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REVIEW

Terahertz spectroscopy as a method for investigation of hydration shells of biomolecules

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Abstract

The hydration of biomolecules is one of the fundamental processes underlying the construction of living matter. The formation of the native conformation of most biomolecules is possible only in an aqueous environment. At the same time, not only water affects the structure of biomolecules, but also biomolecules affect the structure of water, forming hydration shells. However, the study of the structure of biomolecules is given much more attention than their hydration shells. A real breakthrough in the study of hydration occurred with the development of the THz spectroscopy method, which showed that the hydration shell of biomolecules is not limited to 1–2 layers of strongly bound water, but also includes more distant areas of hydration with altered molecular dynamics. This review examines the fundamental features of the THz frequency range as a source of information about the structural and dynamic characteristics of water that change during hydration. The applied approaches to the study of hydration shells of biomolecules based on THz spectroscopy are described. The data on the hydration of biomolecules of all main types obtained from the beginning of the application of THz spectroscopy to the present are summarized. The emphasis is placed on the possible participation of extended hydration shells in the realization of the biological functions of biomolecules and at the same time on the insufficient knowledge of their structural and dynamic characteristics.

Keywords Hydration of biomolecules · Hydration shells · Biomolecules · Water structure · THz spectroscopy · THz-TDS

Introduction

The idea that "water is the matrix of life" is widely known. It was expressed by such classics as Paracelsus (Jacobi 1988) and Szent-Györgyi (Drost-Hansen and Clegg 1979) and is often repeated by modern researchers (Ball 2008; Nibali and Havenith 2014). But what is behind this statement is a debatable question. It is quite obvious that water as a solvent allows various molecules to move and interact, which is necessary for living matter. It is also obvious that water is an important factor in the formation of the native structure of biological molecules. Classic examples are protein folding (Privalov and Gill 1988), the formation of a lipid bilayer (Tanford 1973), and the formation of the B-form of DNA (Saenger et al. 1986), all of which are a consequence of hydration. However, the concept of hydration is not limited to the influence of water on the structure of biomolecules, but also includes the influence of biomolecules on the structure of water, which is otherwise called the formation of hydration shells. A biomolecule with its hydration shell is a mutually consistent system, while the study of the structure of biomolecules is paid much more attention than their hydration shells.

Practically all methods used to study hydration shells are sensitive to strongly bound water molecules from the first, at most the second, hydrate layer. These are methods such as X-ray diffraction, neutron diffraction, NMR, DSC, IR spectroscopy, and Raman spectroscopy. Because of this, experiments have been conducted for decades with slightly hydrated biomolecules in the form of powders or films (Wittlin et al. 1986; Westhof et al. 1988; Lavalle et al. 1990; Haxaire et al. 2003; Whitmire et al. 2003; Wyttenbach and Bowers 2009; Gruenbaum and Skinner 2011; He et al. 2011). And the rest of the water was removed so as not to hinder, as it seemed, with

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the registration of the main effect of hydration in the nearest hydrate layer.

Hydration shells are often represented as static structures of water molecules. In fact, they do not stop, but only slow down the molecular dynamics from the time of the settled life of pure water molecules ~ several picoseconds (Ellison 2007) to ~ 10–100 ps (Furse and Corcelli 2008; Magazù et al. 2008; Gruenbaum et al. 2011; Perticaroli et al. 2013), ~ 1–10 ns (Phan et al. 1999; Oleinikova et al. 2004), and even up to ~ 1 μs (Nandi and Bagchi 1997; Kaieda and Halle 2013) in depending on the localization and degree of binding to the biomolecule. The distribution of characteristic times of the molecular dynamics of water in a hydration shell is one of the most important characteristics of the hydration of biomolecules.

