

Liquid Scintillation Determination of Surfactants on Cotton Fabric

B. E. GORDON, W. T. SHEBS, D. H. LEE and R. U. BONNAR,
Shell Development Company, Emeryville, California

Abstract

A method for the determination of anionic surfactants on cotton fabric has been developed using S^{35} and H^3 labelled compounds. The method is simple, involving immersion of the white fabric in liquid scintillation counting solution and counting. No interference by quenching or self-absorption is encountered. Accuracy and precision are better than $\pm 2\%$ relative. Replacing the internal standard method by the pulse height shift method of obtaining disintegration rates simplifies the analysis and increases its precision.

Combination of the pulse height shift method with automatic data handling has permitted the analysis of several hundred samples per week with little increase in operator time.

Introduction

THE NEED FOR A RAPID METHOD to measure the concentration of surfactant on cotton fabric has been discussed in a previous report (10). Because it was not known whether retained surfactant (after a wash-dry cycle) could be readily extracted from these fabrics nor the exact concentration range to be expected, the development of an analytical procedure based on extraction appeared to be somewhat time-consuming. It seemed reasonable for both speed and expediency to explore the use of radioactively labelled surfactants both for analyses during the development part of the program and as a referee method when developing routine analytical procedures to be used in the field. Such approaches have been reported by Meader and Fries (1) and Boyd (2).

Because both rinsability and adsorption studies generate large numbers of samples, amounting to several hundred per week during peak operation, it was decided to use liquid scintillation counting as the analytical tool. The modern liquid scintillation counter is well suited to such loads because of its large sample capacity (200) and automatic count-print-change features which permit unattended operation for long periods. Other advantages of this method over end-window Geiger counting (1,2) are avoidance of, or at least reduction of, self-absorption effects and greater sensitivity. The direct counting of cotton fabric samples rather than wash water analyses was desirable in the rinsability studies since uncertainties in water volumes plus the fact that most of the surfactant is not adsorbed might lead to gross errors.

This report describes a method for the determination of tagged anionic surfactants adsorbed on cotton fabric.

Experimental

Reagents

1. Dioxane liquid scintillator solution—4 g diphenylloxazole, 0.1 g dimethyl POPOP, 80 g naphthalene, 100 ml water per liter of dioxane.

2. Toluene liquid scintillator solution—6 g diphenylloxazole, 0.1 g dimethyl POPOP per liter of toluene.

Equipment

Packard Model 3214 Tri-Carb liquid scintillation counter (Packard Instrument Company, Downers Grove, Illinois) coupled to IBM Model 026 card-punch via Packard Model 540 card-punch control.

Synthesis of Labelled Compounds

Four labelled compounds were prepared, two alkyl sulfates- S^{35} , one alkyl benzene sulfonate- S^{35} , and the same sulfonate- H^3 labelled. The purpose of two isotopically labelled sulfonates was to make possible a direct competitive rinsability study when using a mixture of alkyl sulfate and the alkyl benzene sulfonate. Liquid scintillation spectrometry permits one to simultaneously analyze samples containing both tritium and sulfur-35.

The alkyl benzene was a commercial linear alkyl benzene containing the following carbon-atom distribution in the side chains:

C_{11} —	8%
C_{12} —	31%
C_{13} —	34%
C_{14} —	26%

Two linear primary alcohol fractions were used to make the labelled alkyl sulfates. The first, Neodol 25, had a carbon-number range of C_{12-15} and an average molecular weight of 207. The second, Neodol 45, had a carbon-number range of C_{14-15} and an average molecular weight of 220.

Tritiated alkyl benzene sulfonate was prepared by exchanging tritiosulfuric acid (95%) with the alkyl benzene for 11 days at room temperature while stirring. After separation of the tritiated hydrocarbon, it was sulfonated by reaction with SO_3 in methylene chloride at ca 17°C (9). After neutralization, the product in benzene-tetrahydrofuran solution was decolorized with Norit A charcoal and dried by azeotropic distillation with toluene. The final yield was 35 mc in 82 g of surfactant.

