more than one metabolic pool, the distinction being on the basis of fatty acids in each.

Customarily, phospholipids are considered to be rich sources of highly unsaturated fatty acids, and triglycerides of animal origin are considered to be not especially rich in PUFA. However, the triglycerides of some of the reproductive tissues are notably rich in PUFA. Although beef testis triglycerides contain only 26% PUFA, pork testis triglycerides contain almost 47%. Beef graafian follicle triglycerides contain 61% PUFA whereas pork graafian follicle triglycerides contain only 21%. Beef and pork ovary triglycerides contain 57% and 49% PUFA, respectively. In all six instances, the major portion of the PUFA are of the ω6, the linoleate family.

In the course of this investigation, a number of PUFA have been identified. For 16 the identification is based upon ozonolysis-reduction of the isolated substances. For several others the identification has been by means of retention time data obtained in GLC analysis. The means of identification, and the data for a large body of individual PUFA are given in an accompanying publication (23). A number of acids still unidentified by these means were detected and are characterized in the tables by equivalent chain length. Of the several acids identified, two, to our knowledge, have not been reported before, 10,13,16-Docosatrienoic acid (22:3ω6) and 9,12,15,18-tetracosatetraenoic acid (24:4ω6) were isolated from beef testis lipid, the structures determined via ozonolysis, and characterized by retention time data on GLC analysis.

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REFERENCES

1. Evans, H. M., S. Lepkovsky and E. A. Murphy, J. Biol. Chem. *106*, 445 (1934).
2. Burr, G. O., and M. M. Burr, J. Biol. Chem. *86*, 587 (1930).
3. Holman, R. T., and S. E. Greenberg, JAOCS *30*, 600 (1953).
4. Aaes-Jørgensen, E., and R. T. Holman, J. Nutrition *65*, 633 (1958).
5. Miller, L. D., The Lipids of Bovine Spermatozoa, Ph.D. Thesis, University of Missouri, 1960, University Microfilms, Ann Arbor, Michigan.
6. Reiser, R., J. Nutrition *44*, 159 (1951).
7. Lustig, B., and E. Mandler, Biochem. Z. *261*, 132 (1933).
8. Rewald, B., Biochem. Z. *202*, 99 (1928).
9. Fullerton, B. and F. W. Heyl, J. Am. Pharm. Assn. *131*, 194 (1924).
10. Marchetti, E., Rass. Clin. Terap. Sci. Affini *48*, 1 (1949).
11. Benoit, J., and A. Wenslaw, Compt. Rend. Soc. Biol. *102*, 45 (1929).
12. Cortland, G. F., and M. C. Hart, J. Biol. Chem. *66*, 619 (1925).
13. Hart, M. C., and F. W. Heyl, J. Biol. Chem. *72*, 395 (1927).
14. Schlenk, H., and J. L. Gellerman, JAOCS *38*, 555 (1961).
15. Schlenk, H., J. L. Gellerman and D. M. Sand, Anal. Chem. *34*, 1529 (1962).
16. Peifer, J. J., and B. Ahluwalia, personal communication.
17. Folch, J., M. Lees and G. H. Sloane-Stanley, J. Biol. Chem. *226*, 497 (1957).
18. Mangold, H. K., in "Dünnschicht-Chromatographic. Ein Laboratoriumshandbuch," E. Stahl, ed., Springer, Berlin, 1962.
19. Privett, O. S., and C. Nickell, JAOCS *39*, 414 (1962).
20. Evans, H. M., S. Lepkovsky and E. A. Murphy, J. Biol. Chem. *106*, 431 (1934).
21. Mead, J. F. and D. R. Howton, J. Biol. Chem. *229*, 575 (1957).
22. Howton, D. R., and J. F. Mead, J. Biol. Chem. *235*, 3385 (1960).
23. Hofstetter, H. H., N. Sen and R. T. Holman, JAOCS *42*, 537–540 (1965).

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Alkaline Methylene Blue Method for Determination of Anionic Surfactants and for Amine Oxides in Detergents

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Abstract

The concn of anionic surfactants in alkaline media has been determined by modification of the methylene blue titration. The method is based on the oxidation of methylene blue chloride to dimethylthionoline, a red dye, in the presence of chloroform and sodium hydroxide. This method in conjunction with the acid methylene blue titration, also may be used to determine the amt of amine oxides in formulated products. For concns of less than 100 ppm a spectrophotometric method was used.

