
Ice Formation in Biological Medium

There are more things in heaven
and earth, Horatio,
Than are dreamt of in your
philosophy.

William Shakespeare

The target of the action of low temperatures is the water contained in large amounts in the biological objects, thus the phase transitions of water are of primary importance. A typical cryosurgery operation lasts between several minutes and an hour [838]. Cryoaction in medicine consists of a single or several (in cryosurgery) cycles “freezing–exposition–thawing” [346, 474, 745, 782, 838]. The interval between the cycles in cryosurgery considerably increases the destruction of the living tissues, leaving the tissue for a longer time in the hypotonic state and providing the sufficient time needed for the blood microcirculation failure to develop [78, 190].

Cryoaction could be modulated in time (the so-called “dynamic” cryosurgery [822] or cryocycling [791]); as experiments show, the frequency of the temperature oscillations, caused by cryoprobe, is conserved throughout the entire region, while its amplitude decays with distance [791].

2.1 Amorphous Ice

The possibility of a fast transfer of the object to the glassy state without crystallization (*vitrification*, from Latin vitri – glass; B.J. Luyet used also the term “amorphization” [599]) [132, 362, 598, 722, 853, 893] is determined by the ability to attain the high enough cooling rate to pass through the ranges of the crystallization temperatures within the time interval shorter than that is needed for the formation of ice crystal nuclei [362], thus vitrification is easier

to accomplish for a small object [27].¹ Usually, vitrification of the biological objects is performed in the presence of the cryoprotective agent (CPA). Type of the cryoprotectant, its concentration, as well as the protocols of the tissue saturation and of a removal of the CPA [474, 562] influence the outcome of the cryoaction. Partial vitrification could occur during the conventional cryopreservation when the residual solution becomes so concentrated that could vitrify in the presence of ice [132].

Two phases of amorphous ice are known for a long time² – the low-density amorphous ice (LDA), also called the amorphous solid water (ASW) or the hyperquenched glassy water (HGW), and the high-density amorphous ice (HDA) [614]; it is under discussion whether the very-high-density amorphous ice (VHDA) that could be obtained from the HDA by heating at the high pressure [354] should be considered as the “true” independent phase [234, 582]. All three phases have a tetrahedral structure of the hydrogen bonds net, but the HDA (VHDA) has one (respectively, two) additional molecules between the first and the second shells of neighbors [354]. Numerical experiments show the possibility of an existence of the fourth phase of amorphous ice [134].

Recent studies indicate that the HDA is a metastable phase while there are only two stable phases – the LDA and VHDA [355, 937]. A possible porous structure of the different phases of amorphous ice (and, hence, the ability to absorb and retain gases) is of great importance for physics of the extraterrestrial objects – the comets, the satellites of the outer planets, and the interstellar dust [634, 734]. The phase transitions from the vapour, the liquid, and the hexagonal ice I_h into the amorphous ice as well as the transitions between the different phases of the amorphous ice are considered in detail in the recent reviews by Debenetti [234] and Loerting and Giovambattista [582].

¹ A probable example of vitrification in the (very) large scale is an anticipated supercooling of the far (with respect to the Jupiter) hemisphere of the Europe [730].

² Polymorphism of the glassy state (“polyamorphism” [778]) is observed not for the water only, but for some other materials also (Si, SiO₂, GeS₂) [582]. The difference between the structure of the crystal and amorphous ice is reflected in the properties of the large water clusters. V. Buch et al. [141] studied the clusters of size n from $n = 20$ to $n = 931$ numerically and from $n = 200$ to $n = 10^6$ experimentally. The large clusters are characterized by the presence of the crystal core, while the outer layers are disordered due to the surface reconstruction aimed at the reduction of the number of the hydrogen bonds. The clusters containing less than about 200 water molecules are too small to support the stable crystal core and have the amorphous structure. Dynamics of the hydrogen bond in water clusters was studied by F.N. Keutsch and R.J. Saykally [485] using terahertz vibration–rotation–tunneling spectroscopy and mid-infrared spectroscopy. D.A. Anick [30] performed a formal analysis of the polyhedral water clusters. Using “rules” that define the possible number of bonds between hydrogen and oxygen atoms, the author applied the graph theory methods to the structure and general properties of the clusters, organized as a polyhedron.

On the relation of the polyamorphism of water to the interpretation of the freezing phenomena in the solutions, see the paper by O. Mishima [665].

The term “order parameter” frequently used in description of the glassy state has a somewhat different meaning than in physics of critical phenomena and phase transitions [413]; it is even proposed to use “order” parameter in the issues relevant to the glassy state to avoid confusion [48]. The order parameters are related to the analysis of the rare transitions between the stable or metastable states in the free energy surface [807]. Various activated events could be described in terms of the transitions between stable (global minimum) and metastable (local minima) basins in the free energy landscape separated by the free energy barriers (transition states) as a set of paths connecting the relevant basins. The order parameters are useful for the classification of the different metastable states accounting for their characteristics such as symmetries associated with different phases and understanding of the mechanisms of transitions analyzing the evolution of order parameters along the paths in the free energy landscape as well as of the equilibrium properties of the system dependence on the relation of the free energy to order parameters. The latter relation could be obtained in two ways:

- By construction of phenomenological theories of phase transition (*top-down approach*); the free energy functional is usually constructed using symmetry considerations;
- By the coarse graining (*bottom-up approach*) of the microscopic Hamiltonian [807].