Sulfur-35 labelled alkyl benzene sulfonate was prepared as above using $S^{35}O_3$ to react with unlabelled alkyl benzene. The yield was 11.3 mc in 125 g of surfactant.

The alkyl sulfates were prepared using chlorosulfonic acid- S^{35} as the sulfating agent. The main radioactive contaminant, $Na_2S^{35}O_4$, was removed by repeated shaking, with inactive sodium sulfate of a benzene-tetrahydrofuran solution of the surfactant. After six such treatments the filtered sodium sulfate showed no radioactivity. The yield from the C_{14} - C_{15} alcohol was 9.4 mc in 69 g and from the C_{12} - C_{15} alcohol was about 9.5 mc in 84 g. All of the above preparations were of somewhat higher specific activity, in mc/g, than required for the rinsability tests, hence, some dilution with unlabelled surfactant was required. Because such dilution aggravates problems of radioactive contamination, since it dilutes the surfactant but leaves the contaminant at its original specific activity, care was taken to establish that any radioactive contaminants present would not adversely affect the results.

Radiochemical Purity

The radiochemical purity of the surfactants was determined by paper chromatography. In this procedure 1 μ l of a 10% solution was placed on a strip of filter paper (1-in. \times 6-in.). This was placed in a tank above a solution of 60 parts ethyl acetate, 15 parts methanol, 5 parts concentrated ammonium hydroxide v/v/v. After one hour equilibration, the labelled end of the paper was dipped in (spot not immersed) and development proceeded until the liquid front reached the top of the paper. After drying the paper was sprayed with a 0.05% solution of pinacryptol yellow in ethanol to develop the color of the surfactant. The paper was then cut into sections and these were counted by immersion in dioxane counting solution. In all four cases, the migrated spots contained all the activity. Because the surfactants were mixtures rather than pure compounds, the spots were considerably broader than one would expect from a single compound. However, the important point, the absence of a strongly adsorbed, immobile contaminant, was indicated. Additional tests which demonstrated the rapid exchange of the adsorbed surfactant with the same surfactant unlabelled in solution also supported the belief that no radioactive contaminant was present which would distort the results.

Development of the Method

For the method development the internal standard method of liquid scintillation counting was used. Aliquots of labelled surfactant solution, or swatches of cotton fabric impregnated with stock surfactant solution were added to 20 ml of the appropriate liquid scintillator and counted in the Tri-Carb counter. Instrument settings were chosen to give optimum values of efficiency²/background as recommended by the manufacturer. After each sample was counted a spike of a standard solution of carbon-14 labelled toluene was added to the counting vial and a second count obtained. The difference between the two counts yielded the count rate of the standard which, when divided by its disintegration rate, gave the efficiency of counting the particular sample. Then one could calculate the disintegration rate of the sample. Carbon-14 labelled toluene was used as the internal standard for sulfur-35 since β^- energies from both isotopes are similar. Tritium labelled toluene was used as the internal standard for the tritium labelled surfactant.

The disintegration rate divided by the specific activity (in dpm/g) of the pure surfactant gave the weight of surfactant in grams.

Several procedures have been described in the literature for counting radioactivity on paper chromatograms (3-6). Among these is one by Blair and Segal (5) who simply immersed the paper in scintillator solution and counted the heterogeneous mixtures. Since this would offer a simple solution to the analyses, this approach was tried using increasing weights of white cotton (diapers) fabric in 20 ml of toluene scintillator solution each containing the

TABLE I
Effect of Weight of Cloth on Counting Efficiency of C¹⁴

Grams cloth	Counts per minute
0	12,200
0.1	12,500
0.2	12,200
0.3	12,100
0.5	12,400
1.0	10,700

TABLE II
Test for Self-Absorption on Impregnated Swatches

Surfactant	dpm/ml of Stock surfactant solution		
	In solution	Impregnated diaper	Impregnated towel
Alkyl sulfate-S ³⁵	14.7×10^6	14.5×10^6	14.3×10^6
Alkyl benzene sulfonate-H ³	16.6×10^7	16.2×10^7	16.7×10^7
Alkyl sulfate-S ³⁵ (toluene scintillator)	13.9×10^6	10.3×10^6

same amount of standard carbon-14 labelled toluene. The results are in Table I.

