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A SYSTEMATIC STUDY OF THE VARIABLES INVOLVED IN THE REVERSE-PHASE THIN-LAYER CHROMATOGRAPHY OF OXYETHYLATED ALKYL SULFATE SURFACTANTS

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SUMMARY

Reverse-phase thin-layer chromatography was used to separate a homologous series of oxyethylated alkyl sulfate surfactants derivable from waste animal fats. The separations were designed on the basis of a systematic study of the variation in the R_F values and spot shapes and areas of the anionic surfactants with the major variables of the chromatographic system. The best separations were obtained with commercially available glass plates covered with a 250-μm layer of Alumina H, Alumina G or Silica Gel G impregnated with a 3–5% (v/v) solution of *n*-dodecanol ethanol, at 15–30° using tanks pre-equilibrated and developed with a 3:2 (v/v) methanol–ammonia solution solvent system. The use of pinacryptol yellow (0.05% w/v in water) together with an ultraviolet viewing chamber was found to be the most satisfactory spot detection procedure. Sample sizes of 0.1–10 μg in 0.5–2.0 μl of solution were applied with a Hamilton syringe or capillary micropipets 2 cm above the base of the plate. A 100-ml volume of solvent was added to the tanks and the solvent was permitted to run a distance of 15 cm from the spotting line. The present study lays the foundation for effecting excellent separations of other homologous series of anionic surfactants.

INTRODUCTION

Mangold and Kammereck[1] were among the first to investigate surfactants containing alkyl groups by thin-layer chromatography (TLC). Separations of alkyl amines, sulfonic acids and fatty acid–triethanolamine condensation products were reported. Alkyl sulfates and sulfonates have been separated on Silica Gel G layers containing 10% (w/w) of $(NH_4)_2SO_4$ using a 1:4 (v/v) acidic methanol–chloroform solvent system[2]. Bey[3] was able to separate anionic from non-ionic surfactants but experienced difficulty in separating both types until reverse-phase chromatography with a 2% dodecanol-impregnated diatomaceous earth was used with a 50:1:50 (v/v) methanol–formic acid–ammonia solution mobile phase. Desmond and Borden[4] found that pinacryptol yellow developed plates gave distinctive colors for individual classes of surfactants under ultraviolet (UV) light. Hofmann (see ref. 2) used an iso-amyl acetate–propionic acid–1-propanol–water (4:3:2:1, v/v) system to separate anionic surfactants. Alkyl sulfates were separated from alkyl sulfonates on hydroxy-

apatite by using a di-*n*-butyl ether–methanol–acetic acid (5:5:1, v/v) solvent system[2]. Chromatographic studies on acid sulfates of aliphatic alcohols with alkyl chains from C_8 to C_{18} have been carried out on paper impregnated with a 5% solution of lauryl alcohol in ethanol with a methanol–ammonia solution (1:1) mixture as the mobile phase. Streaking occurred at temperatures below 35°, compounds remained at the origin and only reasonably differentiated R_F values were reported[5]. Other chromatographic studies on surface-active agents have been reported[6–9] but none of the studies has dealt with a systematic investigation of homologs by TLC. Papers of related interest have been published[10–20].

The surfactants (Table I) used in this investigation were synthesized by The U.S. Department of Agriculture, Eastern Utilization and Development Division, Animal Fats Research Laboratories, Philadelphia, Pa. 19118[21–25]. Our research program was undertaken when attempts by this laboratory to develop a TLC procedure for the separation of these compounds had proved unsuccessful. However, excellent procedures for the gas chromatographic analysis of the fatty alcohols and ether alcohols prior to sulfonation had been developed.

TABLE I

OXYETHYLATED ALKYL SULFATE SURFACTANTS* STUDIED

General structure: $R_1-(OCH_2CH)_x-OSO_3Na^+$ ($x = 1$ or 2)
R_2

No.	Structure	Code No.*	No. of C atoms
1	$C_{12}H_{25}OC_2H_4OSO_3Na$	AF-484-118	14
2	$C_{12}H_{25}(OC_2H_4)_2OSO_3Na$	AF-484-121	16
3	$C_{14}H_{29}OC_2H_4OSO_3Na$	AF-484143	16
4	$C_{14}H_{29}(OC_2H_4)_2OSO_3Na$	AF-484127	18
5	$C_{14}H_{29}OCH_2CH(C_2H_5)OSO_3Na$	AF-484104	18
6	$C_{18}H_{37}OCH_2CH(CH_3)OSO_3Na$	AF-484-152	21
7	$C_{16}H_{33}(OC_2H_4)_2OSO_3Na$	AF-484113	20
8	$C_{18}H_{37}OCH_2CH(C_2H_5)OSO_3Na$	AF-484-94	22
9	$C_{16}H_{33}[OCH_2CH(CH_3)]_2OSO_3Na$	AF-484-99	22
10	$C_{18}H_{37}(OC_2H_4)_2OSO_3Na$	AF-484-145	22
11	$C_{16}H_{33}[OCH_2CH(C_2H_5)]_2OSO_3Na$	AF-484-101	24
12	$C_{18}H_{37}OCH_2CH(CH_3)OSO_3Na$	AF-484-155	24

* All surfactants were synthesized by the Animal Fats Laboratory of the Eastern Regional Utilization and Development Division, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, Pa. 19118. The code numbers refer to their synthesis records.