A very important stage in the study of hydration shells of biomolecules was the spread of terahertz (THz) spectroscopy in the 2000s. This method turned out to be much more sensitive to hydrated water than other methods. It has been shown that hydration shells are not limited to 1–2 layers of strongly bound water on the surface, but also include more distant regions of the aqueous phase with altered molecular dynamics. For this reason, in a number of articles, they were called dynamic hydration shells (Ebbinghaus et al. 2007; Born et al. 2009; Heyden et al. 2010; Nibali and Havenith 2014). Their thickness can reach several nanometers from the surface of the biomolecule (Leitner et al. 2008; Heyden et al. 2012; Sushko et al. 2015; Penkov et al. 2021). It also turned out that in the hydration shell of biomolecules, the dynamics of water molecules can not only slow down, but also accelerate (Tielrooij et al. 2009; Qin et al. 2016; Penkov 2021; Penkova et al. 2021). The effect of the dynamics of hydration shells on the intramolecular dynamics of proteins (Born et al. 2009; Heyden et al. 2010; Pezzotti et al. 2023) and even on some of their biological functions (Ebbinghaus et al. 2008, 2012; Meister et al. 2013) has been shown. There is evidence of a possible role of extended hydration shells in the interaction between biomolecules (Grossman et al. 2011; Nibali and Havenith 2014; Xu and Havenith 2015; Adams et al. 2020). Hydrated shells of biomolecules are sometimes called "biological water" (Pal et al. 2002; Zhang and Durbin 2006; Zhong et al. 2011), suggesting that they, making up most of the water in a living cell, may have some biological meaning (Ball 2008). The phenomenon of the formation of extended hydration shells of biomolecules involved in biological processes is at a new level consistent with the statement of the classics: "water is the matrix of life."

THz spectroscopy for 20 years of application has demonstrated outstanding sensitivity and informative value in the study of hydration shells of biomolecules. However, the data obtained are fragmentary, and require systematization and generalization. This is complicated by the lack of generally accepted and universal approaches to the use of THz spectroscopy for the study of hydration.

The purpose of this review is, firstly, to summarize the data on the hydration of biomolecules of all main types (proteins, phospholipids, nucleic acids, carbohydrates) obtained by THz spectroscopy from the beginning of its application to the present. Secondly, an attempt is made to identify the most promising approaches based on THz spectroscopy for studying the hydration of biomolecules. To achieve these goals, first of all, the necessary consideration is carried out of the fundamental specificity of the THz frequency range, which contains information about the structural and dynamic characteristics of water that change during hydration.

The review examines the hydration shells of biomolecules in aqueous solutions, since it is in aqueous solutions that all the characteristics of hydration are most fully manifested. The study of biomolecules themselves in a dry or weakly hydrated form is not considered and can be found, for example, in reviews (Markelz 2008; Wei et al. 2018). Also, a wide range of recently popular issues related to the biomedical application of THz spectroscopy is not touched upon. This is sufficiently fully presented in the review (Smolyanskaya et al. 2018).

Spectroscopic methods applicable in the THz range

The THz range is a very small frequency range of 0.3–3 THz (10–100 cm^{-1}) from the huge scale covered by modern spectroscopy from 10^{-6} to 10^{20} Hz. At the same time, it was the THz range that turned out to be the most difficult for spectral measurements. From a technical point of view, both electronic measurement methods applied from the low-frequency side and optical methods applied from the high-frequency side of the THz range are poorly applicable in the THz range itself.

The method of dielectric spectroscopy, which allows measuring the spectra of complex dielectric permittivity, has an upper limit of ~ 0.4 THz (Buchner et al. 1999; Kremer and Schönhals 2003), and above 0.1 THz with very low sensitivity. Fourier-transform infrared spectroscopy (FTIR) (Nishizawa et al. 2005; Lee 2009) and Raman spectroscopy (Urabe et al. 1998; Kraiskii et al. 2020) allow measuring THz spectra, but their sensitivity is low and they cannot directly measure dielectric permittivity spectra. Spectrometers with laser radiation sources based on various physical principles have been developed for the THz range (Bergner et al. 2005; Nishizawa et al. 2005, 2006; Bunkin and Pershin 2019). Their advantage is a high spectral resolution (up to ~ 10^{-3} cm^{-1}), but the spectral range is not very wide or even consists of discrete

lines. Also, these spectrometers do not measure dielectric permittivity spectra. The use of spectrometers with THz sources based on synchrotron radiation is well known (Miller et al. 2003; Lee 2009). But this technique has a low signal-to-noise ratio, usually does not cover the entire THz range, and does not measure dielectric permittivity spectra. Spectroscopy based on sources of the backward-wave-oscillator (Volkov et al. 1985) is limited to frequencies of no more than 1 THz.

