peroxide to water and oxygen is exothermic by 23.9 kcal. Considering the uncertainty in the N—O bond energy, this mechanism appears consistent with the activation parameters.

$$R_3N{-}O \ldots HOH \rightarrow R_3N \ldots HOOH \rightarrow R_3N + H_2O + \tfrac{1}{2}O_2$$

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REFERENCES

1. a) Cope, A. C., "Organic Reactions," Vol. 11, John Wiley & Sons, Inc., New York, 1960, p. 317; b) DePuy, C. H. and R. W. King; Chem. Revs. *60*, 431 (1960).
2. a) Cram, D. J., M. R. V. Sahyun, and G. R. Knox, JAOCS *84*, 1734, (1962); b) Sahyun, M. R. V., and D. J. Cram, *Ibid. 85*, 1263 (1963).
3. a) Brooks, R. T., and P. D. Sternglanz, Anal. Chem. *31*, 561 (1959); b) Wimer, D. C., *Ibid. 34*, 873 (1962).
4. Hoh, G. L. K., D. O. Barlow, A. F. Chadwick, D. B. Lake, and S. R. Sheeran, JAOCS *40*, 268–271 (1963).
5. Frost, A. A., and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, 1953, p. 147.
6. Link, W. E., R. A. Morrissette, A. D. Cooper, and C. F. Smullin, JAOCS *37*, 364–366 (1960).
7. Reference 1a, p. 370.
8. Cope, A. C. and N. A. LeBel, J. Am. Chem. Soc., *82*, 4656 (1960).
9. Paquette, L., J. Org. Chem. *27*, 2870 (1962).
10. Reference 5, p. 97.
11. Walling, C., "Free Radicals in Solution," John Wiley and Sons, Inc., New York, 1957, p. 48.

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The Kinetics of Methyl Linoleate Emulsion Autoxidation in the Presence of Polyhydroxy Compounds[1,2]

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Abstract

The kinetics of the autoxidation of methyl linoleate emulsions activated by carbohydrates likely to be present in meat, with special reference to the effects of functional groups, number of carbon atoms and configuration have been investigated by the rate of oxygen uptake. On the basis of equimolar concentrations of aldoses in the system, oxidation rate of methyl linoleate increases as the number of carbon atoms in the sugar molecule decreases, reaching a maximum in the presence of glyceraldehyde. Configuration of the aldose has a slight effect on the oxidation rate of methyl linoleate emulsions.

At comparable molar ratios of hexose to methyl linoleate, the rate of oxidation was found to be: ketohexose > aldohexose > hexahydroxy alcohol.

Replacement of the primary alcohol group in an aldohexose with a methyl group decreases the oxidation rate of methyl linoleate emulsion. An opposite effect is observed when the primary alcohol group is substituted with a carboxyl group, i.e., in the presence of sodium glucuronate.

2-Deoxy-D-glucose and 2-deoxy-D-ribose exhibit a lesser effect on the autoxidation of methyl linoleate emulsion than glucose and ribose, respectively.

Oxidation rates in the presence of reducing disaccharides, maltose, lactose and cellobiose, are more rapid than in the presence of the non-reducing disaccharide sucrose.

Introduction

OF ALMOST A HUNDRED possible monosaccharides containing from two to seven carbon atoms, less than a score have been found in animal cells (6). Glyceraldehyde is found as the phosphate ester in the breakdown product of carbohydrate metabolism. Dihydroxyacetone has been similarly identified. Tetroses have not been found in natural products, but a reduced form, a tetrahydroxy alcohol, erythritol, exists. It has been assumed that the nonexistence of tetroses is due to their extreme reactivities. Most probably tetroses are not synthesized directly but the alcohol is the decarboxylation product of a five-carbon sugar acid. The presence of carbohydrate alcohols in nature may be accounted for by a direct reduction of the reducing sugar or through decarboxylation of the corresponding higher aldonic acid. However, there is no evidence for either mechanism in nature. D-ribose is found in nucleic acids. D-ribulose, D-lyxose, xylulose, glucose, fructose, sedoheptulose and glyceraldehyde are present as phosphoric acid esters in the products of carbohydrate metabolism (17). L-fucose is a constituent of blood group substances which contain galactose, and mannose. By paper chromatography, glucose, fructose, ribose and other unidentified sugars were found in adductor muscle of calves (7). Lilyblade and Peterson (16) identified inositol, glucose, sedoheptulose, mannose, fructose, ribose, ribulose, arabinose and xylose in chicken muscle. During storage under refrigeration, ribose of beef increases from less than 1γ/g to 120γ/g in 16 days, while cold storage at −20C for a month shows 60γ/g (5).