“Physical” order parameter is indeterminate at the critical point (the point of the phase transition); transition to the glassy state is not a genuine phase transition, but a dynamic phenomenon and the glass transition temperature is not strictly defined. The meaning of the order parameters in the glassy state context is rather just a set of variables that define the properties of the glassy state. There is a discussion on how many such order parameters are sufficient for the complete description of glass, which was started in the middle of the 20th century with the appearance of the theory by R.O. Davies and G.O. Jones (that idealized the glass transition by treating it as a true phase transition) and is not finished yet. The question “how many” could be reformulated to the constraints on the so-called Prigogine-Defay ratio Π defines as

$$\Pi = \frac{\Delta c_p \Delta \kappa_T}{T_g (\Delta \alpha_p)^2},$$

where Δc_p is the difference between liquid and glass isobaric specific heat per unit volume at the glass transition temperature T_g , $\Delta \kappa_T$ is the liquid–glass difference of isothermal compressibilities, and $\Delta \alpha_p$ is the liquid–glass difference of isobaric thermal expansion coefficients.

It should be noted that the Prigogine–Defay ratio Π is not strictly defined since, first, the parameters entering this criterium are usually obtained by extrapolation to the glass transition temperature that is not strictly defined

itself, and, second, the glass state relaxes continuously making its properties a function of time. One of the approaches to the description of glass transition consists in freezing-in of one or more order parameters related to the internal structural degrees of freedom involved in vitrification [340].

The inequality $\Pi \geq 1$ follows from the Davies–Jones theory, turning into the equality if there is exactly one order parameter; the case of the single parameter also implies that disturbances should decay to the equilibrium exponentially [48].

Experiments show that for the most glass-forming liquids, $2 < \Pi < 5$ and the relaxations almost always are nonexponentials, seemingly supporting the need for several order parameters. However, the recent study of this issue by N.P. Bailey et al. reported in the just cited paper (the authors analyzed the frequency-dependent thermoviscoelastic response functions of the metastable liquid) showed that properties of many liquids, in particularity those in which intermolecular interaction are dominated by the van der Waals forces, could be determined by the single order parameter (no claim is made that the complete description of the molecular structure is provided by this parameter), while the description of the properties of the hydrogen-bonding liquids such as water requires more parameters.

The process of the amorphous ice aging is of more interest for cryobiology, since it defines the conditions and longevity of the storage of the vitrified biological objects. The most important aspect of aging is the formation of critical embryo/nucleus in the metastable supercooled liquid and their extinction upon heating. Experiments on the supercooled organic glass-forming liquids [719] showed that generation of crystal nuclei is possible at temperatures over a hundred degrees lower than the glass transition temperature.

The amorphous ice by the water vitrification was obtained a half century later than by the deposition from the vapour phase on the cold substrate at low pressure by the method resembling modern molecular beam epitaxy used to grow the semiconductor heterostructures. Crystallization in the biological objects differs greatly from the growth of the inorganic semiconductor crystals [112, 717] for a number of reasons: the anomalous properties of a water itself, the effect of the hydrophobic and hydrophilic biological surfaces on the water behavior, and the peculiarities of the crystallization in the heterogeneous media.

2.2 Water

Water alone contrives things.

Titus Lucretius Carus

First of all, pure water in both liquid and solid phases (the last known phase of water, ice XII, was discovered in 1998 [1025]) possess a number of anomalous

properties³ [300, 317, 479, 936, 941]; a complete list of them contains over 50 entries [164]. The anomalies manifest themselves more strongly when the water is supercooled [234, 317, 666, 866] (the term *supercooled* is used more frequently than the more exact one *undercooled*).

Water has uncommon properties also as a solvent – for example, the mobility of the solvated ions nonmonotonically depends on the radius of the ion and do depend on the sign of the charge, in contrast to continuum theory of the ion behavior in the solution [812].

Probably the most known and certainly the most important water anomaly is that it is liquid at the normal conditions, in contrast to many similar small molecules. Water molecule itself is “an unremarkable small molecule” [317]. All water anomalous properties are defined by interactions between water molecules. Perhaps the simplest explanation is of its liquid state at the ambient temperature: unusually high strength of interaction between two water molecules (about 20 kJ mol^{-1} is that is an order of magnitude larger than the typical thermal fluctuation at room temperature ($kT_{\text{room}} \approx 2 \text{ kJ mol}^{-1}$). In contrast, many other small molecules interact via the much weaker van der Waals forces (the typical energy of the interaction is of the order of kT).

Most of the anomalies of the water originate from the ordering of the water molecules in the different states (the ice crystals, the liquid water, and the gas clathrates⁴); this order, in turn, is just a manifestation of the cooperative effect of the structurally and dynamically nonuniform net of hydrogen bonds [317, 499, 585, 614].

The thermodynamic water properties are determined first and foremost by the static inhomogeneities [937] – a local structure, namely, the fourfold (in the average – computations using the different interaction potentials show a considerable number of the water molecules having the coordination number from 3 to 6 [602]) net coordination – the organization of the water molecules in tetrahedra [318, 939]. The angle between H–O bonds in the water molecule (HOH angle) is well established to be 104.52° [87], which is close to both the tetrahedral angle (circa 109.5°) and the internal angle of the pentagon (108°),

³ There is, however, an opinion that the word *anomalous* is not a correct one [970]: the water is *unique*, but all its properties logically follow from this uniqueness of its structure provided by the unique nature of hydrogen bonds.

⁴ In the clathrates (also called the gas clathrates, the gas hydrates) a molecule of gas is surrounded by the water molecules that form the stable cage using the hydrogen bonds [498].

The best known clathrate is probably the methane clathrate (“flammable ice”) – a solid form of water that contains a large amount of methane within its crystal structure. The size of the oceanic methane clathrate reservoir is poorly known, but is estimated to exceed 2–10 times the currently known reserves of conventional natural gas. It is also considered as a source of the atmosphere of Titan [587]. Structure and electronic properties of clathrates including the behaviour of hydrogen bonds under pressure change were studied using the density functional method by Iitaka and Ebisuzaki [425].

providing thus the ability of water molecules, in addition to tetrahedral structures, to form near-planar pentagonal ring structures. The standard simple model of the water molecule having two regions of positive charge close to the positions of the hydrogen nuclei and two regions of negative charge (“lone pair electrons”) tetrahedrally displaced with respect to the positive charges is, as high quality quantum mechanical computations show, an oversimplification [317]. The negative (lone-pair) regions appear to be much closer to the molecular center than the positively charged regions to the hydrogen atoms and the overall charge distribution is near trigonal rather than tetragonal. The molecule’s repulsive core is nearly spherical; however, deviation from the sphericity may be important in the assemblies of the water molecules [317].

