ENZYMATIC ACTIVITY, IN DESIZING TEXTILES, AS INFLUENCED BY BIODEGRADABILITY AND MOLECULAR STRUCTURE OF COMPANION SURFACTANTS

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ENZYMATIC ACTIVITY, IN DESIZING TEXTILES, AS INFLUENCED BY BIODEGRADABILITY AND MOLECULAR STRUCTURE OF COMPANION SURFACTANTS

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GLOSSARY OF ABBREVIATIONS AND SYMBOLS

ml	milliliter
mg	milligram
gm	gram
Mμ	millimicron
A	Absorbance
DV	Dextrinizing Value
BAU	Bacterial Amylase Unit
e.o.	ethylene oxide
^{o}C	degrees Centigrade

SUMMARY

The quantitative determination of starch in desizing solutions by colorometric techniques involving the starch-iodine complex was investigated. The influence of various commercial ethylene oxide containing surfactants on the activity of amylolytic enzymes used in desizing textiles was studied.

These research investigations disclosed that a correlation exists between the degree of biodegradability of surfactants and the compatibility of those surfactants with amylolytic enzymes. It was found that readily biodegradable ethoxylated surfactants inhibit the starch dextrinizing activity of amylolytic enzymes to a greater extent than the non-biodegradable analogs. The degree of inhibition of enzymatic activity is dependent upon the concentration of biodegradable surfactant, and the inhibition is significantly increased at high surfactant levels. The inhibition of enzymatic activity by surfactants is essentially independent of time and temperature.

CHAPTER I

INTRODUCTION

Amylolytic enzymes, or amylases, are complex organic chemicals which exhibit the unique and specific capability of degrading starch polymers. The amylolytic enzymes may be of malt, pancreatic, or bacterial origin.

In order to facilitate textile weaving operations, the warp yarns are normally treated with a protective coating prior to the weaving operation. With rare exceptions, the coating is of a polymeric nature. It is usually applied from a water solution. The process for applying this protective coating is called "slashing." Colloidal solutions prepared from starch have historically been employed in slashing. In recent years, some of the starches have been modified chemically to give improved sizing and desizing properties. Also, starch is often used as an extender for other sizing materials such as polyvinyl alcohol and sodium carboxymethyl cellulose. Starches are still the most widely used textile sizing agents.

In most cases, the size must be removed from the fabric after weaving. Removal of the size is necessary in order that the fabric may be dyed and finished without interference. Because the size coating is only a temporary finish, the size employed should be removable by easy and inexpensive methods.

Various techniques and different chemicals are used for removing

a starch based size from textiles, including mineral acid degradation, sodium bromite, and other oxidizing chemicals. However, enzymatic desizing remains the most universal technique. The exact mechanism of the catalytic hydrolysis of starch by amylase is not well understood, largely because the complex structure of the various starches have not been completely determined. However, it is known that amylases degrade specific linkages of starch[1], causing little or no damage to cellulosic fibers. Enzymatic desizing is generally considered to be the least harsh of the various desizing processes. The degraded starch fragments are relatively low in molecular weight, and they can be removed by washing or solubilized by a subsequent treatment with caustic soda. A typical preparation procedure for cotton fabric might be as follows:

1. Singeing to remove protruding fibers.
2. Saturation with enzyme solution.
3. Lay over or steaming.
4. Washing.
5. Saturation with caustic soda.
6. Lay over or steaming.
7. Washing (hot wash followed by cold wash).
8. Saturation with bleach.
9. Lay over.
10. Mercerization.
11. Neutralization and/or soaping off.
12. Finishing or dyeing operations.

This paper is concerned with steps two and three of the above process routine.

The enzyme bath normally contains sufficient wetting agent or penetrant to assure complete and uniform penetration of the enzyme solution into the fabric. The enzyme cannot degrade the starch unless it is thoroughly wetted out so that intimate contact of starch with enzyme can be made. The sized fabric is somewhat water-repellant in nature due to the presence of natural waxes in the fiber as well as those size additives which are used to improve surface lubricity. In addition to the wetting agent, the enzyme bath may also contain sodium chloride or some salt of calcium which serves to improve the stability of the enzyme to high temperatures. It is therefore apparent that the enzyme and the wetting agent or surfactant must be compatible, i.e., they must be capable of working together without having an adverse effect upon each other.

The years of 1964 and 1965 were marked by two significant developments in the textile industry. It seems to the author that some aspects of the developments during those two years have not been adequately resolved.

The first major event in question was the advent of "permanent press" or "durable press" finishes. The high resin add-ons necessary for permanent press caused excessive strength loss on all-cotton fabrics. As a direct result of the invention of permanent press finishes, polyester and cotton blend fabrics began to gain public acceptance very rapidly, and they soon began to displace all-cotton fabrics. The presence of the hydrophobic polyester fiber in most fabrics led to innovations in both sizing and preparation techniques. In sizing, more and more polyvinyl alcohol and sodium carboxymethyl cellulose were used with

starch as an extender. More modified starches were used in sizes. A typical desize procedure still employed enzymes; but in order to clean the polyester fibers, new surfactant and solvent emulsion systems began to be used. Where previously only "wetting" concentrations of about 0.1 percent surfactant were used in the enzyme bath, it became common to use up to 2 percent of surfactant in the bath to emulsify the solvents. Higher surfactant and enzyme concentrations also came about as a result of the trend toward high-speed continuous preparation operations involving "flash desizing." Some surfactants which were known to be compatible with enzymes at the lower concentrations were found to be detrimental to enzymatic activity at the higher "emulsification" concentrations of surfactant and enzyme.

The second important change which came about during this time was the change from "hard" or non-biodegradable surfactants to the new synthetic "soft" or biodegradable products. Manufacturers of detergents published extensively concerning the relative surface active properties of the new surfactants as compared to the hard synthetic surfactants. The soft synthetic surfactants have been shown to be equivalent or superior to the hard surfactants in most applications. However, there has been a void in the literature concerning the compatibility of the new soft synthetic surfactants with amylolytic enzymes. Nevertheless, the biodegradable surfactants have been widely used as penetrants and emulsifiers in enzyme desizing.

Enzymatic desizing of textiles is one of the few natural or biological processes employed in the textile finishing industry. Biodegradation of surfactants is a biological process. It seems reasonable to

suspect that an interrelation may exist between these two processes when highly biodegradable surfactants are used in conjunction with enzymes. The object of this research was to search for a correlation between the degree of biodegradability of surfactants and the compatibility of these surfactants with enzymes used for desizing textiles. In order to limit the scope of this investigation, the types of surfactants studied were limited to ethylene oxide condensates and their anionic derivatives.

CHAPTER II

BIODEGRADABILITY OF SURFACTANTS

At the present time, standard methods for the evaluation of biodegradability of surfactants have not been established; but a number of generally accepted tests are in use. One of these tests is the shake-culture test[2]. The shake-culture test is not designed to simulate field conditions. The batch (semi-continuous) activated sludge test[3] is designed as a miniature activated sludge waste treatment process. The river die-away test or the Federal Water Pollution Control Administration method[4] is designed to approximate natural aerobic conditions in rivers. The analytical tests used in conjunction with the above tests utilize methylene blue colorimetry for the anionics and a cobalt thiocyanate colorimetric procedure for the nonionics.

It has been reported that the cobalt thiocynante method can give misleading results[5], and there is still controversy over the utility of the method. Thin layer chromatographic techniques[6] may overcome the difficulty in measuring residual nonionics.

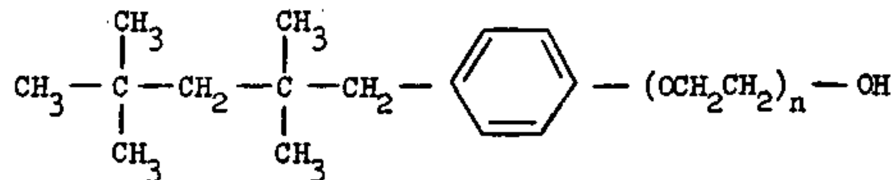
As a confirmation of the extent of biodegradation, the biochemical oxygen consumption during the test procedure may be measured using the "Warburg" Respirometer.

Controversy still exists as to what types of surfactants are acceptably biodegradable, that is to say, where the line can be drawn. However, it is possible by surveying the literature, or by a combination

of the various test methods, to obtain a clear picture of the relative biodegradability of various surfactants. The relative rates of biodegradation are no longer questioned. The nonionic surfactants may be described as very hard, hard, soft, and very soft. The chemical structures conforming to these descriptions are, respectively: branched alkyl phenol ethoxylates, linear alkyl phenol ethoxylates, secondary linear alcohol ethoxylates, and primary linear alcohol ethoxylates[7]. The degree of biodegradability is also a function of the amount of ethylene oxide present in the molecule[8]. Very long ethylene oxide chains reduce the biodegradability of each type of hydrophobe, so that some overlapping of properties does occur between the various classes of hydrophobes, as shown in Figure 5 in the Appendix. The ethoxysulfates parallel the nonionics in regard to their biodegradability (Figure 6 in the Appendix).