The long-term aims of the study were (1) to study systematically the experimental TLC variables so as to ascertain their effect on the R_F values and spot characteristics of the surfactants; (2) to develop, on the basis of (1), methods for quantitatively separating surfactant mixtures; (3) to attempt to correlate the R_F values of the surfactants with experimental parameters of the chromatographic system; and (4) to extend the techniques developed to other homologous series of surfactants.

EXPERIMENTAL

Solvents

ACS reagent-grade solvents methanol (1501), ammonia solution (800), sulfuric acid (600) and 2-propanol (1901), supplied by Corco Chemical Co. (Fairless Hills, Pa. 19030), absolute ethanol from U.S. Industrial Chemical Co. (New York, N.Y.) and 1-propanol (PX1815 from Matheson, Coleman and Bell, East Rutherford, N.J.) were used without further purification.

Adsorbents

Commercially prepared TLC plates of dimensions 20 × 20 cm, coated with 250-μm layers, were used.

Alumina G, Alumina H, Silica Gel G, Silica Gel GF and cellulose (Merck) were obtained from Brinkman Instruments Inc. (Westbury, N.Y. 11590), and Alumina G (3011), Alumina H (3111) and Silica Gel G (1011) distributed by Anal. Tech. Inc. (Newark, Del. 19711) were also used.

Polyamide 11 Bakerflex was supplied by Arthur H. Thomas (Philadelphia, Pa. 19104).

Developing chambers

Rectangular glass jars with inner dimensions of $10^3/_4$ × $2^3/_4$ × $10^1/_4$ in. with sealed glass lids (Thomas-Kolb, 2751-B-10, supplied by Arthur H. Thomas) were used. All sides of the tank were lined to a height of 9 in. with Whatman No. 1 filter-paper, except for a 1 in. window at the front of the tank. A 100-ml volume of the solvent system was added to the chambers, which were usually equilibrated overnight. It is also suggested that the lids be greased with a silicone and a metal weight placed on the cover plate so as to minimize the escape of solvent vapor.

Impregnation of plates with n-alkanols

1-Octanol (11508), 1-nonanol (16403), 1-decanol (20484), 1-undecanol (19704), dodecanol (4483), 1-tetradecanol (6463), 1-hexadecanol (4269) and 1-octadecanol (17036), supplied by K&K Laboratories (Plainview, N.Y. 11803), were used as received. Solutions of concentration 1–25 % (v/v) in absolute ethanol were prepared.

The TLC plates were impregnated by spraying, dipping and chromatographing procedures. After air-drying the plates for 1 h, they were stored on a drying rack (Brinkman 041010) in a large desiccating chamber (Boekel–A. H. Thomas 3744-F-20) with no desiccant, until required for use. Surfactants 1 to 6 were run on 250-μm Alumina G plates impregnated with a 3 % (v/v) solution of dodecanol in ethanol and prepared by the three methods of impregnation. A 4:6 ammonia solution (28 %)–methanol solvent system was used. Samples of 1 μl of surfactants were applied to the plate with disposable capillary pipets and the samples were run at 27° for a distance of 15 cm. The most satisfactory results were obtained with plates impregnated by the TLC procedure; the R_F values for the sprayed and dipped plates were very much lower and the spot areas were less compact. Uniformity of plate impregnation appears to be characteristic of the spraying procedure, whereas dipping causes flaking of the adsorbent layer together with a higher concentration of dodecanol towards the bottom of the plate.

Detection of surfactants

The detection reagents investigated are listed in Table II. All of these reagents proved to be excellent detection reagents in the absence of the impregnating agent, but only pinacryptol yellow gave excellent spot formation with the impregnated plates (Table III). The plates should be air-dried for no more than 1 h. The colors and intensities vary markedly with the drying time before spraying and with the time between spraying and viewing in the UV chamber. If the plate is sprayed soon after removal from the TLC chamber and viewed immediately, color variations and intensities are maximal. Re-spraying the plate tends to restore the chromatographed spots.

TABLE II

DETECTION REAGENTS FOR SURFACTANTS

No.	Reagent	Spot color	Comments*
1	Rhodamine B	Orange	Under UV lamp
2	Rhodamine 6G	White	Under UV lamp
3	Bromocresol green	Yellow	
4	Iodine vapor	Brown	
5	Silver nitrate Sodium fluoresceinate	Light yellow, green background	
6	Sulfuric acid	Black	
7	Pinacryptol yellow	Various colors on light blue background	Under long-wave UV lamp

* With plates impregnated with long-chain alcohols all of the detection reagents interacted with the alcohols except pinacryptol yellow.

