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Introduction: The Great Variety of Spectroscopic Techniques Available for the Analysis of Fatty Acids and Lipids[1]

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Abstract

A consideration of the electromagnetic spectrum from the high energy gamma radiation, through x-ray radiation, the electronic regions of the ultraviolet and visible, the vibrational region of infrared and the rotational region of microwaves, to the lower energy of radio frequencies are now used in resonance techniques. A consideration of how these various radiations can be used in emission, absorption, secondary emission (or fluorescence) and various scattering techniques, show that there are approximately 40 different ways in which spectra can be used as a potential tool for the analytical chemist. However, not all of them are applicable to the analysis of fats and lipids. Six methods will be reviewed with emphasis on the most up-to-date techniques and analyses used in fatty acid or lipid chemistry. In addition, two papers, on gas-liquid chromatography and a description of the important development of computer aided chemical spectroscopy will be presented.

Introduction

The initial problem encountered in arranging a symposium on the subject "Applications of Spectroscopy to the Analysis of Fatty Acids and Lipids," is a decision as to what applications are to be included and which techniques are to be selected. We can all think of several titles, X-Ray Diffraction, Ultraviolet Absorption, Infrared Absorption, Gamma Ray Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, and many others. However, a selection of techniques must be made in arranging a specific symposium, thus, we should probably start with a definition of analytical spectroscopy. For this purpose I have selected the often quoted definition that analytical spectroscopy is "any use of any portion of the electromagnetic spectrum in any manner whatsoever for the purpose of qualitative identification, quantitative determination, or for the elucidation of molecular structure."

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TABLE I
Subdivisions of Analytical Spectroscopy

Nuclear	X-Ray	Electronic	Vibrational	Rotational
Beta and Gamma Ray Radioactive nuclides Activation analysis Tracer analysis Nuclear Magnetic Resonance Wide line High resolution Spin echo Electro Paramagnetic Resonance Electron spin resonance Nuclear Quadruple Resonance	Emission Fluorescence Diffraction Absorption	Ultraviolet and Visible |><| Atomic and Molecular \ / Emission Absorption Fluorescence Scattering Raman effect Reflectance	Infrared Emission Absorption	Microwave Absorption

Our first consideration, then, is an examination of the electromagnetic spectrum and of its various divisions which include, in a descending order of energy, from the high energy gamma radiation, through x-radiation, the electronic regions of the ultraviolet and visible, the vibrational regions of infrared and the rotational region of microwaves, to the lower energy of radio frequencies which have assumed new interest in connection with resonance techniques.

One might object that these divisions are merely separations of a single spectrum, the electromagnetic, into arbitrary units based on differences in wavelength, frequency or energy. While it is quite true that the entire electromagnetic spectrum is a manifestation of the same phenomena, from fundamental and practical considerations, the divisions indicated are real. From a fundamental viewpoint, each of the radiations indicated arise from a different source, a completely different transition within atoms or molecules. Furthermore, from a practical viewpoint, we need quite different instruments to produce different radiations and entirely different devices to detect them or to measure them quantitatively. Finally, and probably of most importance to the analytical spectroscopist, the data which can be obtained from the various uses of radiation in each of these major divisions of the electromagnetic spectrum are quite different and provide entirely different facts regarding the analytical sample. Thus, while convenience alone might justify a division of the electromagnetic spectrum into various divisions, actually such a division does have real fundamental and practical significance.

Along with these seven divisions of the electromagnetic spectrum, we need to consider the various ways each of these radiations can be put to use by the analytical spectroscopist. These are emission, absorption, fluorescence (or secondary emission) and scattering, including reflectance, Raman effect, diffraction and Rayleigh scattering. According to our definition of analytical spectroscopy, we have merely to multiply these seven means of using radiation by the seven different types of radiation and arrive at the fact that there are theoretically, at least, 49 potential methods of analytical spectroscopy. Considerations of available energy would eliminate some of these potential techniques from practical application. For example, one could not imagine investigations of the Raman effect with microwaves. On the other hand, there are factors which increase the number of potential techniques. Within the electronic region of the electromagnetic spectrum we must consider atomic and molecular spectroscopy. With a specific radiation used in a particular manner, these two can be quite different and actually have nothing in common as far as the analytical spectroscopist is concerned. For example, there is little similarity between ultraviolet atomic absorption, a simple and sensitive method for the quantitative determination of most metallic and nonmetallic elements, and ultraviolet molecular absorption, the very popular technique for identification and determination of organic molecules which contain some degree of conjugation or aromaticity. Nor does ultraviolet atomic emission—better known as "spectrochemical analysis" and certainly the oldest and probably still the most popular method for the determination of trace amounts of metallic elements—have much in common with ultraviolet molecular emission, a relatively little known technique for the characterization of organic molecules. Thus, to the seven divisions of the electromagnetic spectrum, we must add atomic and molecular spectra in the ultraviolet and visible (electronic) regions. Considering nine specific regions and seven methods of using the radiation, we would arrive at a potential number of 63 different techniques available to the analytical spectroscopist. Eliminating some as unreasonable for energy considerations, the number of practical techniques might be expected as somewhere between 45 and 63.