The only method that allows spectral measurements in the entire THz range with high sensitivity is terahertz time-domain spectroscopy (THz-TDS) (Nishizawa et al. 2005; Lee 2009; Theuer et al. 2011). The method is based on the principle of coherent generation and detection of pulses of broadband terahertz radiation using femtosecond lasers. It was described in 1989 (Fattinger and Grischkowsky 1989; Grischkowsky et al. 1990), but it became widespread only in the 2000s. THz-TDS is most often used in the transmission mode, but there is another configuration—attenuated total reflection (ATR) (Hirori et al. 2004). THz-TDS has a huge dynamic range (up to 7 orders of magnitude), and the spectral resolution reaches 0.1 cm^{-1}. The indisputable advantage of THz-TDS over other methods is the ability to measure not only absorption spectra, but also the spectra of complex dielectric permittivity, which have much more informative value.

The relationship of the structure of aqueous solutions with their dielectric permittivity in the THz range

The hydration shell of a biomolecule is a region of water with a modified structure near the surface of a biomolecule. Therefore, when analyzing the structure of hydration shells, it is necessary to start from the structure of undisturbed water (pure water at the same temperature). The modern understanding of the structure of water and aqueous solutions was formulated in the works of Bernal and Fowler (1933), Frenkel (1946), Samoilov (1965), and Fisher (1964). According to this, the concept of the structure of water not only reflects the mutual arrangement of its molecules, but also depends on the time of registration. This is directly related to the presence of several types of molecular dynamics of liquid water, realized at different times. Given the different characteristic times of various research methods, the result of determining the structure of aqueous solutions with their help may differ, which is quite natural for associated liquids. THz spectroscopy, in turn, is sensitive to certain types of structure and dynamics of water, realized at times of the order of ~ 1 ps.

THz-TDS, like absorption THz spectroscopy, registers processes associated with changes in the dipole moment of a substance (Peiponen et al. 2013). Water molecules have a dipole moment and usually make up most of the solution, which causes the high sensitivity of the THz spectra of aqueous solutions precisely to water. The spectral contribution of dissolved molecules is usually more than 2 orders of magnitude weaker (Son 2014). At the same time, it is important to understand exactly what information about water is contained in the spectra. To do this, we need to consider all known types of molecular dynamics of water, manifested in the THz range:

1. Orientation relaxation of bound water molecules (Debye relaxation) (von Hippel 1988a, b; Barthel et al. 1990; Lyashchenko and Lileev 2010). The act of reorientation of the molecule is realized after obtaining the activation energy sufficient for release from the bound state, followed by binding in another position. In the spectrum of aqueous solutions, this process manifests as an intense wide band with an absorption maximum of about 1 cm^{-1} (strongly depends on temperature and solutes Wei and Patey 1991; Ellison 2007)).
2. High-frequency relaxation is detected in the spectra of aqueous solutions as a weak band with a maximum of about 20 cm^{-1}. This type of molecular dynamics is attributed to the process of orientational relaxation of free water molecules (Barthel et al. 1990; Yada et al. 2008; Penkov et al. 2013b; Shiraga et al. 2017). Free molecules are understood to be molecules that are not bound by hydrogen bonds and are not in a strong polarizing Coulomb field of ions. It is possible that this fraction of molecules includes some part of weakly bound water molecules, for example, molecules with a single hydrogen bond (Buchner et al. 1999; Tielrooij et al. 2009), but this question is still open in world science.
3. Intermolecular stretch vibrations of water molecules bound by hydrogen bonds (Yuhnevich 1973; Hasted et al. 1985; Walrafen et al. 1986), which manifest themselves as a wide intense band with a maximum of about 200 cm^{-1}.

