Near-Infrared Absorption Spectroscopy—A New Tool for Lipid Analysis

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A REVIVAL of the use of near-infrared spectroscopy for chemical analysis, prompted mainly by the introduction of improved instrumentation which permits absorption in this region to be measured rapidly, simply, and accurately, has been accompanied by its introduction into the analysis of lipids.

Several specific procedures have been suggested or recommended as offering definite advantages over either chemical methods or instrumental methods employing other regions of the electromagnetic spectrum. Thus near-infrared spectroscopy has become, during the past five years, a new tool for the lipid chemist.

Among specific procedures in which near-infrared spectroscopy appears to offer decided advantages are included determinations of hydroxyl number, epoxy contents, hydroperoxide value, cis-unsaturation, etc. The Spectroscopy Committee of the American Oil Chemists' Society is now actively engaged in collaborative investigations, involving several laboratories, of recommended procedures for the determination of hydroxyl value by means of near-infrared spectra, with a view of establishing an official method. The Committee is also planning similar collaborative testing of recommended procedures for epoxy value and for the determination of cis-unsaturation to complement the infrared procedure for trans-unsaturation, now an AOCS Tentative Method. Thus near-infrared spectroscopy appears destined to join the ranks of other branches of spectroscopy and offer to the lipid chemist new and additional instrumental methods of chemical analysis.

Contrary to popular opinion, chemical applications of near-infrared absorption spectroscopy are not new. Many chemists are under the impression that these techniques date back only some five or six years. Actually this is the date of a revival of near-infrared absorption spectroscopy, motivated by the introduction of instruments which make measurements in this region simple, rapid, and accurate, and spurred by the excellent review on the subject by Wilbur Kaye in 1954 (19). The history of spectroscopy shows however that applications of infrared spectroscopy had their start in the region now called the near-infrared before the beginning of the present century.

It is of historical interest that, following the original discovery of infrared radiation by Sir William Herschel in 1800, first application to chemical analysis was made in the field of emission. However when investigations in absorption revealed group frequency relations and methods for characterizing or identifying organic molecules, emission was abandoned and, although still of some potential usefulness, has never been revived for the purposes of chemical analysis. A review of the development and potentialities of emission infrared spectroscopy, for anyone interested, has been published by C.J. Humphreys (17).

As early as 1881 Abney and Festing (1) published the infrared spectra of 52 compounds to 1.2 μ, the limit of the sensitivity of their photographic plates. Fifteen years later Donath (6) published some half dozen spectra to 2.7 μ, covering what he considered the region of "practical significance." Puccianti (21) in 1900 showed that a band at 1.7 μ is observed in the spectra of all compounds in which a carbon atom is directly combined to a hydrogen atom and that in benzene molecules additional bands appear at 2.18 and 2.49 μ. He made the important observation that "the absorption depends upon the groups of atoms which exist in the molecule." This observation was forerunner of the "group frequency theory" of chemical analysis which was to follow shortly, that is, that the identification of a functional group can be made from inspection of the infrared spectra of the molecule.

One of the earliest, and in several ways one of the greatest, contributions of infrared absorption spectroscopy was the detection of hydrogen bonding. Early papers describing these techniques were limited to near-infrared absorption. In the original work of Hilbert et al. (12) with compounds containing hydroxyl groups, the conclusion is given "Since the absence of characteristic OH absorption is found to be closely correlated with the presence of the hydrogen bond as indicated by evidence from other sources, it has been inductively concluded that the absence of such OH absorption constitutes a good criterion for the presence of a hydrogen bond." The band is described as disappearing (rather than shifting to longer wavelengths) on hydrogen bonding as the limit of the sensitivity of photographic plates did not permit detection of the hydrogen bonded –O–H . . . . stretching above 3.0 μ.

Thus not only were early measurements of absorption made in the near-infrared region, but they were restricted to this region, then known as the "prism-optics" region. With the advent of direct reading detectors, i.e. radiometers, bolometers, and later thermocouples, rock salt optics were introduced and the development of analytical applications of infrared absorption spectra took rapid strides in this rock salt region from about 2.5 to 15 μ led by the group frequency theory developed by Julius (18) and by growing collections of spectra for "fingerprint" identifications, such as those published by Coblentz in this country (4). The prism-optics region (near-infrared) was forgotten in the many applications that were made, and in the increasing attempts to extend spectra to longer and longer wavelengths.
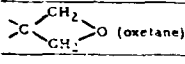
Near-infrared absorption spectroscopy lay more or less dormant until about five years ago when its potentialities were pointed out in the aforementioned excellent review of Wilbur Kaye (19) and the almost simultaneous announcement of the availability of instruments to measure near-infrared absorption rapidly and accurately. It is of passing interest that extension to the near-infrared did not come by development of

modern double-beam infrared spectrophotometers to lower wavelengths, but by extension of ultraviolet-visible instruments to longer wavelengths, mainly by incorporation of interchange lead sulfide detectors. Thus modern near-infrared absorption spectroscopy is again "prism-optics" region infrared. Applications of near-infrared to lipid analysis followed shortly after its reintroduction by Kaye. As these applications have been introduced to the lipid chemist within the past four or five years, near-infrared spectroscopy can accurately be described as a "New Tool for Lipid Chemistry."

