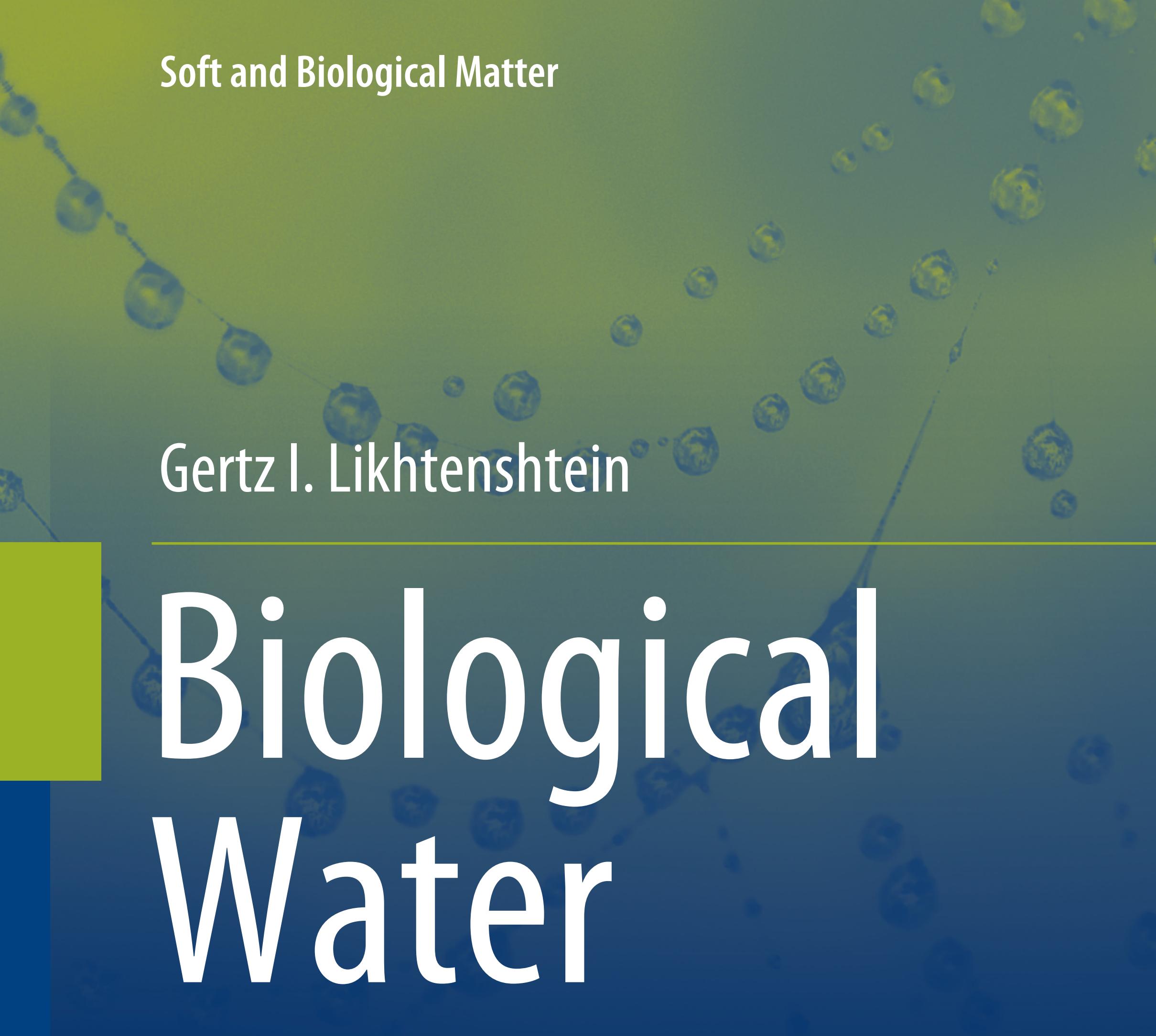

Soft and Biological Matter

Gertz I. Likhtenshtein

Biological Water

Physicochemical Aspects

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Preface

The role water, which is a crucial key component in the development of all aspects of life on Earth, in chemistry, biology, industry, and everyday life of human being cannot be overemphasized. Water provides an important chemical contribution to the functioning and degradation of biological systems. Water represents on average 55–60% of the human body weight and is distributed throughout the body and organs. Health, life expectancy, daily comfort, and mood of every human being largely depend on the body water balance. Water plays a central role in regulating cell volume, nutrient transport, waste removal, and thermal regulation and provides a medium for biological reactions Adequate hydration is crucial in maintaining optimal physical and mental functioning, and the need for a fast and reliable hydration status assessment in behavioral medicine research has become increasingly important.

Water is fascinating in all its phases, forms, and states of aggregation and is unique in its number of anomalous properties. Seventy four anomalous properties of liquid are connected to its ability to form up to four well-defined hydrogen bonds allowing for different local structural arrangements. Classical and modern physical chemistry, chemical physics, chemical kinetics, organic, inorganic, and quantum chemistry provide an arsenal of physical methods and establish a basis for the investigation of structure and action mechanism of processes involving water.

Research in this area which combines fundamental importance of human welfare and intellectual fascination with exploring the unknown will promote solving exciting and complicated problems in biochemistry, biophysics, and medicines related to biological water (здесь еще бы подумать, звучит не очень).

The book is a view on the subject from a physical chemist with the long and broad expertise in chemical biophysics, enzyme catalysis, and physical methods of studying structure and dynamics of biological systems including hydrated biological molecules, in particular. The book is not intended to provide an exhaustive survey of each topic but rather a discussion of their theoretical and experimental background and recent developments. The literature on water is so vast, with so many scientists having made important contributions in the area that it is impossible in the space allowed for this book to give a representative set of references. In fact, for each section in this area, one can write several books. The author apologizes to those he has not been able to include.

Chapter 1 of the monograph is a brief outline of water basic properties and activity. Chapters 2 and 3 present a brief review of physical methods used for the study of biological water. Common and specific features of structure and molecular dynamics of neat supercooled and supercritical water and ice are discussed in Chaps. 4 and 5. Hydration of simple biological molecules and ions is a subject of Chap. 6. Chapter 7 describes protein hydration. Water in enzyme catalysis as a promoter and chemical reagent is in focus of Chap. 8. Hydration of nucleic acids, cells, and tissues biomembranes constitute the contents of Chaps. 9–11. Chapter 12 is devoted to clinical aspects of water.

The book, as a whole, and separate chapters can be used as a subsidiary manual for instructors and graduate and undergraduate students of university chemistry, physics, biophysics, biochemistry, physiology, and medicine.

The department of Chemistry, Ben-Gurion University of the Negev and Institute of Problem of Chemical Physics, Russian Academy of Science, provided excellent conditions for writing this book for which the author is extremely grateful.