Thus, quenching by the cloth is no problem at least up to 0.5 g. When the experiment in Table I was repeated with tritium a slight *increase* in count was found up to 0.5 g. This was due simply to the improvement in photon collection in the presence of a white reflector. Therefore, it was decided to use approximately 0.5 g sample in all analyses. While this test demonstrated that photon absorption by the cloth caused no interference, self-absorption of the β^- particles by the sample was a possibility. This was tested by impregnating 0.5 g swatches with known aliquots of standard surfactant solutions. The swatches were then dried under infrared lamps, immersed in 20 ml of dioxane scintillator, and counted. These results were compared with those obtained by directly dissolving aliquots of the same solutions of labelled surfactants and counting them. Dioxane scintillator was chosen because it is a much better solvent for the surfactants than toluene. However, for comparison one analysis was made with toluene scintillator solution. Two conclusions emerge from Table II. First, toluene is a poor solvent and high self-absorption losses are apparent. Second, quantitative recovery of the activity was obtained with dioxane indicating that either the surfactant is entirely dissolved in this solvent or that self-absorption is minimized by scintillator solution penetration of the cloth fibers. To test which effect was predominant, the cloth swatches were removed from the dioxane counting solution and immersed in fresh counting solution. Correction was made for the entrained solution (by weighing) and the net count in the second vial determined. It was found that 15% of the alkyl sulfate-S³⁵ and 6% of the alkyl benzene sulfonate-H³ remained adsorbed in the cloth. Thus, the absence of a significant self-absorption effect is due mainly to solution.

The accuracy and precision of the method were obtained by impregnating 0.5 g cloth swatches with varying amounts of labelled surfactant solution, drying, and counting. The weight of surfactant on each sample was calculated from the specific activity of the stock solution.

The accuracy and precision are satisfactory (see Table III). The accuracy seems to reflect a small systematic negative error due possibly to a small amount of self-absorption not readily noted in the test described for Table II.

The Pulse-Height-Shift Method

The ISM, with automatic sample handling and data printout, is adequate only for moderate numbers of samples. It is not satisfactory when large numbers of samples are to be processed, for two reasons. First, it is apparent that the step of adding the internal standard requires double handling of each sample, with corresponding delay, which accumulates when large numbers of samples are handled. At sample loads of several hundred per week, a de-

TABLE III
Precision and Accuracy of Method

Quantity surfactant added, μg	Quantity found, μg		Relative std. dev., %	Accuracy, % ^a
		Mean		
Alkyl sulfate-S ³⁵				
9.4	9.0, 9.4, 9.6	9.3	3.3	-1.0
23.6	23.2, 23.3, 23.7	23.4	1.1	-0.8
47.2	44.7, 46.2	45.4	2.3	-3.8
94.4	91.2, 94.2	92.7	2.3	-1.8
236	228, 229	228	0.3	-3.4
472	467	467		-1.1
Alkyl benzene sulfonate-H ³				
3.9	3.7, 3.7, 4.0, 4.1	3.9	5.3	0
9.6	9.3, 9.7, 9.8, 9.9	9.7	2.7	+1.0
19.3	18.3, 19.0, 19.2, 19.5	19.0	2.7	-1.6
38.6	36.6, 37.6, 37.6, 38.2	37.5	1.8	-2.8
96.4	92.8, 92.9, 93.8, 94.1	93.4	0.7	-3.1

^a Accuracy = difference of mean from known.

lay of 1 to 2 days occurred between the first and second counts. In this time instrument drift or change in sample quenching properties sometimes led to appreciable errors. Second, each sample required a 5-step calculation, resulting in an inordinate time spent on calculations.

The pulse-height-shift method (PHSM) of Baillie (7), further studied by Bush (8), seemed well suited to overcoming the limitation noted for the internal-standard procedure. In this method, the β -spectrum of the isotope of interest is divided into two energy channels, with recording of the counting rates for each channel. The ratio of these count rates is related to the efficiency; a calibration curve or function can be readily obtained by measuring a set of standards having a range of efficiencies (i.e., degrees of quenching). The principle is schematically illustrated in Figure 1 and a typical function shown in Figure 2 together with the analytical function describing the curve. Thus only one counting period is required, eliminating the effect of drift. Furthermore, the data are obtained in a form amenable to automatic data processing.

