surfactant complex is precipitated in the usual manner and is then dissolved in concentrated sulfuric acid. The addition of hydroquinone to this solution produces a red color, due to soluble tungstate, which is measured at 500 mμ. Anionics which form precipitates with $BaCl_2$ interfere, as do substances, such as proteins and cationics, which form complexes with heteropoly acids.

Gatewood and Graham (10) have developed a method of assaying sorbitol esters by saponifying them first to release the sorbitol, then oxidizing the sorbitol with periodate, coupling the formic acid produced in this oxidation with chromotropic acid, and measuring the resulting wine color at 570 mμ.

Finally, Hill and co-workers (14) have improved the methylene blue method for determining alkylbenzenesulfonates in water supplies by using a chromic-sulfuric acid mixture to oxidize interfering substances, such as organic sulfates and phosphates. Under the conditions of the procedure, the alkylbenzenesulfonate is unaffected by the oxidation mixture.

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Principles and Utility of Nuclear Magnetic Resonance in Structure Determination of Surfactant Chemicals

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THE THEORETICAL basis for the phenomenon of nuclear magnetic resonance (NMR) has been understood for some 17 years now since the original experiments by Purcell, Torrey and Pound (1) at Harvard and by Bloch, Hansen, and Packard (2) at Stanford University were first published in 1946. In the intervening years, the technique of high-resolution nuclear magnetic resonance has evolved into a research tool of rapidly increasing importance to chemists. It is only within the last three years, however, that sufficiently reliable commercial instrumentation has become available so that NMR can now be considered practical as a routine analytical tool.

The Principle

The information which can be gained by this method is ideally suited for determination of average molecular structures for a variety of raw materials, intermediates, and products in the area of surfactant chemicals. Since the physical and functional properties of these materials are determined primarily by their molecular structures, NMR provides the detergent chemist a unique method of gaining insight into the basic nature of the molecules with which he deals. The purpose of this discussion is to describe some of these areas in which NMR has proven particularly useful. The discussion will be divided into two parts. In the present paper, a general review of the basic principles of the technique will be given. The emphasis will be on application as a general tool for qualitative and quantitative analysis and structure determination of hydrogen-containing molecules. In a second paper, the application of the NMR method to specific problems of structure in the area of surfactant chemicals will be discussed and illustrated by several examples of applications which have been made in our laboratories, some of which have been described elsewhere (3,4). Those who wish to go more deeply into the subject are referred to several excellent books which treat the theory with varying degrees of sophistication (5, 6,7,8,9,10).

The Principle of NMR Measurements

A. *Background*—All atoms whose nuclei contain odd numbers of protons or neutrons possess the property of nuclear spin, which makes their nuclei behave somewhat similar to very small spinning magnets. Actually, these magnetic nuclei obey the laws of quantum physics rather than classical physics, but the analogy is close enough for a working comparison.

The most common examples of nuclei which can be studied by using this property are ^1H, ^{19}F, ^{31}P, ^{11}B and ^{13}C. Atoms with an even number of protons and neutrons—^{12}C and ^{16}O, for example—do not exhibit magnetic properties. Unless otherwise stated, further comments will refer only to the hydrogen

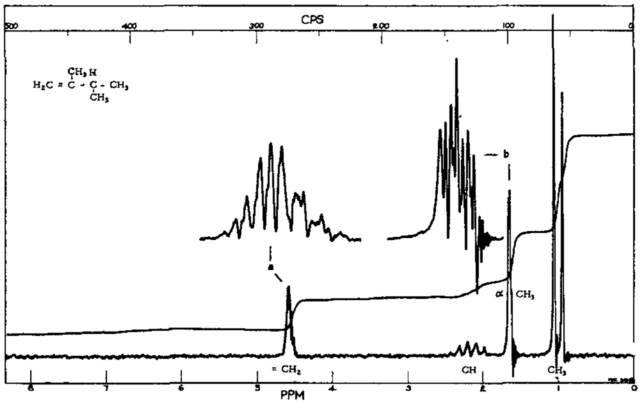

FIG. 1. 60 mc proton NMR spectrum of 2,3-dimethyl-1-butene.