Beer-Sheva, Israel
Chernogolovka, Russia

Gertz I. Likhtenshtein

Contents

Chapter 1
Water Basic Properties and Activity

Abstract The role of water in chemistry, biology, industry, and everyday life of human being impossible to overstate (Szent-Györgyi in Science 124:873–875, 1952 [1]; Bagchi in Water in Biological and Chemical Processes: From Structure and Dynamics to Function, Cambridge University Press, 2013 [2]; Purdie Salas in Water Can Be…, Millbrook Press, 2014 [3]; Paul in Water Is Water: A Book About the Water Cycle (Hardcover), Roaring Brook Press, 2015 [4]; Mondal et al. in J. Phys. Chem. Lett. 8:4878–4882, 2017 [5]; Ball in Nature 452:291–292, 2008 [6]; Popkin et al. in Nutr. Rev. 68:439–458, 2010 [7]; Emoto in Secret Life of Water, Simon & Schuster, 2011 [8]; Makulski et al. in Phys. Chem. Chem. Phys. 20:22468–224769, 2018 [9]). Water is a crucial key component of the development of all aspects of life on earth. It is a very good solvent for salts and all major components of cells like proteins, deoxyribonucleic acid (DNA), or polysaccharides. Water provides an important chemical contribution to the functioning and degradation of biological systems. Most of the physical properties of water are quite atypical. Water has unusually large values of viscosity, surface tension, heat of vaporization, entropy of vaporization, and all in all more than 70 abnormalities. These abnormalities can be ascribed to the extensive hydrogen-bonding interactions present in liquid water. The "open" structure of ice that allows for maximum hydrogen bonding can explain why solid water is less dense than liquid water. Water molecules are prone to under coordination in clusters, droplets, nanocavities, nanotubes, and glasses. A huge number of technological, agricultural, and medical research activities are based on the use of water, water solutions, or water embedded in different environments.

1.1 Introduction

Despite the exceptional simplicity of the chemical structure of water due to the branched system of hydrogen bonds, its physicochemical properties are many-sided (Fig. 1.1) and their understanding requires an integrated approach using a whole cascade of methods (Fig. 1.2) [1–9].

G. I. Likhtenshtein, *Biological Water*, Soft and Biological Matter,
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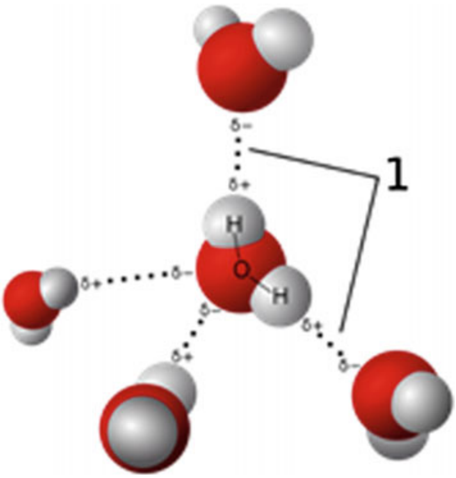


Fig. 1.1 Model of hydrogen bonds between molecules of water. https://en.wikipedia.org/wiki/Water

Figure 1.3 gives an overview of the time windows of several physical techniques. Combining the results by all these and others methods can give a phenomenological picture of the structure and cooperative dynamics in neat water, ice, and state of water in biological objects.

1.2 Water and Life

Water, the main basis for life on Earth continuously circulates through one of Earth's most powerful systems, that is, the water cycle (Fig. 1.4). Water flows endlessly between the ocean, atmosphere, and land. Earth's water is finite, meaning that the amount of water in, on, and above our planet does not increase or decrease.

Since ancient times of the Greek philosophers and during all stages of human civilization, the problems of water have attracted the minds of great scientists and philosophers (Fig. 1.5).

Water represents on average 55–60% of the human body weight and is distributed throughout the body and organs (Fig. 1.6). Health, life expectancy, daily comfort, and mood of an each and every human being largely depend on the water balance of his body.

1.3 Quantum Mechanics of Water

Molecular orbital diagram of water and their corresponding energy is presented in Fig. 1.7.

"and so this men of Indostan
Disputed loud and long,
Each in his own opinion
Exceeding stiff and strong,
Though each was partly in the right
And all were in the wrong!"

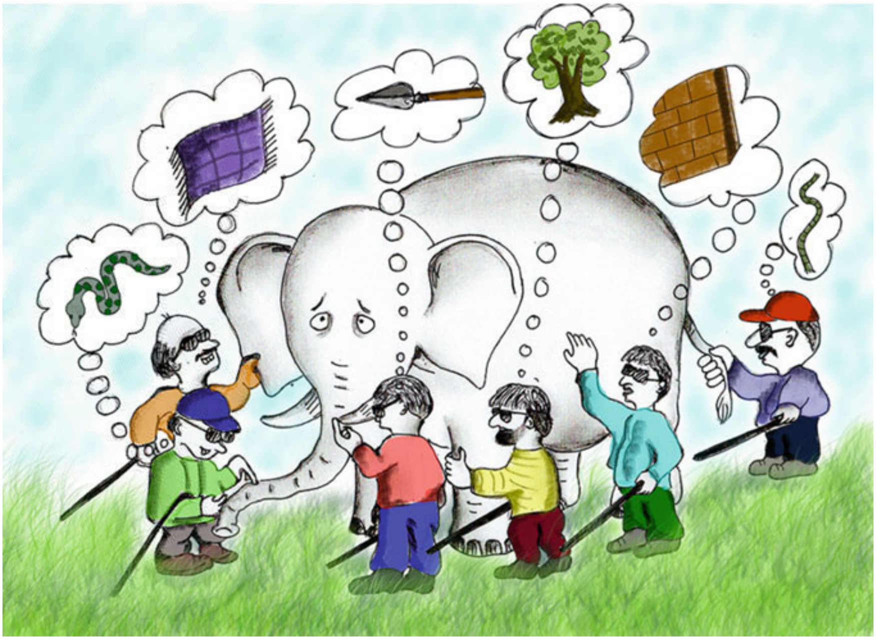


Fig. 1.2 Stanza and representation of the poem, The Blind Men and the Elephant by John Godfrey Saxe. The elephant can be thought of as a protein and those men as the various experimental probes that provide information that are true but partial. We, hence, need to combine all possible techniques in order to understand the real scenario [5]. With permission from American Chemical Society

In the diagram, at the bottom, the low-energy orbitals are occupied by two electrons each in the ground state of the molecule while at the top are listed some unoccupied high-energy orbitals. These are mostly antibonding (or non-bonding). The highest occupied orbital ($1b_1$) is non-bonding and highly localized on the oxygen atom. The next lowest orbital ($2a_1$) is a non-bonding orbital. Above the highest unoccupied molecular orbital (HOMO) is the largest unoccupied molecular orbital (LUMO), which does not contain any electrons in the ground state, as is the case for the orbitals at higher energies. Data on the investigation of the valence orbital electron momentum distributions of H_2O were described [12].