TABLE III

IDENTIFICATION OF SURFACTANTS WITH PINACRYPTOL YELLOW IN UV LIGHT

Surfactant*	Air-dry 15 min; spray		Air-dry 3 h; spray	
	Observe immediately**	Observe 1 h later	Observe immediately	Observe 1 h later
1	Y (6) LB (1)	DB	PY	LB-G
2	B (6) LB (1)	DB	PY	LB-G
3	BY (6) G (1)	DB	PY	LB-G
4	DB (6) B (1)	DB	PY	LB-G
5	LB (7)	DB	PY	LB-G
6	DB (6) G (1)	DB	PY	LB-G
7	O (4) OB (3)	DB	PY	LB-G
8	G (7)	DB	PY	LB-G
9	LB-G (7)	DB	PY	LB-G
10	BRY(2)YB(2)LB(3)	DB	PY	LB-G
11	LB (7)	DB	PY	LB-G
12	Y(2)LB(3)BY(1)YB(1)	DB	PY	LB-G

* See Table I.
** Numbers in parentheses indicate the number of times the indicated color was observed. Yellow (Y); bright yellow (BY); pale yellow (PY); blue (B); bright blue (BB); pale blue (PB); gray (G); orange (O); orange with blue rim (OB); yellow and blue center (YB); blue and yellow center (BY); brownish yellow (BRY). See also ref. 4, p. 553.

Rhodamine B (17470), Rhodamine 6G (17863), bromocresol green (14335), sodium fluoresceinate (13877) and pinacryptol yellow (16858) were supplied by K&K Laboratories. ACS reagent-grade iodine, silver nitrate and sulfuric acid were used. A Chromato-vue C-5 UV viewing chamber (Ultra-Violet Products Inc., San Gabriel, Calif.) equipped with 366 nm, 254 nm and white-light lamp sources was used to locate the surfactant spots. The developed TLC plates were sprayed with a 0.05% (w/v) solution of pinacryptol yellow in water; previous investigators used ethanol as the solvent. Thomas-John spray bottles (J-10-2753, A. H. Thomas) or Camag Uni-Spray Aersol Pak cans (26-510) were used in a hood to spray the plates.

Humidity studies

A Camag (25-400) Vario KS chamber and sulfuric acid–water solutions with known humidities were used.

Sample application

Samples were applied with Hamilton syringes (701-N or 7001-N), Camag capillary micropipets (27-130 or 27-140), Camag micropipets (27-112) or Drummond Microcaps.

Samples were spotted on unactivated TLC plates 1.5 cm from the bottom edge, 1.5–2.0 cm apart, and the running distance was usually 15 cm. A sample size study using 0.25–10 μl of 1% (w/v) of selected surfactants in methanol indicated that the most satisfactory results were obtained with 0.5–2.0 μl of sample containing 5–20 μg of surfactant. Alumina H gave the smallest spot areas.

Standard conditions

All runs were carried out at 25 $\pm$ 2° without special humidity control in "saturated tanks"; in most instances, 2–4 runs were carried out for each variable studied. The deviations of the average R_F values were $\pm$ 0.01–0.05 for each study with an overall mean deviation of $\pm$ 0.02. The solvent system was changed every third run. Usually, two plates were placed in tanks containing 100 ml of solvent. Timing was carried out with a Time-It (69235, minute model, Precision Scientific Co.). No reference standard was used.

RESULTS AND DISCUSSION

In order to ascertain the optimum conditions for designing quantitative separations of the surfactants, the following variables were studied: (1) the variation of the solvent system; (2) the variation of the reverse-phase impregnation agent; (3) the variation of the adsorbent; (4) the variation of temperature; and (5) the variation of humidity.

Variation of solvent system

Six selected surfactants (1, 3, 5, 6, 7 and 8, Table I) were spotted on Silica Gel GF plates, impregnated with a 3% (v/v) solution of 1-dodecanol in ethanol and run with ammonia solution (28%)–methanol solvent systems containing 2:8, 3:7, 4:6, 5:5, 6:4 and 8:2 (v/v) ratios of the solvents. The data are summarized in Table IV. Graphs of R_F values against increasing amounts of ammonia in the solvent system are

TABLE IV

VARIATION OF R_F VALUES WITH SOLVENT SYSTEM ON SILICA GEL GF

Adsorbent, Silica Gel GF (Brinkman); impregnating agent, 5% (v/v) *n*-dodecanol in ethanol; solvent system, NH$_3$ (28%)–methanol; detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; temperaute, 27°; sample size, 1 μl; time, 85 min.

Surfactant	Ratio of NH$_3$ to methanol (v/v)					
	2:8	3:7	4:6	5:5	6:4	8:2
1	1.0	0.73	0.68	0.52	0.38	0.00
3	1.0	0.80	0.52	0.33	0.15	0.00
5	1.0	0.81	0.43	0.21	0.10	0.00
6	1.0	0.82	0.38	0.16	0.06	0.00
7	1.0	—	0.26	0.06	0.00	0.00
8	1.0	0.89	0.17	0.05	0.00	0.00