The definition, as we have been using it, provides us with a more or less systematic method for naming each of the 45 to 63 individual spectroscopic techniques. We first name the region of the spectra and then how the radiation is employed, inserting, if we are dealing with electronic spectra, the additional reference to either atomic or molecular spectroscopy. Thus we have x-ray diffraction, x-ray fluorescence, ultraviolet atomic emission, ultraviolet molecular emission, infrared absorption, microwave absorption, and so on. The major divisions of analytical spectroscopy are: nuclear spectroscopy; x-ray spectroscopy; electronic spectroscopy; vibrational spectroscopy; and rotational spectroscopy. In Table I the specific techniques under each major division are tabulated. Obviously the simple systematic method of naming each spectroscopic technique has not been followed completely.

When figures such as 45 or 63 are mentioned as the number of individual methods available to the analytical spectroscopist, we find most chemists and many spectroscopists skeptical about accepting them. Most chemists and spectroscopists find these figures surprisingly high and accept them, in a temporizing way, as some sort of theoretically calculated number of possible techniques. As a matter of fact, however, a thorough search of the literature reveals that most of these potential methods have been described in some detail in at least one or two isolated papers.

We do not propose to describe each of the 45 to 63 different techniques available to the spectroscopist in this symposium. Furthermore, while most of the methods have been at least mentioned in the literature, they have not at all been of equal importance. Some are in constant use, almost indispensable to daily operations, others can be found described only briefly in an isolated technical report. In addition, while we believe that a sufficient search will reveal an application in each possible area, it by no means follows that this application will be within the area of lipid research or fatty acid processing.

We have selected six techniques which appear to have been the most active, and in which the greatest advances have been made during the past few years, especially in their application to lipid and fatty acid chemistry. Among them we have selected neutron activation analysis and atomic absorption spectroscopy, two techniques for identifying and quantitatively determining small traces of metallic and nonmetallic elements now being introduced into fatty acid and oilseed analyses. Two more are the resonance techniques of wide line NMR and high resolution NMR spectroscopy, among the newer techniques for examination of organic molecules; both are being used by fatty acid, lipid chemists and oilseed processors. The final two are the familiar x-ray diffraction and infrared absorption techniques, which have been used in fatty acid and lipid chemical research for a considerable period of time, but which have found a continuing list of newer and additional applications.

To these six methods we have added three papers which go beyond our definition of analytical spectroscopy. The first of these is mass spectroscopy, which does not involve use of any portion of the electromagnetic spectrum and therefore does not fall within the limit of our definition of analytical spectroscopy.

In mass spectrometry, the molecules of the sample being analyzed are bombarded by a stream of electrons. The sample must be in the gas phase and at low pressure, and the energy of the electrons must be greater than needed for the removal of an electron from the molecules, i.e., their kinetic energy must be greater than the ionization potential of the molecule. Under these conditions the molecules will lose an electron and the molecule ions formed in the excited states will decompose very rapidly into a number of fragments. The process by which these fragments are formed by bombardment of organic molecules is very complex and in many cases not entirely understood. During the impact with the stream of electrons, ionized and neutral fragments are formed. Mass spectrometry is concerned only with the positively charged particles. These particles are separated, either by the path they follow in a magnetic field or by the time they require to traverse a free field, according to the ratio of their mass to their positive charge, m/e. The magnetic or free field may be considered comparable, or at least performing a similar task, to the prism or diffraction grating in optical spectroscopy.

If the fragments with different m/e values are brought successively to a slit and their intensities measured, a spectrum with one parameter the value of the ratio m/e and the other the intensity, will be obtained. Although not employing radiation in the electromagnetic spectrum, such a pattern has always been known as a mass spectrum, with x-y parameters intensity versus mass rather than the usual intensity versus frequency. Mass spectroscopy, although not new, has taken on a new interest to the fatty acid or lipid chemist as improvements in instrumentation permit, with the use of double-focussing, high resolution spectrometers and measurements of large molecules approaching molecular weights of 2,000.

Another subject discussed in this Symposium is gas-liquid chromatography. It is rather difficult to envision an analytical spectroscopic laboratory of any size which would not be concerned with separations and analyses by means of gas-liquid chromatography; there have been considerable advances in this technique in its application to the analysis of fats and fatty acids and their derivatives in recent years. These applications have covered both the analysis of fatty acid material for its composition and the identification of vegetable oils or animal fats, or both, in admixtures.

Finally, we have included a paper on what is probably the most exciting development in the analytical spectroscopy laboratory-computerization. We have accepted the fact that analytical chemistry has moved largely away from so-called "wet" methods to instrumentation and the fact that instrumental methods are being highly automated. Now we are to learn that data from the automatic instruments will be computerized for much more efficient, simple, rapid and economical analyses.