phosphate procedure is simple, very rapid and is capable of high accuracy and precision. In addition, the method is relatively free from common phosphate interferences. The pH, concentration of reagents, and integration time can be manipulated for analysis of a wide range of phosphate concentrations.

The results of blood serum analyses indicate the suitability of the new procedure to the analysis of biological samples. In addition, the method should be applicable to other phosphate-containing samples.

Although the determination of phosphate was chosen to illustrate the application of fast reaction-rate measurements for routine analyses, many other applications are possible. There are many fast reactions that could be utilized for analyses by the millisecond-range rate measurements. The investigation of other rapid reactions suitable for analytical procedures is in progress.

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Determination of Ethanolamides in Mixtures by Differential Saponification Rates

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The fatty alkanolamides can be saponified in alcoholic KOH to give two moles of weak base for every mole of KOH reacting with one mole of the amides. This serves to distinguish them from amines, amine soaps, amine esters, and the ester function of amide esters, and other by-products present in the commercial alkanolamides which yield only one mole of weak base per mole of KOH consumed during saponification. This formation of extra base has been used to follow the saponification of mixtures of commercial mono- and diethanolamide and makes possible the application of the differential reaction rate technique to these systems. The pseudo-first-order rate constant for lauric diethanolamide was found to be 70 times that of lauric monoethanolamide, which condition makes the determination of these two materials by this technique inherently precise.

THE COMMERCIAL ETHANOLAMIDES, especially the diethanolamides, are mixtures of some complexity; they contain, in addition to the amide, appreciable amounts of the free amine, amine soap, amide esters, amidoamines, and possibly also amine esters (*1*). The specifications on these materials generally recognize these facts (*2*). The presence of these other functional groups thus precludes the use of the standard hydrolytic methods for amides unless corrections can be made for contributions by these other constituents (*3*). The ion exchange method for nonionic content gives a reliable figure for the total amide content of such materials; however, it does not distinguish between different kinds of amides such as the mono- and diethanolamides in mixtures. Similarly, the published infrared methods (*4*, *5*) do not provide for the determination of the individual alkanolamides in mixtures.

The differential reaction rate technique has been used rather extensively for determining the individual components of binary mixtures of compounds having the same functional group. The work of Livengood and Johnson (*3*) and Ranny *et al.* (*6*) indicated a considerable difference in the rates of saponification of mono- and diethanolamides. Consequently, a method based on differential saponification rates seemed feasible. Siggia, Hanna, and Serencha (*7*) reported on the analysis of mixtures of primary amides by a differential hydrolysis rate technique; however, their technique of distilling the ammonia from the reaction mixture as it is formed and titrating the distillate is not applicable to the ethanolamides for the amines formed are not readily volatile.

The chemical reactions to be considered in the saponification of commercial ethanolamides in alcoholic KOH and subsequent titration of the weak bases produced are as follows:

$$R\text{CON(CH}_2\text{CH}_2\text{OH)}_2 + \text{OH}^- = R\text{COO}^- + \text{HN(CH}_2\text{CH}_2\text{OH)}_2 \quad (1)$$

$$R\text{CON(CH}_2\text{CH}_2\text{OOC}R\text{)}_2 + 3\text{OH}^- = 3\,R\text{COO}^- + \text{HN(CH}_2\text{CH}_2\text{OH)}_2 \quad (2)$$

$$\text{HN(CH}_2\text{CH}_2\text{OH)}_2 + \text{H}^+ = \text{H}_2\text{N}^+\text{(CH}_2\text{CH}_2\text{OH)}_2 \quad (3)$$

$$R\text{COO}^- + \text{H}^+ = R\text{COOH} \quad (4)$$

$$\text{H}_2\text{N}^+\text{(CH}_2\text{CH}_2\text{OH)}_2 + \text{OH}^- = \text{HN(CH}_2\text{CH}_2\text{OH)}_2 + \text{H}_2\text{O} \quad (5)$$