shown in Fig. 1. At concentrations of methanol above 65% (v/v), the compounds were all compressed near the solvent front owing to the decreased polarity of the system, which increased the mobility of the long-chain aliphatic compounds. On the other hand, the highly polar water–ammonia medium in solvent systems containing more than 65% (v/v) of 28% ammonia solution resulted in a decrease in the mobility of the surfactants. The polar–polar and non-polar–non-polar interactions among the surfactant, solvent system, impregnated dodecanol and adsorbent must be optimally balanced in order to achieve highly differentiated R_F values for the surfactants. The results suggest that solvent systems containing 40–60% (v/v) of 28% ammonia so-
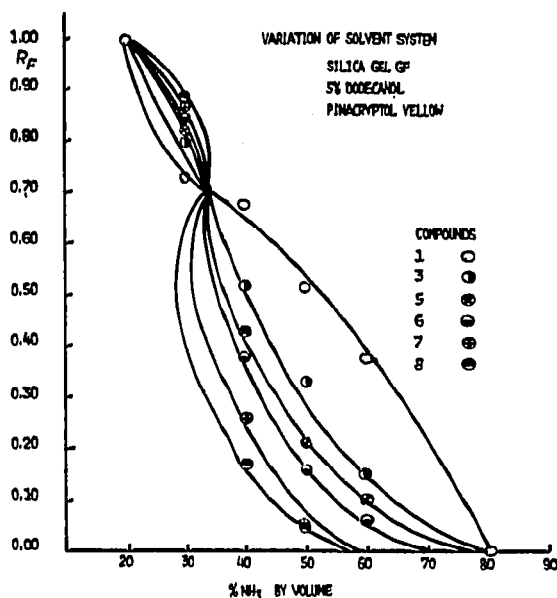

Fig. 1. Variation of the R_F values of surfactants with the percentage by volume, of 28% ammonia solution in the methanol–28% ammonia solution solvent system. Formulae for the surfactants are given in Table I.

TABLE V

VARIATION OF R_F VALUES WITH SOLVENT SYSTEM ON ALUMINA H

Adsorbent, Alumina H (Brinkman); impregnating agent, 3% (v/v) n-dodecanol in ethanol; solvent system, NH$_3$ (28%)–methanol; detection spray, 0.05% pinacryptol yellow in water; run length, 15.5 cm; temperature, 27°; sample size, 1 µl.

Surfactant	Ratio of NH$_3$ to methanol (v/v)				
	2:8	4:6	5:5	6:4	8:2
1	0.69	0.65	0.71	0.60	0.31
3	0.62	0.57	0.38	0.39	0.04
5	0.66	0.50	0.23	0.22	0.06
6	0.66	0.40	0.14	0.10	0.00
7	0.68	0.17	0.06	0.00	0.00
8	0.68	0.14	0.00	0.00	0.00
Separation*	P	E	G	G	P

* E = excellent; G = good; P = poor.
The 2:8 system gave spots which all moved with the head of the spot at the solvent front.

lution should give excellent separations, and the results in Table V support this conclusion.

A second series of runs was made in order to investigate the effect of changing the alcohol used in the solvent system. Ethanol, 1-propanol and 2-propanol were substituted for methanol in the 2:3 (v/v) ammonia solution (28%)–methanol solvent system and the results are given in Table VI. Ethanol gave very erratic results. Some compounds could not be located with the detection reagent and very irregular secondary solvent fronts were formed, most likely due to the chromatographing of the dodecanol impregnating layer. The isomeric propanols increased the mobility of the surfactants to such an extent that the compounds all moved as one band near the solvent front. Further variation of the alkanol concentrations could possibly lead to the discovery of other suitable solvent systems, but this parameter was not studied. The methanol–ammonia solvent system was concluded to be the system of choice.

TABLE VI

EFFECT OF VARIATION OF ALCOHOL IN SOLVENT SYSTEM ON R_F VALUES

Adsorbent, Silica Gel G (Brinkman); impregnating agent, 5% (v/v) n-dodecanol in 95% ethanol; solvent system, ammonia solution–alkanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; sample size, 1 µl.

Surfactant	Methanol	Ethanol	1-Propanol	2-Propanol
1	0.73		0.91	1.0
3	0.61		0.91	1.0
5	0.55		0.93	1.0
6	0.47		0.88	1.0
7	0.28		0.91	1.0
8	0.15		0.96	1.0

Variation of the impregnating agent

Table VII summarizes the results of a series of runs in which the concentration of dodecanol in 95% ethanol was varied from 3 to 25% (v/v). In this study, a mixture of all six surfactants (1, 3, 5, 6, 7 and 8, Table I) and the individual components of the mixture were chromatographed on the same plates. Separations were evaluated on the basis of both R_F values and compactness of the spots. Excellent separations were obtained with plates impregnated with 3 or 5% dodecanol. The R_F values decreased and the elongation of the spots increased with increasing concentration of the impregnating agent. High concentrations of dodecanol probably overload the plate, and also increase interactions between the long aliphatic chains of the surfactant and dodecanol molecules.

TABLE VII

EFFECT OF VARIATION OF IMPREGNATING AGENT CONCENTRATION ON R_F VALUES

Adsorbent, Alumina G (Brinkman); impregnating agent: *n*-dodecanol in 95% ethanol; solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; temperature, 27°; sample size, 1 µl.