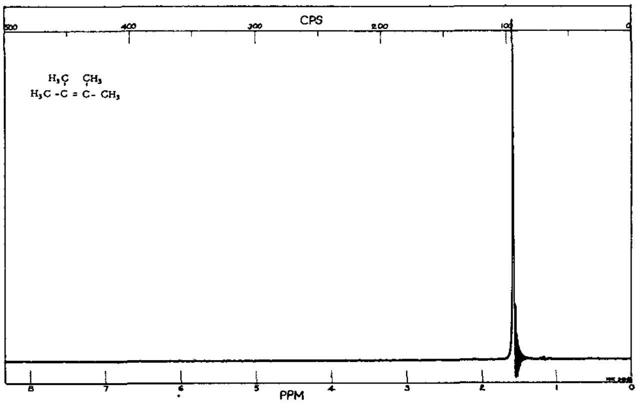

FIG. 2. 60 mc proton NMR spectrum of 2,3-dimethyl-2-butene.

nucleus, which has been studied by this technique more than all other nuclei for three reasons: 1) more compounds contain hydrogen than any other element, 2) the magnetic moment of the hydrogen nucleus is favorably large, and 3) commercial instrumentation suitable for routine analysis is available for this nucleus at present.

B. *Basic Theory*—When a hydrogen atom is placed in a powerful, homogeneous magnetic field, its magnetic nucleus tends to align itself in one of two theoretically permissible directions, i.e., parallel or antiparallel to the strong magnetic field. These two orientations correspond to slightly different energies; and at room temperature the parallel or lower energy state will be favored by a slightly higher population of nuclei, governed by a Boltzman distribution. Since it is also spinning, the torque to which a given nucleus is subjected by the field causes it to execute precessional motion around the axis of the field much as a toy top or gyroscope precess around a vertical axis due to the torque of the earth's gravitational field. The basic equation due to Larmor, which describes the angular precession frequency of the nucleus, ω, and its relation to the strength of a strong applied magnetic field, H, is given by the simple equation

$$\omega = \gamma H \qquad [1]$$

where γ is a physical constant of the nucleus called the gyromagnetic or magnetogyric ratio. The value of γ depends on the magnetic moment and the angular momentum of the nucleus and is identical for all nuclei of the same mass and charge. We see then that the natural precessional frequency of the hydrogen nucleus is directly proportional to the strength of the applied magnetic field. If we now impose a second weak magnetic field at right angles to the first and let this second field oscillate with a frequency, ν, in the radio frequency, rf, range with a value

$$\nu = \omega / 2\pi$$

it will be exactly in phase with the angular precession frequency, ω. When this condition is satisfied, the magnetic nucleus interacts with the weak oscillating field and by either absorbing or emitting a quantum of energy, $h\nu$, it can reverse its direction of orientation. This is the basic phenomenon of nuclear magnetic resonance.

Transitions from lower to higher or higher to lower nuclear energy state are equally probable, but because the lower nuclear energy state has a slightly higher population, there results a net absorption of energy from a sample containing a large number of nuclei. This absorption of energy can be detected electronically in one of several ways and the detected signal can then be amplified and recorded. The electronic details need not concern us here, since instrumentation has become pretty well standardized.

It is from such signals, then, that we obtain information on molecular structure; but to understand how this is possible it is first necessary to expand our simple model a little further. Nothing that has been said thus far suggests that the chemical environment of a hydrogen atom has any effect on its precessional frequency in the fixed magnetic field. In actuality, the rapidly moving electrons which circulate about the atoms of a molecule generate small local magnetic fields of their own which partly screen the hydrogen nuclei from the full force of the strong applied field, H_0. Thus, chemically different hydrogens experience slightly different local fields for any fixed value of the applied field; and, therefore, according to equation [1] they precess at slightly different frequencies. Taking this into account, we can re-write equation [1] to include a small screening constant σ, which depends on the particular chemical environment of the hydrogen in question.