To obtain the disintegration rate from the efficiency the sum of the counting rates in the two channels is divided by the efficiency found from the curve of efficiency vs channel counts ratio (Fig. 2). The disintegration rate is then readily converted to the final desired data (i.e., weight, volume, concentration, etc.).

This calibration function was obtained by counting a series of standards having a fixed quantity of carbon-14 in toluene scintillator plus increasing quantities of CCl_4 as quencher. Previous experience had established that the calibration curve from such a series could be applied to dioxane scintillator solu-

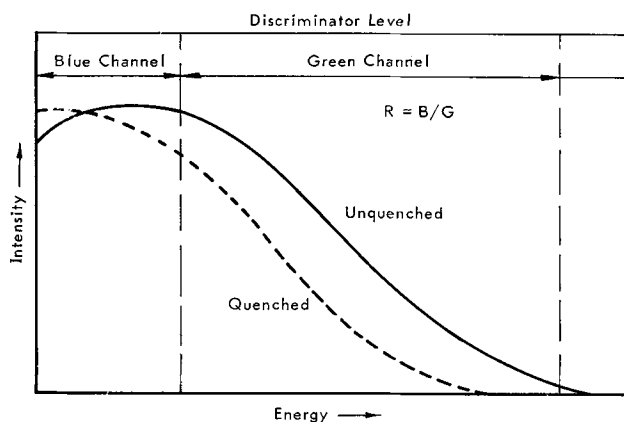


FIG. 1

TABLE IV
ISM vs. PHSM

Sample No.	ISM, ppm	PHSM, ppm
1	784	793
2	799	807
3	819	815
4	799	793
5	805	810
6	806	816
7	815	814
8	813	813
9	753	815
10	870	812
11	806	814
Avg.	806	810
σ	28	6.8
σ /Avg.	3.5%	0.8%
Accuracy ^a	1.3%	0.9%

^a Known = 817 ppm.

tion containing cloth samples or dissolved surfactant. Indeed, such a calibration curve also holds for a wide variety of samples and other scintillation solvents provided that no strong color is introduced into the system.

The PHSM, requiring no additional sample handling, is obviously more convenient than the ISM. However, the relative precision of the two methods was of interest and this comparison was obtained by analyzing a set of surfactant-impregnated cotton swatches by both methods. The results are in Table IV and demonstrate that while the accuracy of both methods is comparable, the precision with the PHSM is superior.

One weakness in the PHSM described in the literature is that frequent recalibration of the curve (see Fig. 2) is required due to instrumental shifts with time. In fact, for most accurate work, it has been recommended that a set of quenched standards be run with every set of samples (8). Even with a computer program this is inefficient and without one it is tedious. Because the most probable cause of instrumental drift in the Packard Tri-Carb is somewhere in the amplification system (i.e., photomultiplier tube, high voltage, amplifier), an attempt was made to adjust operation of the liquid scintillation counter to a common calibration function by using one of the set of quenched standards and adjusting the amplifier gain control for each channel until the count rate in both channels was the same as previously. It was thus hoped that the entire curve would be restored. To see if this was effective, the entire calibration curve was then rerun using the complete set of quenched standards after adjusting the instrument to one standard.

Figure 3 presents the calibration curves before and after adjustment and clearly demonstrates the effectiveness of this simple approach. It is now common

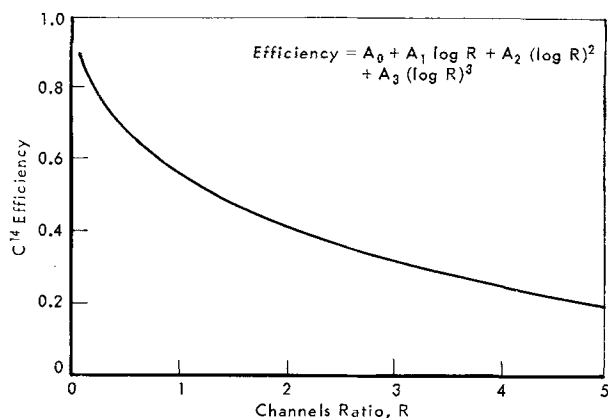


FIG. 2. Efficiency as a function of R.