Holman and Edmondson published (14) results of a general survey of the near-infrared absorption spectra of several fatty acids, esters, glycerides, and other lipids. They discuss bands found in the region 0.9 to 3.0 μ, and their correlations with C–H stretching of methylene, methyl, double bonds, and triple bonds, and with O–H stretching of carboxyl, hydroxyl, and carbonyl groups. They concluded that, in several instances, the near-infrared spectra could simplify the characterization of many molecular structures and would be valuable in the solution of many problems in lipid chemistry. It should be possible to

TABLE I
Spectra-Structure Correlations and Average Data for Near-Infrared Region *

Compiled from data from authors' laboratories and published data, mostly obtained in CCl₄ solution.   Units are liter/mole-cm.

identify, and very probably quantitatively measure, *cis* double bonds,, terminal double bonds, hydroxyl groups, amine groups, hydroperoxides, methyl and ethyl esters, acids, and methylene and methyl groups.

Goddu and Delker (11) published a table showing absorption bands in the near-infrared and their correlations with vibrating groups. See Table I.[2] The near-infrared region of the electromagnetic spectrum is the region of overtones and combinations of fundamentals which appear at longer wavelengths. As can be seen from Table I almost all of the absorption bands observed in this region arise from hydrogenic stretching vibrations. The observation, that all of the bands in the near-infrared region arise from a single type of vibration, the stretching of hydrogen attached to carbon, oxygen, halogens, nitrogen, sulfur, phosphorus, etc., may at first seem rather surprising. However, considering that the near-infrared region covers a range of only about 2 μ, the assignment of vibrations in this region to hydrogenic stretchings is no more improbable than the assignment, for example, of the region about 6 μ to double bond stretchings or the region about 4 to 5 μ to triple bond stretchings, etc. Fortunately, as can be seen from the table, the restriction of vibrations to hydrogenic stretching vibrations does not particularly limit the applications of the near-infrared region in problems of interest to the fatty acid chemist.

As the bands in the near-infrared region of the spectrum are overtones and combination bands, they are almost without exception considerably weaker in intensity than the fundamental bands of the rock salt region. For this reason measurements in the

[2] Reprinted from Analytical Chemistry, *32*, 140–141 (1960) by permission of the American Chemical Society and of the authors.

near-infrared are made, if possible, from more concentrated solutions or, if solubility or other factors such as hydrogen bonding, make more concentrated solutions undesirable or impossible, longer cell paths are employed. It is common to see near-infrared spectra measured from cells 5 or 10 cm in length rather than the 0.2- to 1.0-mm. cells used frequently in the rock salt region. A limiting factor in increasing cell path is, of course, the transparency of the solvent. As bands in the near-infrared region arise mainly from hydrogen stretchings, solvents which contain no hydrogen atoms are most satisfactory. Carbon tetrachloride is one of the most common solvents and, if it can be used, it is transparent over all the near-infrared region in 10-cm cells. Carbon disulfide is available over almost the entire region, but not as transparent as carbon tetrachloride from about 2.0 to 2.5 μ. Completely chlorinated hydrocarbons, such as tetrachloroethylene, are also used with considerable success. Goddu and Delker (11) published a table showing the most common solvents, the portions of the near-infrared spectrum over which they can be used, and the maximum path length which can be tolerated in qualitative work without causing the slits to be too wide for applicability of Beer's law. Their table is reproduced in Table II (2).

Determination of Hydroxyl Number

In 1958 Bayzer, Schauenstein, and Winsauer (2) described a technique for the determination of hydroxyl content of C_{18} fatty acids and their esters by use of near-infrared spectroscopy. From spectra of pure hydroxystearic acids they computed ratios of absorbancy of the O–H stretching vibration at 3130

TABLE II

cm^{-1} (3.20 μ) to that of the C–H stretching at 2920 cm^{-1} (3.42 μ). The ratio of E_{OH}/E_{CH} was 0.443, 0.885, 1.033, and 1.19 for 12-monohydroxy-, 9,10-dihydroxy-, 9,10,12,13-tetrahydroxy-, and 9,10,12,13,15,16-hexahy-droxystearic acids respectively. From a plot of these ratios against the concentration of OH a calibration curve is obtained from which a quantitative determination of OH content in an unknown can be obtained.