$$\omega = 2\pi\nu = \gamma H_0(1 - \sigma) \qquad [2]$$

In practice, the value of ν is usually held constant and the value of H_0 is increased slowly in a linear manner by several parts per million (ppm) of the original magnetic field strength until the resonance condition is satisfied for each of the chemically different types of hydrogen in a sample in turn. The resulting record, called an NMR spectrum, is a plot of detected signal intensity vs. change in the magnetic field strength in ppm. Measurements are most commonly made at a fixed rf of 60.0 mc, which requires a magnetic field strength of about 14,100 gauss. More recently, as the design of high field magnets has improved, instruments designed to operate at 100 mc and 23,490 gauss have become available. In either case, to give the x-axis of the NMR spectrum an origin, the resonance signal for the hydrogens in some arbitrary reference substance (usually tetramethyl silane) is assigned the value 0 ppm. Peak separations, measured in terms of ppm change in the magnetic field from this reference point, are called "chemical shifts." The chemical shift of a given functional group is characteristic of that group and its neighboring functional groups. Moreover, the area under a given signal is directly proportional to the number of hydrogen atoms of that kind which are present in the sample, provided certain conditions of measurement to be described in more detail later are satisfied. Many spectrometers are equipped with electronic integrators for measuring the areas under signals automatically.

Such qualitative and quantitative functional group data alone is enough to provide considerable information on the molecular structure of a compound. In addition, however, neighboring hydrogen nuclei frequently interact according to predictable rules to produce so-called fine structure or spin-spin splitting of the individual resonance peaks into multiplets with specific spacing and intensity distributions. In the simplest case of first-order splitting, the number of peaks in a given multiplet is $\prod_i (n_i + 1)$ where n_i is the number of equivalent nuclei of a specific i^{th} kind which are coupled either directly or indirectly via bonding electrons to the nucleus in question. The rules for more complex second-order splitting which occurs when the chemical shifts for the coupled protons are quite small are known (7,8,9), but their detailed description is beyond the intent and scope of this brief introduction. Suffice it to say at present that these patterns can frequently be interpreted to determine which of the various functional groups are neighbors or next-nearest neighbors.

C. *Examples*—As a simple illustrative example, let us consider the NMR spectra of two isomeric olefins, both of empirical formula C_6H_{12}. The compounds in question are 2,3-dimethyl-1-butene and 2,3-dimethyl-2-butene. Their 60 mc proton NMR spectra are shown in Figures 1 and 2, respectively.

The former shows four separate and distinct bands due to the four magnetically non-equivalent types of hydrogens which are found in the molecule. From low to high field (left to right) these may be identified unequivocally on the basis of the relative peak areas as due to the olefinic protons, the single methine hydrogen, the three methyl hydrogens on the carbon alpha to the olefinic carbon, and the six hydrogens of the two terminal methyl groups, respectively. The integral of the spectrum is superimposed over the absorption spectrum and the integral steps are in the theoretical ratio of 2:1:3:6. This spectrum also shows both first and second-order splittings. The peak for the terminal methyl hydrogens is split into a doublet by first-order coupling to the single methine H which in turn splits the methine hydrogen peak into a seven-fold multiplet with the same frequency separations due to the interaction with the six identical terminal methyl hydrogens. The peak intensities in this seven-fold multiplet should approximate the ratio of the binomial coefficients, i.e., 1:6:15:20:15:6:1. Actually, the two weakest peaks are lost in the baseline noise. The bands due to the olefinic hydrogens and the alpha methyl hydrogens show complex multiplets due to second-order splitting. Such bands can be interpreted, but the calculations are best done on a computer (9). The complex nature of these bands is shown by the inserts at a ten-fold expansion of the horizontal scale.

The NMR spectrum of the 2,3-dimethyl-2-butene (Fig. 2), on the other hand, shows only a single peak due to the twelve hydrogens of the four methyl groups which due to the symmetry of the molecule and rotation around the C-C single bonds are magnetically equivalent. The chemical shift of this peak (−1.6 ppm) is very close to that observed for the alpha methyl group in the previous example (−1.7 ppm). Notice that the horizontal scale is calibrated in both ppm of the magnetic field and in cycles/sec. The two are related through equation 2 and are alternative ways of expressing peak separations. It is customary to specify true chemical shifts in ppm and coupling constants producing spin splitting in cps, because in this way the numerical values are independent of the applied magnetic field or radiofrequency at which the measurements are made.