$$R\text{COOH} + \text{OH}^- = R\text{COO}^- + \text{H}_2\text{O} \quad (6)$$

The first equation represents the saponification of the amide itself, and the second, the complete saponification of the amide ester. Equations 3 and 4 represent the conversion of the weakly basic components of the sample to their conjugate acids. Equations 5 and 6 represent the neutralization of these conjugate acids. The significant thing about all these reactions is that 5 and 6 both yield one mole of weak base for every mole of strong base consumed, whereas the saponification of the amide constituents (Equations 1 and 2) yields an extra mole of weak base for every mole of amide present. Thus, if the basic components of a sample are first converted to their conjugate acids before saponifying the mixture in alcoholic KOH, a determination of the milliequivalents of base over and above that added as KOH would allow a determination of the milliequivalents of amide saponified.

(1) H. L. Sanders, *J. Amer. Oil Chem. Soc.*, **35**, 548 (1958).
(2) Toilet Goods Association Standards, Spec. No. 80, Washington, D. C., June 27, 1960.
(3) S. M. Livengood and C. H. Johnson, Proceedings of the Chemical Specialties Manufacturers Association Symposium on Analytical Methods for Surfactants, Hollywood, Fla., Dec. 1957 p 113.
(4) M. M. Miller, ANAL. CHEM., **30**, 1884 (1958).
(5) M. F. Mallery, *ibid.*, p 1884.
(6) M. Ranny, J. Prachor, and J. Novak, *Prumysl Potravin*, **14**, 211 (1963).
(7) S. Siggia, J. G. Hanna, and N. M. Serencha, ANAL. CHEM., **36**, 277 (1964).

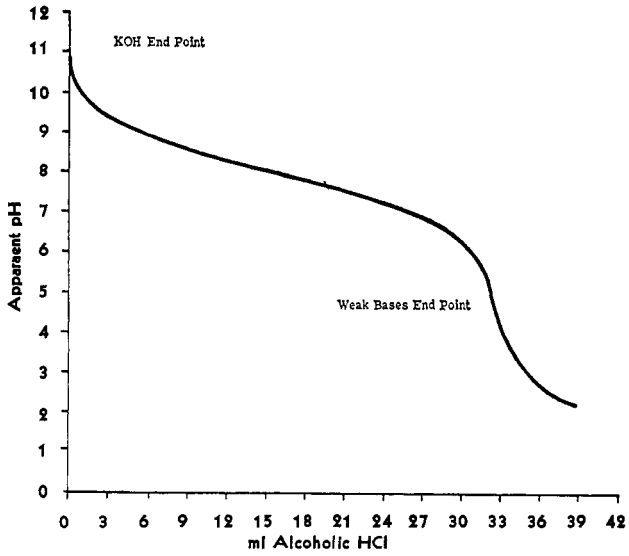

Figure 1. Titration of saponified commercial diethanolamide with 0.5*N* alcoholic HCl

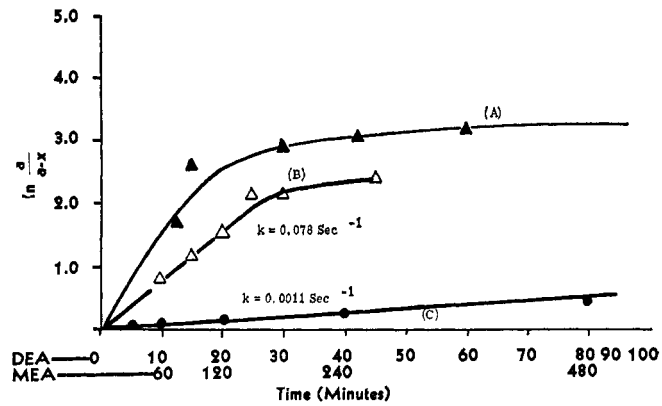

Figure 2. Saponification of lauric monoethanolamide and lauric diethanolamide

A—**Lauric diethanolamide (0.100*M*) in boiling 0.50*N* alcoholic KOH; *B*—Lauric diethanolamide (0.0503*M*) in boiling 0.25*N* alcoholic KOH; *C*—Lauric monoethanolamide (0.0620*M*) in boiling 0.25*N* alcoholic KOH**