Introduction

ANIONIC SURFACTANTS in alkaline solutions are used industrially in applications such as textile processing, metal cleaning, bottle washing, and fruit and vegetable peeling. In order to maintain the concn of surfactant in the cleaning bath, a quick method of analysis is desirable. The total anionic surfactant historically has been determined by titration with quaternary surfactant using methylene blue as an indicator (2), in an acidic system. Longwell and Maniece (4) reported that with aqueous solutions about pH 7 the chloroform layer turned various shades of pink and blue interfering with the end point. To destroy these colors successive extractions with buffered salt and neutral methylene blue indicator (1,4) were required. A spectrophotometric technique using methyl green (5) as the indicator also required the surfactant solution to be acidic. A titrimetric method using bromocresol green (3) as an indicator for the measurement of anionic surfactants in alkaline media has been published.

An investigation of methods for determining the amt of anionic surfactant in highly alkaline systems led to a modification of the methylene blue titration. The method proposed has been extended to include an analysis for amine oxides in formulated products, based on the cationic nature of amine oxides at low pH and nonionic nature at high pH. Concn ranges investigated titrimetrically were 10 to 0.01% actives. Spectrophotometrically the procedure appears sensitive to 10 ppm.

Experimental

Reagents

Methylene Blue Indicator. Dissolve 0.03 g methylene blue chloride (methylthionine chloride) in 50 ml water. Add 6.6 ml 96% sulfuric acid and mix. Add 20.0 g sodium sulfate (anhydrous) and dissolve. Add water to make up to 1 liter.

Hyamine 1622 Solution (ca. 0.0040 N). Dissolve 1.814 g Hyamine 1622, di-isobutyl phenoxy ethoxy ethyl dimethylbenzyl ammonium chloride (Rohm and Haas), in water and dilute to 1.0 liter. Standardize

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solution against 0.0040 *N* Aerosol OT, 100 %, sodium di-2-ethylhexylsulfosuccinate (American Cyanamid) (2).

Procedure

The weighed sample is dissolved and diluted to 250 ml with water. A 10 ml aliquot is pipetted into a 100 ml glass stoppered graduate containing 15 ml chloroform and 25 ml methylene blue indicator. The stoppered graduate is shaken vigorously. A 15 ml volume of 15% sodium hydroxide solution is added and the stoppered graduate is shaken again. The mixture is then titrated with Hyamine 1622. After each addition of titrant, the graduate is shaken vigorously. The chloroform layer is a dark blue and the water layer is a lighter blue initially. As the titration proceeds, the water layer becomes colorless and the chloroform layer goes from blue, blue violet, violet, to red violet. The end point is reached when the chloroform layer is a clear red or pink containing no blue hue. As the end point is approached, the separation of the chloroform-in-water emulsion is rapid. To give a reasonable titer, it may be necessary to vary the concn of the Hyamine 1622 solution or increase the size of the aliquot of detergent solution.

Calculation

$$\% \text{ Anionic} = \frac{H \times N_H \text{ meq} \times 100}{W'}$$

H = ml Hyamine 1622 solution
N_H = normality Hyamine 1622 solution
meq = milliequivalent weight of anionic
W′ = sample weight defined as

$$W' = \frac{\text{ml in aliquot} \times \text{sample weight (W)}}{\text{solution volume}}$$

When the procedure defined above is used the calculation may be simplified to the following:

$$\% \text{ Anionic} = \frac{H \times N_H \times Eq \times 2.5}{W}$$

Eq = equivalent weight of anionic

Discusion and Results

The basis for the determination of the amt of anionic surfactant by the acid methylene blue titration is the solubility of the anionic-cationic complex in chloroform. Initially, acidic methylene blue reacts with the anionic surfactant to give a chloroform soluble complex. Titration with a stronger cationic releases the methylene blue to the water layer, to complex with more anionic. This process is repeated until an end point is reached, which is defined as the even distribution of the blue color between the water and chloroform layers.