Surfactant	R_F *values*[*]				
	% n-Dodecanol by volume[**]				
	3	*5*	*10*	*15*	*25*
1	0.69	0.67	0.60	0.56	+
3	0.74	0.51	0.43	0.46	+
5	0.45	0.40	0.35	0.34	+
6	0.35	0.28	0.27	0.21	+·
7	0.18	0.15	0.12	0.13	+
8	0.12	0.10	0.09	0.10	+
Separations[***]	E	E	F	F	P

[*] Average of at least three values.
[**] + Poor irregular spots. Best zones with 3% and 5% *n*-dodecanol.
[***] E = excellent; F = fair; P = poor.

Two additional studies were carried out in order to determine the effect that the length of the carbon chain in the impregnation agent had on the R_F values and spot characteristics of the surfactants. The results are given in Tables VIII and IX. It was concluded from these experiments that: (1) the best impregnating agents for our chromatographic system were 1-dodecanol, 1-tetradecanol and 1-hexadecanol; (2) the optional concentration range for the impregnating alkanol was between 2 and 5% (v/v) in 95% or absolute ethanol; and (3) alkanols with chain-lengths below 12 and above 16 gave unsatisfactory separations and/or spot characteristics and/or irregular secondary solvent fronts.

Variation of the adsorbent

Alumina G, Alumina H, Silica Gel G, Silica Gel GF, cellulose and Polyamide 11 adsorbents (Table X) were evaluated as possible stationary phases for the surfactant separations. Multiple runs were carried out with the seven adsorbents with the

TABLE VIII

EFFECT OF VARIATION OF IMPREGNATING AGENT ON R_F VALUES

Adsorbent, Alumina G (Brinkman); impregnating agent concentration, 5% alkanol (v/v for liquids, w/v for solids); solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; temperature, 27°; sample size, 1 μl.

Surfactant	R_F values*			
	No. of carbon atoms in the impregnating alcohol			
	12	14	16	18
1	0.67	0.72	0.75	0.77
3	0.51	0.52	0.60	0.05
5	0.40	0.40	0.49	0.00
6	0.28	0.27	0.26	0.00
7	0.15	0.11	0.11	0.00
8	0.10	0.05	0.11	0.00
Separation**	E	VG	F	P

* Average of at least three values. The stearyl alcohol available was technical grade. The dodecanol gave the most satisfactory spot characteristics. The tetradecanol gave faint spots; the 1-hexadecanol gave irregular spots.

** E = excellent; VG = very good; F = fair; P = poor.

TABLE IX

EFFECT OF VARIATION OF IMPREGNATING AGENT ON R_F VALUES

Adsorbent, Alumina H (Brinkman); impregnating agent, n-alkanol in 95% ethanol; solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; temperature, 27°; sample size, 1 μl.

Surfactant	No. of carbon atoms in n-alkanol*								
	8	11	12	12	12	12	14	14	16
	n-Alkanol concentration in ethanol (% v/v)								
	5	5	5	3	2	1	5	3	3
1	0.80	0.65	0.63	0.64	0.59	0.62	0.71	0.59	0.57
3	0.73	0.63	0.50	0.56	0.54	0.59	0.54	0.47	0.52
5	0.73	0.58	0.40	0.49	0.52	0.52	0.36	0.37	0.50
6	0.70	0.56	0.35	0.43	0.52	0.38	0.19	0.24	0.36
7	0.64	0.41	0.19	0.28	0.39	0.21	0.02	0.03	0.20
8	0.60	0.33	0.12	0.14	0.34	0.16	0.00	0.00	0.00
Spot character**	F	F	E	E	G	P	G	F	G

* The 1-nonanol and 1-decanol gave very irregular chromatograms.

** P = poor; F = fair; G = good; E = excellent.

other variables maintained constant. The R_F values for the comparative study are summarized in Table X. As would be expected in reverse-phase chromatography, the differences in R_F values are not large with the different adsorbents, but there were very marked differences in the trial separations and the spot characteristics. All of the aluminas and silica gels gave good separations, but the runs with Alumina H were significantly better.

TABLE X

EFFECT OF VARIATION OF ADSORBENT ON R_F VALUES

Impregnating agent, 3% or 5% (v/v) of n-dodecanol in 95% ethanol; solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; temperature, 27°; sample size, 1 μl.

Surfactant	Adsorbent*						
	I	II	III	IV	V	VI	VII
1	0.67	0.63	0.73	0.68	0.81	0.73	0.69
3	0.51	0.50	0.61	0.52	0.61	0.64	0.59
5	0.40	0.40	0.55	0.43	0.43	0.56	0.53
6	0.28	0.35	0.47	0.38	0.40	0.58	0.48
7	0.15	0.19	0.28	0.26	0.37	0.37	0.29
8	0.10	0.12	0.15	0.17	0.21	0.24	0.19
n-Dodecanol	5%	5%	5%	5%	3%	3%	3%
Separation**	VG	E	G	G	P	P	G

    * I = Alumina G (Brinkman); II = Alumina H (Brinkman); III = Silica Gel G (Brinkman); IV = Silica Gel GF (Brinkman); V = Cellulose (Merck); VI = Polyamide 11 (Bakerflex); VII = Alumina G (AnalTech).
    ** P = poor; G = good; VG = very good; E = excellent.