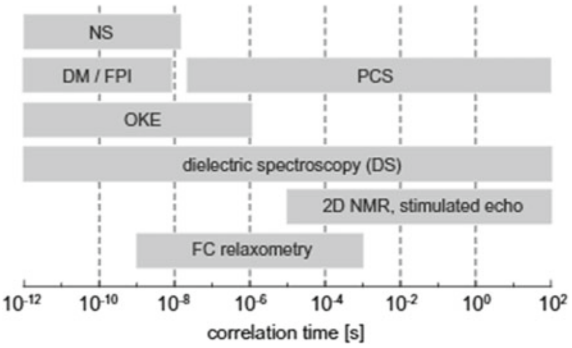


Fig. 1.3 Time windows of the most important methods probing dynamics in dense molecular liquids and comparison with NMR methods: neutron scattering (NS), double monochromator/Fabry–Perot interferometry (DM/FPI), photon correlation spectroscopy (PCS), and optical Kerr effect (OKE) [10]. With permission from Elsevier

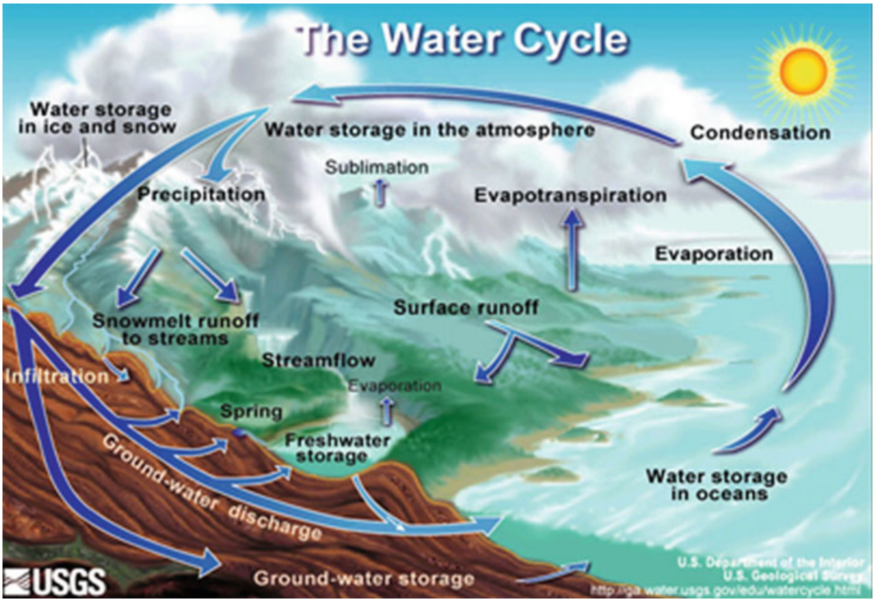


Fig. 1.4 Water circle in the Earth. https://www.usgs.gov/special-topic/water-science-school/science/water-cycle?qt-science_center_objects=0#qt-science_center_objects

1.4 Water Basic Physicochemical Properties

Main [1–9] are molar mass 18.0151 g per mole, melting point 0.00 °C, boiling point, 100.00 °C, maximum density (at 3.98 °C) 1.0000 g per cm^3, heat capacity (C) 75.385 J/(mol K) vapor pressure (25 °C) 23.75 torr, heat of fusion (0 °C) 6.010 kJ per

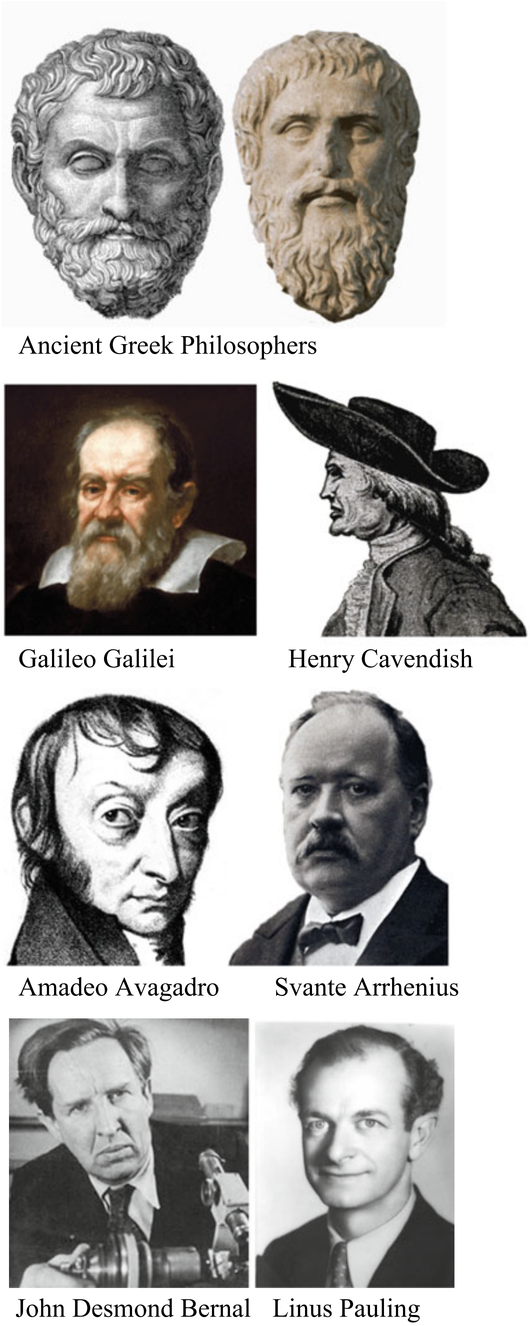


Fig. 1.5 Great philosophers and scientists who have made a fundamental contribution to understanding the role of water and its physicochemical properties. With permission of Dr. Martin Chaplin