Aliphatic polyhydroxy compounds such as glycerol, sucrose, sorbitol, glucose and fructose act as weak antioxidants (3,13,14,23,25).

Franke (4) reported that autoxidation of linoleic acid is catalyzed by glyceraldehyde and dihydroxyacetone. In a study on the oxidation of tetralin, Robertson and Waters (22) found that ethylene glycol and *tert*-butyl alcohol increased the initial rate of oxidation, while α-tetralol, benzyl alcohol, *n*-butyl alcohol, isopropyl alcohol, cyclohexanol and allyl alcohol inhibited the initial rate. Secondary alcohols are oxidized to ketones in inert solvents by tertiary peroxides (8). Co-oxidation of aliphatic aldehydes and oleic acid gave ca. 20% 9,10-epoxystearic acid and 50–70% aliphatic acid (24). Higher yields of 9,10-epoxystearic acid (70–80%) were obtained by using benzaldehyde. The low yield in the case of aliphatic aldehydes is attributed to preferential oxidation of excess aldehyde.

A close relation to the autoxidation of unsaturated hydrocarbons with the participation of RO'_2 is shown by the oxidation of carbohydrates by hydrogen per-

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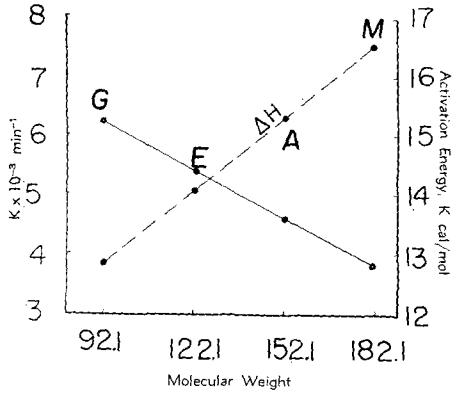

FIG. 1. Effect of mol wt of polyhydroxy alcohols on oxidation rate constants of methyl linoleate emulsions. Initial pH 7.00, polyhydroxy alcohol concentration 6.31 x 10^{-4} mole/mole linoleate, 50C. G—glycerol, E—*i*-erythritol, A—D-arabitol, M—mannitol.

oxide in the presence of divalent iron (ferrous sulfate), i.e., Fenton Reagent (2). The system $H_2O_2 + FeSO_4$ is the source of the free hydroxyl radicals formed by the reaction:

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\cdot} + Fe^{3+} + OH^-$$

Oxidation rate constants of fructose by Fenton reagent are proportional to hydrogen peroxide concentration but not quite proportional to the sugar concentration (12). Mabrouk and Dugan (18) found that oxidation rate constants of methyl linoleate emulsions are not proportional to glucose, fructose and sucrose at concn above 6.31 x 10^{-4} mole/mole linoleate.

Aldonic acids are oxidized with Fenton reagent to yield the next lower aldose sugar (2).

During autoxidation of methyl linoleate in the presence of alcohols and protons, accumulation of the hydroperoxides does not occur since the hydroperoxides are decomposed as they are formed (26). Methyl oleate gave a mixture of $Me-(CH_2)_n-CHO$ and $MeOOC-(CH_2)_n-CHO$, where n is 6,7 or 8.

The important role of sugars in the oxidative rancidity of meat products needs to be elucidated. In a previous publication (19) from this laboratory, the results of a kinetic study of the oxidation of methyl linoleate emulsions in the presence of glucose, fructose and sucrose were presented. The present investigation was performed to determine the effect of polyhydroxy alcohols, aldoses, ketoses, deoxy sugars, reducing disaccharides, raffinose, gluconic, glucuronic and saccharic acids on the autoxidation of methyl linoleate emulsions.

Experimental Procedures

The details of experimental procedures have been described in previous papers (18,19).

Materials Used. Methyl linoleate was obtained from The Hormel Institute Foundation. Its chemical analysis showed an iodine value of 172.5, with only a trace of conjugated diene.

DL-glyceraldehyde, dihydroxyacetone, D-erythrose, *i*-erythritol, D-arabitol, 2-deoxy-D-ribose, 2-deoxy-D-glucose, L-fucose, L-sorbose, D-cellobiose were obtained from Nutritional Biochemical Corp.