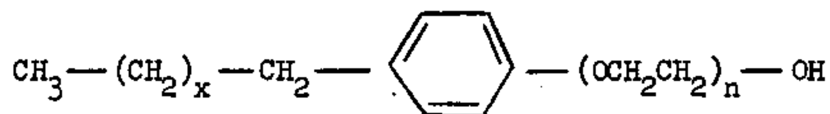
Nonionic surfactants and their anionic derivatives are the most popular textile surfactants. By nonionic surfactants, reference is made to those products containing an ethylene oxide chain. There are five basic types of nonionics used for preparation in the textile industry, and each type contains a different hydrophobe. The five types of hydrophobe for ethoxylation include branched chain alkyl phenols, linear chain alkyl phenols, branched chain fatty alcohols, random secondary linear alcohols, and primary linear alcohols. Typical molecular structures of the five types of nonionics are:

Type 1:



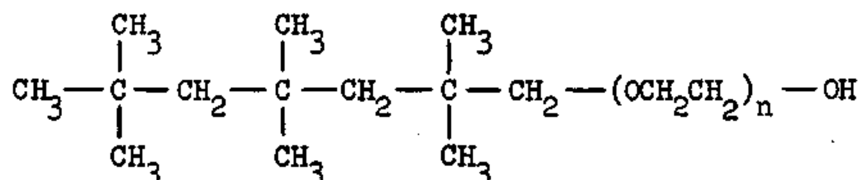
Branched chain alkyl phenol ethoxylate

Type 2:



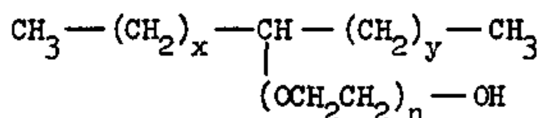
Linear chain alkyl phenol ethoxylate

Type 3:



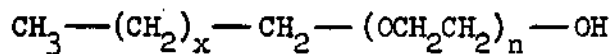
Branched fatty alcohol ethoxylate

Type 4:



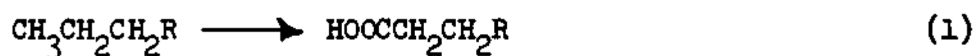
Random linear secondary alcohol ethoxylate

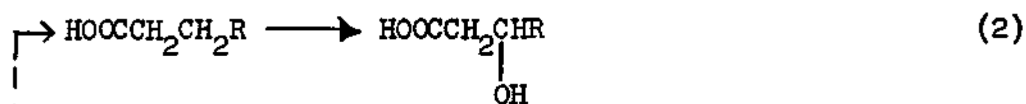
Type 5:

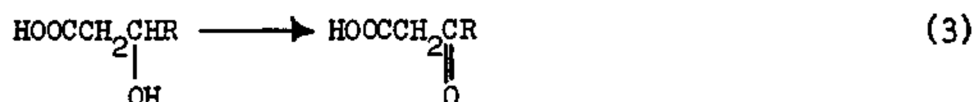


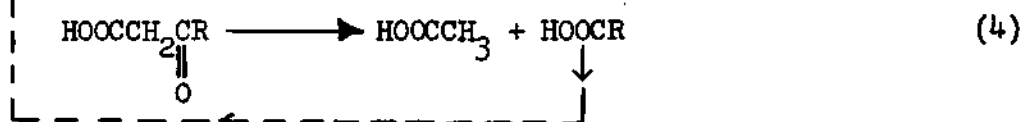
Primary linear fatty alcohol ethoxylate

Hammerton's observation[9] that straight chain anionic detergents are biologically soft and the corresponding branched chain materials are biologically hard is apparently true for nonionic surfactants as well. This is true because both hydrophobes are degraded through a beta oxidation process:









The illustrations consider only the degradation of the hydrophobe. In actual practice, the ethylene oxide hydrophile is also slowly degraded. As shown, the first step in biological degradation is the oxidation of the terminal methyl group to the carboxylic acid. The next step is beta oxidation or oxidation at the beta carbon atom. The chain then undergoes fission to yield acetic acid and a new acid containing two fewer carbon atoms than the starting molecule. It is easily seen that branching at or near the beta carbon atom can sterically hinder or block beta oxidation, which in turn can greatly reduce the capacity of the molecule to be degraded biologically.

It has been reported by Lashen, E., et al., that branched chain alkyl phenol ethoxylates are readily degraded under natural conditions by properly acclimated microorganisms[10]. Booman, K., et al., reported that the microorganisms readily adapt themselves to the branched chain alkyl phenol ethoxylates[11]. Other studies[12] indicate that the microorganisms responsible for biodegradation are very selective, completely consuming a favored kind of material before attacking others. The

microorganisms do not prefer the branched synthetic detergents, and the rate at which these are consumed is largely determined by the availability of other foods[13]. Other references confirm the superior biodegradability of straight chain primary and secondary alcohol ethoxylates as compared to the branched chain products[14,15,16].

It has been established and accepted in the industry that both the rate and the completeness of biodegradability is dependent upon the degree of linearity of molecular structure. For graphic illustrations of the relative biodegradability of the various types of ethylene oxide condensates, see Figures 6, 7, 8, and 9 in the Appendix.

CHAPTER III

SURVEY OF AMYLASE ASSAY PROCEDURES

There are two basic methods for measuring the enzymatic degradation of starch. It is necessary to measure the strength or potency of an enzyme solution by an indirect method, namely, the ability of the enzyme to degrade starch.

The first of the aforementioned enzyme assay techniques is based on measurement of the viscosity change of a starch solution being acted upon by an enzyme. While this viscosity test method is probably very meaningful in the practical terms of correlation with textile practice, it is nevertheless tedious and time consuming. It does not seem applicable for monitoring the activity of a desizing bath in industry. A shorter and more direct method of assay is desirable.

The second common technique involves measurement of the color in a starch-iodine complex. The American Association of Textile Chemists and Colorists (AATCC) has adopted a Tentative Test Method 103-1962T for the assay of bacterial amylases used in desizing. Premier Malt Products, Inc. has developed a similar test method entitled "Determination of Dextrinizing Enzymatic Activity."

The AATCC Tentative Test Method 103-1962T calls its unit of measure the Bacterial Amylase Unit (BAU), which it defines as that quantity of the enzyme that will dextrinize one milligram of starch substrate solution under the specified experimental conditions. The test method

proposes dilution procedures and temperature and pH controls. The analyst is directed to add one milliliter aliquots of the starch-enzyme solution to aliquots of a standard iodine solution at appropriate time intervals. The end point (elapsed time measurement) is taken as the point at which the color developed by the starch-iodine complex is equivalent to a standard alpha-amylase color disc or the No. 17 varnish color in the Hellige Comparator. The alpha-amylase content of the sample is then calculated using the formula:

$$\text{BAU per gram} = 40\ F/T$$

where:

F = dilution Factor = Total dilution volume/sample weight in grams, and

T = Dextrinizing Time

The amylase content of the sample is reported as Bacterial Amylase Units (BAU per gram).

The Premier Malt Products procedure uses the Dextrinizing Value (DV) as the unit of measure. One DV unit is defined as the amylase activity which will dextrinize 20 milligrams of Lintner starch in 30 minutes at 30° C and pH 6.6 under the conditions of the standard assay procedure as described in the test method. The Premier Malt Products procedure permits the use of the Hellige comparator or the colorimeter for analytical purposes.

As in the AATCC procedure, the enzymatic activity using the Hellige comparator is based on determining the time required for the digestion mixture to reach a standard reference color when reacted with

iodine. The analysis of dextrinizing enzymatic activity using a colorimeter is based on the measurement of the color produced when the digestion mixture is reacted with iodine after a digestion time of 20 minutes. For the Hellige comparator, the dextrinizing value of the sample is calculated using the formula:

$$\text{DV units per gram} = 60\ \text{DF}/\text{DT}$$

where:

DF, or the Dilution Factor = the total volume in milliliters to which the enzyme is diluted divided by the weight of the sample in grams, and

DT = the elapsed time of digestion.

