Polyethoxylated Alkyl Phenols: Relationship of Structure to Biodegradation Mechanism[1]

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Abstract

Using various isolation procedures and measurement by infrared spectroscopy, the mechanism of biodegradation of the polyethoxylated alkyl phenols (ABE) in the river water die-away test has been ascertained. Degradation is shown to proceed by carboxylation of the alkyl chain and, in certain cases, by degradation of the ethylene oxide chain. Degradation of the ether chain has been found to take place only when the chain contains ten or less units of ethylene oxide. The analytical evidence points to the degradation of the ether chain by a hydrolysis mechanism.

The effect of ether chain degradation on the measurement of residual surfactant by ultraviolet spectroscopy and the cobalt thiocyanate procedures has been demonstrated. Ether chain degradation causes the ABE results to be high by ultraviolet measurement and low by the cobalt thiocyanate procedures.

Results of this investigation emphasize the necessity of understanding the mechanism by which degradation takes place, and the significant importance of that degradation, in the various biodegradability tests, before the applicability and reliability of any test for measurement of the residual surfactant can be evaluated.

Introduction

ALTHOUGH NUMEROUS PAPERS have been published on the subject of the biodegradation of alkylbenzene sulfonate (ABS) and linear alkylate sulfonate (LAS), relatively few articles have appeared on the biodegradability of nonionic surfactants of the polyethoxylated alkyl phenol type (ABE). At the time the present work was undertaken (early 1964) there did not appear to be a convenient chemical or spectroscopic method for following the progress of degradation of this type of surfactant.

In the work of Blankenship and Piccolini (1), surface tension measurements were used to follow the progress of degradation using the river water die-away technique. Their work suggests that the initial attack on a degradable nonionic surfactant is an attack on the hydrophobic component of the molecule following the route advocated by Swisher (2) for alkyl aryl sulfonates.

Recently Steinle, Myerly and Vath (3) have reported on the measurement of the rate of biodegradation of ABE's using river water die-away tests and the cobalt thiocyanate colorimetric procedure of Crabb and Persinger (4). A different version of the cobalt thiocyanate procedure has been reported by Greff, Setzkorn and Leslie (5).

Frazee, Osburn and Crisler (6) have recently reported an infrared (IR) procedure for measuring the level and aliphatic ether chain length of residual surface-active ABE isolated from river water die-away samples.

The findings reported to date (1,3) regarding the biodegradation of ABE's are as follows: 1) Less branching of the alkyl chain leads to a faster rate of degradation. 2) Increased ethylene oxide content decreases rate of degradation. 3) Position of attachment of the phenol ring to the straight alkyl chain has a large effect on degradability; primary attachment leading to faster rate than secondary.

The above findings have been based on various die-away tests with the rate of degradation being measured by surface tension and the cobalt thiocyanate procedures. The studies reported in the literature have been concerned with the effect of the alkyl group and the length of the ethylene oxide chain on the overall rate and extent of degradation. Apparently, little attention in any of the investigations has been directed towards the mechanism of biodegradation of the alkyl group and ethylene oxide chain of the ABE molecule.

Our work has been pointed towards the development of a satisfactory analytical procedure for the determination of residual surface-active ABE in the standard river water die-away test, as well as the elucidation of the mechanism of biodegradation in regard to structure of this type of surfactant. Increased knowledge of this mechanism should be helpful in the development and evaluation of analytical procedures for measuring the degradation rate.

Experimental

Test Methods

The river water die-away test (7) was used throughout the degradation studies covered by this report. The water was taken from the Ohio River at a point several miles below Cincinnati, Ohio. The test ABE was added at a level of 20 ppm and the test solution thoroughly mixed with a magnetic stirrer to avoid foam. At periodic intervals over a period of 30–35 days samples were withdrawn while the solution was stirred. All of the ABE's used in this investigation were commercial products and were used as received.

Adsorption chromatography, chloroform extraction and foam stripping were examined as isolation procedures. Both UV and IR spectroscopy were employed for measurement of the surfactant isolated from the die-away sample.

The chromatographic procedure was carried out by shaking 250–500 ml portions of the test solution with IRN-150 monobed ion exchange resin followed by adsorption of the ABE on activated carbon from which it was eluted with methanol and chloroform. This procedure was a modification of one undergoing test by the Soap and Detergent Association (8).