In discussing the behavior of methylene blue, D. C. Abbott (1) reported that in the presence of alkaline solutions (above pH 9.5) and chloroform, methylene blue chloride is readily oxidized to dimethylthionoline giving bright red solutions. In this investigation when 15% sodium hydroxide, methylene blue and chloroform were shaken, an immediate red color developed in the chloroform layer. When surfactant solution, methylene blue, chloroform and 15% sodium hydroxide were shaken, the chloroform layer was blue indicating that the anionic surfactant had complexed with the methylene blue preventing its oxidation to dimethylthionoline. The complexing of the methylene blue and anionic appeared to be preferential to the oxidation of the indicator. Therefore, the titration for the determination of anionic surfactant was carried out in alkaline solution using the formation of the red color as the end point.

TABLE I

Determination of Concentration of 0.2% Anionic Surfactant by Acid and Alkaline Methylene Blue Titrations

Surfactant	NaOH, %	Type of titration	Concentration, % (experimental)
Sodium 2-ethylhexyl sulfate[a]	0	Acid	0.202
	2	Acid	0.211
	5	Alkaline	0.205
	10	Alkaline	0.189
	15	Alkaline	0.205
Sodium lauryl sulfate[b]	0	Acid	0.196
	2	Acid	0.193
	5	Alkaline	0.198
	15	Alkaline	0.195
Sodium dodecyl diphenyl ether disulfonate[c]	0	Acid	0.199
	2	Acid	0.200
	5	Alkaline	0.200
	10	Alkaline	0.202
	15	Alkaline	0.205
Sodium alkyl benzene sulfonate[d]	0	Acid	0.193
	2	Acid	0.192
	5	Alkaline	0.195
	10	Alkaline	0.194
	15	Alkaline	0.191

[a] Tergitol Anionic 08 (Union Carbide Corporation).
[b] Sipon PD (Alcolac Chemical Corporation).
[c] Benax 2A1 (Dow Chemical Company).
[d] Ultrawet 60K soft (Atlantic Refining Company).

Table I gives the results of the analyses of surfactant solutions (calculated as 0.2% anionic) by methylene blue titrations. With increasing percentages of sodium hydroxide (i.e., 2, 5, 10, 15%) the end point in the chloroform layer changed from matching blue colors for 2%, to red-violet for 5%, to red for 15%. With 5% sodium hydroxide, the water layer was blue, with 10% light blue, and with 15% almost colorless. For a theoretical concn of 0.200% anionic the range of experimental values was 0.189 to 0.211 (Table I). Since the red color of the end point depends on the demethylation-oxidation of the methylene blue, the higher levels of sodium hydroxide provide a more rapid titration and a more definitive end point. Some surfactants cause the chloroform layer to retain a milky appearance throughout the acid methylene blue titration making the matching of blue colors difficult. Using the alkaline titration provides a means to obtain a sharper end point. This analysis was found to be applicable for compounds of high surface activity as well as those of borderline surface activity.

The alkaline methylene blue titration was employed over a wide concn range for sodium 2-ethylhexyl sulfate (Tergitol Anionic 08). For the concns other than 0.05 to 0.2%, the concn of the quaternary solution and/or the volume of detergent solution was adjusted to obtain a reasonable titer. The results are given in Table II. The precision for 100 ppm and below was not good. Deviation from the theoretical concn was 9% for 100 ppm; for the higher concns 4%or less.

By using both the alkaline methylene blue titration and the acid methylene blue titration, detergent formulations containing amine oxides were analyzed. Based on the behavior of alkyl dimethylamine oxides

TABLE II

Titrimetric Results over Concentration Ranges of Sodium 2-Ethylhexyl Sulfate by Alkaline Methylene Blue Titration

Theoretical active concn, %	Experimental active concn, %
0.005	0.0042
0.010	0.0107
0.050	0.0515
0.100	0.100
0.200	0.205
2.00	2.08
10.0	10.4

TABLE III

Determination of Percentage of Anionic Surfactant and Amine Oxide in Detergent Formulations

Ingredients	*Formulations (parts on 100% active basis)*		
	I	*II*	*III*
Sodium alkyl benzene sulfonate[a]	....	20	20
Sodium alkyl ethoxy sulfate[b]	25	....	5
Lauryl dimethyl amine oxide[c]	5	5	5
Sodium xylene sulfonate	....	10	5
Tetrapotassium pyrophosphate	....	5	5
Analytical results			
Anionic, %	25.4	19.9	26.5
Amine oxide, %	4.6	5.2	5.9

[a] Ultrawet 60 K soft (Atlantic Refining Company).
[b] Tergitol Anionic 15-S-3S (Union Carbide Corporation).
[c] Ammonyx LO (Onyx Oil and Chemical Company).

as nonionic surfactants above pH 7, and as cationic surfactants below pH 3 the anionic surfactant was measured by alkaline methylene blue titration and acid methylene blue titration measured the anionic surfactant not complexed with amine oxide. The difference in the amt of anionic found by these two titrations was calculated as the amt of amine oxide in the formulation. The results given in Table III show that this technique is applicable to the analysis of detergent formulations containing amine oxides.

