr period. Certain of the extraction conditions required control, e.g., the pH and ionic strength. The pH of the aqueous PNS solution contributes markedly to the efficiency of the extraction. Solutions that were either strongly basic or strongly acidic did not show a perceptible extraction using the cobalt color as an indicator. However, solutions that were made intentionally acidic or basic followed by neutralization with $NaHCO_3$ gave results that were comparable with the results obtained by extraction from distilled water. Consequently, it is concluded that the extraction proceeds most favorably in neutral solutions.

The ionic strength of the PNS solution was increased by adding 1 g KCl in order to enhance the ether extraction. However, the salt concn appears to be critical, for when 10 g KCl were used, the extractability of the PNS was dropped to zero. This unexplained phenomenon showed that the extraction of PNS from solutions of high ionic strength is not practical.

Examination of the blue cobalt-PNS complex in the chloroform extract showed two adsorption max at 318 and 620 mμ, respectively. The absorption of these max previously reported by Brown and Hayes (6) also showed that increased sensitivity can be obtained by measuring the absorbance at 318 mμ. However, the ether extract of solutions containing high concn of bacteria contained chloroform soluble material that absorbed strongly at this wavelength. Fortunately, no interference was encountered at 620 mμ and the limits of detectability was increased by using a five cm absorption cell.

Calibration curves obtained for nonylphenol ethoxylates, TERGITOLS, and alkylalcohol ethoxylates (Fig. 2) illustrate that the procedure will give linear results from approx 0.2–5 mg PNS/100 ml water. The reproducibility of the procedure at the 5,10 and 20 parts/million level for a 12-mole ethoxylate of dodecyl phenol shows in Table I.

Observations of the color intensity of the cobalt complexes of equi-molar solutions of PNS containing varying numbers of moles of ethylene oxide/mole of hydrophobe indicated that the color intensity increased as the polyether chain became longer. It was necessary to evaluate this observation in order to determine if the procedure was a general one or if specific calibrations were necesesary. Since PNS samples with a single mol wt were not available, equimolar solutions of nonylphenol PNS and polyethylene glycols were prepared using the number average mol

wt of the nonionic determined by the reaction of the primary alcohol with phthalic anhydride. The absorbance of the chloroform extracted cobalt complexes is plotted in Figure 3 vs. the M concn of ethylene oxide in the solution. This plot shows that at least six moles ethylene oxide/mole PNS is required before a color is formed. This figure also shows that there is a linear relationship between the absorbance of the cobalt complex and the M concn of ethylene oxide in the solution.

The differences in slopes between the polyglycols and the TERGITOLS may be ascribed to a difference in the partial M volume of the two complexes. The TERGITOLS, containing the bulky nonylphenol group, will have a larger partial M volume, a lower extinction coefficient, and consequently a less steep slope. These factors of differing slope and an absorbance that varies with the length of the polyether chain make it necessary to calibrate the procedure on a representative sample of the PNS being studied.

Structure of the Cobalt-PNS Complex. The change in the absorbance of the cobalt-PNS complex can be explained on the basis of the proposed structure of this complex. The proposed structure consists of a cobaltous ion surrounded by at least six moles ethylene oxide. Five of the coordinate positions of the cobalt ion are occupied by the oxygens of the polyether chain and the sixth position by either an ether oxygen or the terminal hydroxide. The hydrophobic portion of the PNS molecule remains freely mobile. The plus two charge of the first member of a complex series is neutralized by $Co(SCN)_4^{2-}$. The series expands by multiples of six moles ethylene oxide and $Co(SCN)_4^{2-}$. This would give the following structure:

$$[Co(R-O-CH_2-CH_2-O)_{n6}H]^{2+}[Co(SCN)_4]_n^{2-}$$

Since PNS with a single mol wt were unavailable at this writing, and since attempts at separating the complexes chromatographically were not successful, the arguments supporting this structure are based on the following observations: 1) no color developed when less than six moles of ethylene oxide were present in the PNS molecule (satisfying the coordination number of octahedral cobalt); 2) a linear curve resulted when moles of ethylene oxide was plotted vs. absorbance; and 3) octahedral cobalt is red with a small absorption coefficient, whereas tetrahedral cobalt thiocyanate is blue (the color of the chloroform extract) with a large absorption coefficient with max at 318 and 620 mμ. Consequently, this means that the quantitative absorption measurements are based on the charge neutralizing cobalt thiocyanate, which increases in concn coincidently with the number of units of six in the cobalt-PNS complex. An additional, but as yet unresolved observation, is the well defined sharp adsorption band that is found for the complex in the IR at 4.8 μ. This wavelength is very near to that found for cobalt carbonyl.