The extraction procedure consisted of extracting 250–500 ml of the test solution, which had its ionic strength increased by the addition of 8 g NaCl per 100 ml, with three 100 ml portions of chloroform.

[1] Presented at AOCS Meeting in Cincinnati, October 1965.

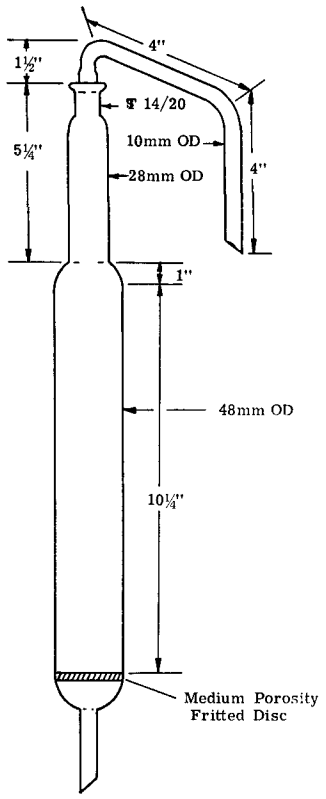


FIG. 1. Foam stripping apparatus.

Although the above isolation procedures have merit for investigational purposes, foam stripping became our procedure of choice for the major portion of the die-away studies carried out during this work. The choice was based on the effective separation of surface-active from non-surface-active ABE, as well as the speed and simplicity of the operation. Foam stripping was accomplished by passing a stream of air through the foam stripping apparatus (Fig. 1) containing 100–400 ml of the test solution. When less than 400 ml of the test solution was used the remainder of the 400 ml volume was made up with distilled water. The foam generated in this manner was removed through the overflow tube and collapsed with chloroform. Foaming was continued until no further foam was produced upon increasing the air flow rate.

The ABE isolated using any of the above procedures was freed from all solvents used in its isolation by evaporation on a steam bath under a stream of nitrogen or clean, dry air. The residue was then taken up in chloroform and diluted to a suitable volume for UV measurement. The solvent was then removed and the residue examined using the IR method (6).

Results and Discussion

The chromatographic isolation procedure was investigated first as a means of separating and concentrating the ABE from a river water die-away sample

TABLE I

Degradation of Ethylene Oxide Chain of Nonyl ABE_{10} (B.C.[a])

Days	Residual ABE		Ethylene oxide
	UV	IR	units/mole
0	17.3 ppm	18.9 ppm	9.6
14	15.5	14.7	10.0
21	5.5	4.7	6.3
28	6.0	3.0	5.1
35	6.3	3.1	4.8

[a] B.C. = Branched chain alkyl group.

TABLE II

Effect of Resin Treatment on Level of $CHCl_3$ Extractable ABE

Days	ppm ABE (UV)		Units ethylene oxide	
	No resin	Resin-treated	No resin	Resin-treated
0	22.3	21 5	9.5	8.9
6	21.8	16.6	8.7	8.9
13	18.9	7.2	6.1	7.5
15	16.7	4.3	4.6	4.8
17	15.4	5 5	4.5	4.0
22	14.3	3.9	4.3	4.3
24	13.3	3.3	4.1	4.5
27	12.7	3.0	3.5	3.9

for UV measurement. A branched chain nonyl phenol containing approximately 10 units of ethylene oxide per mole (ABE_{10}) was used as the test surfactant. The results of this test, plotted as a die-away curve, showed little change in the first two weeks of the test, a loss of approximately 50% of the initial ABE level between the second and third weeks, and a leveling off during the final two weeks of the test. The abnormal appearance of this die-away curve suggested that degradative changes were taking place in the ABE molecule which were not measurable by UV absorption spectroscopy. Ultraviolet measurement is related only to the aromatic ring of the molecule and does not reflect changes in the hydrophobic or hydrophilic structures of the molecule.

Ether Chain Degradation

Since the IR spectrum of a material is a multivalued physical property which can provide much information regarding structure, it appeared ideally suited to the investigation of structural changes in the ABE molecule. The qualitative IR spectrum of the ABE isolated from the above 0-day test showed a normal spectrum of ABE_{10}, whereas the spectrum of the 34-day isolate closely resembled that of a nonyl ABE_4. As a result of this finding the recently reported IR procedure (6) was developed for the measurement of both level and ethylene oxide chain length of the isolated surfactant.

