Infrared Spectroscopy of Fats and Oils

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SPECTROSCOPIC MEASUREMENTS are essentially measurements of the intensity and wavelength of radiative energy. A pattern of wavelengths at which energy is radiated is an emission spectrum. Alternatively, radiation varying continuously in wavelength may be passed through the sample material. It is then found that energy has been absorbed by the material at certain wavelengths. A pattern of these wavelengths, with an indication of the proportion of energy absorbed at each wavelength, is an absorption spectrum.

The spectroscopist is generally concerned with both *wavelength* and *frequency* as characteristics of the quality of electromagnetic radiation. Frequency is more significant than wavelength for interpretation of spectra. A convenient measure of frequency, used particularly in the IR region, is the *wave number*. It is defined as the inverse of the wavelength in centimeters and is the number of waves/centimeter. The unit is cm^{-1}. Both wavelength and wave number have been widely used in the literature. Because wave number is the more fundamental, many spectroscopists are now using it rather than wavelength.

The regions of the electromagnetic spectrum that are of interest to analysts are divided into far IR, IR, near IR, visible, UV and X-ray region. The IR region primarily used in analysis is that region having wavelengths just longer than the visible red up to perhaps 100 cm^{-1}(100 μ). The region from 14,000 cm^{-1}(0.7 μ) to roughly 3,000 cm^{-1} (3.3 μ) is called the near IR. This region, together with the region from 5,000 cm^{-1}(2 μ) to 625 cm^{-1}(16 μ), is primarily used in the analysis of fats and oils, although some methods include up to 250 cm^{-1}(40 μ).

In the short wavelength part of the spectrum, the waves have high energy, and when a molecule absorbs radiation in this region, large energy changes occur. Energy transitions in the visible and near IR regions can be either electronic or vibrational-rotational. For most materials, the visible spectra represent electronic transitions while the near IR reflect vibrational-rotational energy changes. Within the IR region, transitions are due to vibrational and rotational energy. The far IR region is characterized by rotational energy transitions, although some vibrational transitions of low frequency may also occur.

IR spectra are usually recorded on chart paper and presented in graphic form. The wave number or wavelength is represented along the abscissa, and some function of the amount of radiation absorbed (or transmitted) by the sample at each wave number is shown along the ordinate. Accordingly, a spectrum is a graphical presentation of the amount of radiation absorbed as a function of wave number or wavelength.

Although IR radiation was discovered by Herschel in 1800, very little use was made of it in lipid chemistry until the years following World War II. Julius in 1892 was probably the first to show that IR spectra of compounds containing a specific group exhibited that same absorption maximum. His empirical correlation of vibrating groups with specifically observed absorption maxima suggested the possibility of chemical identification and even of quantitative analyses. However, prior to 1940 only two publications appeared that made use of these early observations in the field of fats and oils. In the last 15 years a large number of publications have been made in this field.

IR applications to lipid chemistry have been both of a qualitative and a quantitative nature. An extensive study of the identification and measurement of the *cis-trans* isomers is perhaps one of the most important quantitative analysis made in this field. In the early days of IR, it was shown that a strong band at 980 cm^{-1} appeared in the IR spectra of all unsaturated compounds which contain a *trans* C=C group and that this band does not appear unless it is a *trans* isomer. This band arises from the C—H deformation about a *trans* C=C in the internally unsaturated group RHC=CHR. Collaborative testing of this observation by the Spectroscopy Committee of the AOCS led to the development of a procedure which has been adopted as an Official Method of the Society. The recommended procedure provides for the determination of isolated *trans* content of long chain triglycerides, fatty acid esters and fatty acids. Secondary standards were used to standardize the IR instruments to avoid the tedium of preparing highly purified primary standards otherwise required.

From the spectra of pure *trans*-10, *trans*-12 and *trans*-10, *cis*-12 conjugated linoleates, it became evident that *trans-trans* conjugated linoleate is characterized by a band at 989 cm^{-1} and *cis-trans* conjugated linoleate by a doublet at 982 cm^{-1} and 948 cm^{-1}. The C—H deformations about the *cis* C=C groups are quite variable in position with changes in molecuar environment and are in all cases very weak and consequently, not satisfactory for diagnostic purposes. However, measurement of the *cis*-unsaturation is possible in the near IR region. *Cis-trans* compounds alone or in the presence of *trans-trans* conjugation may be determined directly by absorption at 948 cm^{-1}. In the absence of *cis-trans* isomers, conjugated *trans-trans* octadecadienoates may be determined directly from the absorption at 989 cm^{-1}. But, in the presence of *cis-trans* isomers, *trans-trans* content can be obtained only after applying a correction for the contribution of the *cis-trans* isomers to the 989 cm^{-1} absorption of the *trans-trans* material.

Application of IR absorption spectroscopy to detect the presence of, or to measure the concentration of isolated *trans*-isomers or conjugated *cis*- and *trans*-configurations, has been elaborated in many research reports. Studies on elaidinization or *trans*-isomerizations were involved primarily with IR absorption. For example, it has been used to follow the *cis-trans* interconversions of methyl oleate, linoleate and linolenate when treated with oxides of nitrogen. The *trans*-isomerization of oleic acid and potassium oleate during irradiation with cathode rays was followed by means of IR absorption. The material obtained by prolonged heating of methyl linolenate in alkaline ethylene glycol solution was shown to be free of either *trans-trans* or *cis-trans* conjugation by IR in the 1,000–909 cm^{-1} region.

There is abundant literature wherein IR was used to follow the formation of *trans*-isomers during hydrogenation. The selectivity of the hydrogenation had more influence on the formation of *trans*-isomers than it had on the formation of positional isomers. Thus, by use of IR spectroscopy it was possible to follow the course of the reaction and appraise the influence of temperature, catalyst, pressure and other factors.

The autoxidation of pure fatty acid materials is a field of interest for many workers. In one study, the hydroperoxides formed by autoxidation of methyl oleate and methyl linoleates were decomposed under vacuum, and the volatile product was analyzed. The disappearance of *trans* absorption in the residue of methyl oleate and the appearance of *trans* absorption in the product from methyl linoleate was evident through IR spectroscopy. It was also used for hydroxyl determinations in the products of thermal dimerization of fatty ester hydroperoxide. IR spectroscopy was applied to various fractions of thermal decomposition or autoxidized soybean methyl esters of the polymeric fraction, and in thermally decomposed autoxidized safflower methyl esters. Changes were followed by observations of the IR band of hydroxyl at 3,390 cm^{-1}, of carbonyl at 1,724–1,695 cm^{-1} and of isolated *trans* conjugated *cis-trans* and conjugated *trans-trans* groups at 1,000–909 cm^{-1}. Dimers isolated

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