EXPERIMENTAL

The following materials were prepared and/or purified for use in the experimental work. Lauric diethanolamide and lauric monoethanolamide were prepared by purifying the commercial 95%-pure amides; solutions of the amides in $CHCl_3$ were slurried with Monobed ion-exchange resin to remove free amine and amine soaps, and, after removal of the $CHCl_3$, were recrystallized twice from iso-octane. Elemental (C, H, N) analysis indicated a purity greater than 99% for both materials. Titration with standard acid showed the presence of 3.9% amine soap in the purified diethanolamide, and corrections for this were employed in subsequent experimentation. *N*-(2-Lauroylethyl)lauramide was prepared by refluxing lauric acid and monoethanolamine in a 2/1 mole ratio in toluene in the presence of a little H_2SO_4 as catalyst. The amide ester was then isolated by distilling off the toluene and purified by redissolving in ether and washing the ether solution free of unreacted amine. The ether was removed by evaporation, the product redissolved in benzene, and the mixture chromatographed on silica gel containing 5% H_2O using 10% ether in benzene as eluent. *N,N*-bis-(2-lauroylethyl)lauramide was prepared by dissolving 0.1 mole lauric diethanolamide and 0.75 mole lauric acid in toluene and refluxing in the presence of a small amount of sulfuric acid. The amide diester was isolated and purified by the procedure just described for *N*-(2-lauroylethyl)-lauramide. The infrared spectra of these amide ester products were recorded and compared to the spectra obtained after repeating the chromatographic purification step. The results showed that the *N*-(2-lauroylethyl)lauramide was essentially pure after the first purification, while the *N,N*-bis-(2-lauroylethyl)lauramide contained appreciable amounts of lauric acid and a small amount of diethanolamide. The second chromatographic fractionation reduced these impurities to a level where their presence could no longer be detected by IR spectrophotometry which was satisfactory for our purposes.

A trial experiment was run to determine whether the strong and weak base components of a saponification mixture could be determined by titration in alcoholic solution with alcoholic HCl. The titration curve of a diethanolamide saponification mixture is shown in Figure 1. Both equivalence points can be accurately detected potentiometrically; however, the presence of even modest amounts of water, such as would result from use of an aqueous titration solution, ruins the weak base end point. In the proposed scheme for analyzing the saponification mixtures, it is necessary to detect only the second end point.

In the kinetic experiments, the saponifications were carried out in a conical flask submerged in a boiling water bath on a stirring hot plate to provide a constant temperature source of heat and provide stirring during the saponifications. A seven-fold excess of KOH was used to maintain an approximately constant concentration of hydroxide ion during the saponification, and the reaction was carried out under an efficient reflux condenser. At the end of the reaction period, the reaction mixture was immediately cooled in an ice bath placed on another magnetic stirrer, and a carefully measured aliquot of the titrant solution sufficient to neutralize all of the excess KOH was added as soon as the mixture had cooled. The titrations were completed in the original reaction vessel with a micrometer buret, and the end point was detected potentiometrically with a probe electrode and pH meter.

The general scheme employed for differential kinetic analysis of amide mixtures was as described by Kolthoff and Lee (*8*) for a system conforming to first order rate laws. A calibration curve was established by analyzing mixtures of pure monoethanolamide and diethanolamide. The procedure employed for these and all samples analyzed was to weigh a sample containing about 15 millimoles of total amide, put it into solution in 3A alcohol, and adjust to an apparent pH of 4.8 with alcoholic HCl. The solution was then diluted to exactly 200 ml with 3A alcohol. A 50-ml aliquot of this solution was transferred to the dry saponification flask which contained a stirring bar. Stirring was started, a 50-ml aliquot of 0.50*M* alcoholic KOH (equal to a seven-fold molar excess) was added to the flask, and the flask was immediately attached to the condenser and immersed in the boiling water bath. The timer was started and the saponification allowed to proceed for exactly 25 minutes. At the end of the saponification period the reaction was quenched and the saponification mixture titrated as described above. A blank was run on the alcoholic KOH in an identical manner, and the difference between sample and blank titrations times the normality of the HCl gives the milliequivalents of amide saponified.