D-arabinose, L-arabinose, D-ribose, D-lyxose, D-xylose, D-galactose, D-mannose, sedoheptulose anhydride, D-maltose, D-raffinose, sodium gluconate, sodium glucuronate, saccharic acid (potassium salt) were from Pfanstiehl Laboratories, Inc. Lactose was Merck Reagent. Glucostat was Worthington Biochemical Corp. preparation of glucose oxidase.

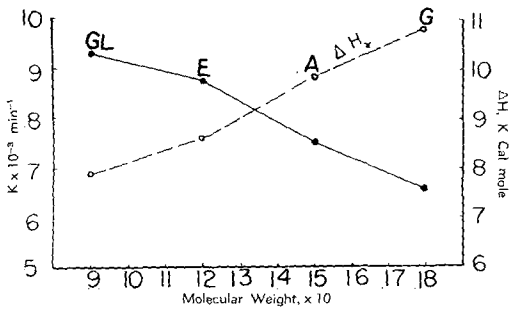

FIG. 2. Effect of mol wt of aldoses on oxidation rate constants of methyl linoleate emulsions. Initial pH 7.00, aldose concn 6.31 x 10^{-4} mole/mole linoleate, 50C. GL—DL-glyceraldehyde, E—D-erythrose, A—D-arabinose, G—glucose.

Carbohydrate solutions were prepared by dissolving weighed quantities in 0.1M KH_2PO_4/Na_2HPO_4 buffer of pH 7.00. These solutions were always freshly prepared for each experiment.

Oxygen absorption by methyl linoleate emulsions in the presence of 6.31 x 10^{-4} mole polyhydroxyl compound/mole linoleate at 50C was measured in a Warburg apparatus. The final volume of the reaction mixture was 4.0 ml. Oxidation rate constants of replicate experiments agreed with less than ±3%.

Spectrophotometric Determination of Glucose with Glucose Oxidase: The method used was that described by Keilin and Hartee (9,10,11).

Peroxide Value Determination: Peroxide contents of oxidized methyl linoleate emulsions were determined according to the method described by Privett et al. (21).

Discussion

Effect of Molecular Weight of Polyhydroxy Alcohols, Aldoses and Ketoses on Oxidation Rate Constants of Methyl Linoleate Emulsions. The effect of mol wt of polyhydroxy alcohols and aldoses on the autoxidation of methyl linoleate emulsions is illustrated in Figures 1,2, respectively.

The curves in both Figures show clearly that oxidation rate constants decrease with increasing mol wt of polyhydroxy alcohols and aldoses. In the presence of 6.31 x 10^{-4} mole polyhydroxy alcohol/mole linoleate a straight line relationship exists between oxidation rate constants and mol wt of the alcohol. Deviation from a similar linear relationship in the presence of aldoses may be attributed to the high reactivity of erythrose (6). Activation energy for the oxidation of methyl linoleate emulsions is dependent on the mol wt of the compound in the system. Oxidation rate constants found in the presence of other compounds were:

Dihydroxyacetone	7.72 x 10^{-3} min^{-1}
Sorbose	7.25 x 10^{-3} min^{-1}
Sedoheptulose anhydride . .	6.78 x 10^{-3} min^{-1}

The lower oxidation rate constant in the presence of dihydroxyacetone in comparison with glyceraldehyde (9.30 x 10^{-3} min^{-1}) is most probably because dihydroxyacetone easily undergoes autocondensation resulting in the formation of higher mol wt and higher mp compounds (15).

Effect of Configuration of Monosaccharides on Oxidation Rate Constants of Methyl Linoleate Emulsions. At comparable molar ratios of sugar to methyl linoleate, structural and optical isomerism has only a slight effect on the oxidation rate constants of methyl linoleate emulsions (Table I).

TABLE I

Oxidation Rate Constants of Methyl Linoleate Emulsions in the Presence of Polyhydroxy Compounds
(6.31 x 10^{-4} mole polyhydroxy compound/mole linoleate)

Polyhydroxy compound	K x 10^{-3} min^{-1}	ΔH, K cal/mole
A—*Monosaccharides*		
Sorbose	7.25	10.40
Fructose	7.16	
Glucose	6.60	
Galactose	6.67	10.75
Mannose	6.86	10.90
D(−) Arabinose	7.41	9.82
L(+) Arabinose	7.50	9.76
Xylose	7.55	
Ribose	7.19	
Lyxose	7.45	
B—*Oligosaccharides*		
Maltose	7.44	12.16
Lactose	7.05	
Cellobiose	7.34	
Sucrose	6.00	16.00
Raffinose	4.46	16.11
C—*Deoxy sugars*		
2-Deoxy-D-ribose	6.22	11.74
2-Deoxy-D-glucose	5.35	13.50
L-Fucose	5.65	13.72
D—*Sugar acids*		
Na gluconate	6.80	9.88
Na glucuronate	7.23	9.59
Saccharic acid, dipotassium salt	6.64	14.07