The determination of hydroxyl numbers of poly-esters and polyethers by near-infrared absorption has been described by Hilton (13). He started with the assumption that total absorption of these materials at 2.83 μ can be expressed as:

$$a = a_x X + a_y Y + a_{1-x-y} (1 - X - Y)$$

where a is the absorptivity of the unknown polyester, a_x, a_y, and a_{1-x-y} the absorptivities for the OH, COOH and the remainder of the molecule at 2.83 μ and X, Y, and $1 - X - Y$ are the fractional per cents of each of these groups in the unknown. Absorptivities were evaluated from a known polyester-ethylene-propyl-ene adipate, where OH and COOH concentration had been determined chemically. Substituting these values in the equation and solving for % OH gives the expression:

$$\% \text{ OH} = 40.5 \text{ A}_{s\,2.83} - 0.15\% \text{ carboxyl} - 1.18.$$

From a chemical determination of the % carboxyl and determination of the absorptivity of the unknown sample at 2.83 μ, ($A_{s\,2.83}$), the per cent hydroxyl can be obtained by use of this equation. For a polyether containing no carboxyl the equation can be simplified:

$$A_s \text{ polyether} = A_{s\,OH} X + A_{s\,\text{remainder}} (1\text{-}X)$$

and by evaluation of the absorptivities from investigations of polyethers of known OH content:

$$\% \text{ OH} = 39.2 \, a_{s\,2.87} - 0.58$$

As hydroxyl number is defined as the number of milligrams of potassium hydroxide equivalent to the acid consumed in esterifying the hydroxyl groups present in 1 g of sample, and acid number is defined as the number of milligrams of potassium hydroxide required to neutralize 1 g of sample, the per cent hydroxyl can be converted to hydroxyl number and the equations expressed in terms of hydroxyl number and acid numbers by multiplying by 561/17.0, or 33.0:

Hydroxyl No. (polyesters) = 1337 A$_{s\,2.83}$ – 0.396 Acid No. – 38.94

Hydroxyl No. (polyethers) = 1294 A$_{s\,2.87}$ – 19.1.

A comparison of results by chemical methods and the near-infrared procedure (as reported by Hilton) are given in Table III. Duplicate chemical analyses on a single sample require about 1.5 man hours and 3 hrs. elapsed time. The near-infrared procedure requires about 0.5 man hours and 2.5 hrs. elapsed time, per duplicate determination. Thus the spectrophotometric method effects a considerable saving in time and, as shown in this table, gives comparable results.

The overtone band at 1.4 μ was used by Crisler and Burrell to obtain the hydroxyl value of alcohols by means of near-infrared spectroscopy (5). They measured the near-infrared spectra of a number of long-chain fatty alcohols and plotted the measured absorbancies against the known OH content, expressed

TABLE III
Hydroxyl Content of Polyethers

Sample	% Hydroxyl (Chemical)	A$_{s\,2.78}$ Polyether	A$_{s\,2.78}$ Hydroxyl	% Hydroxyl Near-IR
LG42	1.45	0.05154	2.561	1.44
	1.43	0.05210	2.600	1.46
		0.05158	2.564	1.44
LG67	2.28	0.07451	2.625	2.34
	2.29	0.07431	2.616	2.33
		0.07392	2.599	2.32
LG112	3.76	0.11107	2.577	3.77
	3.75	0.11098	2.574	3.77
		0.11088	2.572	3.76
LB240	7.11	0.19650	2.569	7.12
	7.08	0.19504	2.549	7.06
	7.13	0.19587	2.561	7.09
			Av. 2.581	

as mg of OH per ml of sample. Such curves were linear (obey Beer's law) only at low concentrations. However for the alcohols octyl to octadecyl they are coincident up to concentrations corresponding to 2 mg of OH per mol of solution in tetrachloroethylene, and from a single calibration curve a hydroxyl value method is possible. Both carbon tetrachloride and tetrachloroethylene were used as solvents. The latter is recommended as it has lower toxicity, higher boiling point, greater chemical stability, and contains less water when saturated. The presence of water in the solvent was shown to affect the results; saturation of the tetrachloroethylene amounts to an additional hydroxyl content of 0.01 mg per ml and of carbon tetrachloride of 0.02 mg per ml. This error can be eliminated by addition of anhydrous sodium sulfate to the sample solution. In Table IV hydroxyl values obtained by the near-infrared procedure are

TABLE IV
Comparison of Near-Infrared with Chemically Determined Hydroxyl Values
Hydroxyl Value, Mg. OH/G. Sample