(8) I. M. Kolthoff and T. S. Lee, *Ann. N. Y. Acad. Sci.*, **53**, 1093 (1951).

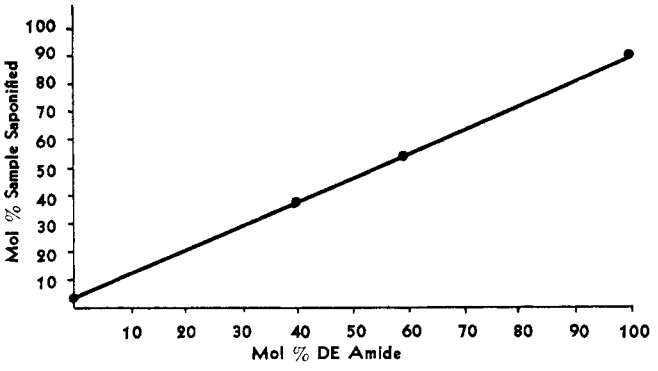

Figure 3. Standard curve for saponification of mixtures of monoethanolamides and diethanolamides

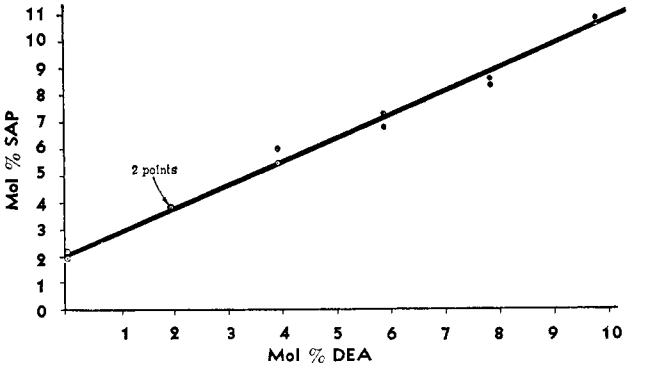

Figure 4. Standard curve for saponification of ethanolamide mixtures low in diethanolamide content

Three times normal sample size employed

RESULTS

The rates of saponification of pure lauric diethanolamide in 0.50*M* and 0.25*M* alcoholic KOH and of lauric monoethanolamide in 0.25*M* alcoholic KOH were measured as a check on the kinetic behavior of the reaction and to enable us to choose the optimum reaction time for the analysis of mixtures. The data are shown plotted in first order rate form in Figure 2, where, in the function plotted as ordinate, a is the initial concentration of the amide and $a-x$ is the concentration remaining at time t. The pseudo first order rate constants are shown next to the graphs for convenience; the ratio $K_{\text{Diethanolamide}}/K_{\text{Monoethanolamide}}$ in 0.25*M* alcoholic KOH under the conditions stated above is 70, a condition very favorable for the analysis of binary mixtures of these two amides by a differential rate method. Using this ratio and the equation developed by Kolthoff and Lee (*8*), one calculates the optimum time for analysis to be 55 minutes. Examination of the rate curves shows that the system deviates from first order kinetics before this time; however, the rate constants are sufficiently different that a 25-minute reaction period is almost equally satisfactory.

The departure from 1st order kinetics for diethanolamide represented by the plateau region of the curve was studied in an attempt to learn the reason for it. In one experiment we exhaustively saponified (120 minutes in boiling 0.25*M* alcoholic KOH) pure diethanolamide by itself and in the presence of approximately equimolar quantities of lauric acid and diethanolamine to determine whether an equilibrium state was being set up. The results showed a shift from 93% saponification to 84% saponification upon adding the saponification products; however, this change is small relative to the shift one would predict for a system at equilibrium. Put another way, the equilibrium constants calculated from each of these two saponifications were widely different. In other experiments we saponified pure diethanolamide for 25 minutes in 0.25*M* KOH using a 3.5-fold molar excess and the usual sevenfold molar excess and found no difference in the mol % of the amide saponified. This indicates that the observed deviation from 1st order kinetics is not the result of an insufficient excess of KOH to maintain pseudo first order conditions during the reaction. We believe that an unidentified side reaction takes place without the production of weak base or the consumption of strong base which produces a product which liberates extra base at a much slower rate than diethanolamide itself.