In order to obtain quantitative evidence of the degradation of the ethylene oxide chain a second die-away test was carried out in the same manner as the first, with measurements being made by UV and IR. The results of this test are given in Table I. This evidence of degradation of the aliphatic ether chain was repeated numerous times in subsequent tests.

Degradation of Alkyl Group

The effect of biodegradation on the hydrophobic alkyl group was next investigated. Carboxylation, the initial step of the mechanism proposed by Swisher (2) for the biodegradation of LAS, would result in a methyl group being transformed to a carboxyl group. If such were the case the molecule would have been rendered ionic and as a result would have been removed from the test solution by the monobed ion exchange resin in the chromatographic procedure used in the first two tests. In order to obtain evidence to support this mechanism a die-away test was carried out with the surfactant being isolated by chloroform extraction from duplicate aliquots, one of which had undergone a batchwise treatment with monobed ion exchange resin. The results of this test are given in Table II, and again show the ethylene oxide chain to have degraded to approximately 4 units/mole. The significant difference in levels of ABE between the resin treated and untreated samples gave strong support to the postulated carboxylation mechanism.

Further evidence of carboxylation was obtained

TABLE III
Recoverability of ABE Using 400 ml Foam Stripping Apparatus

Mg ABE		ppm ABE		% Recovery
Known	Found	Known	Found	
1.88	1.70	4.7	4.3	90.4
1.17	1.03	2.9	2.6	88.0
0.97	0.83	2.4	2.1	83.0
0.70	0.57	1.8	1.4	81.0
0.47	0.35	1.2	0.9	75.0

using a branched chain nonyl ABE_4. The test solutions were examined by our standard foam stripping procedure and by choloroform extraction from acidic and alkaline solutions. The levels based upon ultraviolet measurement (ref. 6, Table III) were the same for the chloroform extraction from alkaline solution and for the foam stripping. The nearly constant difference between the amounts of ABE extracted from neutral and strongly acidified solutions was postulated as being due to partial neutralization of the carboxylated ABE with calcium and other cations in the test solutions. These results leave little doubt that carboxylation of the alkyl chain is one of the routes by which biodegradation proceeds.

Foam Stripping

In the above test a foam stripping apparatus of 100 ml capacity was used. The quantity of ABE, at low levels, which could be recovered was found to be inadequate for measurement by IR with any degree of confidence, leading to the construction and testing of the 400 ml apparatus used in all subsequent tests. In all tests of surface-active ABE recoverability using the 400 ml apparatus recoveries of over 90% resulted for levels ranging from 5–20 ppm. Recoverability of levels below 5 ppm, using the branched chain nonyl ABE_{10} as the test surfactant, are given in Table III. The nearly constant loss of approximately 0.14 mg of ABE (equivalent to 0.35 ppm) over the range tested is postulated as being the threshold level of foaming for the nonyl ABE_{10} in the apparatus tested.

Using foamability as a measure of surface activity, it is apparent that only surface-active ABE is isolated by foam stripping. Conversely, carboxylated ABE, which is not removed by foam stripping, has lost its surface active properties. The degradation of the ether chain to approximately 3 units/mole will, in general, result in loss of surface activity, making it evident that ABE having the ether chain degraded to such extent will likewise not be isolated by foam stripping.

In order to confirm earlier findings using the chromatographic and extraction procedures, another die-away test was carried out on the branched chain nonyl ABE_{10} using foam stripping, with measurements being made by IR and UV absorption spectroscopy. In this test the ethylene oxide chain was again found to have degraded to approximately 4 units/mole, which is in agreement with the previous findings for this surfactant. Figure 2 illustrates by tracings of both UV and IR spectra the decrease in level of surface activity (residual ABE) with time. The IR spectra likewise illustrate the degradation of the ethylene oxide chain as evidenced by the decrease, with time, in the ratio of the aliphatic ether band at 1120 cm^{-1} to the aromatic ether band at 1250 cm^{-1}. Figure 3 shows the IR spectrum of the foamed 34-day sample in which carboxylation is not evident as well as the spectrum of a chloroform extract from acid solution in which the carboxyl band at 1700 cm^{-1} is