Under experimental conditions, the rate of oxidation of methyl linoleate emulsions in the presence of pentoses decreased slightly in the following order: xylose, L(+)arabinose, lyxose, D(−)arabinose and ribose. Among the tested aldohexoses, mannose was the most reactive followed by galactose and glucose. The keto sugars (sorbose and fructose) were more reactive than the sugars with aldehyde groups.

The greater reactivity of pentoses in the oxidation of methyl linoleate emulsions over those of hexoses may be attributed to the presence of the so-called "free aldehyde" or aldehydo structure. The amount of reducible form for hexoses is much less than the configurationally corresponding pentoses (1). However, differences between the reactivity of individual sugars of both classes appear to be small and do not coincide with their free aldehyde contents, but may be due to structural variations and dissociation constants (20).

Oxidation Rate Constants of Methyl Linoleate Emulsions in Presence of Oligosaccharides. The higher values of oxidation rate constants of methyl linoleate emulsions in presence of reducing disaccharides (Table I) may be attributed to the attack of linoleate hydroperoxides on the reducing end units and other oxidized sites (27). In presence of trisaccharide (raffinose), the oxidation rate of methyl linoleate emulsion was 75% of that in presence of sucrose.

Oxidation Rate Constants of Methyl Linoleate Emulsions in Presence of Deoxy Sugars. 2-Deoxy-D-ribose, 2-deoxy-D-glucose and L-fucose differ from pentoses and hexoses, respectively, by having a deficiency of one oxygen atom. Table I shows that oxidation rate constants in the presence of deoxy sugars are approximately 80% of the values in the presence of aldoses. If the carbon atom deficient in oxygen is number 6, i.e., if the primary hydroxyl group of an aldohexose has been reduced to a methyl group (e.g. fucose) the oxidation rate constant is slightly higher than in the presence of 2-deoxy-D-glucose.

Oxidation Rate Constants of Methyl Linoleate Emulsions in Presence of Polyhydroxy Compounds with Different Functional Groups. Data in Table I indicate that methyl linoleate oxidation is markedly activated by the presence of polyhydroxy compounds. As the functional group becomes more oxidized, the oxidation rate constant of methyl linoleate increases. At comparable molar ratios of polyhydroxy compounds to linoleate, the oxidation rate constants were found to be: $-CH_3 < -CH_2OH < -CHO < =CO < -COOH$, (according to the functional group present).

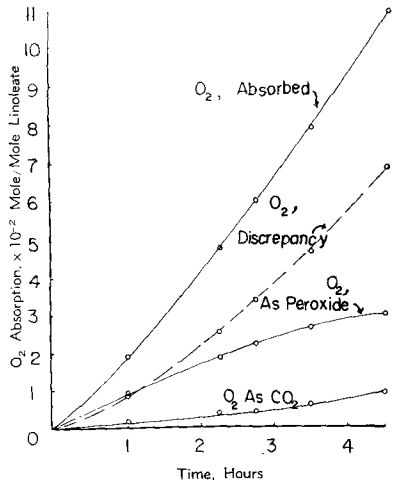

FIG. 3. The distribution of oxygen absorbed by methyl linoleate emulsions in the presence of 6.31 x 10^{-4} mole glucose/mole linoleate, 50C, initial pH 7.00.

The Distribution of Absorbed Oxygen by Methyl Linoleate–Glucose System. Oxygen absorbed, oxygen present as hydroperoxide, and carbon dioxide formed were determined during the oxidation of methyl linoleate in the presence of glucose at 50C. The complete time curve for the absorption of oxygen could be obtained by a single experiment, while the amounts of peroxides and carbon dioxide could only be obtained for a particular instant by one experiment. Therefore, it was necessary to perform several experiments over varying intervals of time in order to secure data for the construction of the oxygen present as hydroperoxide and carbon dioxide time curves. The absorption time curves shown in Figure 3 were taken from a series of experiments in the presence of 6.31 x 10^{-4} mole glucose/mole linoleate.