Near-infrared	Chemical cooperative average	Difference
90.9	90.8	0.1
85.0	84.6	0.4
111.4	111.5	0.1
115.4	115.8	0.4
104.3	104.3	0
91.4	91.4	0
90.3	90.0	0.3
Av. difference	......	0.19
Std. dev.	......	0.27

compared to the average of chemical values obtained cooperatively in a number of laboratories. The standard deviation, 0.27, is better than the standard deviation of the chemical method, 0.77, for OH values in the concentration range 85 to 115 mg of OH per g of sample. The authors' investigations show that a separate calibration curve should be prepared for each type of alcohol for, as expected, the band positions and intensities are dependent on the structure of the compounds. The OH overtone band shows a shift to higher wave lengths in the order, primary-secondary-tertiary-phenol. In a series of primary alcohols a consistent increase in molar absorptivity is observed for the branched alcohols, the value increasing as the position of branching approaches the 1 position. Phenols exhibit a wide variation in absorptivity and wave length, explained to some extent by consideration of the various effects of the other substituents on the aromatic ring.

Goddu (9) has described an investigation of the near-infrared spectra of various phenols showing that it is possible to analyze mixtures containing hindered and free phenolic hydroxyl. Samples containing as many as two or three different types of intramolecularly bonded phenolic hydroxyl compounds in addition to free and/or hindered phenolic hydroxyls can also be analyzed. Phenolic mixtures containing up to four components were successfully analyzed with measurements over the region 2.7 to 3.0 μ with rock salt optics. In favorable cases six or more phenolic components could be determined simultaneously. Sensitivity in detection to about 0.05 ppm of phenolic hydroxyl in carbon tetrachloride solution was possible using 10 cm absorption cells. Traces of water however make this sensitivity difficult to achieve and a practical limit of detection might be better placed at 0.25 ppm of phenolic hydroxyl, i.e., in a 1% solution of a sample in carbon tetrachloride this would mean a detection of the order of 25 ppm of phenolic hydroxyl. This limit could be increased by use of larger samples.

The procedure affords a more rapid analysis than conventional methods and offers considerably more selectivity. The information obtained on the orientation of the hydroxyl groups within the molecule could be of considerable aid in structural analysis in addition to the qualitative and quantitative aspects.

Near-infrared absorption data in the region 2.75–3.00 μ for eleven different classes of polyhydroxyphenols were presented by Goddu to illustrate how relatively small changes in structure of a compound can have a large effect on its free and intramolecularly bonded hydroxyl stretching vibrations. Examples of how these changes in free and intramolecular bonded hydroxyl stretching vibrations, expressed as the relative intensity of the free and intramolecularly bonded hydroxyl peaks or as frequency of wave length separation between these two peaks, can be used to differentiate structural differences, such as ortho-, meta-, and para-orientation, are discussed in detail. See Table V.

The use of near-infrared spectroscopy for the determination of hydroxyl concentration of polypropylene glycols has been described by Burns and Muraca (3). They established the expression:

$$A = A_{OH} + A_{H_2O} = a_{OH}c_{OH} - a_{H_2O}c_{H_2O}$$

(where A and a are absorbancies and absorptivities respectively and c is the concentration) to account for absorption at 2.82 μ as arising from –OH stretching and from water. By varying the concentration of the water and determining its content by Karl Fischer titration, an expression of the absorptivity of –OH stretching at 2.84 μ, corrected for water content was obtained:

$$a_{OH} = A_{OH}/c_{OH} = [A - (0.0755)(\% H_2O)]/c_{OH}$$

From this equation, a Karl Fischer determination of water content of the sample and measurement of the absorbance at 2.84 μ in the near-infrared spectra, permit a determination of the –OH concentration. Examination of 14 different polypropylene glycols by the four-hour acetylation procedure and for water content by the Karl Fischer technique showed, after measurement of the absorbance at 2.84 μ, that the ratio A_{OH}/c_{OH} is nearly constant with a value of 0.273