To describe these three processes, we can use the well-developed theory of dielectric spectroscopy (Kaatze 1983; Ellison 2007; El Khaled et al. 2016), according to which relaxation bands are described by Debye functions, and vibrational bands are described by Lorentzian terms:

$$\varepsilon_w^* = \frac{\Delta\varepsilon_1}{1 - i\omega\tau_1} + \frac{\Delta\varepsilon_2}{1 - i\omega\tau_2} + \frac{A_1}{\omega_1^2 - \omega^2 - i\omega\gamma_1} + \varepsilon_\infty + i\frac{\sigma_0}{\varepsilon_0\omega}, \quad (1)$$

where $\tau_{1,2}$ is the relaxation time of bound and free water molecules; $\Delta\varepsilon_{1,2}$ is the contribution of these relaxation processes to the overall dielectric response; A_1 is the amplitude of the vibrational band; ω_1 is the resonant frequency; γ_1 is a parameter that makes sense of the bandwidth; ε_∞ is the

high-frequency dielectric constant (on the high-frequency side of the vibrational band); ω is the cyclic frequency, and *i* is the imaginary unit. In the case of ionic conductivity of an aqueous solution, it is described by the last term, where σ_0 is the dc-conductivity, and ε_0 is the dielectric constant of vacuum. Figure 1 shows the decomposition of the dielectric permittivity spectrum of water.

In addition to intermolecular stretch vibrations, water also exhibits intermolecular bending vibrations (Hasted et al. 1985; Guardia et al. 2015). The band of these vibrations with a maximum of about 50 cm^{-1} is clearly visible in the Raman spectra (Walrafen et al. 1986; Nielsen 1993). However, in dielectric permittivity spectra, its contribution does not exceed 5% of the total dielectric losses (Fukasawa et al. 2005; Shiraga et al. 2017), and this band can be excluded from consideration without compromising accuracy (Yada et al. 2009). In some cases, the band of intermolecular stretch vibrations is also not taken into account if the consideration is carried out in the low-frequency part of the THz range, where its contribution is small (Tielrooij et al. 2009; Son et al. 2012; Adams et al. 2020). But to describe the spectrum of the entire THz range, taking into account this band is obviously necessary (Fig. 1). If additional data from the GHz range are available, sometimes the fraction of strongly bound water molecules is taken into account by a separate relaxation band (van der Post et al. 2013), but its contribution to THz spectra is negligible. When expanding the spectral range towards the mid-infrared, the libration band (Yada et al. 2009) is additionally taken into account, which also relates to the intermolecular dynamics of water. However, its manifestation in the THz range is negligible (Fukasawa et al. 2005). As a result, it can be stated that within the THz range, the description of dielectric permittivity spectra by Eq. (1) is sufficient and generally accepted for aqueous solutions (Yada et al. 2008; Shiraga et al. 2015a, b; Cherkasova et al. 2020), including solutions of biomolecules (Nazarov et al. 2016; Penkov et al. 2021; Penkova et al. 2021).

By an amazing coincidence, almost all processes of intermolecular dynamics of liquid water (except librations) manifests in a fairly narrow THz range. Since full-fledged spectral methods in the THz range have appeared relatively recently, this gives a good opportunity to study the structure of aqueous solutions in a very informative frequency (time) window. Using THz-TDS, it is possible to obtain spectra of complex dielectric permittivity in the THz range, which makes this method truly unique in its capabilities for analyzing the structure of aqueous solutions.

Principles of studying hydration shells using THz spectroscopy

THz spectroscopy from the beginning of its application has shown outstanding sensitivity in the study of hydration shells. This can be demonstrated by comparing the hydrate numbers of a sucrose molecule determined by different methods: calorimetry, 6 (Kawai et al. 1992); viscometry, 11 (Branca et al. 2001); acoustic methods, 14 (Branca et al. 2001); THz spectroscopy, 35 (Arikawa et al. 2008). The hydrate number determined by THz spectroscopy corresponds to the thickness of the hydration shell of the disaccharide of more than 0.5 nm, and for larger molecules it reaches several nanometers (Heyden et al. 2012; Sushko et al. 2015; Penkov et al. 2021; Singh et al. 2021). This significantly exceeds the values determined by other methods (no more than 3 Å).

Considering this, it is necessary to study the hydration shells of biomolecules using THz spectroscopy not on weakly hydrated samples, but on aqueous solutions, and not at high concentrations. For example, it has been shown that in solutions of sugars with an increase in their concentration above 3–5 wt%, a decrease in the hydrate number is observed (Arikawa et al. 2008; Shiraga et al. 2013, 2017). This indicates the overlap of hydration shells, mainly their outer parts. That is, the higher the concentration, the lower the sensitivity of THz spectroscopy to the least studied outer part of hydration shells.