In any case it is apparent, from the analytical point of view, that a 25-minute saponification in boiling, 0.25*M* KOH is quite suitable, in that it provides a controllable reaction time and temperature and maximum saponification of the amides.

While a 50-fold excess of reagent is recommended for maintenance of strict pseudo first order conditions, use of such a large excess in this reaction/analysis system would make it very difficult to maintain something approaching constant temperature over a measurable time period. In addition, because what is measured is an increase in the total base content of the system we wanted to keep the KOH content low enough so that the increase (due to saponification of amides) would be a significant portion of the total in order to give the necessary precision in its measurement. These conditions were accordingly selected for the analysis of mixtures of mono- and diethanolamide.

Thus, the choice of a seven-fold excess of KOH is a compromise, which for this system at least, gives linear first order calibration curves in keeping with the theory developed by Kolthoff and Lee (*8*) for a first order reaction system. A typical calibration curve is shown in Figure 3. The mol % of amide saponified was calculated from the measured millimoles of amide saponified and the theoretical total millimoles of amide taken for saponification. The graph is linear in keeping with the theory developed by Kolthoff and Lee for a first order reaction system. Diethanolamide is approximately 90% saponified under these conditions.

A separate calibration covering the range 0–10% diethanolamide was prepared for the analysis of samples having a mol % diethanolamide in that range. For this calibration and for the analysis of such samples, solutions containing three times the concentration of total amide (about the maximum permitted by the solubility of the amides of interest) were saponified by the same procedure described above. Such a low-range calibration curve is shown in Figure 4. It should be pointed out that, while the molar excess of KOH is theoretically reduced by trippling sample size, in practice, because the sample composition is restricted to less than 10% of the faster-reacting diethanolamide, the maximum amount of KOH consumed by the larger sample is no greater than that for a normal-size sample of pure diethanolamide. The data points define a good straight line with a slope of 0.90 in excellent agreement with the value (0.89) found for the 0–100% calibration line (Figure 3).

To test the effects of the various minor components known to be present in the commercial amides on the accuracy of the method, known mixtures of pure mono- and diethanolamide containing one or more of the minor components were analyzed. The results for diethanolamide are shown in Table I. The % monoethanolamide in each case is equal to 100 − % diethanolamide. The method is accurate in the range 10 to 100% diethanolamide, and the standard deviation has been estimated from the data on the four samples in that range at ±3.4% relative. Small amounts of free amine and

Table I. Recovery from Mixtures of Pure and Commercial High-Active Lauric Monoethanolamide and Lauric Diethanolamide

Sample composition	Mol % diethanolamide Taken	Found	% Recovery
Lauric monoethanolamide, and lauric diethanolamide only	9.63	8.75	91
		10.0	104
	24.3	23.5	97
		24.5	101
	84.4	82.5	98
	49.0	49.2	100
		49.0	100
	4.98	4.00	80
		3.25	65
Lauric monoethanolamide, lauric diethanolamide, lauric acid (5%) diethanolamine (10%)	33.6	34.5	102
Lauric monoethanolamide, lauric diethanolamide and *N,N*-bis-2(lauroylethyl)-lauramide (13%)	49.4[a]	48.1	97
High active lauric diethanolamide/lauric monoethanolamide	8.94[a]	12.0	134
		12.5	140
	27.4[a]	30.2	110
		27.5	100
	57.0[a]	60.2	105
		58.8	103
	Mean recovery (10–100% Range)		101

[a] *N,N*-Bis-(2-lauroylethyl)lauramide counted mole-for-mole as diethanolamide.

amine soap and amide ester do not cause any error, although the latter is counted as amide by this method.

For the mixtures of commercial high-active mono- and diethanolamides, the total millimoles of amide in each sample were obtained as the difference between the total millimoles of nitrogen (by a Kjeldahl analysis) and the millimoles of amine + amine soap as determined by the preliminary titration of the sample to pH 4.8. The results demonstrate the accuracy of the method for such commercial samples containing 25% or more diethanolamide, and, at the same time indicate the unsuitability of the method for the precise quantitative analysis of mixtures containing less than 10% diethanolamide.