Variation of temperature

    As most of the work was carried out during the summer in a room that was not air-conditioned but well-shielded from the sun, it was decided to carry out a preliminary investigation of the effect of temperature on the R_F values. The runs below 27° were carried out in the inner chamber of a Sorvall refrigerated centrifuge; the run at 40° was carried out in an incubator. Table XI summarizes the results of this experiment. Except for the run at 40°, the R_F values for the six surfactants are relatively constant at different temperatures, but the spot shapes and sizes clearly suggest that the 5–30° temperature interval gives the most satisfactory separations. In reverse-phase chromatography, one would not expect humidity to play a major role in the

TABLE XI

EFFECT OF VARIATION OF TEMPERATURE ON R_F VALUES

Adsorbent, Alumina H (Brinkman); impregnating agent, 3% (v/v) of n-dodecanol in 95% ethanol; solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05% pinacryptol yellow in water; run length, 15 cm; sample size, 1 μl.

Surfactant	Temperature (°C)					
	−10	5	10	15	27	40
1	0.60	0.67	0.70	0.62	0.65	0.22
3	0.51	0.56	0.59	0.52	0.57	0.14
5	0.40	0.43	0.48	0.43	0.50	0.10
6	0.30	0.36	0.42	0.35	0.40	0.00
7	0.11	0.15	0.25	0.17	0.17	0.00
8	0.00	0.08	0.16	0.10	0.14	0.00
Separation*	F	G	G	VG	E	P

    * E = excellent; VG = very good; G = good; F = fair; P = poor.

mobility of the surfactants because the impregnated surface of the plates is hydrophobic. In adsorption chromatography, the relative humidity would play a more significant role because it alters the activity of the adsorbing surface. With the methanol–ammonia solution solvent system, one might expect a greater loss of solvent from the chromatographic chamber at high temperatures, as the solvent system has a high vapor pressure. Low temperatures give rise to condensation on the walls of the tank.

Variation of humidity

Values for the percentage by weight of H_2SO_4, relative humidity and density of aqueous solutions of sulfuric acid were obtained from the literature[26], and the relative humidity was plotted against the solution density. From this graph, the densities of solutions for systems with relative humidities at 20°C of 10, 20, 30, 40, 50, 60, 70, 80 and 90% were determined by interpolation. The necessary data for preparing the solutions are given in Tables XII and XIII.

TABLE XII

PARAMETERS FOR PREPARING AQUEOUS SULFURIC ACID OF UNKNOWN HUMIDITY

Relative humidity at 20° (%)	Density (g/ml)	H_2SO_4 (% w/v)	H_2SO_4 (g/l)	Conc. H_2SO_4 (ml)
100	1.00	0	0	0
90	1.14	20	227.0	261.7
80	1.21	29	351.0	402.9
70	1.25	34	425.5	489.0
60	1.30	39	504.7	579.7
50	1.34	44.5	597.7	686.5
40	1.39	49	678.8	779.5
30	1.43	53.5	765.0	878.3
20	1.49	59.3	883.0	1013.7
10	1.58	67.3	1063.7	1221.5

TABLE XIII

EFFECT OF VARIATION OF RELATIVE HUMIDITY ON R_F VALUES

Adsorbent, Alumina G (AnalTech); impregnating agent, 5% (v/v) *n*-dodecanol in ethanol; solvent system, 28% ammonia solution–methanol (1:1, v/v); detection spray, 0.05% pinacryptol yellow in water; UV light; run length, 16 cm; temperature, 23°; sample size, 1 μl; time, 129 min.

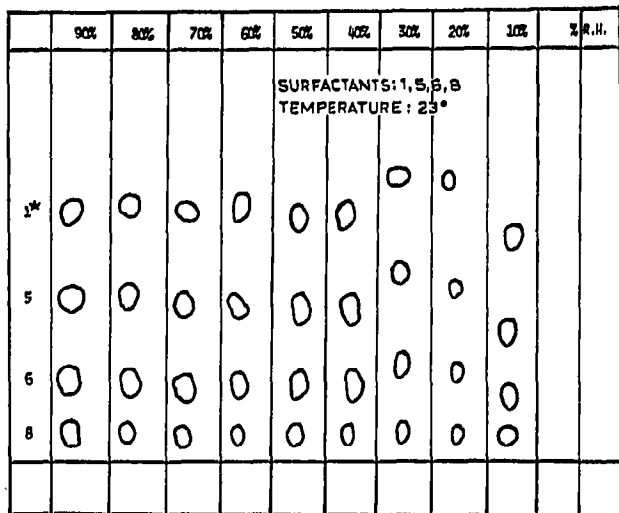| Relative humidity (%)	Surfactant			
	8	6	5	1
10	0.09	0.18	0.33	0.58
20	0.10	0.22	0.43	0.69
30	0.10	0.24	0.46	0.71
40	0.09	0.18	0.37	0.60
50	0.09	0.19	0.38	0.59
60	0.09	0.19	0.39	0.63
70	0.09	0.19	0.39	0.64
80	0.09	0.21	0.41	0.64
90	0.09	0.21	0.41	0.63

Fig. 2. One-dimensional chromatograms of surfactants on 250-μm Alumina G plates impregnated with 5 % (v/v) of n-dodecanol in absolute ethanol with a 1:1 methanol–28 % ammonia solution solvent system. Run length, 16 cm; run time, 129 min. Humidity variation, from left to right: 90, 80, 70, 60, 50, 40, 30, 20 and 10%. Detection reagent: pinacryptol yellow (UV light). * See Table I.