It is possible to obtain information about the hydration shells of biomolecules based on a comparison of the spectra of a solution of biomolecules (where there are hydration

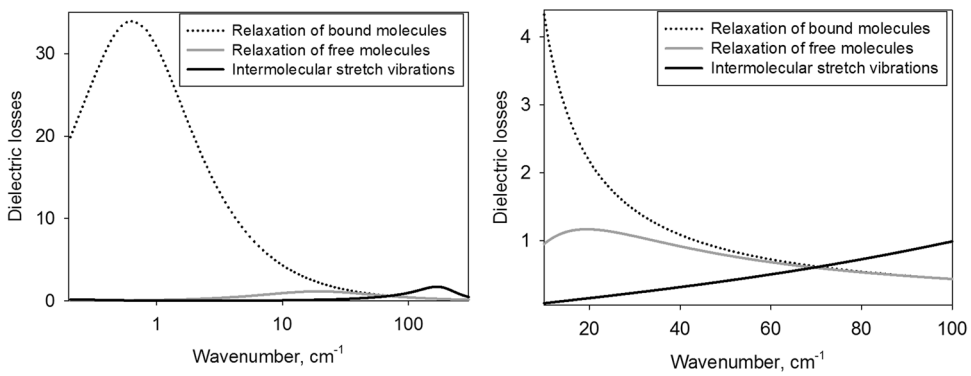

Fig. 1 Decomposition of the imaginary part of the dielectric permittivity spectrum of water at 25 °C into bands corresponding to the orientation relaxation of bound and free water molecules, as well as intermolecular stretch vibrations. Bands in a wide frequency range are shown on the left, and in the THz range on the right

shells) and a solvent (where there are none). However, in practice, it turns out that the THz spectra of aqueous solutions are not characteristic (Xu et al. 2006; Penkov 2021; Penkov et al. 2021). There are no pronounced spectral bands, as is usually observed in the IR range or GHz region. This is the main difficulty in interpreting the spectra of aqueous solutions in the THz range. For dielectric permittivity spectra, the solution to this problem is seen in the fact that it is possible to consider not the spectra themselves, but the parameters of the model (1) determined from these spectra using a fitting. Each of these parameters describes a specific characteristic of the intermolecular structure and dynamics of water.

The Debye relaxation band reflects the dynamics of bound water molecules. When the binding of water increases, the parameter $\Delta\varepsilon_1$ (Kaatze 1983) decreases and the relaxation time τ_1 increases (Barthel et al. 1998; Fuchs and Kaatze 2001; Perticaroli et al. 2013). Thus, when comparing the values of these parameters of a solution of biomolecules and a solvent, it is possible to determine the strengthening or weakening of the binding of water in the hydration shells of biomolecules.

However, when analyzing spectra only in the THz range, it turns out to be almost impossible to determine both of these parameters simultaneously. At frequencies greater than 0.3 THz, the ratio $\omega\tau_1 \gg 1$ is fulfilled; therefore, the first term of Eq. (1) depends on the ratio of the parameters $\Delta\varepsilon_1$ and τ_1. It is necessary either to supplement the spectra with data from the GHz region (Penkov et al. 2015; Samanta et al. 2017), or to use other approaches. A decrease in $\Delta\varepsilon_1$ and an increase in τ_1 have the same meaning, namely, an increase in the binding of water molecules. Therefore, it is possible to register a change in the ratio $\Delta\varepsilon_1/\tau_1$ with an unambiguous interpretation. At the same time, one parameter is excluded from the fitting, which increases the reliability of the mathematical procedure. This approach was used in the works (Shiraga et al. 2015a, b; Nazarov et al. 2016; Penkov et al. 2021; Penkova et al. 2021).

The second term of Eq. (1) describes the orientation relaxation of free water molecules, so $\Delta\varepsilon_2$ is proportional to the number of free molecules. Despite the fact that the meaning of the concept of "free molecules" is not precisely defined in this case, the parameter $\Delta\varepsilon_2$ is quite informative, since it unambiguously reflects the degree of destruction of the structure of water in solution (Penkov et al. 2015; Penkov 2019). The parameter τ_2 determines the time of reorientation of free water molecules, which is associated with the averaged local environment of these molecules (Penkova et al. 2021).