After one hour, it was found that hydroperoxide equivalent to ca. 50% of the oxygen absorbed could be detected. As the reaction proceeded, the peroxide content decreased gradually until it reached ca. 30% of the oxygen absorbed after 4.5 hr. Experimental results show that the presence of glucose prevented the accumulation of peroxide oxygen. The discrepancy between the amount of oxygen absorbed and the oxygen accounted for by analysis may be due to oxygen present in the oxidation products of glucose and in the secondary products of methyl linoleate oxidation.

Co-oxidation of Glucose. Figure 4 is a typical diagram showing carbon dioxide formed and glucose per cent relative to time during oxidation of methyl linoleate-glucose system.

Co-oxidation of glucose proceeds until ca. 60% of its content disappeared, after which glucose degradation products may be preferentially oxidized. Carbon dioxide formed does not correspond to complete oxidation of glucose.

It has been previously shown that autoxidation of methyl linoleate emulsions in the presence of polyhydroxy compounds was effectively reduced by the addition of 1.25 x 10^{-3}M nordihydroguaiaretic acid, propyl gallate and hydroquinone (19). Furthermore, under the same experimental conditions and in the absence of methyl linoleate, glucose solutions did not absorb any oxygen. In view of these findings, it would seem that the role of polyhydroxy compounds is dependent upon the formation of linoleate peroxide. The effect of these compounds rests in the activation

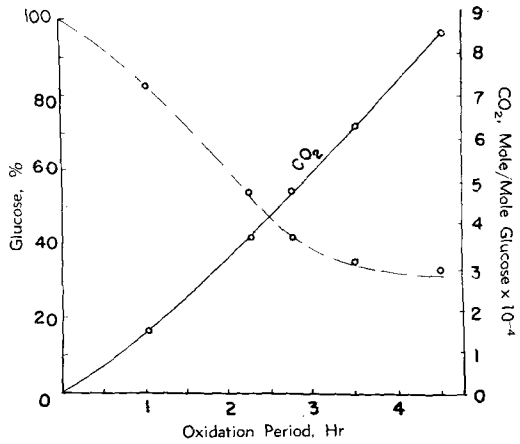

FIG. 4. Co-oxidation of glucose present in methyl linoleate-glucose system, initial concn 6.31 x 10^{-4} mole glucose/mole linoleate, 50C, initial pH 7.00.

of linoleate hydroperoxide decomposition into free radicals and on the acceleration of autocatalysis.

The mechanism for linoleate oxidation in the presence of polyhydroxy compounds may be summarized as follows:

(methyl linoleate RH and its hydroperoxide ROOH)

Initiation: $RH + O_2 \rightarrow ROOH$
$ROOH + \text{Polyhydroxy compounds} \rightarrow ROO^{\cdot}$ or $R^{\cdot}$ + oxidation products of polyhydroxy compounds

Propagation: $ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$
$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$

Chain termination: $ROO^{\cdot} + ROO^{\cdot} \rightarrow$
$R^{\cdot} + ROO^{\cdot} \rightarrow$ } Inactive products
$R^{\cdot} + R^{\cdot} \rightarrow$

In conclusion, all polyhydroxy compounds tested have shown to accelerate the oxidation of methyl linoleate emulsions. The oxidation rate constants of methyl linoleate vary considerably from one polyhydroxy compound to another and can be linked with the functional groups present and the mol wt. Activation energy values for methyl linoleate oxidation in their presence are less than the value for autoxidation, 18.20 K cal/mole (18).