If a water molecule is surrounded by four hydrogen-bonded neighbors, in two of these interactions the central water molecules act as hydrogen-bond donor by directing its positively charged hydrogen regions to negatively charged regions (lone-pairs) of each of the two neighbor molecules. In other two interactions, the central molecule acts as a hydrogen bond acceptor of two neighbor water molecules pointing their hydrogen regions towards the lone-pairs of the central molecule. Water seems to be unique in having this donor:acceptor ratio equal to 2:2, which is considered as a very important attribute with respect to the chemical and biological consequences [317].

A cause of the tetrahedral near order is the sp^3 hybridization of the electron orbits [866]. The dynamical properties – both translational and rotational – are traced down to the defects of the hydrogen bonds’ net [645].

Both experiments and computer simulations show that in the supercooled liquids in some transient regions, relaxation times greatly differ from that in other fluid regions. In the early 1960s, G. Adam and J.H. Gibbs postulated the existence of cooperatively rearranging regions (CRR) whose molecules moves essentially independently of the rest of the system. M.G. Mazza et al. [645] studied this issue in detail using MD simulation of the system comprising 1,728 water molecules interacting via the extended simple point charge potential (SPC/E model) for the temperature range from 350 K down to 200 K. Computations have been used to extract a rotational mean square displacement

$$\langle \varphi^2(t) \rangle \equiv \frac{1}{N} \sum_I (\varphi_I(t) - \varphi_I(0))^2$$

and a rotational diffusion coefficient

$$D_R \equiv \lim_{t \rightarrow \infty} \frac{1}{4t} \langle \varphi^2(t) \rangle.$$

The computations showed that rotational dynamics is spatially heterogeneous. The study of the temporal evolution of the clusters containing more easily rotating molecules compared with that in the bulk (the authors called them rotational heterogeneities (RH)) and the radial distribution function of oxygen atoms within HR, which show the amplitude of the first peak that is

greatly enhanced in comparison with bulk water, showed that there is a strong tendency for the more mobile molecules to be neighbors. These molecules, as a rule, have more than four neighbors and thus represent “defects” in the tetrahedral network of hydrogen bonds. The translational heterogeneities, while not identical to RH, certainly have similar features and the same nature.

The net structure changes significantly in the case of the spatial confinement – for example, for a quasi-one-dimensional water in the single-wall carbon nanotube (SWCN), the coordination number is less than 2 [505]. The ordering in the liquid phase is based on the structures similar to the hexagonal ice I_h – the water hexamers $(H_2O)_6$, which can form a number of configurations with close energy [968]; the pentamers $(H_2O)_5$ observed in the clathrates are grouped into the dodecahedra. Dodecahedral water clusters have also been reported for protein surfaces [165]. Using an elementary cell containing 14 molecules, an icosahedral structure containing 280 molecules could be constructed that can transform between the two configurations with the different density without breaking the hydrogen bonds [165]. This model was developed by combining sheets of boat-form and chair-form water hexamers that are present in the crystallographic lattices of hexagonal and cubic ice.

Water displays a remarkable combination of rigidity and flexibility: while the hydrogen bonds network seems to be “rigid” with respect to the thermal fluctuations at normal temperatures, the large number of coordination defects and water’s ability to vary its hydrogen bonding and participate in cooperative motions provide high molecular mobility [877] and anomalously high proton conductivity in liquid water. The first explanation of the latter was suggested two centuries ago (“Grotthuss mechanism”) using the hydroxonium ion H_3O^+ as a mediator agent in the proton transport; modern experimental evidence is in conflict with this simple scheme; mechanism of the proton conduction based on the *ab initio* computations involving the hydrogen-bond breaking process as a possible rate-controlling stage of diffusion is considered in detail by D. Marks et al. [626]. It should be noted that the high diffusivity of protons in the liquid water is relevant to some biomolecular process; also, it is the basis of the hydrogen energy – fuel cells that directly convert a fuel source energy to electricity heavily rely on this property.

In normal liquids, density and entropy fluctuations decrease with the temperature while in the water, due to the local tetrahedral geometry of the molecule interactions, these fluctuations increase. Quantitatively this behaviour could be described with the spatial (tendency of the molecules to adopt preferential separations) and orientational (deviation from the local tetrahedral arrangement) order parameters that both decrease as the volume is decreased [304] largely due to the increased dispersion in both bond angles and bond lengths [317]. The net of hydrogen bonds serves as a cornerstone for some water models: it is considered as a set of space filling curves in [938]; in [585] the temperature dependence of the water properties is expanded as a series in the structure functions that describe the equilibrium net of hydrogen

bonds (the first function gives an average number of bonds per molecule, the second one describes the deviation from the tetrahedral structure, etc.).

The degree of the water ordering increases (respectively, decreases) when cosmotropic (chaotropic) ions are present. The character of the ion effect on the water depends on the ion radius and the charge sign and manifests itself, *inter alia*, in the viscosity η dependence on the concentration c

$$\frac{\eta(c)}{\eta_0} = 1 + A\sqrt{c} + Bc,$$

where the Jones–Doyle coefficient B is positive for the cosmotropic and negative for the chaotropic ions [260]. On the experimental study of the effect of the polar, nonpolar, and charged molecules on the water structure see the paper by D.T. Bowron [120]. The ordering of the water molecules increases the water thermal conductivity, making it close to the thermal conductivity of ice [306].

