Identification of Polyoxyalkylene-type Nonionic Surfactants by Paper Chromatography[1]

GEORGE L. SELDEN and **J. H. BENEDICT**, The Procter & Gamble Company,
Ivorydale Technical Center, Cincinnati, Ohio 45217

Abstract

Despite the increasing importance of nonionic surfactants, methods for their identification have been limited to specific groups. Accordingly, a general analytical scheme for the identification of polyoxyalkylene-type nonionic surfactants was developed, which uses qualitative tests and two paper chromatographic solvent systems. This procedure differentiates among the ethoxylates of alcohols, thioalcohols, alkyl phenol, fatty acids, polyoxyethylene polyoxypropylene acids, sorbitan fatty esters, fatty amides, polyoxyethylene glycols (PEG) condensates. In addition, individual members of a class can be separated and identified. The method is applicable to a variety of detergent products and to mixtures of more than one nonionic. As little as 7 mg of a nonionic surfactant are sufficient for complete characterization.

Introduction

MANY ANALYTICAL TECHNIQUES have been used for the isolation, identification and quantitative estimation of alkylene oxide adducts. Most of these methods are nonspecific or are specific for certain groups of polyoxyalkylene nonionic surfactants.

Gravimetric, volumetric and colorimetric methods for the quantitative determination of nonionics are widely used for examining detergent products (10,11,14). These methods are limited to the determination of nonionics as a group, and no indication of the class or of the chemical formula is obtained for an unknown product.

Color tests for the detection of polyoxyalkylene nonionics have been devised (4,7,12), but these tests are limited to the detection of nonionics as a group or as a class, such as alkyl phenol ethoxylates. Consequently, they can characterize the various classes but do not give an indication of the specific compound present. Some of these tests require an appreciable amount of sample.

Infrared techniques can be used to determine the class of nonionic surfactant (9). However, only limited distinctions can be made between different nonionics belonging to the same class. The method is subject to interferences because other materials or more than one nonionic are frequently present.

Chromatographic techniques, such as column, paper, and thin-layer chromatography, are capable of resolving members of a homologous series and possess the potential for resolving different types of nonionic compounds. Column chromatography was used (3,8) to separate different alkyl polyoxyethylene (POE) ethers, alkyl phenol POE ethers and alkyl POE esters from other members of the group based upon ethylene oxide content. Thin-layer chromatography has been used to characterize various nonionic surfactants in surface and waste waters and also to estimate the trace levels that are present (2,5,6). None of these procedures, however, provide an overall system for characterizing these materials by type of nonionic surfactant and by size of the polyoxyethylene group.

In the present work, two solvents were developed which permit the paper chromatographic identification of commercially available nonionic surfactants. The differences in R_f value and the responses to color tests serve to divide the nonionics into classes. To cover the entire field of nonionic surfactants, 50 representative nonionics, selected from about 300 commercially available nonionics, were examined. An outline of the method is presented in Fig. 1, and the experimental details follow.

Experimental Procedures

Apparatus

Standard chromatography equipment was used in this work; no special equipment is required. Whatman chromatography paper, grade 1, was used to prepare the chromatograms. Ascending chromatography was used throughout this work.

Reagents

Draggendorff's, Millon's, and the hydroxamate-ferric chloride reagents for the color tests used in this method are described by Block et al. (1). Toennies' reagent (sodium nitroprusside) is an equal mixture of reagents 1 and 2 prepared at double strength according to Block et al. (1).

Solvent A for paper chromatography is a mixture of 20 parts ethanol, 10 parts concentrated HCl and 70 parts water. Prior to chromatography with this solvent, the paper is impregnated with cetyl alcohol by pulling it through a 2% cetyl alcohol solution in acetone and then allowing the acetone to evaporate. Solvent B is a mixture of 65 parts n-heptane, 20 parts glacial acetic acid and 15 parts n-butanol. No impregnation of the paper is required.

Procedure

Inorganic matter, anionics, cationics, fats, fatty acids and hydrocarbons must be removed prior to analysis as described later. The resulting nonionic fraction is then used for classification and identification of the nonionic.

Preparation of the Nonionic Fraction

No complete scheme for the removal of extraneous materials can be given, since the products that contain nonionic surfactants vary widely in composition. In solid detergent products, the organic matter is extracted with ethanol. If only nonionics are present in the alcohol extract, the nonionic is identified directly by the outlined method. If in addition to the nonionics, anionics or cationics are present, the alcohol soluble matter is separated by ion exchange on a monobed resin column (13). The nonionics in the effluent of the column are then classified and

---

[1] Presented at the AOCS Meeting, Washington, D.C., April, 1968.