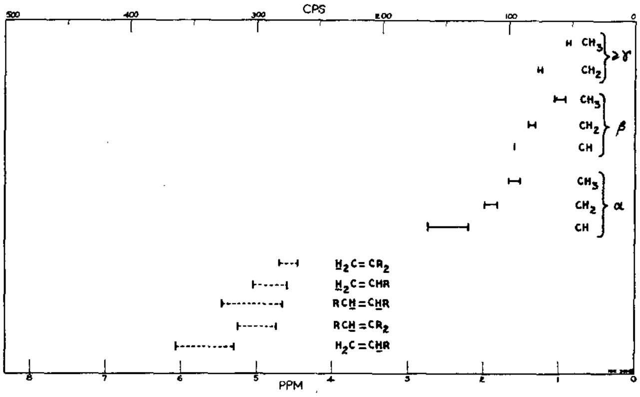

Fig. 3. Correlation chart of chemical shift data for olefins.

Observations on a sufficient number of known compounds have now been made so that correlation charts can be prepared showing the expected chemical shifts of various functional groups. See, for example, Figure 3, which shows such a chart for olefins. The solid horizontal lines on this chart represent the range of chemical shifts observed for the indicated types of hydrogen in a series of twenty-six pure olefins of known structure. The dotted lines on the other hand correspond to the total range of observed band widths for complex multiplets arising from second order splitting of olefinic hydrogen peaks.

Other examples of correlation charts will be presented later in connection with the discussion of specific classes of detergent chemicals (3).

D. *Quantitative Measurements*—The NMR spectrum of every compound is unique, and in this sense, it can serve as a "fingerprint" for the compound. It is much more than just a fingerprint, however, since it gives *quantitative* information about the number, kinds, and relative positions of the various hydrogen-containing functional groups which are present.

From a purely operational point of view, an NMR analysis is nondestructive and requires only about 15 min per sample. One stringent requirement is that the sample be a homogeneous liquid so that local magnetic field irregularities in the sample, which destroy high resolution, are averaged out by molecular motions. Solutions of soluble solids are suitable, with non-protonic solvents such as CCl_4, $CDCl_3$, D_2O, etc., being preferred. Solution concentrations should be as high as possible with at least 5% by weight solute or more for optimum quantitative results. Sample volumes of a few tenths of a milliliter are commonly used in a 5 mm cylindrical glass sample tube, although sample volumes as small as 0.025 ml can be studied with the aid of special spherical sample holders. Since this 0.025 ml can be as much as 75% solvent, actual sample sizes as small as 0.007 ml have been used. This permits the examination of fractions separated, for example, by preparative vapor phase chromatography.

The principal value of NMR to the detergent chemist, who frequently works with mixtures of isomers rather than pure compounds, lies in this qualitative capability of the measurements. One fre-

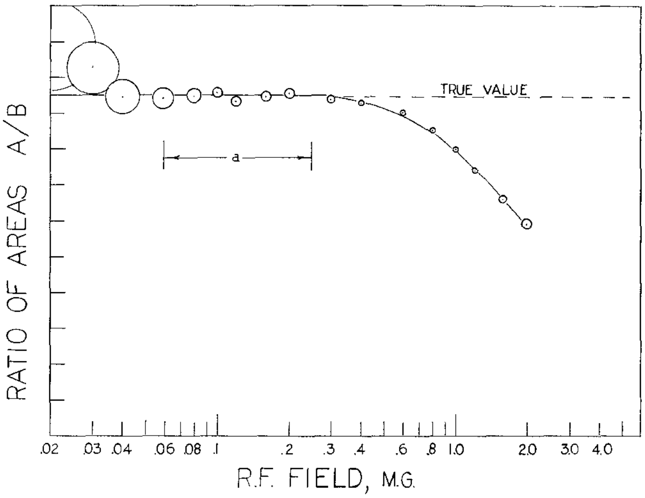

FIG. 4. The effect of saturation on relative peak areas.

quently already knows the qualitative structure of the molecules with which one deals but wants quantitative answers to such questions as: What is the average chain length of the alkyl group in an alkylbenzene sulfonate sample which consists of a mixture of isomers? Or, what is the average degree and kind of branching in the alkyl chains? Or, what are the relative lengths of the hydrophobe and hydrophilic chains of an ethylene oxide nonionic surfactant? Or, what is the ratio of ortho and para isomers in an alkyl phenol?