The colorimeter measurement involves the preparation of a standard curve, which is a plot of DV versus percent transmission obtained in a standard procedure using various dilutions of known DV. In subsequent tests on unknowns, the percent transmission readings are referred back to the standard curve, and the DV is read off the curve. The DV units per digestion are then converted to DV units per gram as a measurement of the sample potency, as follows:

$$\text{DV units per gram} = \frac{(\text{DV units/digestion})(\text{dilution})}{(\text{gms enzyme})(\text{ml diluted enzyme used in digestion})} .$$

CHAPTER IV

EXPERIMENTAL METHODS

A Klett-Summerson Photoelectric Colorimeter Model 900-3, Serial No. 10-535, was employed for recording the scale reading of samples. The scale reading is the Absorbance multiplied by the Factor 500. Divide the scale reading by 500 to calculate the Absorbance. The red filter (640-700 Mμ) was used, and the instrument was standardized to zero Absorbance with deionized water.

Standard solutions were prepared as follows:

1. Iodine-stock solution:

Five and one-half grams of reagent grade crystalline iodine and 11 grams of reagent grade potassium iodide were dissolved in deionized water and diluted to 250 milliliters. The stock solution was stored in an amber bottle and kept in the dark.

2. Iodine-working solution:

Four milliliters of the iodine stock solution and 40 grams of reagent grade potassium iodide were dissolved in deionized water and diluted to 1 liter. The iodine solution, prepared weekly, was stored in an amber bottle.

3. Starch solution:

Ten grams (dry weight) of Lintner starch (11.07 grams total) were slurried in deionized water, then added to 300 milliliters of vigorously boiling and agitated deionized water. The starch

solution was again brought to the boil for 6 minutes, then it was cooled and diluted to 500 milliliters to give a 20 grams per liter starch solution.

Starch Iodine Standard Curve

Both the AATCC test and the Premier Malt Products test method make the assumption that the intensity of color due to the starch-iodine complex is a linear function of the quantity of residual starch at the dilution used in their assay procedures. Browning, et al[17] have demonstrated that the linear relationship exists over a particular dilution range. In order to confirm that a linear relationship exists, a calibration curve was prepared.

The standard starch solution was diluted in stages to 10 grams per liter and 2 grams per liter concentration, and aliquots of the solutions having known starch content were taken. The aliquots were added to 10 milliliters of the working iodine solution in the colorimeter cell. The total volume of the iodine-starch solution was adjusted to 11.0 ml in each test with deionized water. The scale reading of each sample was recorded, and the readings were plotted against the amount of starch in the aliquot. The results are shown in Table 1 and Figure 1. See the Appendix for a sample calculation.

Figure 1 shows that a linear relationship exists between starch concentration and color formation with iodine. The deviation from linearity above 0.8 mg starch was probably due to the tendency of the complex to agglomerate and precipitate at the higher concentrations, with a resultant reduction of the scale reading as expected. Agglomera-

Table 1. Starch-Iodine Calibration

Milligrams Starch	Scale Reading
0.1	36.0
0.2	67.5
0.4	130
0.6	190
0.8	242
1.0	292
1.2	323
1.4	395
1.6	410
1.8	452
1.0	292
2.0	490
3.0	625

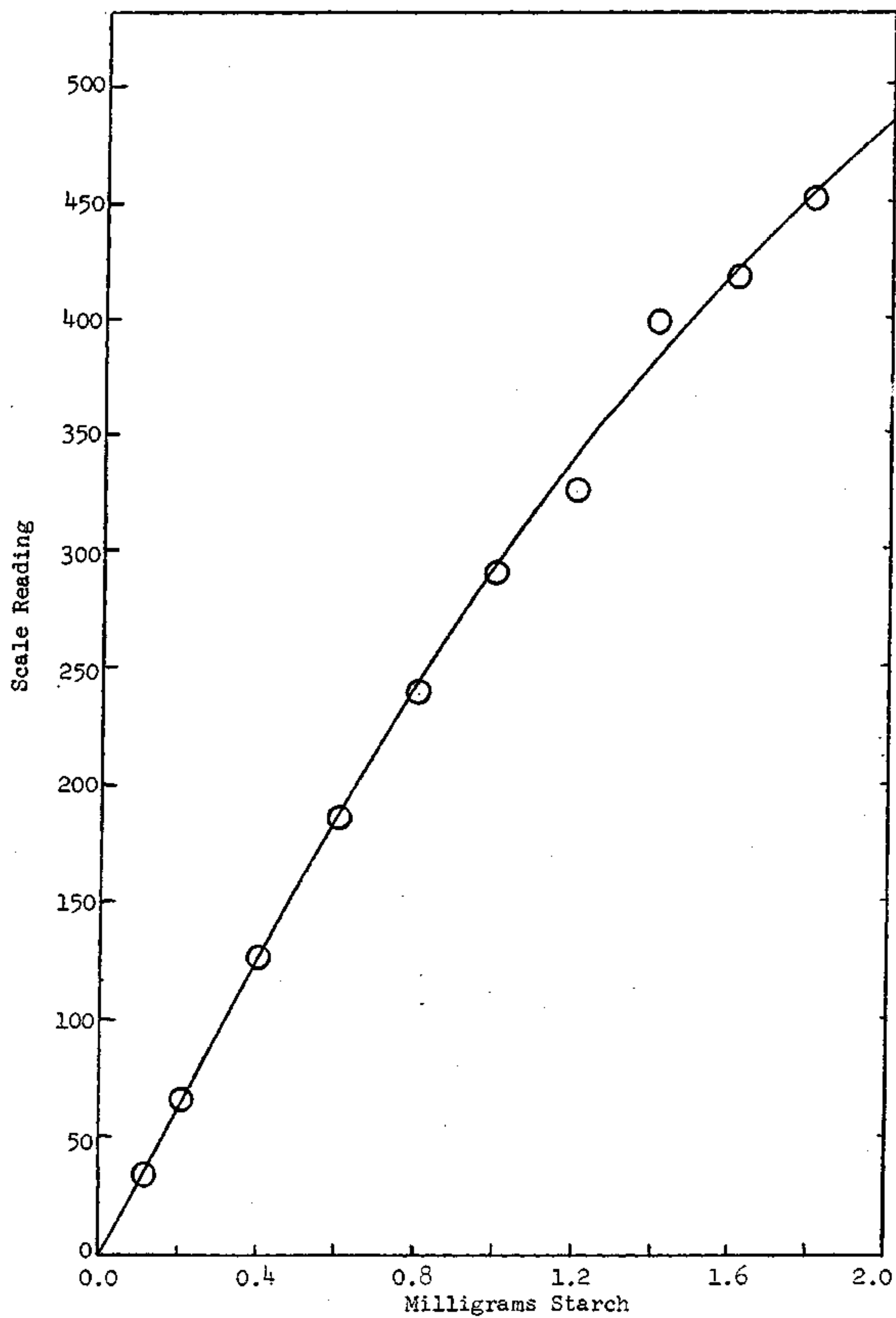


Figure 1. Starch-Iodine Calibration Curve

tion at high concentrations was visually observed. It was concluded that the starch-iodine complex may be used for the colorimetric measurement of residual starch.

Enzymatic Degradation of Starch as a Function of Time

Both the AATCC test method and the Premier Malt Products method assume that the degradation of starch by enzymes is a linear function of time. The definitions of BAU and DV include time in this respect, and it is so treated in all calculations based on the two test methods.

Jalke has reported that enzymes are stabilized by the starch itself during the degradation process[18]. It is to be expected that the concentrations of starch and enzyme would affect the rate of reaction. Because of this question and because other unknown factors might enter into the relationship, it was desirable to check the validity of the assumption that the rate of reaction is a linear function of time.

Exactly two milliliters of enzyme solution (Exsize TX-2H, Premier Malt Products, Inc., 2700 DV per gram) and 1 gram of sodium chloride were diluted to 100 milliliters. A 5-milliliter aliquot was further diluted to 100 milliliters with deionized water. A 5-milliliter aliquot was then added to 10 milliliters of the standard starch stock solution in a constant temperature bath at 30.0 ± 0.1°C. At 5 minute intervals over a 40 minute period, 1-milliliter aliquots of the digestion mixture were added to 10 milliliter samples of the iodine working solution in the colorimeter cells. The scale reading of each sample was recorded and plotted against the elapsed time. The results are shown in Table 2 and Figure 2.