An Alumina G plate impregnated with 5 % of dodecanol in absolute ethanol was spotted with 1 μl samples of a mixture of four surfactants. A ten-section vapor-phase conditioning tray was used in a Camag Vario KS chamber to equilibrate the plate with a relative humidity gradient perpendicular to the direction of flow. After equilibration for 30 min, the sandwich slide was inserted and the plate was developed

TABLE XIV

R_F VALUES FOR QUALITATIVE SEPARATIONS

Impregnating agent, n-dodecanol–ethanol; solvent system, ammonia solution–methanol (2:3, v/v); detection spray, 0.05 % pinacryptol yellow in water; run length, 15 cm; temperature, 27°; sample size, 1–2 μl.

Surfactant	n-Dodecanol concentration in ethanol (% v/v)					
	3	5	3	5	3	5
	Alumina H		Alumina G		Silica Gel G	
1	0.64	0.63	0.67	0.67		0.73
2	0.63		0.61	0.62	0.56	
3	0.56	0.50	0.60	0.51		0.61
4	0.51		0.44	0.46	0.42	
5	0.49	0.40	0.45	0.40		0.55
6	0.43	0.35	0.35	0.28		0.47
7	0.28	0.19	0.18	0.15		0.28
8	0.14	0.12	0.12	0.10		0.15
9	0.16		0.27	0.23	0.20	
10	0.10		0.17	0.17	0.28	
11	0.10		0.22	0.28	0.14	
12	0.06		0.16	0.14	0.11	

with a 1:1 (v/v) methanol–28% ammonia solution system for a length of 16 cm; the run took 129 min. The results are shown in Fig. 2. It was anticipated that humidity control was less important in reverse-phase chromatography than in adsorption chromatography, and Fig. 2 does indicate essentially constant R_F values for the four surfactants, except at lower humidities. In view of the excellent separations obtained up to this point, it was decided that no further attempt would be made to control or study the effectiveness of relative humidity.

Typical separations
        Table XIV summarizes the experimental parameters for designing the best separations for mixtures of the twelve oxyethylated alkyl sulfate surfactants investi-
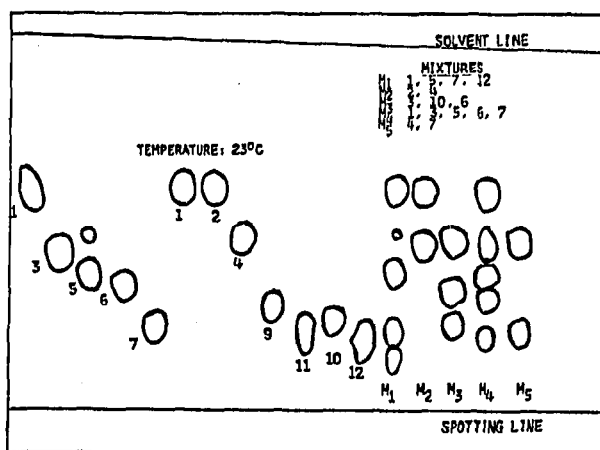
Fig. 3. One-dimensional chromatograms of 12 surfactants and various mixtures on 250-μm Alumina H plates impregnated with 5% (v/v) of *n*-dodecanol in absolute ethanol and developed with a 2:3 (v/v) ammonia solution (28%)–methanol solvent system. Run length, 14.5 cm; run time, 130 min; detection reagent, pinacryptol yellow (UV light).
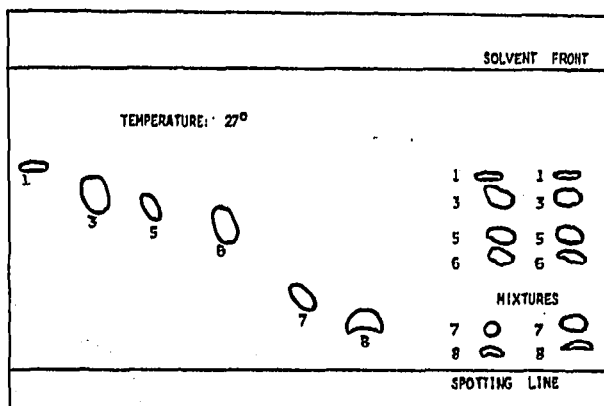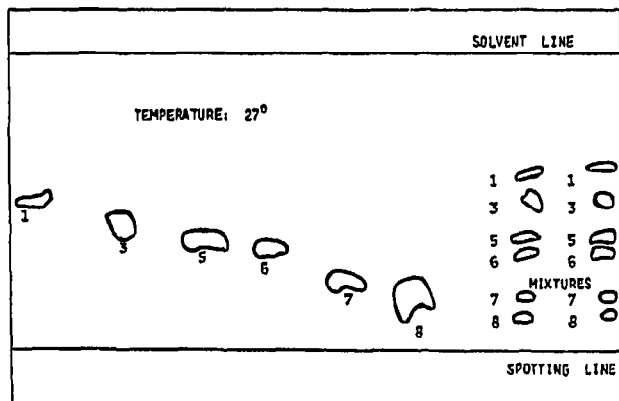