For the determination of anionic surfactant in alkaline media at concns of 100 ppm and less, a spectrophotometric method has been employed. The procedure is similar to that in *Standard Methods—Water and Waste-Water* (6). Measured amts of surfactant, chloroform, methylene blue and sodium hydroxide were shaken until all available methylene blue was oxidized to dimethylthionoline. These dyes are light sensitive and fade on prolonged exposure. Therefore, all measurements were carried out on a comparable time basis. Absorbances of solutions for various concns of sodium 2-ethylhexyl sulfate were taken at 547 mμ. The plot of absorbance versus concn was linear.

REFERENCES

1. Abbott, D. C. Analyst *87*, 286–293 (1962).
2. ASTM Standards, D1681.
3. Lew, H. Y., JAOCS *41*, 297–300 (1964).
4. Longwell, J., and W. D. Maniece, Analyst *80*, 167–171 (1955).
5. Moore, W. A., and R. A. Kolbeson, Anal. Chem. *28*, 161–165 (1956).
6. Standard Methods for the Examination of Water and Wastewater, APHA, AWWA and WPCF, 11th ed., 246–251 (1960).

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Kinetic Studies of Detergency. III. Dependence of the Dupré Mechanism on Surface Tension

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Abstract

The kinetics of the removal of thin films of pure radioactive tristearin from a stainless steel surface by detergent solutions in a circulation cleaning system were used to study the effect of the surface tension of the detergent solution on the Dupré mechanism and the flow mechanism.

Lowering the surface tension reduced the effectiveness of the Dupré mechanism for both species 1 soil and species 2 soil, as expected. Lowering the surface tension increased the efficiency of the flow mechanism for both species of soil, but, at the present time, it is not known how much of the increase can be ascribed to the lowered surface tension, and how much is due to the increased concn of the surfactant that was used to lower surface tension.

Introduction

KINETIC ANALYSIS of the removal of tristearin from stainless steel indicates that two species of soil are present (2). The difference between the two species has not yet been resolved, but can probably be attributed to a difference in the attractive force(s) between the substrate and the two soil species. Both species are removed simultaneously and independently by two soil removal mechanisms: *a*) a time-dependent flow mechanism, and *b*) a time-independent interfacial mechanism (1,3) called the Dupré mechanism (4) in honor of the French scientist, M. Athanase Dupré (1869) who first developed the equations relating surface tension forces at a gas-solid-liquid interface. The force specifically associated with the air-solution interface is surface tension, which is probably responsible for this latter mechanism. The Dupré mechanism, in other words, might be visualized as tearing soil from the surface by the surface tension of the advancing or receding interface.

If this explanation is valid, the effectiveness of the Dupré mechanism should be related to surface tension; the mechanism should be most effective in solutions of high surface tension and diminish in solutions of lower surface tension. The experiment described below was designed to establish the relationship (if any) between surface tension and the Dupré mechanism.

Theoretical Aspects

Although the equations set up in an earlier paper (2) are correct, they do not allow separate consideration of the two cleaning mechanisms. This can be achieved by describing the cleaning process as:

$$-d(A)/dn = K_1(A)$$

where (A) represents the amount of species 1 soil remaining after n washes, K_1 is the rate constant for species 1 soil removal, and n the number of washes, each of constant time t^1.

This can be integrated to give

$$\ln(A) = \ln(Ao) - K_1 n \qquad (1)$$

where (Ao) represents the amount of species 1 soil at zero washes. K_1 represents the cumulative rate of soil removal by both the flow mechanism F and the Dupré mechanism D; i.e.

$$K_1 = F_1 t^1 + D_1$$

where t^1 is the time duration of one washing treatment. By substitution in (1);

$$\ln(A) = \ln(Ao) - (nF_1t^1 + nD_1), \text{ or}$$
$$\ln(A) = \ln(Ao) - (F_1t + nD_1)$$

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