Table 2. Starch Degradation as a Function of Time

Elapsed Time (minutes)	Scale Reading
5	710
10	630
15	395
20	153
25	67
30	23
35	16
40	9

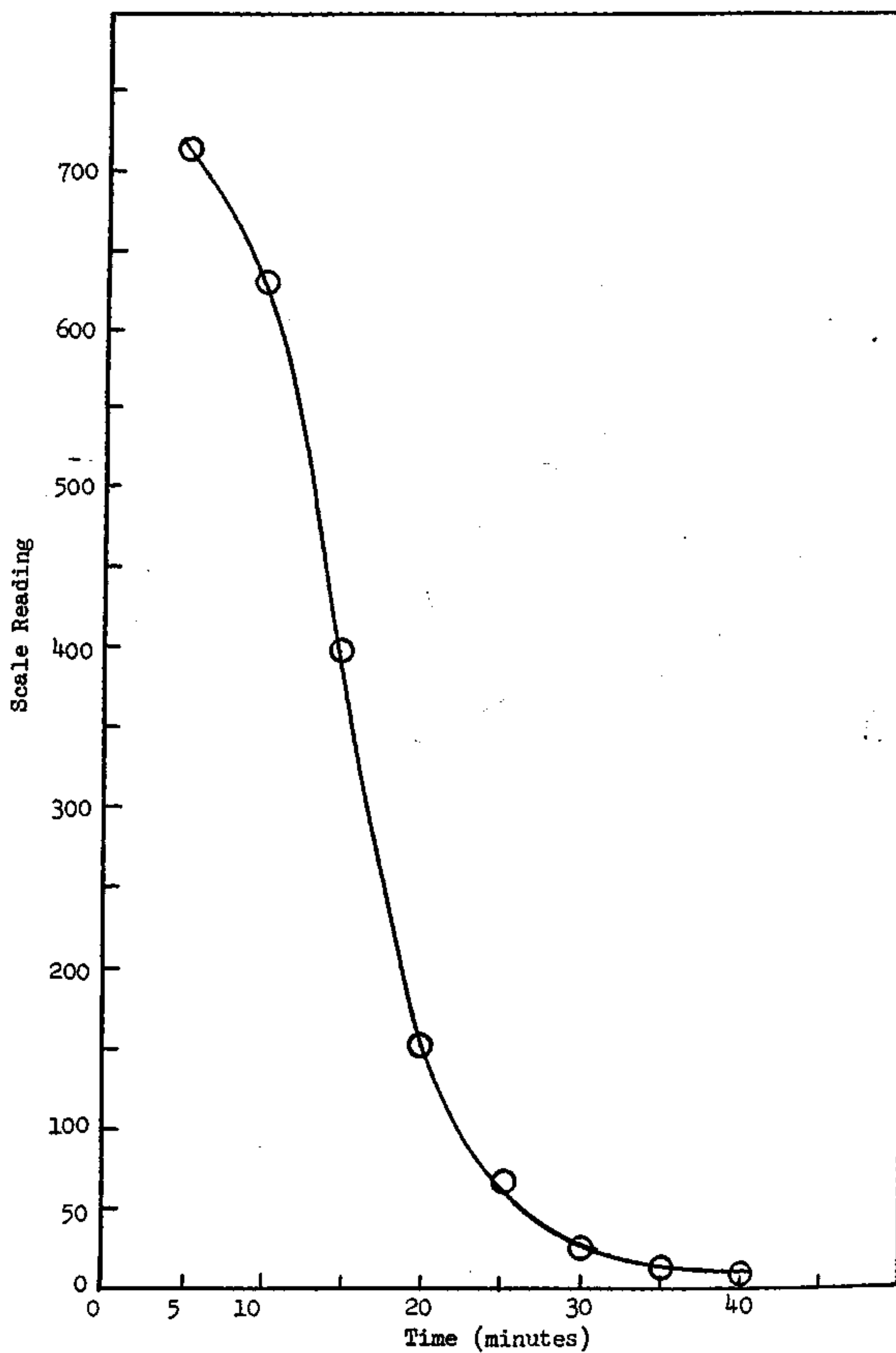


Figure 2. Enzymatic Degradation of Starch as a Function of Time

Figure 2 shows that in the degradation of starch by enzymes, time may not be taken as a linear function except over a narrow and well defined range. Most of the range over which the relationship appears to be linear is in the high Absorbance region where readings are not considered reliable. Furthermore, in order to utilize the linear portion for rate determinations, measurements must be made in the steep portion of the curve where small errors are magnified. It was concluded that the assumptions by the AATCC test method and the Premier Malt Products assay procedure are not valid because the changing concentrations of starch are not considered, and because no consideration is given to the possible changes in enzyme activity during the course of the reaction.

Enzymatic Degradation of Starch in the Presence of Surfactants

The previously mentioned test methods attempt to measure the rate of enzymatic degradation of starch. In checking for interference in the dextrinization of starch, it might be possible to measure not the rate of degradation, but the final residual starch concentration after the rate curve has leveled out. To determine if this is feasible, the preceding work was extended to the case in which a surfactant was added to the enzyme-salt solution. Three nonionic surfactants were used, of the hard, soft, and very soft types. The initial 100 ml solutions were prepared containing 2 percent surfactant, 1 percent sodium chloride, and 2 milliliters enzyme solution. The tests were run exactly as the test in the preceding section, which now became the control. The results are given in Table 3 and Figure 3.

Figure 3 indicates that the residual starch concentration may be

Table 3. Scale Reading as a Function of Time in the Presence of Surfactants

Time (minutes)	Branched C_{13} Alcohol + 9 e.o.	Random Secondary C_{11}-C_{15} Linear Alcohol + 9.e.o.	Primary Linear C_{12}-C_{15} Alcohol + 9 e.o.
5	680	600	460
10	570	540	455
15	305	410	460
20	120	292	455
25	46	244	455
30	21	224	455
35	18	220	455
40	16	215	455

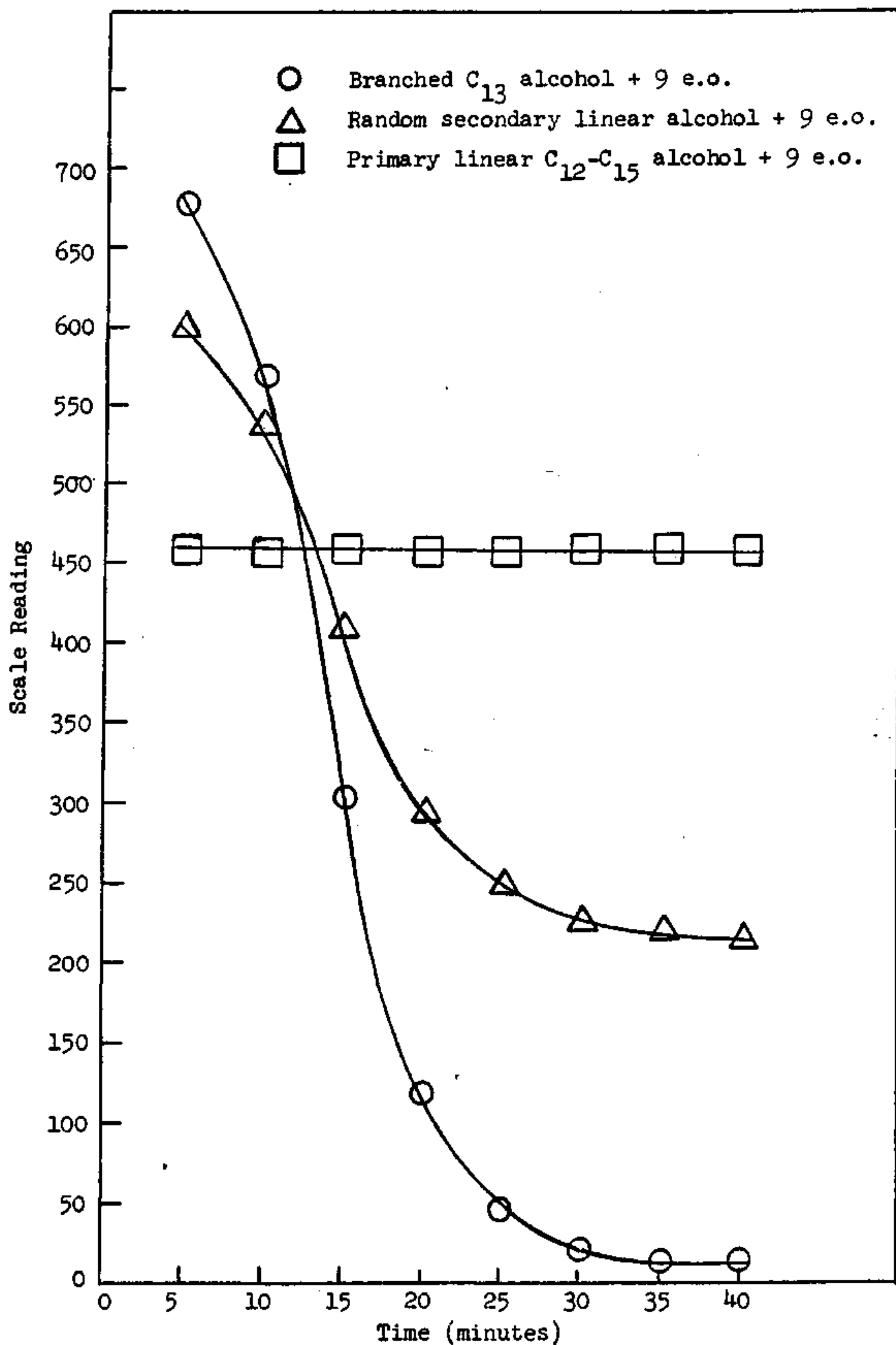


Figure 3. Degradation as a Function of Time in the Presence of Surfactants

measured at 40 minutes reaction time and that it will be a meaningful measure of the loss of enzymatic activity in the presence of surfactants. It is also noted that the hard surfactant caused no loss of enzymatic activity, while the soft and very soft surfactants caused a progressively greater loss of enzymatic activity.