The third term (1) characterizes intermolecular vibrations of hydrogen-bonded water molecules. The higher/lower resonant frequency ω_1 of the biomolecule solution compared to the solvent indicates a higher/lower average energy of hydrogen bonds in the hydration shells of biomolecules. A larger/smaller parameter γ_1 of the biomolecule solution compared to the solvent indicates a larger/smaller width of the distribution of hydrogen bond energies in hydration shells. The difference in the amplitude A_1 reflects the difference in the contribution of intermolecular vibrations of water molecules to the dielectric response of aqueous solutions. According to the meaning of this band, this may be due to two factors: the difference in the number of hydrogen bonds or the difference in the average dipole moment of intermolecular vibrations. An increase/decrease in the dipole moment is associated with an increase/decrease in the amplitude of the vibration, which is directly related to the energy of the hydrogen bond. The greater the binding energy, the smaller the vibration amplitude and vice versa (Scheiner 1997). Since the change in the average energy of hydrogen bonds can be identified by the change in ω_1, for the correct interpretation of the change in A_1, it is necessary to consider it in conjunction with ω_1. If there are no differences in the parameter ω_1, then a change in the parameter A_1 clearly indicates a change in the number of hydrogen bonds. In dielectric spectroscopy, the combined parameter A_1/ω_1^2 is often considered instead of parameter A_1, having the same dimension as the amplitudes of the relaxation bands $\Delta\varepsilon$ (Nazarov et al. 2016; Penkov and Penkova 2021a). When both parameters, A_1 and ω_1, change, it is more correct to consider them separately (Møller et al. 2009; Nazarov et al. 2016; Penkov et al. 2021).

A special issue considered in the literature concerns the relationship of the parameters of model (1) with the number of free water molecules in solution. The simplest calculation of the fraction of free molecules was carried out on the basis of the ratio of the amplitudes of the relaxation bands $\Delta\varepsilon_1$ и $\Delta\varepsilon_2$ as

$$n = {}^{\Delta\varepsilon_2}\!/_{(\Delta\varepsilon_1+\Delta\varepsilon_2)}, \tag{2}$$

or with minor variations (Yada et al. 2008; Tielrooij et al. 2009; Shiraga et al. 2015a, b, Shiraga et al. 2017). However, in a series of theoretical papers (Penkov et al. 2013a, 2014), when considering the process of orientational polarization of free water molecules in aqueous solutions, it was shown that this is not entirely correct. As a result, a theoretically more reasonable ratio was obtained for calculating the proportion of free molecules n (Penkov 2023).

$$n = \frac{\Delta\varepsilon_2\left(2\Delta\varepsilon_2 + 3\varepsilon_\infty\right)}{(\Delta\varepsilon_2 + \varepsilon_\infty)(\varepsilon_\infty + 2)^2} * \frac{\varepsilon_0 9kT}{Nd^2}, \tag{3}$$

where d is the dipole moment of the water molecule, k is the Boltzmann constant, T is the absolute temperature, N is the numerical concentration of water molecules, and the other parameters are similar to Eq. (1).

The parameter n can be used as a criterion determining the average water connectivity in solution (Penkov et al. 2015). Based on a comparison of the values of this parameter for a solvent and a solution of biomolecules, one can see how the number of free water molecules changes during hydration.

Subtraction of the spectral contribution of biomolecules from the spectra of their solutions

When analyzing THz absorption spectra of biomolecule solutions, they are often considered three-phase systems (Heugen et al. 2006; Ebbinghaus et al. 2007; Heyden et al. 2008; Born et al. 2009; Vondracek et al. 2014):

$$\alpha_t(\omega) = \frac{V_b(c)}{V_t}\alpha_b(\omega) + \frac{V_{sh}(c)}{V_t}\alpha_{sh}(\omega) + \left(1 - \frac{V_b(c)}{V_t} - \frac{V_{sh}(c)}{V_t}\right)\alpha_w(\omega) \tag{4}$$

where V_b and $\alpha_b(\omega)$ are the volume and absorption spectrum of biomolecules, V_{sh} and $\alpha_{sh}(\omega)$ are the volume and absorption spectrum of hydration shells, V_t and $\alpha_t(\omega)$ are the total volume and absorption spectrum of the solution, and $\alpha_w(\omega)$ is the absorption spectrum of undisturbed water (outside hydration shells). This is a rather rough approximation (as the authors themselves note), since the hydration shell is obviously not a separate phase with a clearly defined boundary. Nevertheless, knowing the spectra of water and dry matter of biomolecules, it is possible to determine approximate spectral characteristics and the thickness of hydration shells by the concentration dependences of the spectra of solutions.