TABLE V

Near-Infrared Absorption Data for Phenols of Various Structure

	λ Max., μ	Molar absorptivity ξ, liter/mole·cm	
	Phenols	2–5 mM	0.1–0.3 mM
Phenol	2.771	194	192
p-Cresol	2.771	192	196
p-Isopropylphenol	2.771	195	......
p-Nonylphenol	2.771	200	215
p-Phenylphenol	2.774	245	256
4,4'-Isopropylidenediphenol			415
(bisphenol A)	2.772	425	(2 OH's)
m-Cresol	2.773	202	206
m-Isopropylphenol	2.773	186	185
Mono-ortho-Substituted Phenols			
o-Cresol	2.771	172	171
2-tert-Butyl-p-cresol	2.774	173	175
	2.745	14	14
2-tert-Butyl-4-methoxyphenol	2.771	190	......
2,5-Di-tert-butyl-p-cresol	2.775	190	69
	2.746	......	16
2-Isobornyl-p-cresol	2.772	160	157
o-Phenylphenol	2.808	196	201
	2.776	27	21
2-(a,a-Dimethylbenzyl)-p-cresol	2.833	222	220
Diortho-Substituted Phenols			
2-tert-Butyl-o-cresol	2.769	168	169
2,4-Di-tert-butyl-o-cresol	2.768	150	153
2,6-Diisopropylphenol	2.766	147	147
2,6-Di-tert-butyl-phenol	2.746	210	214
2,6-Di-tert-butyl-p-cresol	2.744	180	183
2,4,6-Tri-tert-butylphenol	2.746	186	189
2,6-Diisobornyl-p-cresol	2.771	143	144
2,6-(a,a-Dimethylbenzyl)-p-cresol	2.843	290	280

and a standard deviation of 0.0059 g per millimole.

If the water content is less than 0.25% correction for it could be eliminated without increasing the error within the limits of error of the measurement. It should be possible to measure the water content by use of the 6.06 μ water band in the infrared spectra, but in general this is unsatisfactory because of the low absorption in this region for the determination of water content. A more satisfactory method for eliminating the need for water correction was found to be a half-hour predrying of the samples at 0.1 mm pressure and a temperature of 100°C., where it can be reduced to 0.005% (the limit of the Karl Fischer determination) and correction of –OH absorptivities for water content can be completely eliminated.

Determination of Epoxy and Hydroperoxy Groups

Goddu and Delker (10) investigated the 1.65 and 2.20 μ bands in the spectra of compounds containing

a terminal epoxy, $\overset{\diagdown}{\underset{\diagup}{C}} - CH_2$, group. The band at 1.65 μ

is the first overtone of the fundamental C–H stretching vibration of terminal epoxides, the fundamental band occurring at 3.28 to 3.26 μ. The band occurs at shorter wave lengths and is well resolved from the similar first overtone band of C–H stretching of either the methyl or methylene groups, at about 1.70 μ. The band at 2.2 μ is a combination band characteristic of terminal epoxides. It falls between, and is well resolved from, the combination bands of the aliphatic methyl and methylene groups at 2.3 and 2.4 μ and the terminal methylene band due to unsaturation at 2.1 μ. This epoxide band is readily distinguishable from the aromatic C–H bands in this region and epoxide may easily be detected and measured in aromatic compounds without interference. The band at 2.2 μ is considerably more intense than the overtone band at 1.65 μ and hence could be used to advantage in the determination of epoxide in samples containing a low content of this group. The band

can be used for differentiation and quantitative determination in the presence of other oxygen rings such as oxetanes, furans, and dioxane and in terminal epoxides with a tertiary carbon atom, where difficulty is often encountered in determination of epoxy content by chemical methods.

Absorption bands at 2.07 and 1.46 μ in the spectra of oxidized fatty acids were shown by Holman and coworkers (16) to arise from vibrations of the hydroperoxide group. In all samples examined in which hydroperoxides are known to occur, these two bands, along with the fundamental at 2.82 μ, will be found in the spectrum. More important, probably, all substances in which the hydroperoxide groups are not present failed to reveal these absorption maxima. Thus the bands appear to be very specific for the hydroperoxide group and afford an opportunity to distinguish between hydroperoxides and other substances which also oxidize KI and are therefore measured chemically as peroxide. The fundamental vibration at 2.82 μ can be used for the detection and measurement of hydroperoxides but the resolution, especially with rock salt, is very poor. It is difficult to differentiate, even with quartz optics, the 2.82 μ band arising from the hydroperoxide group from that of the hydroxyl, aldehyde, ketone, acid or ester groups, all of which reveal absorption peaks in this region. Hence the bands at 1.46 and 2.07 μ are recommended. With quartz optics these two bands can be readily differentiated from neighboring hydroxyl absorptions. The maximum at 2.07 μ is probably a combination absorption and that at 1.46 μ a harmonic of the fundamental at 2.82 μ. Consequently, one disadvantage is their relatively low intensity, necessitating the use of high concentrations or long absorption cells.