Table II shows results for the analysis of seven synthetic mixtures of commercial low active mono- and diethanolamides. The data in the top half of the table were obtained by the method previously described, in which the total millimoles of amide taken for saponification are calculated as the millimoles of N found by a Kjeldahl N determination minus the millimoles of amine + amine soap as determined by the preliminary titration of the sample to pH 4.8.

The data in the bottom half of the table were obtained by the same basic method, except that the total millimoles of amide were obtained by determining % nonionic on the sample and % N on the nonionic residue. The total millimoles of amide determined by each of these schemes for the seven samples analyzed are also listed for comparison. The mol % taken was determined from the % nonionic of the individual commercial amides and the average molecular weight of the amides present in each as determined from a knowledge of the average molecular weight of the fatty acids used to make the amides. A recent study of the nonionic method by M. E. Wittekind of our staff completely confirms the accuracy of that method for the total amide content of commercial amides (*9*).

Table II. Analysis of Low Active $C_{10}C_{12}$ Diethanolamide/Coconut Monoethanolamide Mixtures

Total mmoles amide in sample $\left[\frac{(\text{S. W. in mg})(\%\text{ N})}{(14)(100)}\right]$ − (Meq. HCl to pH 4.8)	Mol % diethanolamide Taken	Found	Error
2.851	11.1	10.8	−0.3
		10.5	−0.6
2.674	24.8	20.5	−4.3
		24.0	−0.8
		18.0	−6.8
2.311	54.1	42.2	−11.9
		44.5	−9.6
Std mixture + DEAmine	50.0	49.0	−1.0
2.610	24.7	20.0 ± 0.0[a]	−4.7
	100.0	73.5 ± 0.8[a]	−26.5
	24.5	20.2 ± 0.8[a]	−4.3
	10.9	8.4 ± 0.4[a]	−2.5
		Mean	−6.5
$\frac{(\text{S. W. in mg}) \times (\%\text{NI})(\%\text{N on NI})}{10{,}000}$			
2.532	11.1	12.0	+0.9
		12.3	+1.2
2.254	24.8	24.9	+0.1
		29.0	+4.2
1.821	54.1	53.5	−0.6
		56.1	+2.0
2.262	24.7	23.8 ± 0.2[a]	−0.9
1.367	100.0	95.2 ± 0.6[a]	−4.8
2.227	24.5	21.3 ± 0.7[a]	−3.2
	10.9	10.4 ± 0.4[a]	−0.5
		Mean	−0.8

[a] Average of four saponifications on same sample solution.

Table III. Analysis of Commercial Diethanolamide/Monoethanolamide Mixtures by Two Modifications of the Differential Saponification Rate Method

Mol % diethanolamide Taken	Found	Error
100	96.5	−3.5
	99.2	−0.8
33.3	32.2	−1.1
	34.7	+1.4
11.8	10.0	−1.8
	15.5	+3.7
5.4	3.2	−2.2
	9.5	+4.1
100	96.8	−3.2
33.3	30.8	−2.5
11.8	10.5	−1.3
5.4	3.8	−1.6

An inspection of the data of Table II shows a very significant difference in the total millimoles of amide and the mol %

(9) M. E. Wittekind, The Procter & Gamble Co., Company Technical Report, Cincinnati, Ohio, June 23, 1966.

DEAmide of these samples as determined by the two methods represented. Needless to say, we believe the data based on the nonionic analysis are correct, and that the titration of the unsaponified sample to pH 4.8 does not adequately correct for all of the nonamide nitrogen in these commercial low-active amides.