The *trans*-conformation of the hydrogen bond due to the additional interactions of protons with the nondivided electron pairs that does not participate in the forming of the bond in question proved to be stronger than the *cis*-conformation. As a rule, two OH groups of the same molecule participate in hydrogen bonds of different strength [289]. A model of the continuous net of hydrogen bonds could account for both the strong and the weak bonds [497].

The presence of the far order in water is confirmed by the shape of the radial (pair) distribution function $g(\mathbf{r})$, found numerically in MD computations or experimentally by scattering of the neutrons or the X-rays [499]. These methods are the best currently available means to probe the atomic structure of aqueous solutions [707]. The distribution functions $g_{\alpha\beta}(r)$ of pairs of atoms α and β of the systems are derived using Fourier transformation from the neutron or X-ray scattering patterns obtained experimentally. Knowledge of these functions allows to determine atomic correlation distances, coordination numbers, and the extent of the local order around a particular atom type. An accuracy of the measurements could be enhanced using the so-called *isotopic substitution* to enrich the experimental information by exploiting the differences between the scattering patterns of isotopically labeled samples [707].

Both the distribution of atoms and of the interstitial voids are of interest. The *difference* distribution function $G(\mathbf{r}) = r^2(g(\mathbf{r}) - 1)$ is well suited for visualization of the large distance correlations [1036]. The orientation-correlation function $g(\mathbf{r}, \Omega_1, \Omega_2)$ [120] could not be found directly from measurements and requires post-processing by the method of empirical potential structure refinement (EPSR) that invokes the Monte-Carlo procedures [120]. The total correlation function $g(\mathbf{r}, \Omega_1, \Omega_2, \Omega_{12})$ includes the polar coordinates of the vector connecting the centers of the molecules [602]. It is proposed in the just cited paper to analyze the coordination shells using the correlation functions for the spherical layers $R_{\min} < r < R_{\max}$. While the radial distribution function is routinely obtained in simulations, the determination of the orientational distribution function that depends on bond angles for each distance between

molecules is rather labor-intensive, thus approximations to it are of great interest [540].

The water structure could be described with the aid of integral equation as was pioneered in 1914 by Dutch physicists Leonard Salomon Ornstein and Frederik Zernike. Their idea was to reformulate the equation for the total correlation function $h(r_{12}) = g(r_{12}) - 1$ (g is the radial distribution function), that is, the measure for the “influence” of molecule 1 on molecule 2 at a distance r_{12} by splitting this influence into two parts, direct and indirect. The direct one is given by the so-called direct correlation function $C(r_{12})$, while indirect contribution to the “influence” is weighted by the density and averaged over all the possible positions of particle 3.

Method of Ornstein–Zernike (OZ) integral equations is especially useful in combination with the so-called central force models within which the water is considered as a simple mixture of the oxygen and hydrogen atoms and no distinction is made between the atoms interactions within the same molecule and for the atoms belonging to the different molecules. One is thus relieved from the necessity to describe the orientational properties of the hydrogen bonds and to monitor the orientation of the water molecule. The OZ equation for the pair correlation function of the spatially homogeneous system reads as [866]

$$\gamma(r_{12}) = h(r_{12}) - C(r_{12}) = \rho \int C(r_{13})h(r_{23}) dr_3, \quad (2.1)$$

where

$$h(r) = g(r) - 1 = \exp \left[-\frac{\Phi(r)}{kT} + \omega(r) \right] - 1. \quad (2.2)$$

Here $g(r)$ is the usual radial correlation function defined as

$$g_2(r_1, r_2) = V^2 \iint \frac{1}{Q_N} \exp \left[-\frac{U_N(r_1, r_2, \dots, r_N)}{kT} \right] dr_3 \dots dr_N, \quad (2.3)$$

where Q_N is the configuration integral and U_N is the potential energy of the system, $\omega(r) = \omega(r; T, \rho)$ is thermal potential, and $\rho = V/N$ is the density. In the case of central forces, $g_2(r_1, r_2) = g(|r_2 - r_1|) = g(r)$.

The OZ equation (2.1) is exact. However, its analysis requires the introduction of approximations arising from the generally unknown relation $C = C(h(r))$ that could be reformulated as finding of the so-called bridge-potentials $B(r)$. The latter are expressed as an infinite series of the irreducible diagrams. The problem of finding approximation for the bridge-potential is especially complex if long-range electrostatic interactions are present in the system. If, however, an approximation for the radial correlation function is found, all macroscopic parameters could be relatively easily determined.

A recent review by Sarkisov [866] considers both the phenomenological models of water and the models based on the integral equations for the correlation functions of the Ornstein–Zernike type.

The computations indicate that a first-order phase transition “liquid–liquid” is possible at low temperatures [327, 938, 939, 1091]. This transition

is analogous to the one observed in phosphorus that has a similar structure [669], metal-metalloid melts (Ni-P, Fe-P, Ni-B), some metal alloys (Ni-Zr) [923], presumably, in solutions of some macromolecules [401], and some other fluids [328]. The liquid-liquid transition is also predicted to occur at high pressure in BeF₂ [28]. This transition coincides with a violation of the Stokes-Einstein relation

$$D = \frac{kT}{6\pi\eta R}, \quad (2.4)$$

where D is the diffusion coefficient, η is the fluid viscosity, and R is the hydrodynamic radius of the particle [527]. Since the determination of the viscosity from MD computations is not straightforward, the authors of the just cited paper used instead in their study the time τ_α of the so-called α -relaxation: this variable exhibits the same temperature dependence as η .

It is known [644] that both the Stokes-Einstein relation (2.4) and its analog for the rotational diffusion – the Stokes-Einstein-Debye relation

$$D_r = \frac{kT}{8\pi\eta R^3}, \quad (2.5)$$

that were derived by the combination of classical hydrodynamics (Stokes law for flow around the sphere) and kinetic theory – hold for low-molecular-weight fluid for the temperatures significantly higher than the glass transition temperature T_g : $T \gg T_g$. When liquid is deeply supercooled, these relations greatly overestimate the diffusion coefficients.