Ancient Greek Philosophers

Galileo Galilei

Henry Cavendish

Amadeo Avagadro

Svante Arrhenius

John Desmond Bernal

Linus Pauling

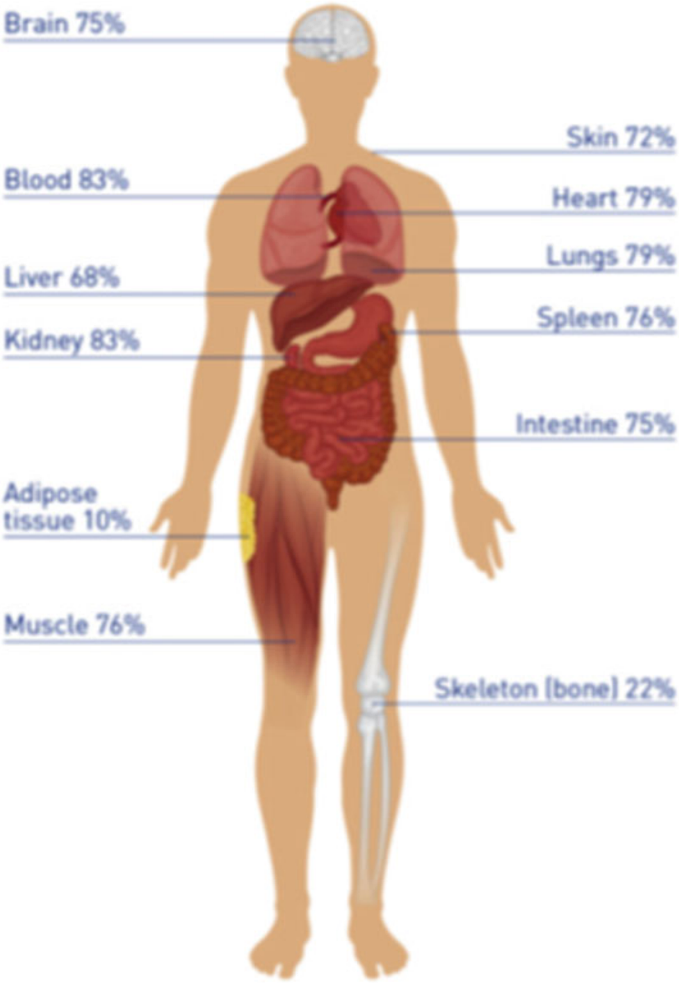


Fig. 1.6 Water composition of tissues and organs by weight [5]. Adapted from Pivarnik and Palmer [11]. Taylor permission request has send

mole, heat of vaporization (100 °C) 40.65 kJ per mole, heat of formation (25 °C), heat of formation (25 °C) 285.85 kJ per mole, entropy of vaporization (25 °C), viscosity, surface tension (25 °C) 71.97 dynes per cm, density 999.9720 kg/m $\approx$ 1 t/m^3 = 1 kg/l = 1 g/cm^3 (liquid, maximum, at ~4 °C) 917 kg/m^3 (solid), magnetic susceptibility (χ) -1.298×10^{-5} cm^3/mol (20 °C, 1 atm), thermal conductivity 0.58 W/m K, refractive index (*nD*) 1.3325, viscosity 1 cP (20 °C), dipole moment 1.85 *D*, dielectric constant 80 (20 °C), specific heat capacity (*C*) 75.375 ± 0.05 J/mol K, std. molar entropy (69.95 J/mol K) enthalpy of formation (ΔfHo 298) −285.83 kJ/mol, Gibbs free energy ($\Delta fG°$) −237.24 kJ/mol, thermal conductivity 0.6065 W/(m K), *ionic product* of pure water, 10^{-14} at 25 °C, *acidity* (pK_a), and *basicity* (pK_b) 13.995,

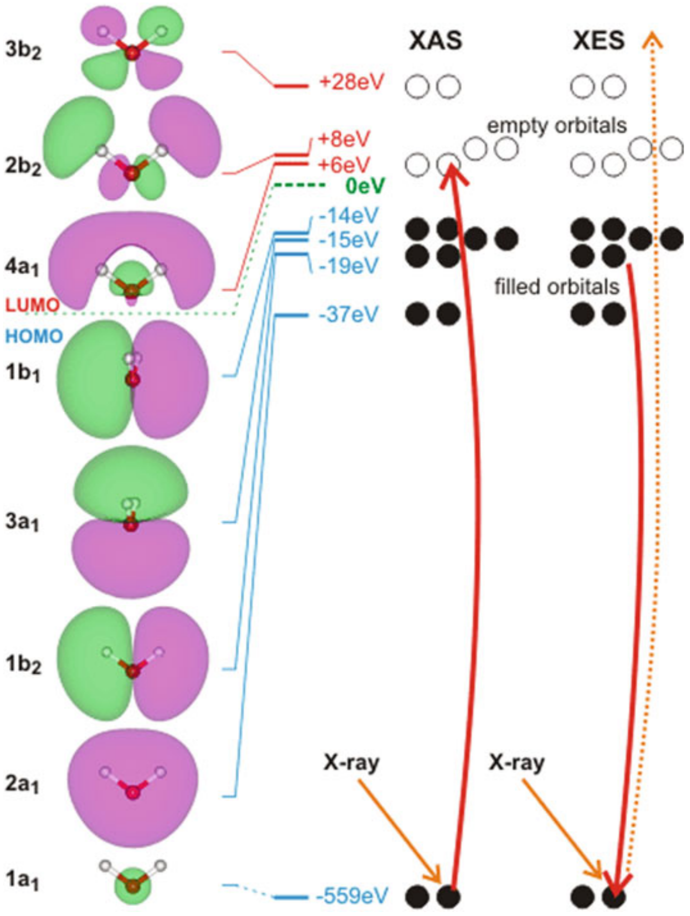


Fig. 1.7 Molecular orbital diagram of isolated water. q=molecular+orbitals+of+water+molecule&oq=molecular+orbitals++of+watervb&gs_l=psy ab.1.1.0i19j0i22i30i19.110325.119281..122686...1.0..0.220.2145.0j12j1..0.1j2..gws-wiz.10..35i39i19j35i362i39j0i203j0i10i203j0i22i30j0i13i30i19.SdJgISkro5k

and ice dielectric constant (ε_r) ~ 3.15 [https://en.wikipedia.org/wiki/Properties_of_water].

[https://www.cambridge.org/core/journals/journal-of-glaciology/article/dielectric-properties-of-ice-and-snowa-review/A4F950358E513838DAE111F6A07EC077/core-reader] "Guideline on the Use of Fundamental Physical Constants and Basic Constants of Water".

Water with one deuterium atom HDO occurs naturally in ordinary water in low concentrations (~0.03%) and D_2O in far lower amounts (0.000003%) Oxygen also has three **stable isotopes**, with ^{16}O present in 99.76%, ^{17}O in 0.04%, and ^{18}O in 0.2% of water molecules [13].

Water is a good solvent, due to its **polarity**. Substances that will mix well and dissolve in water (e.g., salts, ethanol, DMSO) are known as hydrophilic substances, while those that do not mix well with water (e.g., fats and oils), are hydrophobic substances.