IDENTIFICATION SCHEME

1. Isolate nonionic fraction—alcohol soluble, ion exchange, silica gel chromatography, etc.
2. Test for polyether—Draggendorff's reagent
3. Test for reactive groups—spot tests

Positive Spot Test	Type Surfactant	Identify in Solvent System
Millon's	Alkyl Phenol POE	A + B
Toennies'	POE Thio Ether	A + B
Hydroxamate $+FeCl_3$	POE Ester or POE Sorbitan Fatty Ester	B

4. Classify by running in Solvent System A—samples which react only with Draggendorff's Reagent

Location in A	Type Surfactant	Identify in Solvent System
No movement	POE Amide	B
Solvent Front	PEG	B
Definite R_f	Alcohol POE Ether	A + B
Streaks	POP-POE Polymer	A + B

FIG. 1. Analytical scheme for the identification of polyether nonionic surfactants. See text for composition of solvent systems A and B.

identified by the paper chromatographic separations described below.

With liquid detergent products, no separation of organic and inorganic matter is required. Ionic and nonionic materials in liquid detergent products are separated directly by ion exchange on a monobed resin column.

Where nonionics are mixed with fatty materials, water was removed first, and then the nonionic isolated by column chromatography on silica gel.

Classification of Nonionics

First, the presence or absence of polyoxyalkylene nonionic surfactants in the nonionic fraction is established. The nonionic fraction is spotted on filter paper and sprayed with Draggendorff's reagent. If an orange-red color develops, nonionics are present and the following color tests are used to divide the polyoxyalkylene nonionics into classes (Fig. 1).

The nonionic spotted on the filter paper is sprayed with Millon's reagent and heated for 10 min to develop the color. If a red-brown color is observed, an alkyl phenol ethoxylate is present. If the test is negative, another portion of the nonionic is sprayed with Toennies' reagent. When a red-purple color is observed, the nonionic is classified as alkyl thio ethoxylate. In case this test is negative, the nonionic, spotted on filter paper, is sprayed with the freshly prepared alkaline hydroxylamine reagent; after the paper is dried, it is sprayed with ferric chloride. If a red-brown color develops, the nonionic is classified as fatty ester ethoxylate or sorbitan fatty ester ethoxylate.

If the spot test with Draggendorff's reagent is positive, but the other color tests are negative, the nonionic is chromatographed in solvent A. If the nonionic has migrated with the solvent front in solvent A, classification as polyoxyethylene glycol (PEG) is made. If the nonionic forms a streak, either rising up from the origin or trailing up to the solvent front, the classification is a polyoxyethylene-polyoxypropylene condensate. If the nonionic remains at the starting point, it is classified as fatty amide ethoxylate. If the nonionic has a specific R_f value between 0.1 and 0.9 in solvent A, classification as alcohol ethoxylate is made.

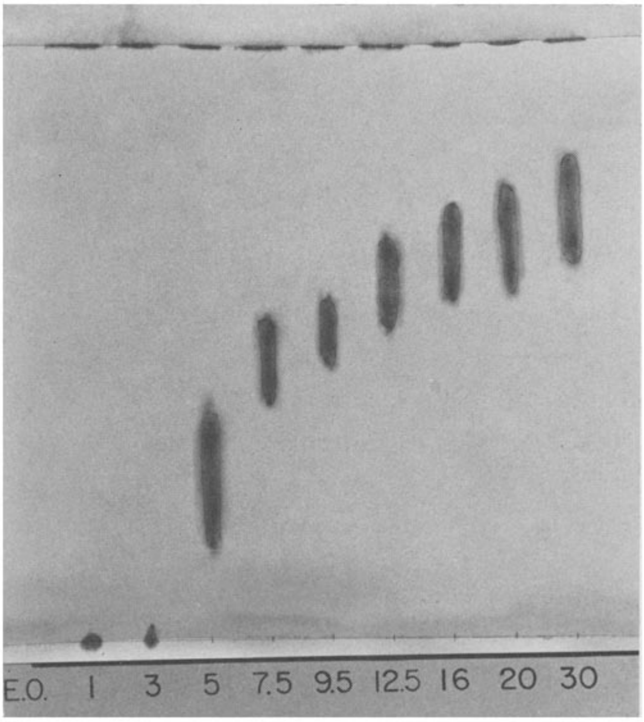

FIG. 2. Chromatogram run in the A system for a series of isooctyl phenol ethoxylates with oxyethylene chain lengths increasing from left to right. The uncombined PEG is the small band at the solvent front.