M.G. Mazza et al. [644] have established that origin of this breakup could be traced down to the dynamic heterogeneities in the supercooled liquid that does not become a glass in the spatially homogeneous fashion. The authors also found that degree of the violation of the Stokes-Einstein (2.4) and the Stokes-Einstein-Debye relation (2.5) varies with temperature, since following from these relations a temperature-independent ratio

$$\frac{D}{D_r} = \frac{3}{4R^2}$$

does change with supercooling.

In the low-density liquid (LDL) an open locally ice-like network of hydrogen bonds is present, while in the high-density liquid (HDL) the local tetrahedrally coordinated hydrogen bond network is not fully developed; the structures of LDL and HDL are similar to those of the corresponding phases of amorphous ice LDA and HDA [616].

The behavior of the disordered media under the temperature variation is usually attributed to their ability to form stable structured objects using the directional bonds [923, 924]. It is turned out, however, that the directional character of the bond is not the necessary feature: the numerous water anomalies could be reproduced assuming the tetrahedral (sp^3) coordination described by the spherically symmetric interaction potential with the soft core

(see [437] and the references therein). Such potential (ramp potential with two characteristic lengths – an impenetrable hard core and a penetrable soft core) is called sometimes the Jagla potential and the corresponding fluid, displaying many water-like structural, dynamic, and thermodynamic anomalies, called the Jagla fluid [143].

Zanotti et al. tested the hypothesis on the two phases of liquid water (“liquid–liquid critical point hypothesis” [616]) experimentally [1112]. Using the differential scanning calorimetry (DSC) and the neutron spectroscopy, the authors observed two states of the supercooled water differing in density and the first-order transition between these states. The existence of this critical point could explain, *inter alia*, the anomalous increase of the isothermal compressibility and isobaric specific heat on cooling [878].

Experimental study of the water molecule dynamics in SWCN [619] showed that at 228 K the temperature dependence of the relaxation time is changed from the Vogel–Fulcher–Tammann law [815]

$$\tau = \tau_0 \exp\left(\frac{B}{T - T_0}\right) \quad (2.6)$$

to the Arrhenius-type dependence

$$\tau = \tau_0 \exp\left(-\frac{E}{kT}\right),$$

where E is the activation energy.

The authors assume that this moment corresponds to the first order transition from the low density ($\rho < 1.02 \text{ g cm}^{-3}$) liquid water to the high density ($\rho > 1.14 \text{ g cm}^{-3}$) liquid water. Similar results on the change of the temperature dependence of the relaxation time were obtained for the water in the pores of 50 nm diameter in the zeolites [444]. It should be noted, however, that dynamic water transition observed by E. Mamontov et al. [619] in SWCN with the inner diameter of 14 Å was not detected in the double-wall carbon nanotubes with the inner diameter of 16 Å.

Since heterogeneous nucleation could be experimentally circumvented, the homogeneous nucleation theory gives an upper bound to the maximum supercooling. A homogeneous nucleation of the pure water is observed at -39°C ; more deep supercooling can be achieved under the high pressure (up to -92°C [329]) and in nanopores [176] (it should be noted that, on the other hand, the glassy state of the water in the pores [848] and at the silica surface of Vycor [86] could exist at the room temperature); the water dynamics corresponding to a liquid state is observed at low temperatures in the carbon nanotubes [505, 629]. When the temperature is lowered further, tube-like solid structures are observed named by the author’s ice-nanotubes (ice-NTs) [629] or a square-ice sheet wrapped into a cylinder inside the carbon nanotube [505].

The water is not unique in rather high degree of supercooling that can be achieved: the ratio of the homogeneous nucleation temperature to the freezing

temperature at normal conditions is about 0.85 for the water while this ratio is 0.79 for phosphorus and 0.75 for ammonia [326].

Note that the value of -39°C as the homogeneous nucleation limit below which even very small droplets would crystallize is in some contradiction with experiments by L.S. Bartell and J. Huang, who observed freezing of droplets formed by condensation of water vapour in the supersonic Laval nozzle to cubic ice I_c at temperatures close to 200 K [366].

Recently the possibility of the existence of ice at room temperature has been established. Computations performed by A. Wissner-Groos and E. Kaxiras showed that a layer of diamond coated with sodium atoms will keep a thin layer of water in the frozen state up to 108 degrees of Fahrenheit (42°C) [779]. This discovery (if confirmed by experiments) will greatly advance biocensors and other implanted devices, since an ice layer covering the nonorganic surface will smooth the diamond surface and prevent clotting proteins from attaching the surface.

The electric field of strength about 10^6 V m^{-1} raises the crystallization temperature of the water in the nanoscale slit to room temperature [188]. Both the restrictions on the water molecule's mobility in the normal to the wall direction and ordering of the dipoles in the electric field help the formation of the stable net of hydrogen bonds and of the critical size embryo. This effect is reversible – decreasing the field strength results in the ice melting.

S. Wei et al. [1053] studied experimentally the effects of dipole polarization of water molecules on ice formation under an electrostatic field with strength E in the range 10^3 – 10^5 V m^{-1} . When a water molecule is in the external electrostatic field, there is force moment M acting on the molecule given as $M = \mu_0 E \sin \theta$, where μ_0 is the electric dipole moment of the water molecule and θ is the angle between the dipole moment direction and the field direction. The water molecules, excluding the case when the dipole moment is parallel to the field direction, tend to turn in the direction of field to reach the stable state. The potential energy of the interaction U is $\mu_0 E \cos \theta$. Assuming the Boltzmann distribution law, the distribution of water molecules could be written as [1053]

$$f(U) = A \exp\left(-\frac{U}{kT}\right) = A \exp\left(-\frac{\mu_0 E \cos \theta}{kT}\right).$$

Evidently, the Boltzmann distribution function attains maximum when all water molecules have their dipoles oriented along the electrostatic field direction that assists in forming the critical nuclei. The authors, however, found that in contrast to the nucleation, the growth time was unaffected by the presense of the field. The effect of the electric field on the nucleation of the ice crystals is known for over one and a half centuries [124]. The electric field could be used to assist the formation of the ice nuclei in the water solution at the given temperature [758] to control the size of grains in the growing polycrystal or the liophyllization time [756].