Timescales of physical methods used to investigate the structure of liquid water and aqueous and biomolecular solutions are as follows: X-ray absorption ($\approx 10^{-15}$ s), infrared, Raman ($\approx 10^{-14}$–10^{-13} s), pump–probe laser ($\approx 10^{-12}$–10^{-10} s), magnetic resonance (10^{-2}–10^{-11} s), and microwave (10^{-9}–10^{-13} s). Some physical processes and its corresponding timescales are indicated in Fig. 1.8. Absorption, infrared, Raman scattering, and NMR of water spectra are presented in Figs. 1.9, 1.10, 1.11 and 1.12.

The experimental spectra of water obtained by some spectroscopic methods.

The overall hydration of the main biological molecules is depicted schematically in Fig. 1.13.

Various aspects related to biological water are discussed in reviews [1–4, 7, 14, 15].

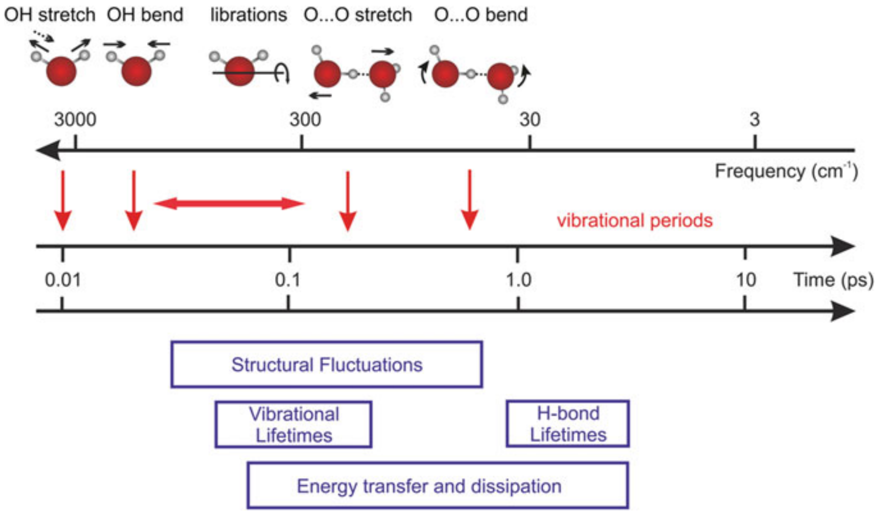


Fig. 1.8 Timescales and processes in bulk H_2O. Red arrows above the logarithmic time axis mark the periods of vibrational and librational degrees of freedom schematically illustrated at the top of the figure. Horizontal boxes below the time axis illustrate the time range covered by particular processes of bulk water dynamics [14]. With permission from American Chemical Society

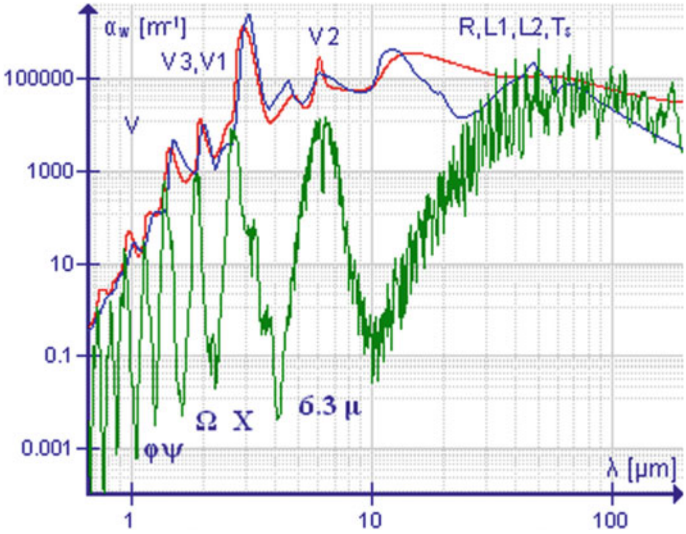


Fig. 1.9 Absorption spectrum (*attenuation coefficient* vs. wavelength) of liquid water (red), atmospheric *water vapor* (green), and ice (blue line) between 667 nm and 200 μm. The plot for vapor is a transformation of data synthetic spectrum for gas mixture "Pure H_2O" (296 K, 1 atm) retrieved from *Hitran* on the Web Information System. https://en.wikipedia.org/wiki/Electromagnetic_absorption_by_water

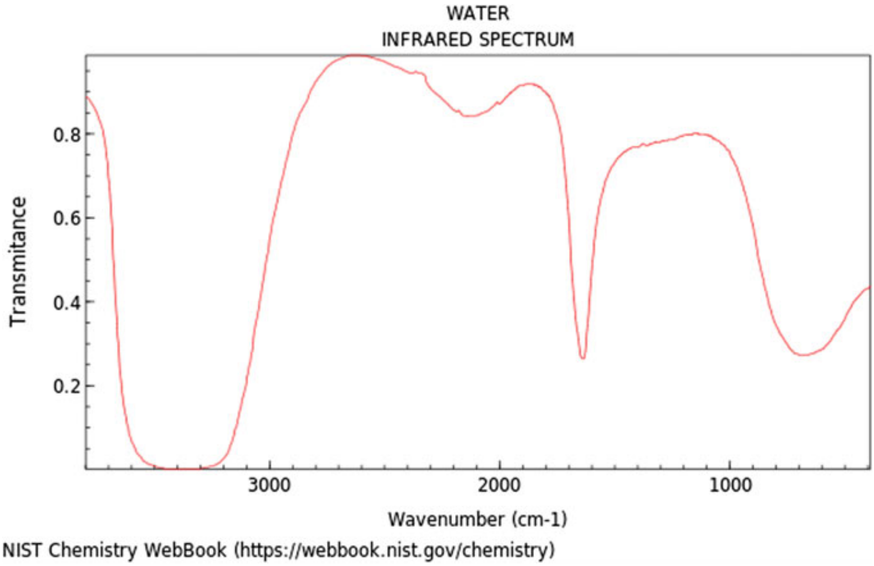
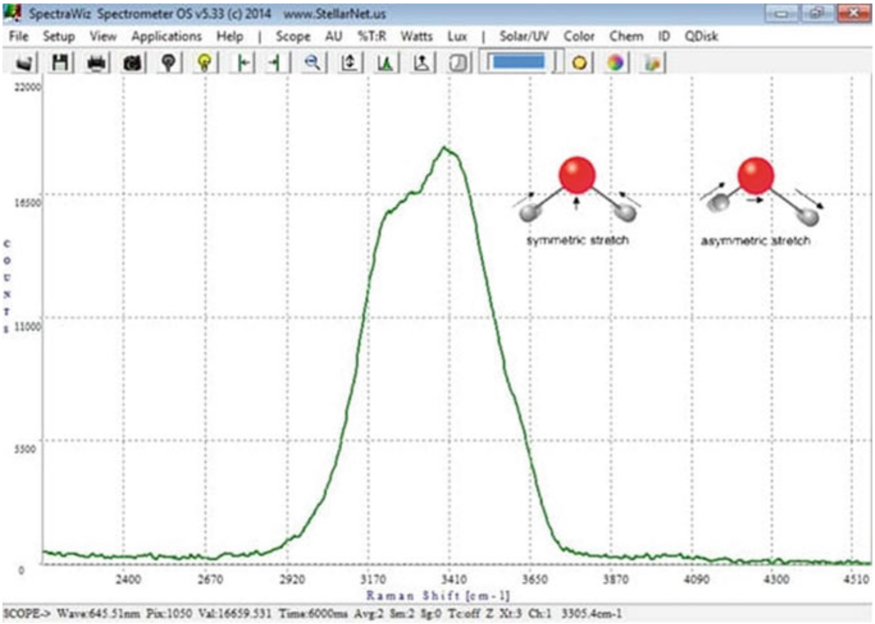