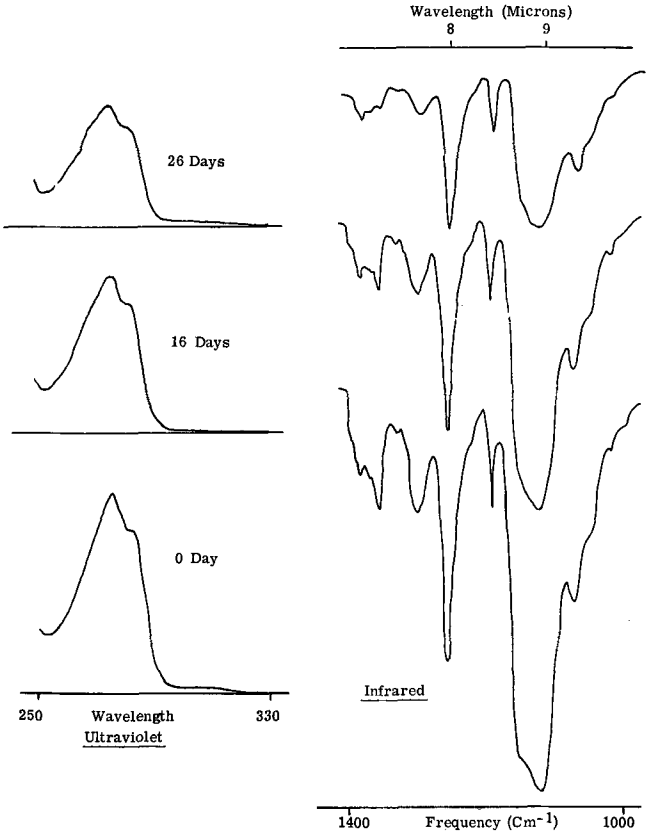


FIG. 2. UV and IR spectra of foam from a typical nonyl ABE_{10} river water die-away test.

very much in evidence. The residual level of ABE and the units/mole of ethylene oxide determined by IR analysis, as well as the apparent level and level corrected for ethylene oxide chain degradation by UV analysis are given in Table IV. The Table IV results clearly show the necessity of correcting the UV results for the ether chain degradation. Since the IR analysis is a prerequisite to accurate UV results, measurement in all subsequent tests was made only by IR.

Proposed Mechanism of Ether Chain Degradation

Although the carboxylation mechanism has been

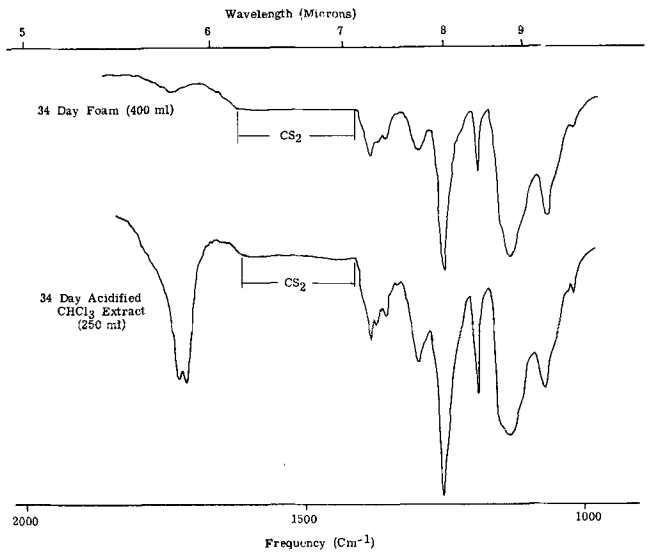


FIG. 3. IR spectra of foam and $CHCl_3$ extract (acid) from nonyl ABE_{10} die-away test.

TABLE IV

Comparison of IR and UV Measurements—ABE_{10} Die-Away

Day	Units EO (IR)	Residual ABE		
		ppm (IR)	ppm (UV)	ppm (UV Corrected [a])
0	9.1	18.4	19.0	17.8
5	8.9	17.5	18.0	16.7
8	8.7	15.7	17.3	15.8
12	7.8	14.7	16.3	13.9
14	7.2	13.2	16.2	13.2
16	7.0	12.5	15.3	12.2
19	6.3	11.2	14.0	10.5
22	5.3	8.7	12.8	8.8
26	4.1	6.2	11.0	6.7
30	4.2	5.3	8.6	5.3
34	3.6	2.4	4.9	2.8