Liquids can exist in metastable supercooled state; in contrast, solids cannot be superheated since in contrast to freezing there is no energy barrier for melting. The temperature of solids is always below their (pressure-dependent) bulk melting temperature T_m .⁵ When solid temperature is close (from below) to T_m , the phenomenon of premelting occurs [225,1059]: mobile liquid is observed on at one facet of a wide class of materials that includes water, solid rare gases, semiconductors, metals [1059]. The premelting is subdivided into the surface melting (at the solid–vapour interface) and the interfacial melting (at the interface between the solid and the foreign substrate). The existence of the interfacial premelting depends on a competition between attraction of the liquid to the solid (called *adhesion*) and attraction of the liquid to itself (*cohesion*) [1058]. The film thickness d is proportional to the deviation of the temperature from its bulk melting value [1058]:

$$d = \lambda(T_m - T)^{-\frac{1}{\nu}},$$

where the parameter ν characterizes the fall-off rate of the intermolecular forces and equals $\nu = 2$ for the electrostatic and $\nu = 3$ for the van der Waals forces. It could be seen that the film thickness increases rapidly as the temperature tends to the bulk melting temperature, that is, the crystal melts from its surface inwards.

Ice exhibits all aspects of premelting, including grain-boundary melting at junctions between the individual crystals in the polycrystal [1059]. Polycrystalline ice contains liquid water due to the impurity and curvature depression of the freezing point [609] in microscopic channels (a few tens of micrometer wide) called veins formed by the tri-grain junctions. Water is also present at nodes separating four grains. Water forms a network distributed throughout the whole polycrystal.

The so-called “growth-melt asymmetry” [149] was observed for pure ice crystals at high pressure (2,000 bar). As the pressure increases, the melting temperature decreases and the system passes through a roughening transition with change of the cylindrical shape into the hexagonal prism due to the formation of the facets. Both growth and melt shapes are hexagonal, but the corners appear to be rotated by 30° . This behavior is explained by the more fast melting of the initial corners where the molecules are weakly bound; the rate of the corners’ recession is considerably larger than that of the facets.

Some of the water anomalies are also exhibited by other tetrahedrally coordinated or simply tetrahedral liquids with different bonding varying from the purely ionic nature in the case of BeF_2 to the purely covalent in the case of SiO_2 [28].

⁵ This statement is not strictly accurate. The superheating of solids is impossible if one means a metastable state as in the case of liquid. However, nonequilibrium state of solid with the temperature higher than the melting temperature could be attained with ultra high speed of heating that is provided, for example, by an intense laser irradiation or a planar shock-wave loading that results in the heating rate up to $Q \approx 10^{12} \text{ K s}^{-1}$ [596].

2.3 Biological Water

Water, taken in moderation,
cannot hurt anybody.

Mark Twain

Second, the behavior of the water molecules near the hydrophobic or hydrophilic surfaces cardinally differs from their behavior in the bulk.

The terms *bond*, *ordered*, *biological* water are used, and the cryobiologists also speak about *unfreezable* water (it is more correct, however, to use the word *unfrozen*, allowing for a nonequilibrium state [1073, 1099]). It is possible to distinguish *buried* [652, 738] or *inner* [386] water that is held by the hydrogen bonds in the inner hydrophobic regions of the biological molecules [76] and sometimes form clusters [302]. Such water is present in most globular proteins; in spite of the permanent exchange with the exterior molecules with the characteristic times from 10 ns to 1 ms [339], it should be considered as an intrinsic element of the protein [386, 556, 738]. The term *vicinal* water (from the word vicinity) is usually used in respect to inorganic surfaces [305].

The importance of the water for the stability and function of biological molecules hardly can be overestimated [811] – the water itself could be considered as the biomolecule and even is called the 21st amino acid [557]. In some cases the normal behavior of the protein molecule is possible only if the water on its surface forms (in terms of the percolation theory) an infinite cluster [721, 921]. The hydrogen bonds from the water to the peptide groups are only slightly stronger than the hydrogen bond between the water molecules [564]. MD computations reproduce the anomalous behavior of the water near the surface (see, e.g., [22, 108, 115, 886]).

To establish the hydrogen bond with the protein surface water either donates its proton to protein (to carbonyl/carboxyl and hydroxyl oxygen atoms) or accepts proton from the protein (from amine, amide, and hydroxyl groups that are present in the protein backbone (NH) and in some side chains (NH, NH₂, NH₃)). Water molecule also could mediate interaction between two protein atoms (“water bridge”). Hydrogen bond contribution to the solvation energy was determined recently by M. Petukhov et al. for several atom types (NH/NH₂-uncharged groups, H in hydroxyl groups, NH₂/NH₃-charged groups, O-uncharged carbonyl/hydroxyl groups, and O-charged carboxyl groups) [764].

The change of the average orientation of the water molecules near the interface could be assessed by the normalized probability distribution for the angle between the water dipole and the normal to the interface and also for the angle between the normal and the vector joining the hydrogen atoms [928]. In addition to the water/solid interfaces of different nature, the water/carbon tetrachloride interface was extensively studied [928], since this system serves as a prototype for hydrophobic interactions and in many respects mimics the biological membrane.