Fig. 1.10 Water infrared spectrum. https://en.wikipedia.org/wiki/Electromagnetic_absorption_by_water



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SCOPE-> Wave:645.51nm Pix:1050 Val:16659.531 Time:6000ms Avg:2 Sm:2 Sg:0 Tc:off Z Xt:3 Ch:1 3305.4cm-1
```

Fig. 1.11 Raman scattering spectrum of pure water. The shoulder at ~3250 cm^{-1} corresponds to the asymmetric –OH stretch, and the most intense feature at ~3410 cm^{-1} corresponds to the symmetric –OH stretch. Even more intriguing, there is a small shoulder at ~3630 cm^{-1} which corresponds to the –OH stretch of a water molecule which is only partially involved in hydrogen bonding—a particularly rare event. https://www.stellarnet.us/application-note-raman-spectrum-water/

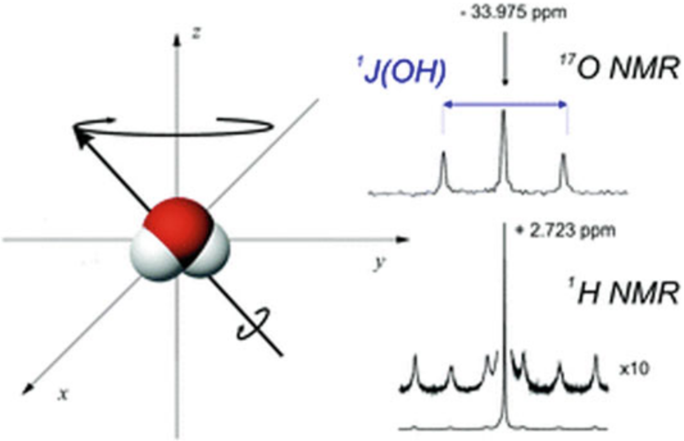

Fig. 1.12 ^{17}O and ^{1}H NMR spectra of isolated water molecules [9]. With permission of Royal Society of Chemistry

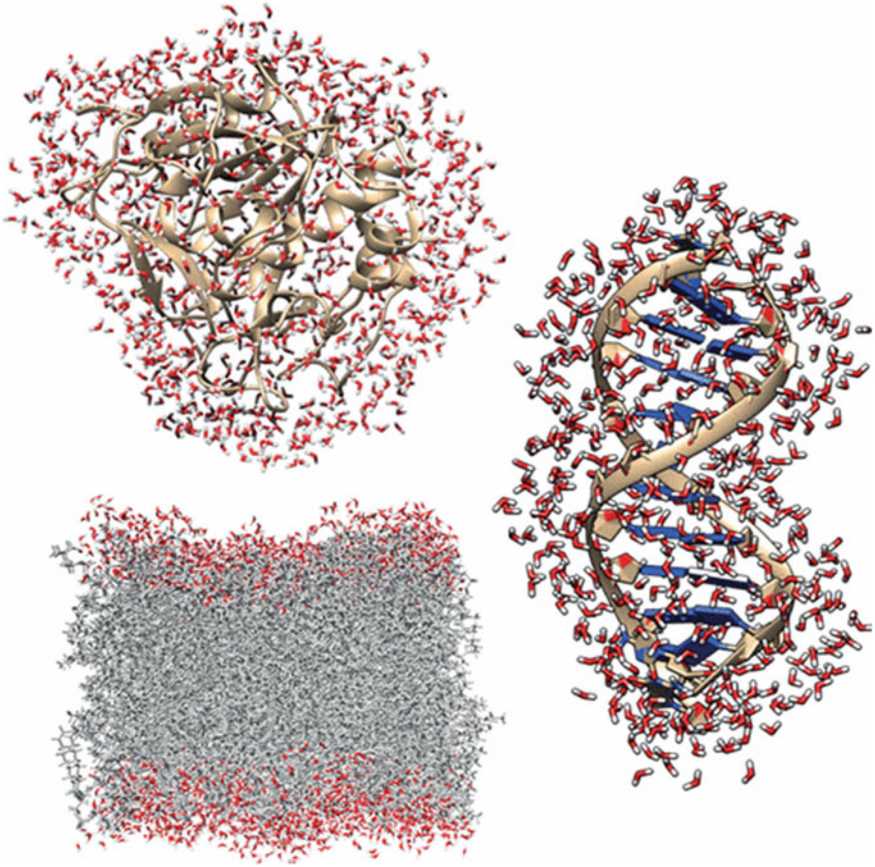

Fig. 1.13 Hydration of a protein, nuclear acid fragment, and biomembrane [13]. With permission from American Chemical Society

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Chapter 2
Physical Methods for the Study of Biological Water. Magnetic Resonance

Abstract This chapter describes the fundamentals of electron paramagnetic (spin) magnetic resonance (EPR, ESR), and nuclear magnetic resonance (NMR). The focus is on phenomena whose quantitative parameters are directly used to characterize the molecular structure, polarity and proticity and dynamics of water and ice, namely NMR chemical shift, the dipolar coupling between nuclear spins, fast-field-cycling (FFC) NMR effect, the isotropic Fermi contact, the nuclear Overhauser enhancement or effect (NOE), dynamic nuclear spin polarization, electron spin dipole–dipole interaction, free induction decay (FID), nuclear and electron spin–lattice and spin–spin relaxation, nuclear magnetization of chemically exchanging systems. The above physical phenomena form the basis of methods such as electron–electron double resonance (ELDOR) or double electron–electron resonance (DEER) or pulse electron nuclear double resonance (ENDOR) or multifrequency ESR (MF ESR) techniques, two-dimensional ESR (2D ESR), two-dimensional electron–electron double resonance (2D-ELDOR), ENDOR with circularly polarized radio frequency fields (CP-ENDOR), electron nuclear–nuclear resonance (double ENDOR), proton electron double resonance imaging (PEDRI), and electron nuclear–nuclear triple resonance (TRIPLE).

2.1 Introduction

Electron and some nuclear possess corresponding intrinsic angular momentums that are quantized. In an external magnetic field, there is a high-energy state and a low-energy state depending on the relative orientation of the magnet to the external field, and in thermal equilibrium, the low-energy orientation is preferred [1–19]. In a constant magnetic field of strength H_0, the electron spin resonance (ESR) phenomena involve the resonance absorption or dispersion of a microwave frequency (0.3–250 GHz) of electromagnetic field (ν) by a system of particles with the intrinsic spin moment of an unpaired electron [1–13].

The absorption leads to magnetization in the excited state of the system. Accordingly, the electron magnetic resonance condition is:

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$$\boldsymbol{h}\nu = g_e \beta_B \boldsymbol{H}_0, \tag{2.1}$$

where g_e is a g-factor, characterizing the value of the intrinsic electron spin moment (free electron g-value is 2.002319), β_B is the Bohr magneton ($9.27400968 \times 10^{-24}$ J T^{-1}). The values of g-factors and magnetic field strength H_0 dictate position of resonance frequencies in the NMR and ESR spectra.

The nucleus-bearing spin also has different energies in a nonzero magnetic field [14–19]. The energy difference between the two states with the nuclear quantum number $m = 1/2$ and gyromagnetic relation γ is:

$$\Delta E = h\nu = \gamma(h/2\pi)H_0 \tag{2.2}$$

and the NMR phenomena involve the resonance absorption or dispersion of a frequency range from around 20 kHz to around 300 GHz of electromagnetic field (ν).

Spin relaxations times are important parameters of electron spin resonance and nuclear magnetic resonance. Two approaches have been used for describing relaxation phenomena. In the frame of the first approach, the characteristic time of recovery of the induced magnetization to the ground state on account of a transfer of magnetic energy to energy of media (lattice) is noticed as spin–lattice relaxation ($1/T_1$). Another type of relaxation, spin–spin or spin–phase relaxation ($1/T_2$), is related to time of return of the spin system to equilibrium in the exited magnetic state as a result of spin–spin interaction with environment.

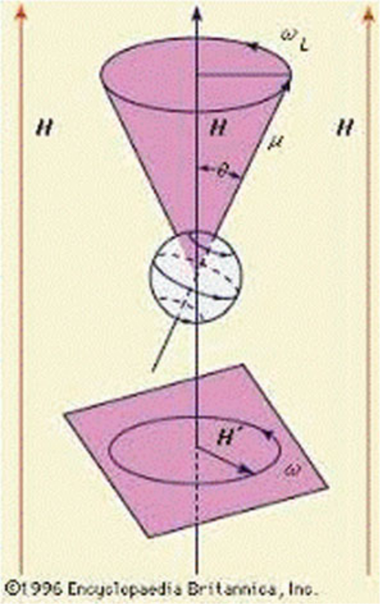