[a] UV Corrected = UV $\times$ $\frac{\text{Mole Wt. of Alkyl Phenol} + (44 \times \text{Units EO/Mole})}{\text{Mole Wt. of Original ABE}}$

shown to be the initial step in the degradation of the alkyl group, the question arises as to the mechanism by which the ether chain degrades. The terminal hydroxyl of the ether chain is evident at 1065 cm^{-1} in all IR spectra, whether of the surface-active isolate or of the nonsurface-active isolate (see Fig. 2 and 3). A further examination of the hydroxyl band shows its ratio to the aliphatic ether band to increase as degradation of the ether chain progresses. On the basis of the above observations it is proposed that the ether chain is degraded by bacterial or enzyme induced hydrolysis, with one unit of ethylene oxide being converted to a mole of ethylene glycol and the ether chain always ending with the terminal hydroxyl group. The only product of such a degradation mechanism would be ethylene glycol which has been reported to be further biodegradable (9).

Biodegradability of Other ABE's

Reported findings of less branching of the alkyl chain leading to faster degradation were confirmed using our test procedure of foam stripping followed by IR measurement. Using a straight chain nonyl ABE_{10} as the test surfactant, the test showed the surfactant to begin degrading to a significant extent during the first week of the test as compared with the slower rate of its branched chain counterpart. The straight chain molecule degraded to approximately 5 units of ethylene oxide per mole in 30 days, with the degradation of the ether chain commencing between the second and third weeks of the test. This is in agreement with the findings for the branched chain structure, indicating that the earlier and more rapid degradation of the straight chain ABE is due solely to carboxylation of the alkyl chain. Chloroform extracts of the strongly acidified sample following foam stripping were made on the 13 and 22-day samples with the IR spectrum of each showing carboxyl bands at 1700 cm^{-1}. These findings show the straight chain structure to degrade by the same mechanism as determined for the branched chain structure. Comparative die-away curves for the straight and branched chain molecules are shown in Figure 4.

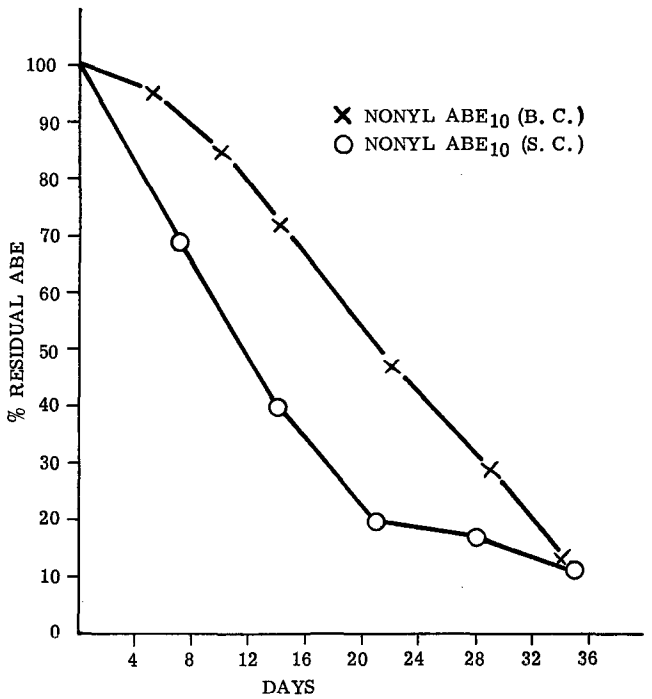


FIG. 4. Comparative die-away curves for straight and branched chain nonyl ABE_{10}.

The effect of biodegradation on ABE's having longer ether chains was first checked using a branched chain nonyl ABE_{15}. The results of this test showed insignificant degradation to have occurred over a period of 35 days, with no degradation of the ether chain taking place. Tests were then carried out with three ABE_{11}'s having different alkyl chains. The results of these tests are shown in Figure 5. In no case was there any evidence of degradation of the ether chain, in the surface-active material isolated by foaming as had been found in the case of ABE_{10}, both straight and branched chain, and branched chain nonyl ABE_4. In fact, the units of ethylene oxide per mole were found to increase in the case of the two straight chain structures. The reason for this apparent increase in ether chain length is not known. However, it is speculated that the ether chain distribution of these surfactants was such that some of the shorter ether chains were degraded resulting in an increase in the average ether chain length of the residual ABE. This speculation is somewhat supported by the finding of an average ether chain of approximately 8 units/mole in the chloroform extracted, carboxylated ABE not removed by foam stripping on the 28 and 35-day samples of the straight chain $C_{12}ABE_{11}$ die-away. Although there was no evidence of ether chain degradation in the surface-active ABE in any of the above cases, degradation by the carboxylation of the alkyl group occurred rapidly and to an appreciable extent in the case of both straight alkyl chain surfactants as shown in Figure 5. The straight chain nonyl ABE was found to degrade more rapidly and to a greater extent than the straight chain $C_{12}ABE$. These findings indicate that the ether chain length has a definite effect on the carboxylation of branched chain alkyl groups, with ether chains greater than 10 units/mole being nearly complete in the suppression of carboxylation. Insufficient testing has been completed to determine at what, if any, point the ether chain length will suppress carboxylation of the straight chain alkyl group.