Fig. 4 One-dimensional chromatograms of 6 surfactants and surfactant mixtures on 250-μm Alumina H plates impregnated with 3% (v/v) of *n*-dodecanol in absolute ethanol and developed with a 2:3 (v/v) ammonia solution (28%)–methanol solvent system. Run length, 11.7 cm; run time, 98 min; detection system, pinacryptol yellow (UV light).

gated in this research. Table XV contrasts the R_F values obtained with pure com-
pounds and with the same compounds in mixtures. Surfactant 5 (see Table I) con-
tains an impurity. Figs. 3–7 illustrate the type of separations obtainable with the
optimal conditions delineated by the systematic study of the experimental variables.
Preliminary results on two other series of surfactants indicate that the results of this
study are applicable to other classes of anionic surfactants. While chromatography
is an art, trial-and-error approaches to developing quantitative chromatographic
separations usually lead to either failure or to procedures that do not give the best

Fig. 5. One-dimensional chromatograms of six surfactants and surfactant mixtures on 250-μm Alu-
mina H plates impregnated with 5 % (v/v) of n-dodecanol in absolute ethanol and developed with a
2:3 (v/v) ammonia solution (28 %)–methanol solvent system. Run length, 11.6 cm; run time, 103 min;
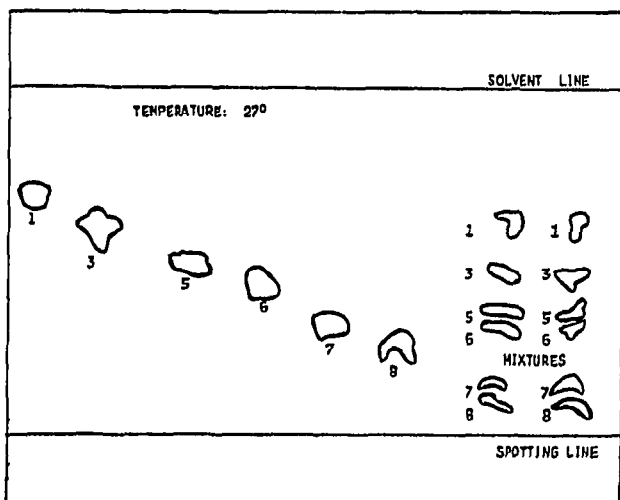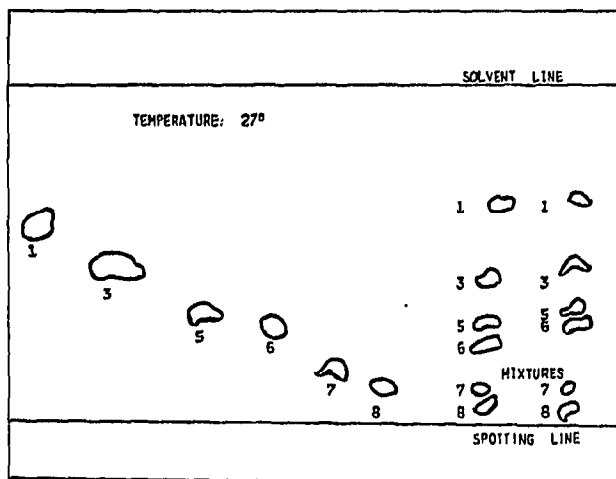detection reagent, pinacryptol yellow (UV light).

Fig. 6. One-dimensional chromatograms of six surfactants on 250-μm Alumina G plates impregnated
with 3 % (v/v) of n-dodecanol in ethanol and developed with a 2:3 (v/v) ammonia solution (28 %)–
methanol solvent system. Run length, 13.8 cm; run time, 115 min; detection reagent, pinacryptol
yellow (UV light).

Fig. 7. One-dimensional chromatograms of six surfactants and surfactant mixtures on 250-μm Alumina G plates impregnated with 5% (v/v) of n-dodecanol in absolute ethanol and developed with a 2:3 (v/v) ammonia solution (28%)–methanol solvent system. Run length, 13.1 cm; run time, 110 min; detection reagent, pinacryptol yellow (UV light).

TABLE XV

R_F VALUES FOR TYPICAL SEPARATIONS

Compound	Pure compounds	Mixtures				
		M_1	M_2	M_3	M_4	M_5
1	0.60	0.61			0.61	
2	0.60		0.60			
3	0.43			0.47	0.46	
4	0.47		0.46			0.47
5	0.36 (0.47)	0.36 (0.47)			0.37	
6	0.33			0.33	0.31	
7	0.22	0.22			0.20	0.22
8						
9	0.28					
10	0.24			0.24		
11	0.32					
12	0.18	0.14				

separations. There is a great need in this field for describing more carefully the details of the experimental procedures.

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