When working with dielectric permittivity spectra, such an additive approach is not applicable in principle. Dielectric permittivity is a concept of electrodynamics of continuous media, according to which an aqueous solution of biomolecules is a heterogeneous system consisting of a continuous medium (aqueous phase) with a phase of inclusions (biomolecules). To separate the dielectric contributions of phases, it is necessary to use effective medium models. The simplest and most versatile models are the Maxwell-Garnett (1904)

$$\frac{\varepsilon^*_{eff} - \varepsilon^*_2}{\varepsilon^*_{eff} + 2\varepsilon^*_2} = f_1\frac{\varepsilon^*_1 - \varepsilon^*_2}{\varepsilon^*_1 + 2\varepsilon^*_2}, \tag{5}$$

Bruggeman (1935)

$$\sum\nolimits_{i=1}^{M} f_i\frac{\varepsilon^*_i - \varepsilon^*_{eff}}{\varepsilon^*_i + 2\varepsilon^*_{eff}} = 0, \tag{6}$$

Landau-Lifshitz-Looyenga (Looyenga 1965)

$$\varepsilon^*_{eff} = \sum\nolimits_{i=1}^{M} f_i\left(\varepsilon^*_i\right)^{1/3}, \tag{7}$$

where f_i and ε^*_i are the volume fraction and dielectric permittivity (in general, a complex function of frequency) of the ith phase, and $\varepsilon^*_{\mathrm{eff}}$ is the effective dielectric permittivity of the entire system, and M is the number of phases under consideration.

Model (5) is applicable for a small fraction of inclusions f_1 and describes two-phase systems (biomolecules in water). Models (6) and (7) can describe systems with more than two phases, for example, taking hydration shells as a separate phase. Models (5) and (6) were developed for spherical inclusions, but their applicability is also proved for non-spherical, but randomly located inclusions (Sushko and Kris'kiv 2009). It is also necessary that the sizes of inclusions are much smaller than the wavelength of radiation (corresponding to THz frequencies), and the absorption of a single inclusion is small (Sihvola 2000). All these conditions are fully satisfied by aqueous solutions of most biomolecules, such as globular proteins, peptides, nucleotides and oligonucleotides, phospholipid liposomes, and mono- and oligosaccharides. Model (7) was developed under the condition of a small difference in the dielectric characteristics of the medium and inclusions, and its applicability to solutions of biomolecules requires additional verification. These effective medium models were used to analyze the dielectric permittivity spectra of aqueous solutions of lactose (Cherkasova et al. 2020), glucose and galactose (Penkov 2021), bovine serum albumin (BSA) (Penkov et al. 2018), and adenosine triphosphate (ATP) (Penkov and Penkova 2021a).

In the study of model cell membranes or cell suspensions, the effective medium model (6) was used (Penkov et al. 2021), as well as more advanced models (Lisin et al. 1996; Polevaya et al. 1999; Asami 2010), taking into account the difference in the dielectric characteristics of the internal and external environment, and the thickness and dielectric permittivity of the shell.

The problem of subtracting the dielectric contribution of extended biopolymers (DNA, polysaccharides, fibrillar proteins, and fibrillar protein aggregates) from the spectra of their solutions deserves special consideration. There are very few such works. Attempts were made to subtract the contribution of DNA using a suspension model of the effective medium (Glancy and Beyermann 2008; Glancy 2015). However, this model was developed for the analysis of clay-sand slurry (Kraszewski et al. 1976), and its use for DNA solutions, on the one hand, is puzzling, and on the other hand confirms the absence of more adequate effective medium models at the time of these works. In 2021, the first theoretically reasonable effective medium

model was developed, applicable for solutions of extended biopolymers (Penkov and Penkova 2021b):