Unsaturation by Near-Infrared Absorption Spectroscopy

From an investigation of the near-infrared spectra Goddu (8) showed that isolated double bonds of two types could be readily detected and determined. Terminal methylene groups may be determined at 1.6 or 2.1 μ with a sensitivity within 0.01% $C=CH_2$, and a precision and accuracy within ±2%. Cis double bonds may be determined at 2.1 μ with a sensitivity of about 1% $-CH=CH-$. Trans unsaturation and saturated C–H groups do not interfere. Thus a mixture containing cis-4-methyl-2-pentene (33.6%), trans-4-methyl-2-pentene (33.8%), and 2-methylpentane (32.7%) was analyzed for the cis compounds and a cis-4-methyl-2-pentene content of 32.6% was obtained. A mixture containing 35% 1-octene and 65% cis-4-methyl-2-pentene was analyzed for both the internal cis unsaturated and the terminal unsaturated components. Values of 35% for the 1-octene and 60% for cis-4-methyl-2-pentene were obtained. Thus mixtures of cis, trans, and terminal double bonds can be analyzed readily for cis and terminal double bonds in the near-infrared.

Holman, Ener, and Edmondson (15) measured the absorptivities of natural fats and oils at 2.15 μ and attempted to correlate these values directly with unsaturation measured by iodine value. Data were scattered about a straight line until trans unsaturation, measured by infrared analysis (in the 10 μ region) was subtracted from the total unsaturation measured by iodine value. Thus the value measured at 2.15 μ

is correlated with cis unsaturation. These authors point out that terminal unsaturation with bands at 2.12, 2.17, and 2.23 μ and methyl and ethyl esters which absorb at 2.14 μ could cause some interferences. A comparison was made between the direct measurement of cis unsaturation from iodine value and trans content from infrared measurements at 10.36 μ. Results, as reported by these authors for a series of hydrogenated fats and oils, are given in Table VI and

TABLE VI

Comparison of Indirect and Direct Methods of Measurement of Cis Unsaturation in Mixed Fatty Acids

Source of sample	Total unsaturation		Trans unsaturation	Cis unsaturation by difference	Cis unsaturation direct
	Iodine value	moles/kg	moles/kg	moles/kg	moles/kg
Margarine					
A	73.6	2.90	0.83	2.07	2.09
B	84.5	3.33	1.35	1.98	2.17
D	77.0	3.03	1.52	1.51	1.71
P	84.0	3.31	1.84	1.47	1.69
SS	59.4	2.34	0.61	1.73	1.65
Shortening					
C	80.0	3.15	0.79	2.36	2.21
S	79.0	3.11	1.36	1.75	1.74
Hydrogenated coconut oil	5.4	0.21	.22	−0.01	0.36
Hydrogenated cottonseed oil	73.5	2.90	1.92	0.98	1.20
Hydrogenated whale oil	70.4	2.77	2.24	0.53	0.08
Peanut butter S	90.0	3.54	0.38	3.16	3.27

indicate a reasonable agreement between the two methods. It is obvious from the data in this table that it would be possible, from a combined determination of cis unsaturation in the near-infrared and a trans determination in the infrared region to obtain an iodine value by an indirect spectrophotometric method without using iodide.

Fenton and Crisler (7), using the band at 2.143 μ, describe in detail a precise method for the analysis of refined oils, hydrogenated oils, and finished shortenings for cis content. In the presence of less than 10% trans isomers analyses were made by a direct-area-measurement procedure in which the approximate area under the absorption band (obtained by multiplying the peak absorbance by the band width at half band height) was used. However if the trans isomer content is above 10% further refinements were necessary. In these cases a base line was chosen. A single point baseline correction was selected and the absorbance difference between the peak at 2.143 μ and a transmission peak at 2.157 μ was used. A series of cottonseed oils hydrogenated to different trans contents were used as standards. The calibration curve was prepared by plotting the absorbance differences between 2.143 and 2.157 μ against cis contents (calculated from iodine value and trans acid data). In this manner an excellent straight-line relation as shown in Figure 1 was obtained, which is independent of the number of multiple double bonds present. The Spectroscopy Committee of the American Oil Chemists' Society is planning to study this procedure for cis content in collaborative investigation for possible recommendation as an Official Method of the Society to complement the recently adopted Tentative Method for Trans Content by measurement in the infrared region at 10.36 μ.
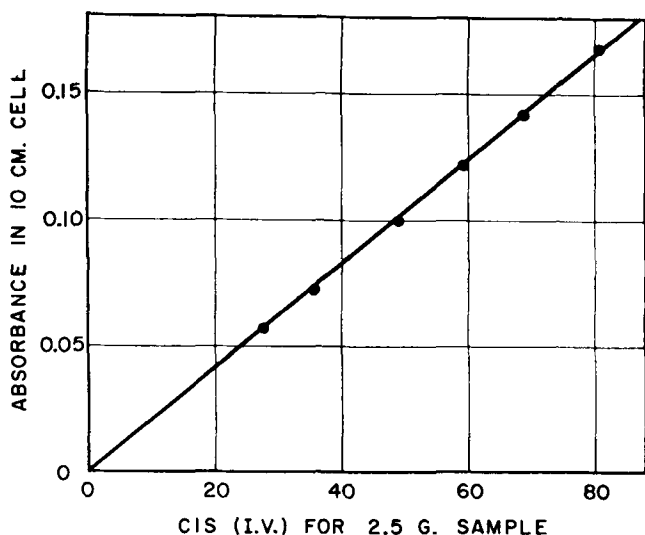
Fig. 1. Calibration curve prepared from hydrogenated cottonseed oils.