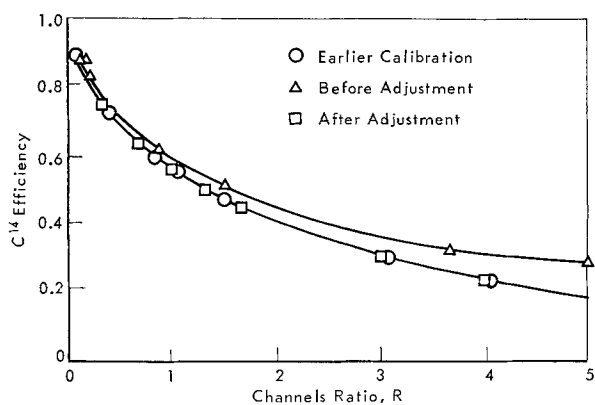


Fig. 3. Effect of adjustment of channels to a quenched standard.

practice to reset the instrument daily, a matter of a few minutes, before proceeding to analysis of samples. Thus the same calibration curve has been in use for several months.

The counting data—sample number, counting time, and counts in each channel are punched into IBM cards after each sample by means of the Packard 540 coupler and an IBM 026 card punch. Descriptive data, including sample weight and specific activity, are also entered on the cards. These cards form load decks for a program for the IBM 7040 computer that performs all calculations and expresses the final result as parts per million of surfactant on the cloth.

Several details of the data-handling procedure are of interest. The first of these is the recycling of the sample series. Each set of samples (40–200 per set) is recycled through the counter several times during an overnight or weekend period; one card is punched each time a sample is counted. The descriptive data is entered *only* in those cards obtained during the *first* cycle of counting.

Samples are counted as backgrounds, calibrations, or unknowns. If the samples are unknowns, the efficiency is found from the channel count ratio by means of the calibration function (Fig. 2), the constants for which are entered via a card at the beginning of a load deck. If the samples form a calibration series (i.e., are standards), the counts are stored until all samples of the series have been entered, then a subroutine is called that finds the constants for the function. At output time, the averages for each calibration sample are likewise processed through the subroutine; the constants are punched into a card for use with subsequent loads of unknowns. Varyingly quenched background samples used with the series of calibration samples are tested for possible response to degree of quench; if such a response is found, the appropriate background count is obtained by in-

terpolation for each sample depending on its quench characteristics.

Data output of the program includes, in addition to the final results, the counts, time, net counting rates, channel count rates ratio, efficiency, dpm, and dpm per gram for each cycle of samples through the counter. Means and standard deviations are found for the more pertinent items. Thus one can, at a glance, obtain information other than the final concentration such as activity, specific activity, counting efficiency, and precision of counting each sample. By comparing the successive counts one can also establish instrument stability or point to the onset of a malfunction. The program contains provisions for expressing the final results in other forms such as volume per cent, weight per cent, mole fraction, etc., if required.

Certain error checks are made by the program; in addition to checking for mechanical errors such as lost cards or erroneous punch positioning, checks are included at several points for channel readings too close to backgrounds to be trustworthy. If such a condition is met, the sample is bypassed and accounted for separately in an error listing.

Discussion

The method as developed serves very well where large numbers of samples are involved. If colored materials are to be counted, a second calibration curve for these samples will probably be required. The efficiency will certainly be more sensitive to sample size with colored samples than with white and this sensitivity will have to be determined before embarking upon an extensive program.

While the PHSM has only been applied to S³⁵ labelled surfactants, its use with tritiated materials poses no problem. Bush (8) has described procedures for determining the best widths for the two channels for different isotopes. An application of this method to adsorptivity and rinsability studies has been reported (10).

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