The variation of the water properties near the biological surface is caused by the regrouping of hydrogen bonds – by ordering of the water molecules [182] – determined by both the nature of the surface (polar or non-polar) and the spatial restrictions on the structure of the hydrogen bonds net (cut-off of the linear correlation scale [528]); the mobility of the water molecules [55, 57, 310, 400, 846] and the water dielectric constant [656, 732] considerably decrease, the water viscosity increases [1134]. The effect of the wall proximity on the crystallization temperature is known from observations on the soil freezing [186, 520] and the water behavior in the pores of minerals [1050]. Zangi [1111] simulated using MD method the water crystallization in the narrow (several diameters of the water molecule) gap for the case of the inert surface that does not form the hydrogen bond with the water molecule. P. Kumar et al. [529] studied the effect of the interaction potential for the gap containing from two to three layers of the water molecules. Two different interaction potentials were considered: Lennard–Jones and purely repulsive potential. The increase of the pressure shifts the water molecules towards the hydrophobic surfaces and increases the value of the tetrahedral parameter; no pressure effect is observed for the case of hydrophilic surface [353].

MD computations using the density functional theory show the existence of the thin water layer of the higher density near the hydrophilic surface [193] and the significant increase of the strength of bonds between the water molecules in the first hydration shell near the nonpolar surface in comparison with the molecules in the neighbor shells [813]. The authors studied the ordering of the water molecules in the hydration shells of benzene and cyclohexane. They stress that correct classification of surrounding water molecules into discrete shells could be done either by using a distance cut-off, measuring the distance between the water oxygen to the nearest solute carbon atom, or by Voronoi tessellation of the simulation domain, the latter being more robust. Atoms in contact could be explicitly defined as those sharing a face of their surrounding polyhedra accounting for that fact that being closer than some specified distance is not sufficient to be in contact.

Zewall [1115] investigated the evolution of the water structure on the model amphiphilic surface (the specially processed silicon surface was used) exploiting the superfast electron crystallography and found an order of magnitude difference between the characteristic times of the decay of the hydrogen bonds structure for the surface layer and in the bulk. On the other hand, the measurements of the rotational mobility of the water molecules in the protein hydration shell and in the bulk using the method of the magnetic relaxation dispersion give only twofold reduction [386].

Anomalous water properties also manifest themselves in the behavior of the fluid viscosity in the layer confined between two close planar surfaces. If the gap thickness is greater than 8–10 molecular diameters, no effect is observed either for the water or for the standard organic liquids. For the gap between five and eight molecular diameters, however, the effective viscosity increases

greatly in the case of normal liquids with much smaller relative change for the water [816].

Computer simulations show that the changes in the dynamic behavior of the water near the surface depend on the morphology of the latter: while the water dynamics typically slow down near the nonattractive *rough* surface, the dynamics speed up near the nonattractive smooth surface [528].

The direct proofs of the anomalous dynamic properties of the *biological* water were obtained in the experimental study of the water near the lactose molecule using the absorption spectroscopy in the terahertz range (with the time resolution about a fraction of picosecond) [402,656] and the water at the protein surface with the femtosecond time resolution [733]; several layers of the water ordered due to the interaction with the heads of lipid molecules are also observed near the surface of the biological membrane [404]. The water molecules adjacent to the lipid bilayer interact strongly with the headgroups of the lipid molecules and this interaction can extend to several molecular diameters from the membrane surface [657]: for the case of 1,2-dipalmitol-*sn*-glycero-3-phosphocholine (DPPC) phospholipids computations based on the local density as a function of the distance from the surface show the existence of four distinct regions of water, the first two corresponding to the first and second solvation shells surrounding the phosphocholine groups [736]. It is anticipated that these water layers can effectively influence the membrane permeability, posing an obstacle to the transport of permeant molecules to the membrane and even be a rate-limiting stage in permeation [404]. The study of the water dynamics in the micelles showed that there is no considerable variation of the water mobility in the core while a significant reduction is registered in the Stern layer that contains the polar groups of the amphiphilic molecules and the counter-ions [266].

Both direct measurements by the terahertz absorption spectroscopy and MD computations of water near the surface of solute (lactose molecule) show over a hundred water molecules beyond the first solvation shell are affected by the molecule, that is, belong to the hydration layer that extends to approximately 5–6 Å from the surface (123 such molecules were found in the experiment, 112 were from computations). Molecules in this hydration layer show slower dynamics in comparison with molecules in bulk. The authors assume that this retardation of water dynamics by solute is the source of the stabilization effect on the proteins and membranes in a dehydrated or a frozen state by solvated mono- and disaccharides.

On the basis of the experimental data and MD computations it was suggested to distinguish three kinds of water molecules [58] – free (1) and bound (2,3) viz.:

1. Molecules bonded to other water molecules only
2. Molecules having a single bond with the macromolecule's polar group
3. Molecules having two bonds with the macromolecule's polar group

Somewhat more formal classification of hydration water molecules was suggested by M. Nakasako in relation to the water present in protein crystals [701]. The four classes are “inside” (water molecules that occupy cavities in the protein molecules), “contact” (molecules that are located outside the SAS of protein and mediate interactions between adjoining molecules in the crystal; these contact-class hydration sites are formed during the crystallization process), “first-layer” and “second-layer.” The electron density in the first-layer hydration shell is greater than that in bulk solvent, thus dielectric properties of hydration water molecules of this class must differ from those in bulk solvent. Hydration water molecules of the first-layer and second-layer classes form aggregates using hydrogen bonds that link together and are indirectly connected to the polar protein atoms forming a network of hydrogen bonds on the protein surface [700].