Application to Analysis of Monoglycerides

In the analysis and structural determination of mono-, di-, and triglycerides, where chemical methods are tedious and time-consuming infrared absorption spectroscopy has been of considerable value (20). The differentiation and characterization of 1-monoglycerides and 2-monoglycerides by infrared methods however have not been satisfactory. Susi, Morris, and Scott (22) have recently described investigations of the –OH stretching vibration of monoglycerides in the near-infrared from 1.3 to 1.5 μ. The absorbances at 1.430 μ of various 1-monoglycerides investigated all fall on a straight line when plotted against concentration; those of all 2-monoglycerides at the same wave length also fit a straight line, but of different slope (Figure 2). The two straight lines indicate that absorption at 1.430 μ is not appreciably influenced by chain length or degree of unsaturation. Evaluation of the absorptivities from these curves gave values of $k_1 = 0.39$ and $k_2 = 0.20$, where k_1 and k_2 are the molar absorptivities of 1-monoglycerides and 2-monoglycerides respectively. The absorbance (A) of a mixture of 1- and 2-monoglycerides at a specific wave length is given by the relationship:

$$A = (c_1 k_1 + c_2 k_2) b$$

where c_1 and c_2 are the molar concentrations of 1-monoglycerides and 2-monoglycerides $(c_1 + c_2 = c)$ and b is the path length in cm. Substituting the evaluated absorptivities at 1.430 μ for a path length of 5.0 cm, the equation can be rearranged:

% 1-monoglyceride $= (c_1/c) 100 = (A - ck_2 b)$
$100/(k_1 - k_2) bc$

and substituting the values for k_1, k_2 and b:

% 1-monoglyceride $= (A - c) 100/0.95 c$

Values reported by the authors by use of this equation for the analysis of known mixtures of 1- and 2-monostearin and 1- and 2-monopalmitin, as shown in Table VII, indicate that very satisfactory results can be obtained by the near-infrared procedure.
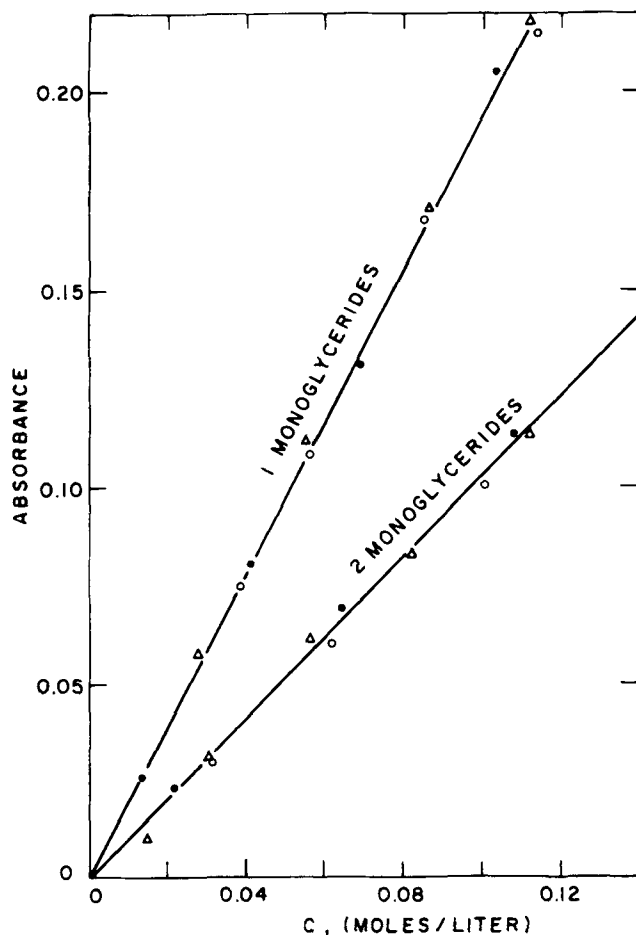

Fig. 2. Absorbance of 1-monoglycerides and 2-monoglycerides at 1.430 microns as a function of concentration. Open circles—monopalmitins; closed circles—monostearins; triangles —mono-oleins.