The second approach is based on the properties of the electron Larmor precession, which is the precession of the magnetic moments of electrons about external magnetic field (Fig. 2.1). The angular momentum vector $\overrightarrow{J}$ processes about the external field axis with an angular frequency known as the Larmor frequency,

$$\omega = -\gamma B \tag{2.3}$$

where ω is the angular frequency, and γ is the gyromagnetic ratio. Magnetic resonance occurs when the frequency of the electromagnetic radiation w_r coincides with the Larmor frequency. In NMR and ESR, the Bloch equations [20] are used to calculate the dynamic of spin magnetization $M = (M_x, M_y, M_z)$, after the microwave resonance absorption, as a function of relaxation times T_1 and T_2. The relaxation times T_1 and T_2 are called as longitudinal and transverse relaxation times, being synonyms of spin–lattice and spin–spin relaxation time, respectively.

The interaction of an unpaired electron, by way of its magnetic moment, with nearby nuclear spins, results in additional allowed energy states. This phenomenon leads to the superfine (hipper fine) splitting of the ESR signal into N lines in accordance with the quantum mechanical rule $N = 2I + 1$. Two common mechanisms by which electrons and nuclei interact are: the Fermi contact interaction and

Fig. 2.1 Larmor precession



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dipolar interaction. The isotropic Fermi contact interaction is the quantum mechanical magnetic interaction between an electron and an atomic nucleus when the electron is inside that nucleus. This interaction leads to the appearance of spin density on s-orbits of atoms bearing nucleus and applies to the case of isotropic interactions which are independent of sample orientation in a magnetic field. The anisotropic dipole–dipole interaction between spin electron and spin of nucleus depends on the sample orientation. Both phenomena serve as a tool for the investigation of structure paramagnetic spices and its rotational or dynamics.

Dynamic nuclear spin polarization is the third mechanism for interactions between an unpaired electron and a nuclear spin. Spin polarization is the degree to which the spin is aligned with a given direction. The alignment of electron spins at a given magnetic field and temperature is described by the Boltzmann distribution under the thermal equilibrium. Dynamic nuclear polarization (DNP) relates to transferring spin polarization from electrons to nuclei, resulted in aligning the nuclear spins to the extent that electron spins are aligned [10–12, 14].

Interaction between two electron spins can occur according to two fundamental mechanisms. The spin dipole–dipole interaction arises because the magnetic dipole of one paramagnetic center induces a local magnetic field at the site of another paramagnetic group. This interaction manifests itself in the dipolar energy splitting (dipolar coupling) and change of spin–lattice (T_1) and spin–spin (T_2) relaxation times. In the frame of the spin exchange mechanism, electron spins are involved in a quantum–mechanical interaction due to the overlapping orbitals bearing spins. The dipolar coupling between nuclear spins through space also resulted in splitting of the

NMR lines, while the interaction through intermediate covalent bonds (shielding) is the cause of the chemical shift of the NMR lines.

The fundamental phenomena described above form the basis of numerous variations of the ESR and NMR methods and their combinations. These methods can serve as powerful tools for studying the molecular structure and dynamics of myriad objects including biological water.

This and the next chapters don't intend to present a rigorous derivation of the ESR and NMR spectroscopies and other physical methods, but rather to describe physical phenomena of various methods related to study of structure and molecular dynamics of water and to present a concise summary of the equations and relations needed for the basic understanding of the corresponding experiment. For more rigorous in-depth treatments, the reader is referred to the excellent books and reviews cited throughout the chapter's text [1–19].

2.2 Nuclear Magnetic Resonance

2.2.1 General

Nuclear magnetic resonance spectra are characterized by a number of parameters available for experimental measurements. Among them, the most important are the position of the spectra, their fine structure, intensity, and relaxation times T_1 and T_2. The combination of these parameters in the object of interest allows to measure the distance between the nuclei, the distance between the electron and the nuclei, and the value of the correlation time τ_c, which is a quantitative characteristic of molecular mobility (Fig. 2.2), chemical exchange and other dynamic processes.

Properties of three isotopes of water nuclei used in NMR and ESR are shown in Table 2.1.

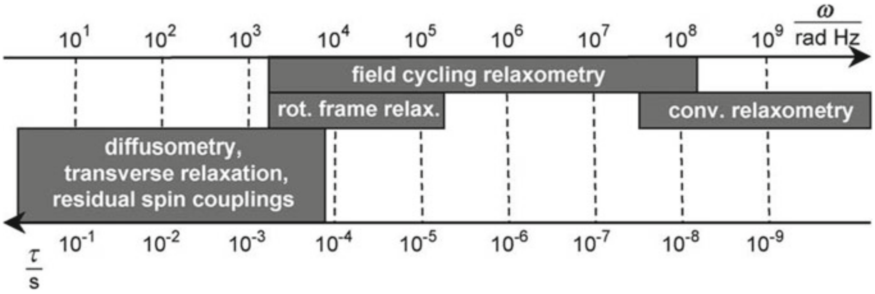