Additional work is planned to elucidate the structure of this complex based upon the isolation of single ethylene oxide adducts of nonylphenol by column chromatography followed by analysis of the complexes formed with these materials and cobalt thiocyanate.

Limitations

The nonionic surfactants examined in this study showed excellent partition into the ether phase; however, the nature of the aqueous solution can cause a reduction in extraction efficiency, e.g., high ionic strength, strongly basic or acidic solutions, and the presence of solid particles from which the PNS could not be removed. The procedure as described is designed to conc by ether extraction PNS with long hydrophobic chains; however, if this chain becomes shortened or oxidized to a carboxylic acid (the polyether chain remaining intact) the ether extraction may be reduced or may fail completely.

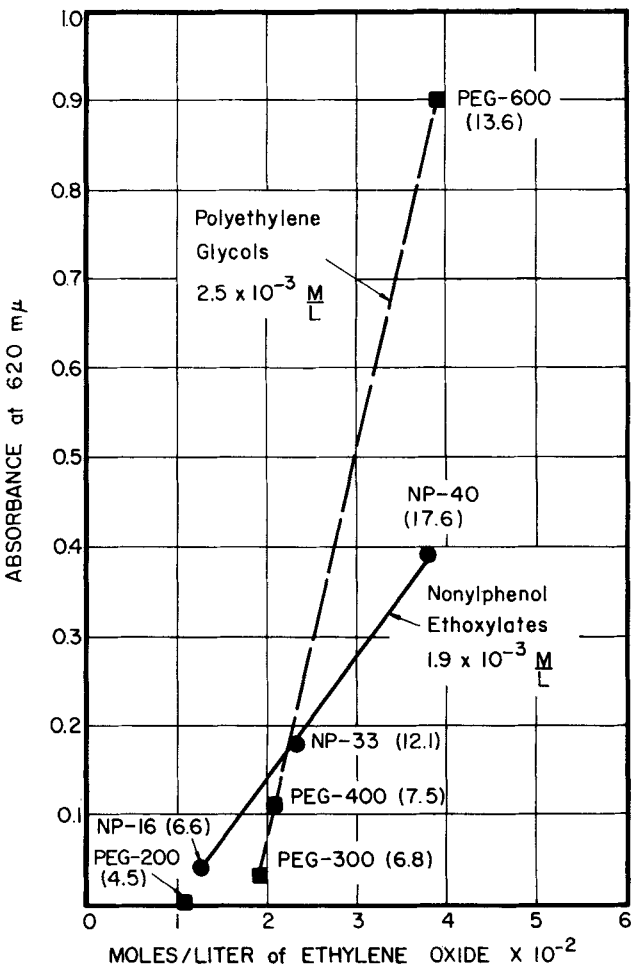

FIG. 3. Absorbance of cobalt—polyoxyethylene complexes as a function of polyoxyethylene chain length.

The formation of the cobalt-PNS complex with a measurable absorbance at 620 mμ is limited to those PNS having at least six moles ethylene oxide/mole of hydrophobe. Those molecules with less than six moles of ethylene oxide will not form a color with cobalt thiocyanate. On the other hand, long polyoxyethylene chains (25–30 moles of ethylene oxide) will form blue precipitates with cobalt thiocyanate that are not soluble in chloroform. Fortunately, long chains of this nature are rarities and were encountered only with compounds similar to CARBOWAX PEG 1000. These limitations will produce low results. High results are possible if the ether extract contains compounds that will yield blue chloroform extractable complexes. Examples of compounds of this type are acetone and quaternary ammonium compounds.

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[Received January 22, 1964—Accepted April 2, 1964]