Because an accurate analysis of mixtures of low-active mono- and diethanolamide requires the isolation of the nonionic residue, it is feasible to run the differential saponification rate method on a portion of the nonionic residue. To evaluate this possible modification, four sample mixtures of these amides were prepared, and the analysis was carried out on the samples directly and on the nonionic residues of the samples. The results are compared in Table III. Again, the direct analysis method appears to be accurate, although the reproducibility is not as good as we thought it should be. Analysis of the nonionic residue appears to introduce a small negative basis for diethanolamide, and we know of no reason why this should be so, for this procedure more closely approximates the calibration system than the commercial amide mixtures themselves. In spite of this small bias, however, the data of Tables II and III demonstrate the applicability of the differential kinetic technique to the analysis of fatty alkanolamide mixtures by saponification when an appropriate method, such as the one which has been described here, for carrying out the saponification and following its course is employed.

Of the various modifications of the basic differential saponification rate method which were evaluated, that employing a prior separation of the nonionic fraction of the sample followed by saponification and Kjeldahl N analysis of the nonionic residue would be the most generally applicable. Its use on finished products containing mixture of these amides seems feasible. The practice of obtaining the total millimoles of amide by a Kjeldahl N analysis on the sample itself and correcting for amine and amine soap would have utility for only very select systems such as the high active amides. The presence of bases other than amines or amine soaps (NH_4Cl or sodium acetate for example) would interfere. The modification employing direct saponification of the sample and a determination of nonionic content of the sample and N content of the nonionic residue to give total millimoles of amide should be rather generally applicable because the method of measuring the extent of saponification is itself rather specific.

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Determination of Nonionic Surfactants by Atomic Absorption Spectrophotometry

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A quantitative method for the rapid determination of nonionic surfactants is described. The surfactant is precipitated as a heteropoly phosphomolybdic acid–barium complex and the molybdenum in the supernatant solution is assayed by atomic absorption. Optimum conditions for the analysis of high levels of molybdenum in the presence of high levels of barium were determined. The effect of other cations on the solution was determined.

THE CONCENTRATION of nonionic surfactant in multicomponent solutions has previously been determined by a method used for biological materials (*1*, *2*). The surfactants were precipitated from solution with phosphomolybdic acid and barium chloride and the precipitate weight was determined. The amount of surfactant in solution was related to the precipitate weight by an empirical factor determined for each surfactant batch and each multicomponent system, since the precipitate is nonstoichiometric. We have achieved a considerable saving in time by isolating the precipitate with centrifugation and measuring the residual molybdenum concentration of the supernatant solution by atomic absorption spectrophotometry, and relating this to the quantity of surfactant by an empirical factor determined for each batch and each multicomponent system. The conditions for measurement of molybdenum in the residual reaction mixture were determined.

EXPERIMENTAL

Instrumentation. A Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with a recorder readout and a Westinghouse WL 22937 molybdenum hollow cathode lamp was used to obtain the experimental data. A premix burner with the short path head attached (Perkin-Elmer, part number 290-1169), rotated 90° from normal to minimize the absorption path length, was used. This configuration was necessary because of the high concentration of molybdenum in the solutions. The fuel-oxidizer mixture used in the burner was highly reducing air–acetylene that gave a white flame on top of small blue base. When the gas regulator gages supplied with the instrument were used to meter the air and acetylene to the premix burner, a very unstable flame resulted. Introducing the solution into the aspirator with a constant injection pump (Harvard Apparatus Co., Model 901) improved the reproducibility of the absorption values found on replicate runs of the same solution. The pump did not improve the reproducibility of absorbance values sufficiently and was cumbersome in routine use, so it was abandoned. The sample injection was controlled with the aspiration air flow rate in the conventional manner and varied from 2 to 4 ml of solution per minute. Sufficient reproducibility was obtained with a gas regulation system modified in the following way (*3*).

Acetylene from a tank (Union Carbide, Linde Division, purified No. WC) passes through a single stage regulator existing at 15 psi (Matheson Gas Co., Model 1L), into a precision rotameter (Brooks Instruments Co., Model 8900

(1) C. Boyd Shaffer and Francis H. Critchfield, ANAL. CHEM., **19**, 32 (1947).
(2) J. Oliver and C. Preston, *Nature*, **4162**, 242 (1949).
(3) J. Johnson, Westinghouse Electric Corporation, Atomic Power Division, Madison, Pa., personal communication, 1967.