Identification of Nonionics

After division of the polyoxyalkylene nonionics into classes, individual nonionics are identified by paper chromatography of the unknown with reference nonionics (Table I and II) of the previously established class in solvent A or B or both. With solvent A

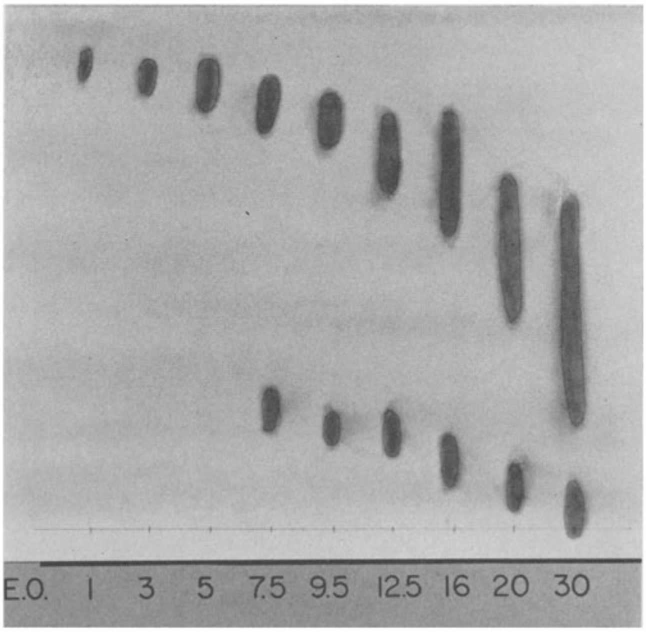

FIG. 3. Chromatogram run in the B system for same series as shown in Fig. 2. The free PEG is the lower spot for each pair.

TABLE I

R_f's of Nonionics Chromatographed in Solvent A[a]

Type and Name	EO[b]	μg[c]	$R_f \times 100$[d]
Alkyl Phenol Ethoxylates			
Isooctyl phenol ether	3	100	8
Isooctyl phenol ether	9.5	10	51
Isooctyl phenol ether	16	10	64
Nonyl phenol ether	4	50	0
Nonyl phenol ether	6	25	18
Nonyl phenol ether	9.5	10	30
Nonyl phenol ether	12	10	31
Nonyl phenol ether	15	10	37
Nonyl phenol ether	30	10	51
Dodecyl phenol ether	10	10	4
Dodecyl phenol ether	48	10	21
Higher alkyl		10	(2),31,(64)
Alcohol Ethoxylates			
Lauryl ethoxylate	12	10	4,35
Tridecyl ethoxylate	12	10	47
Tridecyl ethoxylate	15	10	54
Alcohol ethoxylate		10	50
Alkyl ethoxylate		10	57
Alkyl Thio Ethoxylates			
Tert. dodecyl thio ether		10	70
Alkyl thio ether		10	60

[a] Solvent A is 20:10:70 ethanol:HCL:H_2O; cetyl alcohol impregnated paper.

[b] Units of ethylene oxide per molecule.

[c] Micrograms of nonionic spotted on the chromatogram (optimum working level).

[d] Values in parenthesis are faint spots; unreacted polyoxyethylene glycol appears at or near the solvent front.

about 16 hr (overnight) and with solvent B 2 hr chromatography time are recommended. The nonionics are visualized on the chromatogram by spraying with Draggendorff's reagent.

Discussion

Draggendorff's reagent (bismuth subnitrate and KI in acetic acid) gives an extremely sensitive color reaction with POE groups. Since nonionic surfactants used in detergent products have POE groups attached to the hydrophobic chain, this reagent is very convenient for the detection of nonionic surfactants. With most nonionics as little as 10 μg are easily detectable. The sensitivity of this reaction increases with the number of moles ethylene oxide per molecule. Therefore, nonionics with long POE chains give a more sensitive color reaction than those with short POE chains.