Starch Dextrinization as a Function of the Enzyme-Surfactant Storage Time and Temperature

For the purpose of these studies it has previously been assumed that the inhibition of enzymatic activity by surfactants occurred instantly at the time of addition of the enzyme to the salt-surfactant solution. Aliquots for further dilution and for the assay procedure were taken immediately after preparation.

However, a typical mill procedure would be to make up a surfactant-salt-enzyme mix in a head tank with water at up to 160° F. The prepared mix might stand for a period of hours before being consumed in the enzyme saturator. It is pertinent to this study to determine if the total inhibition occurs instantly or if it is accelerated by time and elevated temperatures.

A 100 ml control solution was prepared containing 1 gram of sodium chloride and 2 milliliters of enzyme. A second solution of 100 ml was prepared containing 1 gram of sodium chloride, 2 grams of a random secondary linear C_{11}-C_{15} alcohol with 9 moles of ethylene oxide, and 2 milliliters of enzyme. Five milliliter aliquots were taken immediately for assay in the previous manner.

The two enzyme stock solutions were placed in a constant tempera-

ture bath at 65.0 ± 1.0°C. Additional aliquots were taken for assay after 1 hour and 2 hours at 65°C.

The assay procedure used in these studies and those that follow is as follows: The 5-milliliter aliquot of enzyme-salt-surfactant stock solution was diluted to 100 milliliters with deionized water. A 5-milliliter aliquot was then added to 10 milliliters of the standard stock starch solution in a test tube in the constant temperature bath at 30.0 ± 0.1°C. After a 40-minute digestion period, a 1-milliliter aliquot of the digestion mixture was added to 10 milliliters of the standard iodine working solution in a colorimeter cell. The cell was twice inverted and the scale reading recorded. The results are shown in Table 4.

The percent starch remaining was calculated from the amount of starch as taken off the graph in Figure 1 corresponding to the scale reading and from knowledge of the initial starch concentration. For a sample calculation, see the Appendix.

Table 4 shows that only a slight deterioration of enzymatic activity occurs due to storage of the enzyme stock solution at 65° C. This slight loss of activity was due to the known effect of temperature on enzymatic activity. It is clearly shown that the important effect on enzymatic activity was due to the presence of the surfactant, and that the inhibition occurred essentially instantly. The inhibition was not enhanced by time or elevated temperature. It was concluded that meaningful assay procedures need not consider the effect of enzyme-surfactant contact time. It was further concluded that temperature considerations of the enzyme-surfactant mixture are not more critical than those limitations placed upon the enzyme alone due to temperature.

Table 4. Enzymatic Degradation of Starch as a Function of Time and Temperature of the Stock Solution

Time at 65°C (minutes)	Control		Random Linear Secondary Alcohol + 9 e.o.	
	Scale Reading	Percent Original Starch Remaining	Scale Reading	Percent Original Starch Remaining
0	9	0.19	225	5.47
60	12	0.22	244	5.92
120	16	0.34	257	6.22

The Effect of Surfactant Concentration on Enzyme Inhibition

Previous tests which include surfactants have been run arbitrarily at the 2 percent surfactant level, simply because as much as 2 percent surfactant is often used with enzyme in the head tank stock solutions and in the enzyme saturator. Before collecting data, it was necessary to determine the effect of varying concentrations of surfactant on enzyme inhibition.

A series of 100 ml solutions containing 2 milliliters enzyme and 1 percent sodium chloride were prepared, containing also from 1/10 percent to 2 percent surfactant. The surfactants used were a random secondary linear C_{11}-C_{15} alcohol with 9 moles of ethylene oxide, and a primary linear C_{10}-C_{12} alcohol with 6 moles of ethylene oxide.

Following the previous assay scheme, allowing a 40-minute digestion period, the runs were conducted. The results are shown in Table 5 and Figure 4.

It is apparent from an analysis of Table 5 and Figure 4 that the surfactant concentration has a very large effect on enzyme inhibition. It was concluded that only slight and probably negligible inhibition occurs at low (penetrant level) concentrations of ethylene oxide condensates. At the 2 percent surfactant level, about the highest level used in the mills, significant inhibition is shown. The inherent enzyme compatibility properties of surfactants should be distinguishable at 1 to 2 percent.

Table 5. Enzymatic Degradation of Starch at Varied Surfactant Levels

Percent Random Secondary Alcohol + 9 e.o.	Scale Reading	Percent Starch Remaining
0.1	16	0.34
0.5	40	0.82
1.0	95	2.18
1.5	157	3.67
2.0	225	5.47

Percent Primary C_{10}-C_{12} Alcohol + 6 e.o.	Scale Reading	Percent Starch Remaining
0.1	49	1.12
0.5	92	2.10
1.0	425	12.35
1.5	460	13.85
2.0	430	12.53

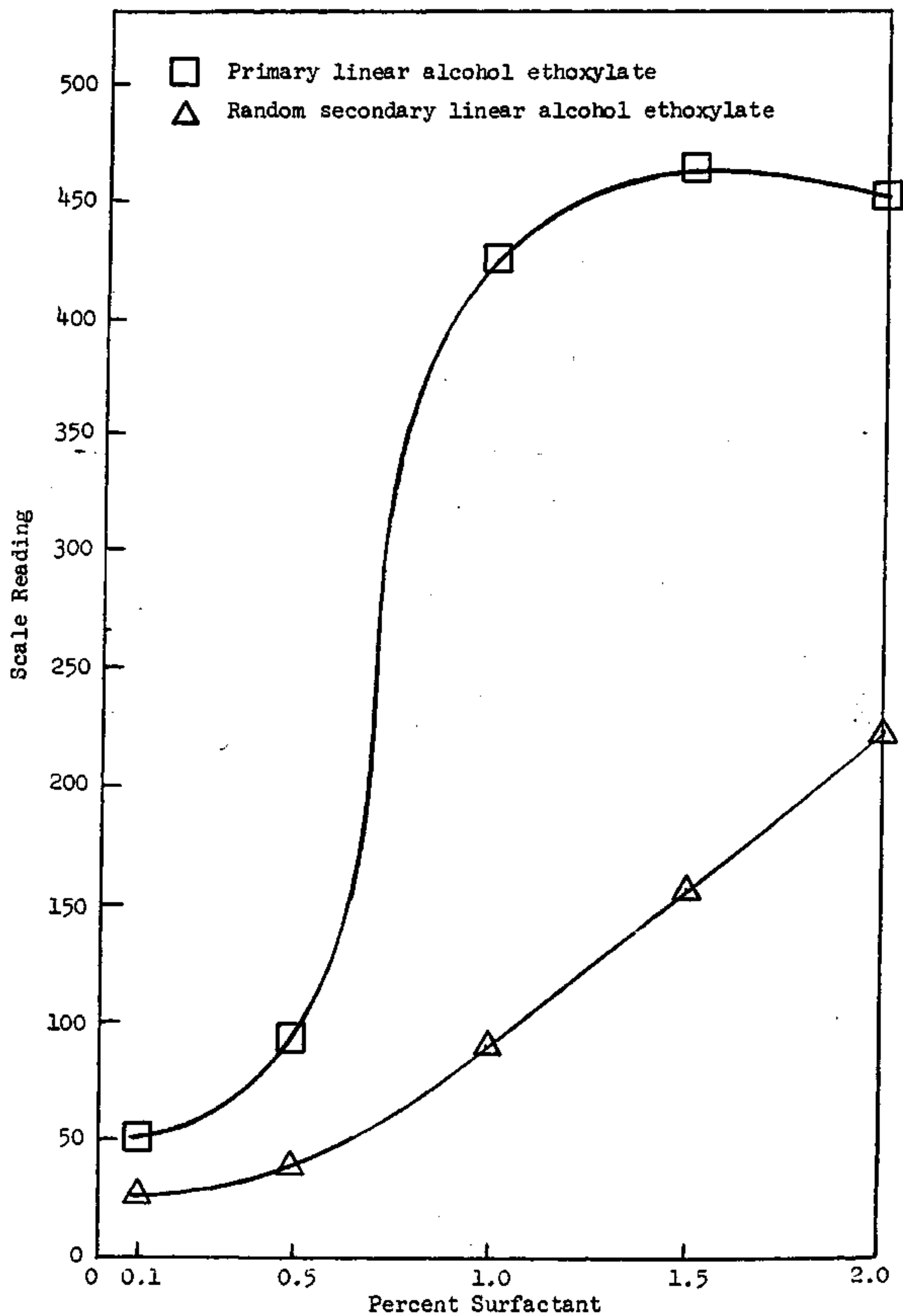


Figure 4. Enzymatic Degradation of Starch as a Function of Surfactant Concentration

Enzyme Compatibility Studies of Various Groups of Ethylene Oxide Condensates

Ethylene oxide-based surfactants of varied structures were evaluated for their effect on the enzymatic degradation of starch. The parameters obtained in previous experiments were employed as guidelines for conducting the comparative studies. Specifically, the following conditions were used:

A. All surfactants tested were commercial products.

B. The nonionic surfactants were all 100 percent active; the anionic derivatives were 58 to 60 percent active, as sold commercially.