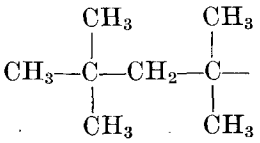
A missing gap in these studies was filled in by a die-away test on a branched chain nonyl ABE_6. As expected, this surfactant degraded by both carboxylation and degradation of the ether chain. After four weeks the ethylene oxide chain was found to have been reduced from 6 to 4 units, degradation being first apparent in the 3-week sample. No surface activity remained after five weeks. A chloroform extract from the acidified fifth week sample showed the ether chain to have degraded to 2.5 units/mole.

TABLE V

Degradation of Isooctyl ABE_9

Days	Isolation procedure	ABE, ppm	Alkyl phenol, Calcd. ppm	Ethylene oxide, units/mole
0	Foaming	17.8	6.3	8.0
5	Foaming	18.5	6.8	8.3
7	Foaming	18.0	6.5	8.5
14	Foaming	13.6	5.9	6.1
21	Foaming	11.3	5.7	4.6
28	Foaming	9.9	5.6	3.6
35	Foaming	7.8	5.0	2.6
35	$CHCl_3$ Extra.	10.1	6.6	2.5

Our findings of degradation of the alkyl chain by the carboxylation mechanism in all cases where degradation of the polyether chain had occurred led to an investigation of the degradation mechanism of an isooctyl ABE. This surfactant was selected on the basis of the structure of the alkyl chain which was confirmed by its nuclear magnetic resonance spectrum as being primarily



Due to the absence of a methyl group connected to a carbon containing a replaceable hydrogen, conditions which are necessary for the initial step of carboxylation, the isooctyl ABE would not be expected to degrade by the carboxylation route.

Application of our test procedure to an isooctyl ABE_9 resulted in a finding that approximately 40% of the surfactant remained after 35 days, with the ethylene oxide chain having been degraded to 2.6 units/mole. This ether chain degradation was first evidenced in the 14-day sample. These findings indicated that ether chain degradation was the principle route of degradation for this surfactant. This was established by making a chloroform extraction of the 35-day sample, which was found to contain slightly carboxylated ABE containing an ethylene oxide chain of 2.5 units/mole, and then calculating the ppm alkyl phenol in each isolate of the die-away test. These results are given in Table V.

It will be noted that the calculated level of alkyl phenol accounted for in the 35-day $CHCl_3$ extract approximates the level present prior to the start of degradation, showing that none of the surfactant had been totally degraded. Since equivalent ethylene oxide chain lengths were found in both the foam stripped and chloroform extracted 35-day isolates, it is presumed that the 24% difference in calculated alkyl phenol levels and the 23% difference in ABE levels of these isolates are a measure of the maximum degradation by carboxylation which could have taken place. Although some carboxylation did occur, as evidenced by the carboxyl band in the IR spectrum of the 35-day chloroform extract, it is proposed that this is attributable to the presence of a low level of some isomer capable of carboxylating; and that the 23–24% apparent carboxylation is most likely a result of the decreased foamability of the ether chain degraded surfactant.

Effect of Ether Chain Degradation on Cobalt Thiocyanate Methods

The findings regarding the degradation of the ethylene oxide chain using the river water die-away test suggest the inapplicability of the cobalt thiocyanate colorimetric procedures in those cases where such degradation occurs. This likelihood has been confirmed on a limited scale. In the case of the isooctyl ABE_9 die-away, equivalent levels were found using the cobalt thiocyanate procedure and the IR procedure prior to degradation of the ether chain. However, following degradation of the ether chain to 2.6 units/mole, the level of surface-active ABE determined using the cobalt thiocyanate method (5) was 2.3 ppm as compared with the 7.8 ppm determined by IR. The same tests when applied to a straight chain $C_{12}ABE_{11}$, in which case ether chain degradation does not take place, resulted in the finding of equivalent levels of residual ABE by both methods. In the above tests the samples for the cobalt thiocyanate test were passed through a column of IRN-150 monobed ion exchange resin to remove any carboxylated surfactant prior to the application of the cobalt thiocyanate procedure.