TABLE VII
Known and Computed Composition of Mixtures of
1- and 2-Monoglycerides

Glyceride mixture	Total concentration (moles/liter)	Known amount of 1-monoglyceride (%)	Computed amount of 1-monoglyceride (%)
1- and 2-monostearin	0.1093	26.8	23.2
	0.1073	47.8	48.1
	0.1052	69.8	73.0
1- and 2-monopalmitin	0.1110	16.4	18.0
	0.1127	40.3	42.0
	0.1139	57.6	57.2
	0.1154	78.9	79.6

Hydrogenic Stretchings Other Than C-H and O-H

Application of hydrogenic stretching bands in the near-infrared region other than those of the C–H and O–H have not been applied to specific problems in fatty acid chemistry but are, obviously, of potential usefulness. Whetzel et al. (23,24,25) have described the use of near-infrared spectra in the differentiation and analysis of primary aromatic amines, mixtures of primary and secondary aromatic amines, and of N-alkyl and N-alkyl-N-hydroxylalkyl aromatic amine mixtures, using N-H stretching bands between 1.0 and 2.0 μ. Such analyses could be applied to measurements of amine derivatives of long chain fatty acids. Similarly S–H and P–H stretching bands in this re-

gion will undoubtedly find usefulness in applications to derivatives containing these groups.

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Recent Progress in the Applications of Infrared Absorption Spectroscopy to Lipid Chemistry

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INFRARED ABSORPTION SPECTRA were introduced to the lipid chemist as an analytical tool during the decade 1940–1950. The first half of the following decade, the years 1950–1955, witnessed widespread growth with a continually increasing number of re-search papers in technical journals describing infra-red spectral procedures for qualitative identifications, quantitative determinations, and for elucidation of structures of lipid materials (91).

Infrared absorption spectroscopy cannot therefore be introduced at this Short Course as a new tool of lipid chemistry. However there have been many ad-vances in applications and many new techniques in-troduced to the fatty acid chemist during the past few years. This paper will describe some of these. Except for earlier references, where necessary to estab-lish essential background, this review will be limited to work reported during the past five years or since the American Oil Chemists' Society's Short Course on "Analytical Techniques" held at the University of Illinois in 1955.

Cis and Trans Unsaturation

One of the most popular applications of infrared absorption spectroscopy to lipid chemistry has been the identification and measurement of *cis-trans* iso-merization. It should be recalled that in 1947 Ras-mussen, Brattain, and Zucco (104) showed that a strong band at 10.3 μ appears in the infrared spectra of all unsaturated compounds which contain a *trans* C=C group and furthermore this band does not appear unless this group is a *trans* isomer. In 1949 Sheppard and Sutherland showed that the band arises from a C-H deformation about a *trans* C=C in the internally unsaturated group RHC=CHR (112). From 1950 to 1953 four papers described techniques that used this band as a means for the quantitative determination of internal, isolated (that is, noncon-jugated) *trans* isomers (1, 53, 113, 122).

The published methods for the determination of isolated *trans* content have been the subject of exten-sive study by the Spectroscopy Committee of the American Oil Chemists' Society. As a result of long collaborative testing the committee has recommended a procedure which has been adopted as a tentative official method of the Society (93). The recommended procedure provides for the determination of isolated *trans* content of long-chain fatty acid esters and of their triglycerides. Long-chain fatty acids can be analyzed directly if the *trans* content is 15% or greater. If below 15%, the recommended procedure requires conversion of the fatty acid to its methyl ester for, at these low concentrations, correction for an absorption arising from the carboxyl band becomes too great for precise measurement. The method pro-poses standardization of infrared instruments with secondary standards to avoid the difficulty of pre-paring the highly purified primary standards other-wise required. Secondary standards for the methyl esters and triglycerides are available, and similar standards for the long-chain fatty acids, with *trans* content greater than 15%, are in the process of col-laborative examination.

Back in 1952 Jackson *et al.* (55) investigated the spectra of pure *trans*-10, *trans*-12, and *trans*-10, *cis*-12 conjugated linoleates. From these spectra they con-cluded that *trans-trans* conjugated linoleate is charac-terized by a band at 10.11 μ and *cis-trans* conjugated linoleate by a doublet at 10.18 and 10.55 μ. Ahlers *et al.* (1) investigated several pure compounds, many containing conjugated unsaturation. They confirmed the assignments of Jackson *et al.* and accounted for the positions of maxima in the spectra of conjugated acids containing *trans* bonds by a hypsochromic shift from the position of the isolated *trans* band, small but significant and consistent with the effect of conjuga-tion on the C-H deformation frequency of the system. O'Conner in 1958 (92) extended this postulation to the isolated *cis*-system (Table I). However the C-H