C. No pH adjustments were made, but the pH was found to be within the range of 6.5 to 7.5 in every case.

D. All surfactants were tested at 2 percent concentration of commercial strength as marketed.

Test Procedure

1. The stock solution was prepared by dissolving 1 gram of sodium chloride and 2 grams of surfactant in deionized water, then adding 2 milliliters of enzyme and diluting to 100 milliliters.

2. A 5-milliliter aliquot of the stock solution was immediately diluted to 100 milliliters with deionized water.

3. A 5-milliliter aliquot of the diluted solution was added to a test tube containing 10 milliliters of the standard starch stock solution. The timer was started.

4. The test tube was inverted five times and placed in the constant temperature bath at $30.0 \pm 0.1^{\circ}$ C.

5. After 40 minutes digestion time, a 1-milliliter aliquot of the digestion mixture was added to 10 milliliters of the working iodine solution in a colorimeter cell.

6. The cell was inverted twice and placed in the colorimeter, and the scale reading recorded. The red filter (640-700 mμ) was used. The colorimeter was adjusted to zero scale reading with deionized water.

The results are shown in Tables 6 and 7. The nonionic and anionic surfactants are grouped in order of increasing biodegradability from top to bottom.

Table 6. Starch Degradation by Enzymes in the Presence of Nonionic Surfactants

Type Surfactant	Moles Ethylene Oxide	Classification	Scale Reading	Percent Original Starch Remaining	Percent Retained Enzyme Activity
Branched chain alkyl phenol	9	very hard	7	0.15	100.0
Branched chain alcohol	12	very hard	12	0.22	99.9
Linear alkyl phenol	9	hard	13	0.24	99.9
Branched chain alcohol	9	hard	16	0.34	99.7
Branched chain alcohol	6	hard	24	0.52	99.5
Random secondary linear alcohol	30	soft	50	1.13	98.9
Random secondary linear alcohol	12	soft	175	4.13	95.9
Random secondary linear alcohol	9	soft	225	5.47	94.6
Random secondary linear alcohol	7	soft	232	5.70	94.3
Primary linear alcohol	9	very soft	410	11.75	88.4
Primary linear alcohol	6	very soft	430	12.53	87.5

Table 7. Starch Degradation by Enzymes in the Presence of Anionic Surfactants

Type Surfactant	Moles Ethylene Oxide	Classification	Scale Reading	Percent Original Starch Remaining	Percent Retained Enzyme Activity
Sulfated branched chain alkyl phenol ethoxylate, sodium salt	4	very hard	21	0.46	99.5
Sulfated linear alkyl phenol ethoxylate, ammonium salt	4	hard	9	0.19	100.0
Sulfated random secondary linear alcohol ethoxylate, sodium salt	3	soft	133	3.08	97.1
Sulfated primary linear alcohol ethoxylate, sodium salt	3	very soft	284	7.31	92.7
Sulfated primary linear alcohol ethoxylate, ammonium salt	3	very soft	280	7.21	92.8
Sulfated primary linear alcohol ethoxylate, ammonium salt	3	very soft	288	7.42	92.6

CHAPTER V

DISCUSSION OF RESULTS

Initial experimental work was done for the purpose of determining the requirements and the limitations of amylolytic enzyme assay procedures. By devising an experimental scheme which related all determinations back to a standard starch-scale reading curve, it was possible to make relatively basic measurements of enzymatic degradation of starch. It was possible to avoid the use of such abstract concepts as Bacterial Amylase Unit (BAU) and Dextrinizing Value (DV), which eliminated the false or doubtful assumptions which frequently accompany arbitrary definitions. The results obtained were reproduceable with good accuracy.

Tables 6 and 7 show that a definite decrease in enzyme compatibility occurs as increasingly biodegradable surfactants are employed. The nonionic or anionic surfactants classed as hard or very hard because of branching and/or the presence of aromatic structures do not significantly inhibit amylolytic enzymes. The hard surfactants allowed 99 percent or higher degradation of the starch by the enzyme as compared to the control solution which contained no surfactant. During the assay, it was noted that with the hard surfactants the starch-enzyme reaction mixture quickly became clear and remained clear. The foam did not break during the 40-minute digestion period.

The random secondary C_{11}-C_{15} linear alcohol ethoxylates are completely biodegradable in that no residue remains after degradation, as

opposed to the branched and/or aromatic-based surfactants[15]. However, the secondary linear alcohol ethoxylates are not as rapidly biodegradable as the primary linear alcohol ethoxylates (Figures 8 and 9 in the Appendix). Just as the random secondary linear derivatives are intermediate between the hard and the very soft surfactants in rate of biodegradability, they also occupy an intermediate plateau in enzyme compatibility. About 94 to 98 percent degradation of the starch was obtained when 2 percent of these products was used. The foam was low during the digestion period, and the digestion mixture did not become completely clear.

The data show clearly the variation of enzyme compatibility of the secondary linear ethoxylates as a function of ethylene oxide content. As more ethylene oxide is added, the surfactant becomes less biodegradable. The increase in ethylene oxide content results in progressively greater enzyme compatibility.

The very soft, very rapidly biodegradable primary linear alcohol ethoxylates are on the lowest plateau of enzyme compatibility. About 87 to 93 percent enzyme activity was retained in the presence of the very soft surfactants. During the 40-minute digestion period, the foam broke completely. It was also noted that some precipitation of conglomerate white particles occurred during the digestion period. It was necessary to shake the digestion samples before taking an aliquot for assay. The solutions did not become clear.

Felgentreff and Stieler[19] report that it is not necessary to remove 100 percent of the size from the fabric to obtain a satisfactory fabric for dyeing and finishing. It is reported by Svoboda[1] that an

evaluation of dextrinization activity is not sufficient basis for making decisions but that a trial on the fabric must be made. Enzyme manufacturers commonly consider surfactants to be compatible with their enzymes if greater than 85 percent activity is retained. Therefore, for practical purposes, the use of even the very soft surfactants would be expected to create no problems in the enzymatic desizing process.

The conditions used for these studies approximated the operating conditions in textile mills where a surfactant-salt-enzyme mixture is prepared in a head tank. Inhibition of amylolytic activity due to exposure to relatively high surfactant concentrations was measured. The method used in these tests placed certain limitations on the dextrinization conditions. The dextrinization was accomplished in solution rather than on a dried film. It was necessary that a colloidal starch solution be used in order to have an exact knowledge of the amount of starch present. Physical limitations of the experiment also required that rather high dilutions be used. The surfactant concentration was only 0.033 percent during the actual digestion period.

The experiments were not designed to investigate the merits of anionic ethoxylated surfactants as compared to the nonionic surfactants for use in enzyme desizing operations. However, the data indicate that the sulfated ethoxylated surfactants are comparable to the nonionic surfactants of the same biodegradability class in the inhibition of enzymatic activity when considered on an equal activity basis.

Szentpaly and Kovacs[20] have developed a photometric method for the determination of the degree of desizing which is based on the hue of the iodine complex formed with amylose degradation products. The

reported color change is from blue to purple to red to yellow. In these studies, the red was filtered out to minimize interference from colors other than the blue starch-iodine complex. While more exact quantitative work would require a very narrow light band at 580 mμ, the equipment was adequate for the purpose of these studies, and only slight deviations from the reported figures would be expected due to the different hues.

Figure 3 presents a problem. Literal interpretation of the plot of scale reading versus time for the primary alcohol ethoxylate would indicate that very rapid dextrinization of starch occurred at first, and no further dextrinization occurred. The hypothesis is advanced that the most biodegradable surfactants preferentially form a colorless iodine complex, thus denying the initial high starch concentration sufficient iodine to form the dark blue starch-iodine complex. Although the initial region of the curve for very soft surfactants may be affected, the meaning of the graph is unchanged. Sufficient starch-iodine complex was formed to establish that inhibition of enzymatic starch degradation occurred to a greater extent in the presence of the most biodegradable surfactants.