The reduction of the water mobility near the solid surface is qualitatively similar to the behavior of the supercooled water; sometimes even an “equivalent temperature shift” is determined: for example, the value of this parameter for the water in the nanogap between two smooth *hydrophobic* surfaces is estimated from MD computations as 40 K [528], while M.C. Bellissent-Funel reported the estimated temperature shift of 20°C that is attributed to the difference life times of the hydrogen bonds [86]. On the other hand, simulation for the case of the *hydrophilic* surface of hydroxide $\text{Mg}(\text{OH})_2$ shows that the confinement effect is more akin to the pressure increase [1047]. When the temperature decreases, similar to the aforementioned experiments with SWCT [619], the change of the relaxation time dependence on the temperature is observed approximately in the same temperature range (222 K and 220 K for the water on the surface of DNA and lysozyme, respectively [175]).

The discrepancies in the dynamical data of the biological water obtained in the different measurements are related to the difficulties of separating the water and the macromolecule motion in some experimental approaches [386]; for example, when quasi-elastic and inelastic neutron scattering is used, the elastic contribution from the biological molecule itself could be significant [86]. B. Halle [386] noted that reduction of the mobility of the macromolecules in the solution in comparison with the mobility of the equivalent (hydrodynamically) body (a sphere, an ellipsoid) is at least partly explained by the significant surface irregularity.

2.4 Crystallization in Heterogeneous Media

Finally, the biological media has a complex microstructure. In the absence of a seed surface, ice formation in the supercooled solution is initiated by the homogeneous [366] or the heterogeneous nucleation. The heterogeneous nucleation dominates if the following nonequality (“wetting criterium” [342]) is valid:

$$\sigma^{\text{vs}} - \sigma^{\text{vl}} < \sigma^{\text{ls}} ,$$

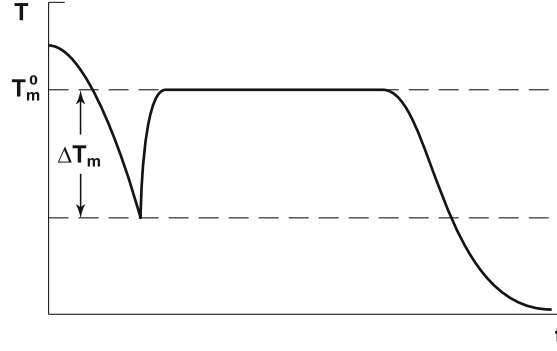


Fig. 2.1. Freezing of pure water

where σ^{ij} is the surface tension on the interface between phases i and j , subscripts v , l , and s denote vapour, liquid, and solid, respectively [480]. The nucleation is observed at the cell membrane [326] or at the objects contained in cytoplasm; the importance of the nucleation on the subcellular objects increases as the temperature decreases [421]; heterogeneous nucleation has not been observed in the cells of some hardwood plants [743] and co-existence of ice and supercooled water is possible [930].

The phase transition in the biological media occurs in the finite temperature interval [18,235,769] (in this respect biological tissues are not unique – the same behavior is observed, for example, in freezing of the soils [186,191,648]). There are two reasons for the finite temperature interval of the crystallization in the biological media: continuous variation of the solute concentrations in the different tissue compartments of finite volume and the presence of the extracellular matrix.

The freezing process of the pure water and aqueous solutions, being cooled externally, is well known. First, the temperature decreases with time until the maximal supercooling ΔT_m is achieved (Fig.2.1). Once nucleation occurs, the rapid crystal growth results in the releasing of the latent heat of fusion faster than it can be removed from the system and rise of the temperature to the equilibrium melting temperature. The system temperature then remains constant for some time until all water is transformed into the ice (the so-called “latent heat plateau”), then (if external cooling is continued) again decreases.

The behavior of the freezing aqueous solution displays a few differences (Fig. 2.2):

- The absolute temperature of supercooling, i.e. the nucleation temperature, is usually higher than that for the pure water, since the presence of solutes promote nucleation [18]
- The temperature rise after nucleation is smaller, since the equilibrium melting temperature of the solution is depressed

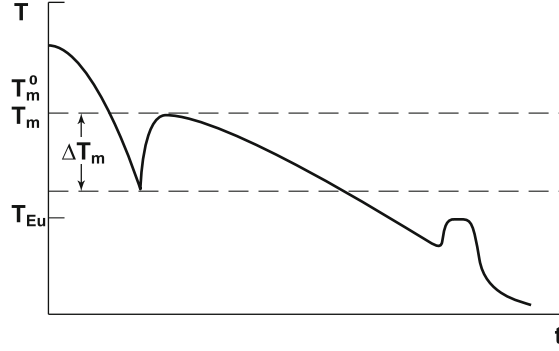


Fig. 2.2. Freezing of solution

- The temperature on the “latent heat plateau” is not constant, but slowly decreases due to the continuous growth of the solute concentration and, hence, continuous lowering of the equilibrium melting point of the solution
- There is another peak on the temperature vs. time curve related to the heat release at the point of the eutectic crystallization; cooling below T_{Eu} will be continued only after the solution has solidified completely.

The behavior of water in porous media, including freezing, is known to differ from the behavior of bulk water (see also section 5.3.3). The relative strength of the fluid–wall interaction to the fluid–fluid interaction determines the freezing point variation: the shift $T_{f,pore} - T_{f,bulk}$ is positive if the fluid–wall interactions are more attractive than the fluid–fluid interaction and negative otherwise [807].

Sometimes it is assumed that mechanical damage due to freezing in the porous material is attributed to the volume expansion at the phase transition in capillars. In fact, the mechanism could be more involved since freezing in the capillars themselves is suppressed by a significant depression of the freezing point when the diameter is small and the curvature of the crystallization front is large (see Sect. 3.3). Ice crystals are first formed in the relatively large voids and are called ice lenses. These crystals are not insulated and the water is supplied through the capillars to support further ice growth. Finally, large enough crystals act mechanically on the porous matrix causing its deformation. In the case of wet soil freezing, this phenomenon is known as frost heave. It alters landscapes, destroys roads, runways, pipelines, building foundations in countries with severe winters, and redistribute methane in the permafrost regions [1058].

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