TABLE II

R_f's of Nonionics Chromatographed in Solvent B[a]

Type and Name	EO[b]	μg[c]	$R_f \times 100$	
			Nonionic	PEG
Fatty ester ethoxylate				
Coconut ethoxylate	5	25	91	....
Coconut ethoxylate	15	25	41, 85	....
Stearate ethoxylate	5	25	77, 92	....
Stearate ethoxylate	10	25	75, 90	....
Stearate ethoxylate	15	25	63, 87	21
Stearate ethoxylate		25	49, 80	15
Stearate ethoxylate	50	25	40, 76	12
Rosin ester ethoxylate	5	25	78, 91	21
Sorbitan fatty ester ethoxylates				
Sorbitan monolaurate POE		25	51, 82	17
Sorbitan monopalmitate POE		25	51, 82	17
Sorbitan monostearate POE		25	51, 82	17
Sorbitan tristearate POE		25	87	....
Sorbitan monooleate POE		25	51, 82	17
Sorbitan trioleate POE		25	87	....
Fatty amide ethoxylate				
Coconut amide ethoxylate	5	25	45, 90	18
Tallow amide ethoxylate	5	25	44, 90	16
Tallow amide ethoxylate	15	25	72	11
Polyoxyethylene glycols				
PEG 200	5	50	....	36
PEG 300	7	25	....	31
PEG 400	9	10	....	28
PEG 600	14	10	....	23
PEG 1500	35	10	....	16
PEG 4000	91	10	....	4
PEG + PPG polymers[d]				
PEG–16	2.5	10	73	....
PPG–16	22	10	46	....
PPG–21	7	10	75	
PPG–21	18	10	50	
PPG–30	4.4	10	76	....
PPG–30	17	10	47	
PPG–30	160	10	3	

[a] Solvent B is 65:20:15 heptane:acetic acid:butanol.

[b] Units of ethylene oxide per molecule.

[c] Micrograms of nonionic spotted on chromatogram (optimum working level).

[d] Polyoxyethylene and polyoxypropylene block polymers.

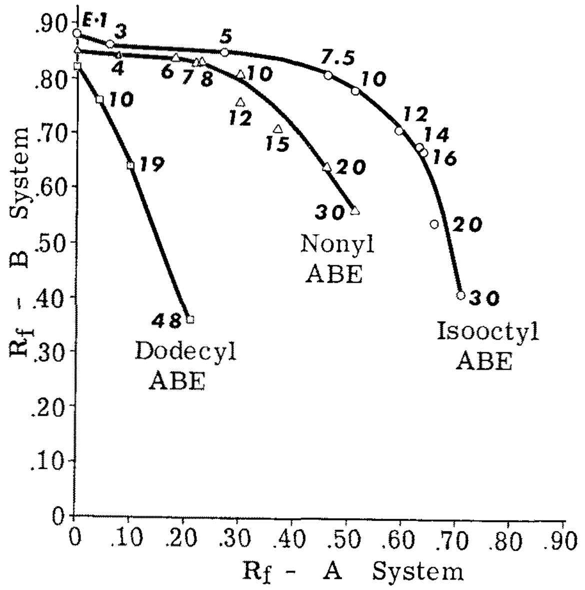

FIG. 4. R_f values for three series of alkyl phenol ethoxylates in solvent system A versus system B. Numerals beside each point indicate the units of ethylene oxide per molecule.

Toennies' reagent (sodium nitroprusside), Millon's reagent (mercuric nitrate in nitric acid) and the hydroxamate-ferric chloride reagent are specific for individual classes of nonionics. Lower limits of detection with Toennies' and with Millon's reagent are 1 mg and with the hydroxamate-ferric chloride reagent 5 mg of the nonionic. Therefore, these reagents are convenient for spot tests on filter paper, where milligram quantities can be easily applied.

Identification of many commercially available ethoxylated nonionic surfactants is made possible by paper chromatography of the nonionic in one of the two solvents. In mixtures where more than one nonionic class is present, the nonionic surfactants can be identified. In simple mixtures of the same class, individual nonionics can frequently be identified.

A typical separation in the A system is illustrated in Fig. 2. The chromatogram consists of a series of iso octyl phenol ethoxylates with increasing oxyethylene chain length from left to right. This is a reversed phase system and migration shows a direct relationship to the oxyethylene-lipophile ratio. The lipophile is fixed in this series and therefore the R_f value increases with increasing oxyethylene chain length.

Conversely, if the molecular weight of the lipophile portion is increased, the R_f values for the entire series is shifted downward. Only the fatty amides and the fatty ester ethoxylates do not move in the A system.