Some type of interference in the starch-iodine color formation is also indicated by Figure 4. The curve for the primary alcohol ethoxylate goes through a maximum at about 1 and 1/2 percent concentration and actually declines to a lower level at 2 percent. The hypothesis of a colorless surfactant-iodine complex can explain this behavior, in that very high surfactant concentrations will require more of the available iodine, which prevents the starch from developing color quantitatively.

The curve as plotted is possibly the resulting combination of two curves. The first curve would represent the surfactant-iodine complex, while the second curve would represent the true starch-iodine complex.

The actual amount of residual starch must be at least as great as the experiment indicates; it could be greater. For the purpose of this study, the exact level of starch remaining is immaterial in this case. There is definite evidence that the very soft surfactants cause greater enzyme inhibition than the less biodegradable types.

CHAPTER VI

CONCLUSIONS

1. A definite correlation was found between the biodegradability of ethylene oxide containing surfactants and the compatibility of the surfactants with enzymes.

2. Biodegradable ethoxylated surfactants inhibit the starch dextrinizing activity of amylolytic enzymes to a greater extent than the nonbiodegradable analogs.

3. The degree of inhibition of enzymatic activity is dependent upon the biodegradable surfactant concentration, and the inhibition is significantly increased at high surfactant levels.

4. The inhibition of enzymatic activity by surfactants occurs essentially instantaneously, and this inhibition is essentially independent of time and temperature.

CHAPTER VII

RECOMMENDATIONS

No hypothesis is offered as to the mechanism by which the highly biodegradable ethoxylated surfactants inhibit amylolytic enzyme activity. No references or other indications were found to indicate that the surface active properties of any ethoxylated surfactants are reduced or altered in the presence of enzymes. The possible biodegradation of linear ethoxylated surfactants under the influence of amylolytic enzymes should be investigated.

The textile industry has the task of desizing textiles efficiently and economically, obtaining goods of the quality required for subsequent processing, and at the same time minimizing stream pollution. The use of biodegradable ethylene oxide-based surfactants in desizing is not detrimental to the stated objectives if they are used in a knowledgeable manner. It is advantageous to carefully choose the biodegradable surfactants best suited for the purpose, in order that the use of excessively high concentrations of surfactants may be avoided. If solvents are to be emulsified in the desize bath, choose a surfactant which is an efficient solvent emulsifier. Particularly for continuous processing, choose a surfactant which is a highly efficient penetrant. Avoid making highly concentrated mixes in head tanks. Because high surfactant concentrations may adversely affect enzymatic activity, selection of surfactants to use in processing is probably more important with biodegrad-

able surfactants than with hard surfactants. By proper selection and process control, surfactant concentrations and costs can often be reduced, and enzymatic activity need not be significantly affected by the use of biodegradable surfactants.

APPENDIX

Calculation of starch in the starch-iodine calibration, Table 1:

$$\text{Concentration of starch (grams/liter)} \times \frac{\text{volume of aliquot (ml)}}{1000} \times 1000 = \text{mgs starch.}$$

For example, $10.0 \times \frac{0.1}{1000} \times 1000 = 1.0$ mg starch.

Calculation of the percent starch remaining after the digestion period, in Tables 4, 5, 6, and 7:

Step 1: Read the amount of starch on the absissa of Figure 1 corresponding to the intercept of the scale reading with the standard curve. For example,

Scale reading = 225, equivalent to 0.73 mg starch.

Step 2: 10.0 ml of 2.0 percent starch solution plus 5 ml enzyme solution contains initially 200 mg starch. A 1.0 ml aliquot contains (initially) 200/15 mgs = 13.333 mgs starch.

Step 3: $\frac{\text{Experimentally determined starch (Step 1)}}{\text{Initial amount of starch}} \times 100 =$ percent starch remaining. For example,

$$\frac{0.73 \text{ mg}}{13.333 \text{ mg}} \times 100 = 5.47 \text{ percent.}$$

Calculation of percent retained enzyme activity, Tables 6 and 7:

$$\frac{\text{Initial amount of starch - actual amount of starch (from Figure 1)}}{\text{Initial amount of starch - actual amount of starch (control, no surfactant)}}$$

x 100 = percent retained enzyme activity.

For example,

$$\frac{13.333 - 0.960 \text{ (scale reading = 280)}}{13.333 - 0.025 \text{ (scale reading = 9)}} \times 100 = 92.6 \text{ percent.}$$

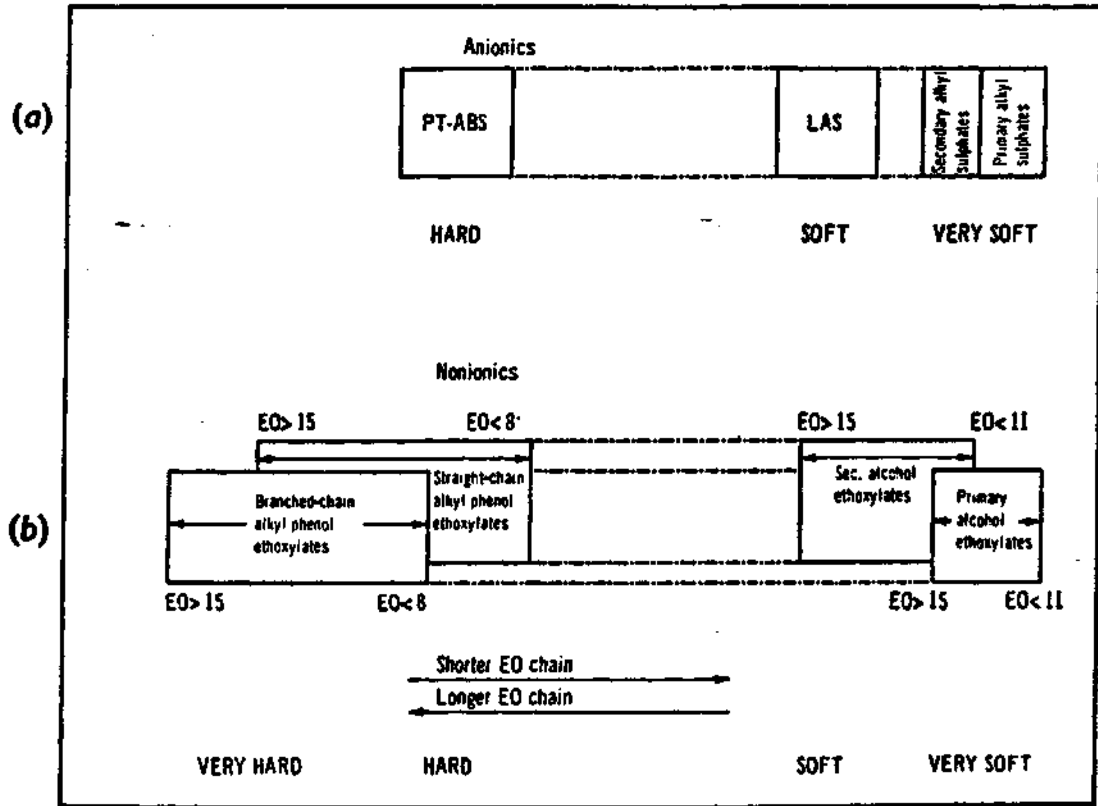


Figure 5. Comparative Biodegradabilities of Some Anionic and Nonionic Surface Active Agents Used in Textile Processing[7]

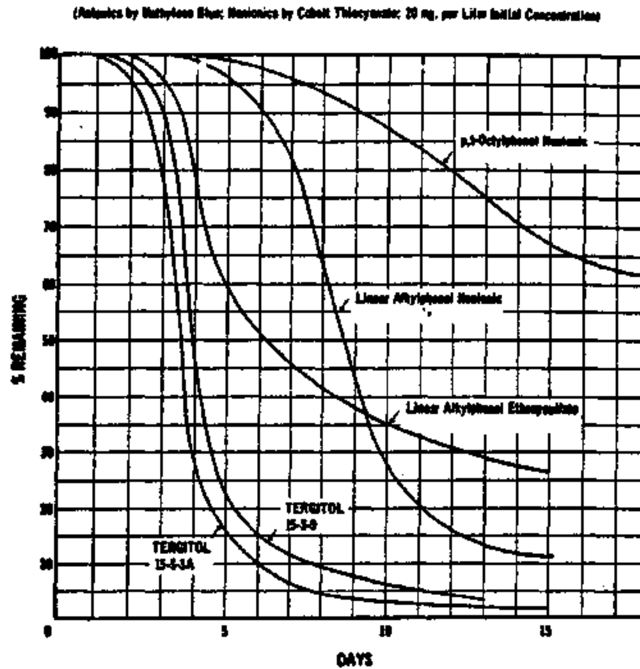


Figure 6. Measurement of Biodegradation by the River-Die-away Test
Source: Union Carbide Corporation Sales Brochure