Fig. 2.2 Schematic representation of the correlation time *t* and angular frequency scales covered by diverse NMR techniques. The ranges indicated refer to proton resonance [21]. With permission from Elsevier

Table 2.1 Properties of three isotopes of water nuclei used in NMR and EPR

Isotopes	^{1}H	^{2}H	^{17}O
Spin states	+½, −½	1, 0, −1	5/2, 3/2, 1/2, −1/2, −3/2, −5/2
Gyromagnetic ratio, rad × T^{-1} × s^{-1}	26.7522×10^{7}	4.1066×10^{7}	-3.6281×10^{7}
Frequency at 2.3488 T (MHz)	100	15.3506	13.5565

https://en.wikipedia.org/wiki/Isotopes_of_hydrogen, https://en.wikipedia.org/wiki/Isotopes_of_oxygen

2.2.2 NMR Chemical Shift

Shielding is a barrier made of inner-shell electrons, and it decreases the nucleus pull on the outer electrons [13, 14, 16, 22]. Electrons orbiting around the nucleus generate a small magnetic field that opposes B_0, and electrons are shielding the nucleus from B_0. Due to the addition of electron density, magnetic induction, or other effects chemical shift has been decreased. Thus, the higher the electron density around the nucleus, the higher the opposing magnetic field to B_0 from the electrons, the greater the shielding. When an atom is deshielded, it means that nucleus chemical shift has been increased due to the removal of electron density, magnetic induction, or other effects. Important factors influencing chemical shift are electron density, electronegativity of neighboring groups and anisotropic induced magnetic field effects. The nucleus also feels stronger magnetic field.

The detected frequencies (in Hz) for ^{1}H, ^{13}C, and ^{29}Si nuclei are usually referenced against TMS (tetramethylsilane). Chemical shift δ is expressed in parts per million (ppm) by frequency. For example, the chemical shift positions of the water signal in several common solvents are: acetone (2.8), benzene (0.4), chloroform (1.6), dimethyl sulfoxide (3.3), methanol (4.8), pyridine (4.9), and D_2O (4.8). The most deshielding solvents are the protic H-bonding ones. Next in effectiveness are those with oxygen atoms with lone electron pairs available for H-bonding. An additional effect on water shifts takes place due to the aromatic ring current [22]. The following results of ab initio density functional theory calculations of the NMR chemical shift of liquid water and hexagonal ice were reported, depending on the structural model used: (1) the calculated isotropic shift of ice Ih with respect to the gas phase is −8.0 or 8.1 ppm for the proton, and −48.6 or −48.1 ppm for oxygen; (2) the proton anisotropy is −33.4 or −33.6 ppm; a gas-to-liquid shift of −5.8 ppm for hydrogen, and −36.6 ± 0.5 ppm for oxygen; and (3) molecules beyond the first solvation shell influence the proton chemical shift predominantly via the electric field generated by their permanent electric dipole moment.

2.2.3 Proton Spin Relaxation!

Due to the fundamental importance of the study of the structure and molecular dynamics of a huge number of objects in chemistry, physics, and biology, a whole cascade of methods for measuring and calculating the quantitative parameters of relaxation T_1 and T_2 was proposed and developed for 70 years [14, 16, 19–32].

Free induction decay (FID) [20] by the rotating component of the magnetization vector in the *x*–*y* plane is a short-lived sinusoidal electromagnetic signal which appears following the 90° pulse in a form $[\sin \omega_0 t]\, \mathrm{e}^{-t/T_2^*}$ (Fig. 2.3). The time dependence of the FID measurement is governed by the relaxation rate $1/T_2^* = 1/T_2 + 1/T_{2\mathrm{IH}}$ where $1/T_2$ is the transverse relaxation rate, while $T_{2\mathrm{IH}}$ describes signal loss due to dephasing in the presence of an inhomogeneous background magnetic field.

The first sequence of two-pulse nuclear spin-echo detection was developed by Hahn [20]. The value of T_2 can be measured by performing a series of spin-echo experiments (Fig. 2.4). In a spin-echo experiment, a $\pi/2$ pulse is applied, followed after a time τ, by a π pulse. A free induction decay is observed immediately after the initial $\pi/2$ pulse. Monitoring the decay of the spin-echo intensity

$$I(2\tau) = I_0 \exp(-2\tau/T_\mathrm{M}) \tag{2.4}$$

allows one to determine T_M, the phase memory time.

In the Hahn three-pulse sequence $\pi/2$–τ–$\pi/2$–T–$\pi/2$–τ–echo, after the first 90° pulse, the magnetization vector spreads out forming a disk in the *x*–*y* plane (Fig. 2.4). The spreading continues for a time τ, and then a second 90° pulse is applied such that the “disk” is now in the *x*–*z* plane. After a further time T a third pulse is applied and a stimulated echo is observed after waiting a time τ after the last pulse. The decay of the echo intensity is fitted to an exponential function of T as

$$I(2\tau + T) = I_0 \exp(-T/T_1) \tag{2.5}$$

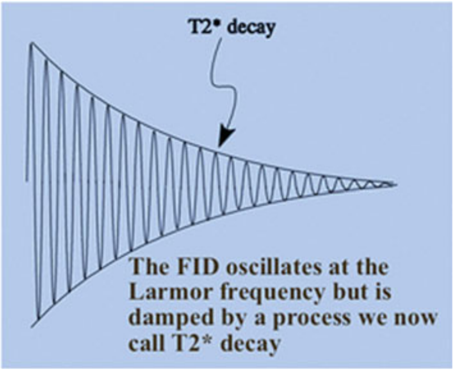

Fig. 2.3 Free induction decay signal. http://mriquestions.com/free-induction-decay.html