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A pulsed NMR study of water and alkali ions in heterogeneous biological systems.

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CHAPTER I. INTRODUCTION AND SUMMARY.

Some 30 years ago the first nuclear magnetic resonance (NMR) signal was observed *(1)*. Since then the NMR technique has evolved into a powerful tool for studies of molecular structure and dynamics in physics and chemistry. A thorough theoretical treatment of NMR can be found in many excellent monographs. The knowledge up to 1960 is presented in Abragam's "bible" *(2)* which still is very usable. A general introduction is given by Carrington and McLachlan *(3)*. More advanced treatises which focus mainly on applications to the solid state are given by Slichter *(4)* and Goldman *(5)*. The theory of and application of NMR to organic molecules in solution is given by Emsley, Finey and Sutcliffe *(6)* and by Bovey *(7)*. Applications of NMR in biochemistry are treated by Dwek *(8)*.

There exist two experimentally different types of NMR. In the continuous wave (CW) methods the perturbing *rf* electromagnetic radiation is applied continuously at sufficient low power level to essentially maintain the spin system in its equilibrium state. The NMR spectrum is measured from the change in *rf* absorption during the passage of a resonant condition. Two types of spectrometers operate on this principle: wide line (WL) NMR spectrometers for the detection of broad resonances in solids; and, high resolution (HR) NMR spectrometers for a good resolution of narrow resonances in solution.

In pulsed NMR methods the nuclear spins are coherently perturbed by short intense *rf* pulses at the NMR resonance frequency, that leave the spin system in a well defined non-equilibrium state. After the pulse is turned off, the spin system may coherently radiate energy while it returns to the equilibrium state. This radiation constitutes the measurable NMR signal. An introduction to the theory of pulsed NMR is given in *II*.

The signal that is emitted after a single pulse contains the same spectral information as that in a CW absorption signal; the two form a Fourier transform pair. The Fourier Transform (FT) NMR technique is based on this principle. There a short *rf* pulse excites the whole spin system simultaneously, whereafter information on all separate resonances present is obtained simultaneously from the signal response. In this way the NMR spectrum can be obtained in much less time than is needed in the CW method in which a lot of measuring time is lost in scanning the spectrum between resonance peaks.

Pulsed NMR is not restricted to single pulse experiments, however, and multiple pulse experiments open a new dimension in NMR that is not accessible by CW NMR techniques. The exploration of the application of well-defined pulse sequences are a challenge to the ingenuity of the experimenter. He can play "tricks" with the nuclear spin system that enable him to separate or characterize specific magnetic interactions. Many multiple pulse sequences are currently in use, ranging from simple two-pulse experiments that measure the relaxation rates of the spin system towards its equilibrium, to rather complex many-pulse sequences that enable one, for example, to resolve chemical shifts in solids by artificially narrowing the broad resonances. An elementary treatment on the uses of pulsed NMR methods is given by Farrar and Becker *(9)*. A very useful text that covers many applications to problems in physical chemistry is the one by Boden *(10)*. The use of multiple pulse sequences in the study of solids has been reviewed by Mansfield *(11)* and Vaughan *(12)*.

Pulsed NMR techniques are especially well suited for the measurement of the nuclear magnetic relaxation rates *(see II)*. The relaxation rates depend on the details of the molecular motions. The calculation of the dependence of the relaxation rates on specific molecular motions needs, in general, a quantum mechanical treatment and is rather complex. Extensive details on relaxation theory can be found in most of the textbooks mentioned above. A textbook devoted exclusively to relaxation in magnetic resonance has appeared *(13)*.

In this thesis studies are described in which pulsed NMR techniques have been used to study the nuclear magnetic relaxation behavior of the nuclei of water and of alkali ions in biological systems. It is of importance in the study of biological systems to keep the total measuring time short; in general, samples are not very stable in time. In addition the NMR signals from biological samples are often weak. Special attention was therefore paid to the improvement of the signal-to-noise ratio of the NMR signals in a short total measuring time. In particular signal averaging of the NMR signals was used and, the pulse sequences,with which the nuclear magnetic relaxation rates are measured, are selected primarily to obtain the maximum amount of measurable signal in a given time span.

Details on the pulse sequences that have been used for the relaxation measurements are given in *II* and *III*. The pulsed NMR spectrometer system is described in *IV*. Special attention is given in *IV* to some of the complications that arise if signal averaging and gated signal integration are used in collecting the relaxation data. In *V* a computer method is described that has been used to analyze the measured relaxation decays.

Nuclear magnetic relaxation studies of water in a *Halobacterium sp.* and in edematous rat brain tissue are described in *VI* and *VII*. Many studies on water relaxation in biological tissues continue to appear. In particular cancerous tissue is receiving much attention. The results of such studies confirm that an empirical relation exists between malignancy of a tissue and its nuclear magnetic relaxation rates. It is concluded that in my opinion this relation is due primarily to the increase of water content in malignant tissue *(see VII)*.

A good understanding of the factors that determine the magnitude of the relaxation rates of water in biological tissues and cells still is wanting. In *VI* measurements are described of the relaxation rates of the ^{1}H and ^{2}D nuclei of water in a *Halobacterium sp.* By comparing the results for the two nuclei it has been possible to obtain information on the relative importance of several effects that contribute to the relaxation rates. The result is disappointing in that no clearcut relation exists between the relaxation rates and the rotational motions of the water molecules. This result is at odds with the theory that is generally used to explain the water relaxation rates in tissue. In such a theory it is assumed that the relaxation rates are completely determined by interactions that are located intramolecularly in the water molecules. A direct relation then exists between the nuclear magnetic relaxation rates and the rotational motions of the water molecules. The result of such an analysis subsequently results in a classification of the motions of the water

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molecules. This classification can involve discrete classes with a certain degree of rotational mobility (e.g. free water, bound water, and hydration water); or, the classification can be given in terms of a particular distribution of correlation times for the rotational motions of the water molecules. The relevance of such a classification seems questionable, in particular if it is obtained from proton NMR measurements only. The results in *Halobacterium sp.* show that such an analysis probably is too simple: proton exchange and slow translational diffusion here give an important contribution to the observed NMR signal.

NMR measurements of alkali ions in biological systems are described in *VIII-XI*. A review on the physical state of diffusible ions in cells was written; the part of this review which treats NMR measurements is reproduced in *VIII*. An explanation is given for the observed signal intensities and relaxation rates of alkali ions in heterogeneous systems. It is shown that the signals are modified by quadrupolar interactions. A more extensive treatment of the theory is given in *IX*, along with measurements of sodium nuclear magnetic relaxation in rat muscle and in Dowex exchange resin. Measurements on alkali ions in a *Halobacterium sp.* are described in *X*; the results substantiate that the intracellular ions in this bacteria are somehow bound. NMR observations of ^{7}Li and ^{23}Na NMR spectra in oriented samples of DNA are described in *XI*.

Finally, the response to some multiple pulse sequences is discussed in *XII* for the NMR signal of mobile components in a heterogeneous, more or less oredered, macromolecular matrix. It is shown that the CPMG sequence may yield relaxation rates that are not the true R_2 values.