As has been previously stated, *under suitable instrument conditions,* the areas under the various peaks of an NMR spectrum are all proportional to the number of hydrogen nuclei giving rise to them. This is the basis for the quantitative usefulness of the observations. Once the various peaks in a spectrum are assigned, then the integrated spectra can be used as an analytical tool to determine mixture compositions or isomer distributions. The proper instrument conditions must be emphasized, however, since it is otherwise possible to obtain misleading results.

An excellent description of the theoretically important factors in determining what the necessary instrument conditions are has been given by Williams (11). Most of the problems he mentions have been subsequently solved by improved electronic instrumentation, but one important pit-fall remains. Since signal to noise ratios increase with the strength of the oscillating radio-frequency (rf) field, it is desirable to operate at high rf field strengths for good precision of measurement. On the other hand, at high rf field strengths, the phenomenon of "saturation" can introduce errors.

Because of its importance to the quantitative usefulness of NMR measurements this phenomenon will be described in more detail below.

As we have already discussed, the total observed NMR signal is due to the slightly higher population of the lower nuclear spin state so that there is a *net* absorption of energy as energy-absorbing transitions from lower to higher energy occur simultaneously with energy-emitting transitions from higher to lower energy. If there were no relaxation process by which nuclei in the upper state could spontaneously return to the lower state, then the resonance experiment would very quickly produce an equalization of the population of the two energy states and the detected signals from this saturated sample would diminish to zero. In actuality the mean lifetime of a nucleus in the upper state is limited by the possibility of radiationless transitions which can occur as a result of local fluctuating magnetic and electric fields in the vicinity of the nucleus, arising from the motions of neighboring atoms and nuclei. This provides an "escape valve" which helps prevent complete saturation of the spin system. The effectiveness of the relaxation processes varies considerably with the chemical environment of the nucleus, making it easier to saturate some types of nuclei than others. The saturation factor, Z, which is the fraction of nuclei in the lower energy state compared to the maximum number which exist there in the typical Boltzman distribution, is given by

$$Z = (1 + \gamma^2 H_1^2 T_1 T_2)^{-1} \qquad [3]$$

where γ is the nuclear gynomatic ratio, H_1 is the strength of the oscillating radio-frequency magnetic field, and T_1 and T_2 are the so-called longitudinal and transverse relaxation times for the nucleus.

In order for the measured area ratio for two NMR peaks to be an accurate representation of the relative concentration of the nuclei producing them, it is necessary either that the term $\gamma^2 H_1^2 T_1 T_2$ be small compared with unity, or that the relaxation time product $T_1 T_2$ be nearly equal for the two kinds of nuclei. The former can be accomplished by making measurements at very low H_1 power levels. On the other hand, it can be shown that the maximum signal to noise ratio is obtained when $\gamma^2 H_1^2 T_1 T_2 = 1$, so that it is desirable to operate at high rf power levels for the maximum sensitivity and best precision of measurement. One is faced then with a source of error due to low signal to noise ratios giving poor precision and low sensitivity at low rf field strengths and with a source of error due to saturation and resulting biased peak areas at high rf field strengths. The situation is represented schematically in Figure 4 for the ratio of two hypothetical peaks A and B, of which A is the more easily saturated, i.e., it has the longest relaxation time, T_1.

It is obvious that there is an optimum region, a, in which the best measurements can be made. It is advisable when developing an analytical method for a new class of compound to establish optimum instrument conditions by means of a plot such as this. Alternatively, one can operate at very low rf power levels, where saturation is always negligible, and improve precision by repetitive measurements.

More detailed examples of the application of quantitative techniques to specific surfactant raw materials, and intermediates are described in a separate paper (3).

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