TABLE VI

Effect of Ether Chain Degradation on Cobalt Thiocyanate Measurement of Residual ABE[a]

Days	Cobalt thiocyanate	Infrared	
	ppm ABE	ppm ABE	E.O. (units/mole)
0	17.3	18.6	10.4
7	17.1	20.2	9.8
14	15.4	16.8	10.0
21	8.0	13.0	7.8
28	3.6	8.8	6.0
34	0.6	5.7	5.3

[a] Test surfactant = Nonyl ABE_{20} (branched chain).

A river water die-away test was then carried out on the branched chain nonyl ABE_{10} and the surface-active material removed by foam stripping at weekly

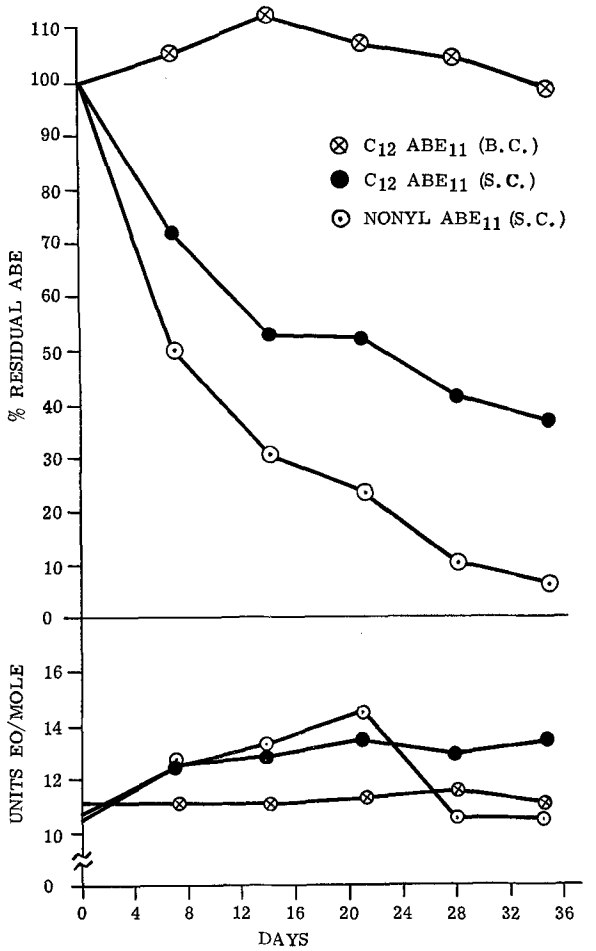


FIG. 5. Effect of alkyl chain structure on degradation of ABE_{11}.

intervals. These isolates were divided into duplicate aliquots, with one aliquot being analyzed by the IR method and the other by the cobalt thiocyanate procedure. In this test the complexing, extraction and measurement of the cobalt complex were carried out as described in (4). The results of this test are given in Table VI.

It is emphasized that the findings of this investigation are based solely on river water die-away tests and do not necessarily apply to other types of degradation studies. In those cases where there is no degradation of the ether chain, measurement of the residual surface-active ABE by UV or a cobalt thiocyanate procedure should give results equivalent to those obtained by IR. An advantage of the IR procedure, however, is that calibration curves are required only for the two types of alkyl chains; i.e., branched and straight chain, whereas both the UV and cobalt thiocyanate procedures require calibration curves for each surfactant tested.

In summary, tests have been described for the analysis of the residual surface-active ABE in the river water die-away test which elucidate the mechanism of biodegradation. Degradation has been shown to proceed by one or both of two routes; namely, carboxylation of the alkyl group and degradation of the ether chain. Test results show both branched and straight chain structures containing ten or less units of ethylene oxide per mole to degrade by both routes, straight chain structures containing more than ten units of ethylene oxide per mole to degrade only by carboxylation of the alkyl group, and branched chain structures containing more than ten units of ethylene oxide per mole to be essentially nonbiodegradable. An exception to the above is the isooctyl alkyl group which, by the nature of its structure, does not appear to be subject to degradation by the carboxylation route.

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