REFERENCES

1. Cantor, S. M. and Q. P. Peniston, J. Am. Chem. Soc. *62*, 2113–21 (1940).
2. Cohen, J. B., "Organic Chemistry for Advanced Students," Part III. "Synthesis," 4th Ed., Longmans, Green & Co., New York, 1923, Chap. 1 "The Carbohydrates," p. 1–67.
3. Cowan, J. C., Patricia M. Cooney and C. D. Evans, JOACS *39*, 6–9 (1962).
4. Franke, I. W., Ann. *498*, 129–65 (1932).
5. Fredholm, H., Acta Chem. Scand. *14*, 437–40 (1960).
6. Gardner, T. S., J. Org. Chem. *8*, 111–20 (1943).
7. Grau, R., H. Gunther and J. Scheper, Fleischwirtschaft *12*, 728–730 (1960).
8. Karasch, M. S., A. Fono, W. Nudenberg and B. Bischof, J. Org. Chem. *17*, 207–220 (1952).
9. Keilin, D. and E. F. Hartee, Biochem. J. *50*, 331–41 (1952).
10. Keilin, D. and E. F. Hartee, *Ibid.* *50*, 341–48 (1952).
11. Keilin, D. and E. F. Hartee, *Ibid.* *42*, 221–29 (1948).
12. Kuchlin, A. Th., Biochem. Z. *261*, 411–24 (1933).
13. Lea, C. H., Rept. Food Invest. Bd. (1935), 38–43.
14. Lea, C. H., J. Soc. Chem. Ind. *55*, 293–302T (1936).
15. Leven, P. A. and A. Walti, J. Biol. Chem. *78*, 23–33 (1928).
16. Lilyblade, A. L. and D. W. Peterson, J. Food Sci. *27*, 245–54 (1962).
17. Leloir, L. E., "The Interconversion of Sugars in Nature," in "Currents in Biochemical Research, Edited by David E. Green, Interscience Publishers, Inc., New York, 1956, p. 585–608.
18. Mabrouk, A. F. and L. R. Dugan, Jr., JAOCS *37*, 486–90 (1960).
19. Mabrouk, A. F. and L. R. Dugan, Jr., *Ibid.* *38*, 692–95 (1961).
20. Perlmutter-Hayman, B. and A. Persky, J. Am. Chem. Soc. *82*, 3809–10 (1960).
21. Privett, O.S., W. O. Lundberg and Christense Nickell, JAOCS *30*, 17–21 (1953).
22. Robertson, A. and W. A. Waters, Trans Faraday Soc. *42*, 201–10 (1946).
23. Roller, P. E., J. Phys. Chem. *35*, 3286–92 (1931).
24. Swern, D. and T. W. Findley, J. Am. Chem. Soc. *72*, 4315–16 (1950).
25. Thompson, P. and D. M. Hegsted, J. Nutr. *60*, 361–5 (1956).
26. Treibs, W., Fette, Seifen, Anstrichmittel *63*, 1151–56 (1961).
27. Whistler, R. L. and Richard Schweiger, J. Am. Chem. Soc. *81*, 3136–39 (1959).

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• *Letters to the Editor*

Infrared Determination of Alkyl Branching in Detergent ABS

IN DETERMINING the level of alkyl benzene sulfonate (ABS) in sewage by the Soap and Detergent Association (SDA) method (1), we have found that the relative amounts of straight and branched chain isomers can be estimated from the intensities of the 1367 cm^{-1} and 1410 cm^{-1} IR absorption bands. The 1367 cm^{-1} band arises from the methyl deformation vibrations of a number of branched structures present in tetrapropylene ABS. The origin of the 1410 cm^{-1} band has not been established; however, it is associated with the sulfonate group. Its position is dependent upon the nature of the alkyl group, occurring at 1410 cm^{-1} in straight chain isomers and at 1400 cm^{-1} in tetrapropylene-ABS.

ABS is isolated by the SDA method as the 1-methyl heptyl amine salt, then converted to the *n*-octyl amine salt and its absorption between 1450 and 1350 cm^{-1} recorded as a 2–3% solution in CCl_4. The absorbance ratio of the 1367/1410 cm^{-1} bands is calculated and the relative amounts of straight and branched chain ABS read from a calibration curve. The octyl amine salt is used rather than the 1-methyl heptyl amine because it avoids an additional methyl branch absorption in the analytical region. Calibration standards are 1) a 99+% pure straight chain sodium ABS (mol wt 348) composed of a mixture of 2,3,4,5, and 6 phenyl isomers, and 2) the SDA standard tetrapropylene ABS. These materials most nearly approximate commercial ABS. Typical IR curves show in Figure 1 and 2.

Method

Reagents. Basic Solution—15 g NaCl, 40 ml 3A alcohol, 25 ml 0.5 N NaOH and 3 ml 0.1% chlorophenol red indicator solution into a 500 ml volumetric flask. Fill to volume with distilled water and mix well.

Phosphate buffer, pH 6.8

Petroleum ether (PE), redistilled

Calibration. Convert the sodium ABS calibration standards to their *n*-octyl amine salts by the procedure described in the SDA method. Prepare a series of mixtures ranging from 5–95% of each standard and dissolve in CCl_4 to make 2% solutions on the sodium ABS basis. Scan the 1450–1350 cm^{-1} spectral region in a 1-mm cell vs. a CCl_4 blank, using a five-fold expansion of the wavelength scale. Measure