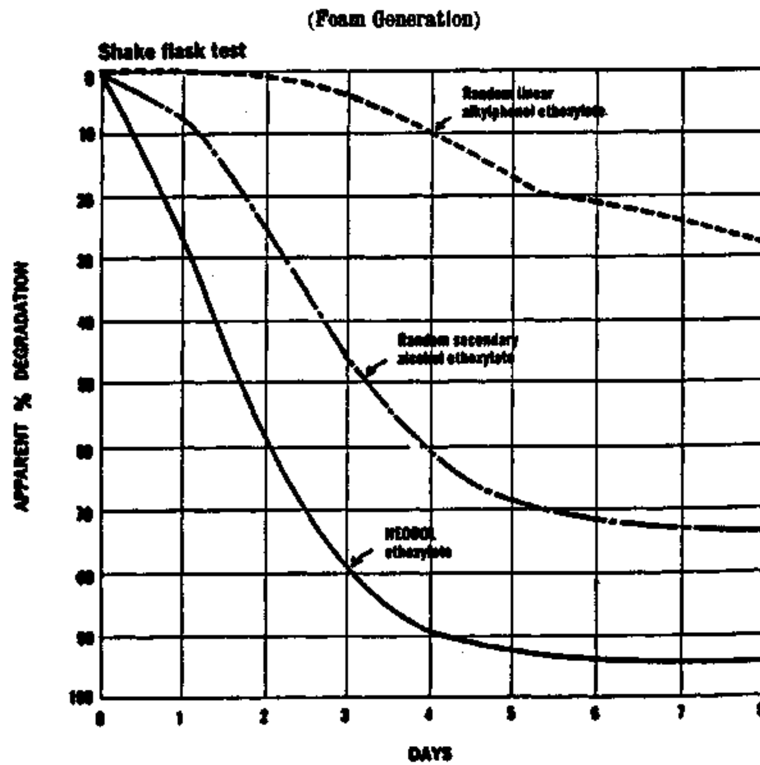


Figure 7. Comparative Biodegradability - Nonionics
Source: Shell Chemical Company Sales Brochure

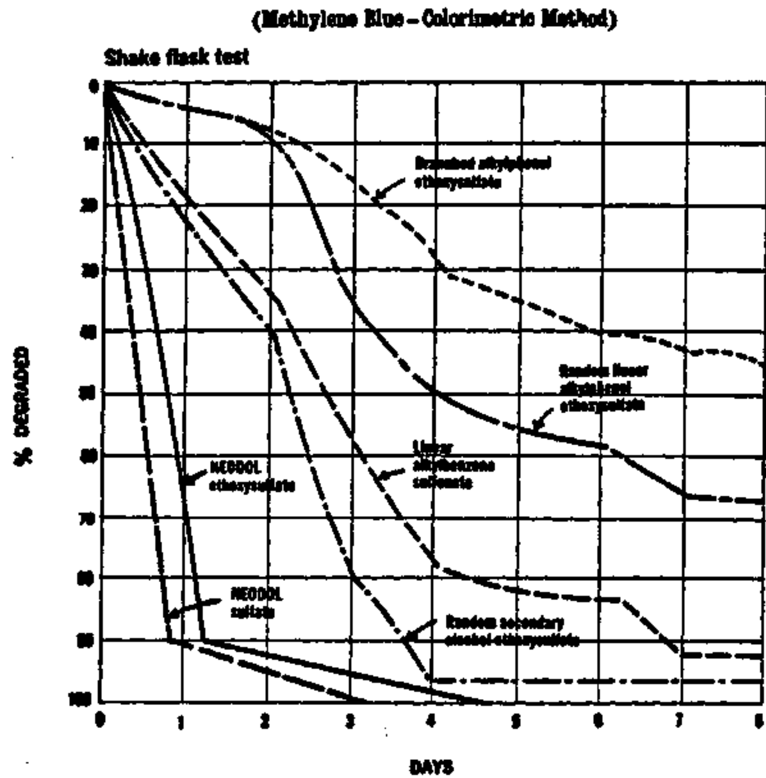


Figure 8. Comparative Biodegradability - Anionics
Source: Shell Chemical Company Sales Brochure

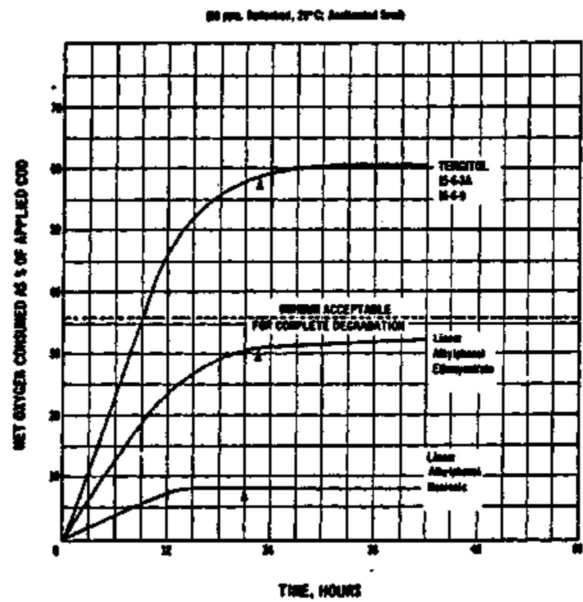


Figure 9. Biological Oxidation as Measured with the "Warburg" Respirometer
Source: Union Carbide Corporation Sales Brochure

LIST OF COMMERCIAL PRODUCTS USED IN THIS STUDY

Name	Source
Lintner Starch	Merck and Company, Inc.
Exsize TX-2H	Premier Malt Products, Inc.
Tergitol 15-S-3S	Union Carbide Corporation
Tergitol 15-S-7	Union Carbide Corporation
Tergitol 15-S-9	Union Carbide Corporation
Tergitol 15-S-12	Union Carbide Corporation
Tergitol 15-S-30	Union Carbide Corporation
Alfonic 1012-6	Continental Oil Company
Alfonic WB-S	Continental Oil Company
Alfonic WB-A	Continental Oil Company
Emulphogene BC-610	General Aniline and Film Corp.
Emulphogene BC-720	General Aniline and Film Corp.
Emulphogene BC-840	General Aniline and Film Corp.
Alipal EO-526	General Aniline and Film Corp.
Alipal LO-436	General Aniline and Film Corp.
Alipal AB-436	General Aniline and Film Corp.
Igepal AB-630	General Aniline and Film Corp.
Igepal LO-630	General Aniline and Film Corp.
Igepal CO-630	General Aniline and Film Corp.
Neodol 25-9	Shell Chemical Company

BIBLIOGRAPHY

Literature Cited

1. Svoboda, F., Textil - Praxis 19, No. 4, 378-384 (1964).

2. Journal of the American Oil Chemists Society 42, 986 (1965).

3. Ibid

4. Journal of Water Pollution Control Federation, 181-187 (February, 1967).

5. Greff, R., Setzkom, E., and Leslie, W., Journal of the American Oil Chemists Society 42, 180 (1965).

6. Patterson, S., Hunt, E., and Tucker, K., Journal Proc. Inst. Sew. Purif. 190, (1966).

7. Barnes, W., and Dobson, S., Journal of the Society of Dyers and Colorists 83, 313, (August, 1967).

8. Ibid

9. Hammerton, C., Journal of Applied Chemistry 5, 517 (1959).

10. Booman, K., Dupre, J., and Lashen, E., American Dyestuff Reporter 56, 82-88 (1967).

11. Lashen, E., Trebbi, G., Booman, K., and Dupre, J., Soap and Chemical Specialties 43, 55-58, 122-124, 126-127 (1967).

12. Brebion, G., Cabridenc, R., and Jullig, T., Manufacturing Chemist 35, 70 (1964).

13. Ibid

14. Garrison, L., and Matson, R., Journal of the American Oil Chemists Society 41, 799-807 (1964).

15. Myerly, R., Rector, J., Steinle, E., Vath, C., and Zika, H., Soap and Chemical Specialties 40, 78-80, 82, 170-171 (1964).

16. Huddleston, R., and Allred, R., Journal of the American Oil Chemists Society 42, 983-986 (1965).

17. Browning, B., Bublitz, L., and Baker, P., TAPPI 35, 1925 (1961).

18. Jalke, H., Textil - Rundschau 20, No. 6, 155-159 (1965). Through Shirley Institute 45, 3673 (1965).

19. Felgentreff, H. and Stieler, B., Z. ges. Textil - Ind. 62, No. 19, 844-849 (1960).

20. Szentpaly, T. and Kovacs, K., Magyar Textiltech 10, 404-405 (1958). Through Shirley Institute 39, 4548 (1959).