The same series of iso octyl phenol ethoxylates, but run in the B system, is shown in Fig. 3. This is a normal adsorption system and migration has an inverse relationship to polarity and molecular weight. Hence, the R_f values decrease with increasing oxyethylene chain length. An increase in the alkyl chain length will also decrease the R_f value. Uncombined polyoxyethylene glycol appears as a small spot in the lower half of the chromatogram below the alkyl phenol ethoxylates.

Some of the commercial grade nonionics contain free polyoxyethylene glycol (PEG), which is not combined with the hydrophobe, and thus they are separated into more than one component during chromatography. In solvent A, the spot with a specific R_f value is the nonionic ether (Table I). The other spot is on the solvent front and is the uncombined PEG. In solvent B, frequently two or more spots are visualized with Draggendorff's reagent. One or more spots are associated with the nonionic surfactant and the other spot is generally free PEG (Table II). Most fatty ester ethoxylates are separated into the monoester and the diester in addition to the one spot for uncombined PEG. Two spots represent the two major reaction products (monoester and diester) in commercial grade POE sorbitan fatty ester. The amide ethoxylates show three spots. The upper spot is probably the monosubstituted amide, the middle spot is the disubstituted amide, and the lower spot is free PEG. As a result, this method for the identification of polyoxyalkylene nonionics can be extended to check the reaction products in the manufacture of nonionic surfactants.

With certain nonionics, elongated spots are observed on chromatograms. This is due to the wide range of different POE chain lengths in the individual nonionic and is not an indication of poor chromatographic separation. This is seen particularly with PEG + PPG block polymers which have two moieties with wide ranges of chain length. The R_f may be obtained in these by spotting diminishing increments of sample and noting the midpoint of the major components.

Application of the Method

Although final identification must be made by direct comparison with standards, it is not necessary to compare a sample with every standard. Paper chromatographic R_f values are sufficiently constant to permit tabulation of R_f values for standards. Thus, a sample may be run, its R_f calculated and three or four standards selected for direct comparison.

For those surfactants which migrate in both solvent systems, this comparison is also useful for identification purposes. Of the six classes of polyoxyethylene surfactants, four migrate in both systems. A typical comparison is illustrated in Fig. 4 which shows the R_f's for a series of alkyl phenol ethoxylates with different alkyl chain lengths. A distinct curve is obtained for those ethoxylates having the same alkyl phenol base.

The applicability of the paper chromatographic method for identification of nonionic surfactants was evaluated on finished products, such as dishwasher and automatic washer products, solid and liquid detergent products, shampoos, creams and lotions. Specific identifications were made in all cases. As little as 7 mg of the nonionic was sufficient for complete characterization.

REFERENCES

1. Block, R. J., E. L. Durrum, G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," 2nd ed., Academic Press, Inc., New York, 1958, pp. 131, 262, 353 and 361.
2. Burger, K., Z. Anal. Chem. *196*, 259 (1963).
3. Kelly, J., and H. L. Greenwald, Jr., Phys. Chem. *62*, 1096 (1958).
4. Kortland, C., and J. F. Dammers, JAOCS *32*, 58 (1955).
5. Patterson, S. J., E. C. Hunt and K. B. E. Tucker, J. and Proc. Inst. Sewage Purif. Part 2, 190–198 (1966).
6. Patterson, S. J., C. C. Scott and K. B. E. Tucker, JAOCS *44*, 407–412 (1967).
7. Rosen, M. J., Anal. Chem. *27*, 111 (1955); Rosen, M. J., and H. A. Goldsmith, "Systematic Analysis of Surface-Active Agents," Interscience Publishers, Inc., New York, 1960, pp. 109–153.
8. Rosen, M. J., Ibid. *35*, 2074 (1963).
9. Sadtler, P., ASTM Bulletin *190*, 51 (1953).
10. Schonfeldt, N., JAOCS *32*, 77 (1955).
11. Steele, A. B., and L. D. Berger, Soap Chem. Specialties *32*(2), 48 (1956).
12. van der Hoeve, J. A., J. Soc. Dyers and Colourists *70*, 145 (1954).
13. Weeks, L. E., M. E. Ginn and C. E. Baker, Soap Chem. Specialties *33*(8), 47 (1957).
14. Weeks, L. E., J. T. Lewis and M. E. Ginn, JAOCS *35*, 149 (1958).

[Received April 15, 1968]