$$(1-f_2)\varepsilon_1+\frac{f_2}{3}\left(\varepsilon_2^t+\frac{4\varepsilon_1\varepsilon_2^n}{\varepsilon_1+\varepsilon_2^n}\right)=\varepsilon_{eff}\left(1-f_2+\frac{f_2}{3}\left(\frac{5\varepsilon_1+\varepsilon_2^n}{\varepsilon_1+\varepsilon_2^n}\right)\right), \tag{8}$$

where ε_2^n and ε_2^t are the dielectric permittivity in the transverse and longitudinal directions, respectively, relative to the axis of the biomolecule. For biomolecules with low anisotropy of dielectric permittivity, such as DNA (Wittlin et al. 1986; Kistner et al. 2007), Eq. (8) can be simplified:

$$(1-f_2)\varepsilon_1+\frac{f_2\varepsilon_2}{3}\left(\frac{5\varepsilon_1+\varepsilon_2}{\varepsilon_1+\varepsilon_2}\right)=\varepsilon_{eff}\left(1-f_2+\frac{f_2}{3}\left(\frac{5\varepsilon_1+\varepsilon_2}{\varepsilon_1+\varepsilon_2}\right)\right) \tag{9}$$

Model (9) was successfully tested on the example of a DNA solution (Penkov and Penkova 2021b) and was used in studies of hydration shells of plasmid DNA (Penkova et al. 2021), as well as dextran and amylopectin polysaccharides (Penkov 2021).

To subtract the dielectric contribution of biomolecules from the spectra of their solutions using effective medium models, it is necessary to have dielectric permittivity spectra of biomolecules in dry form. They can be measured in the form of thin films (Kistner et al. 2007) or powders compressed into pellets with polyethylene (Markelz et al. 2000). However, special attention needs to be paid to the question of how to avoid the formation of crystals when obtaining dry samples of biomolecules. The problem with crystals is that they exhibit phonon oscillations at THz frequencies, and individual biomolecules in solution, whose spectral contribution is subtracted, do not exhibit phonon oscillations. Therefore, it is necessary to form dry samples of biomolecules with their chaotic arrangement, that is, an amorphous phase. If, for example, rapid vacuum drying of solutions of biological macromolecules is used, then crystals are not formed (Fischer et al. 2005). Drying phospholipids in chloroform also does not allow the formation of ordered structures. However, for small molecules such as monosaccharides, it is quite difficult to avoid crystallinity. To solve this problem, procedures for the glass transition of sugars by their rapid cooling from the molten state are described (Walther et al. 2003). Moreover, it is better to do this by preventing the evaporation of water (Penkov 2021) formed during the high-temperature decomposition of sugar (Antal et al. 1990).

For the first time, the need to subtract the dielectric contribution of biomolecules from the spectra of their solutions was mentioned in 2006 (Nagai et al. 2006). Today there are enough examples of the use of effective medium models for solutions of biomolecules of any type. However, despite the obvious importance of this procedure, it is ignored in most works.

Hydration shells of proteins

Of all the types of biomolecules, the study of protein hydration using THz spectroscopy is devoted to the largest number of works. The thickness of hydration shells of proteins was often estimated using three-phase model (4). Thus, for BSA, it is 1.5 nm (Bye et al. 2014); ubiquitin, 1.8 nm (Born et al. 2009); insulin monomer, 1.5 nm; insulin dimer, 1.8 nm (Wang et al. 2019); human lysozyme, 4 nm (Novelli et al. 2017); and lambda repressor protein, 1–2 nm (Ebbinghaus et al. 2007) or even 4–5 nm (Heyden et al. 2012). The use of molecular modeling using THz data confirms the structural correlation of water molecules up to 2 nm from the protein surface (Matyushov 2012). Such hydration shells combine thousands of water molecules around small proteins (Leitner et al. 2008; Wang et al. 2019) and reach a million for large proteins as monoclonal antibodies (Wallace et al. 2015). The described hydration shells include not only strongly bound water molecules located in the primary hydrate layer, but also more distant hydration regions with altered molecular dynamics of water.

In the vast majority of cases, it is concluded that the absorption of hydration shells at THz frequencies is greater than that of undisturbed water. As an exception, there is another point of view, according to which the increased absorption of a protein (myoglobin) solution is due to the influence of water on the polarizability of protein molecules (Zhang and Durbin 2006).

The THz spectra of aqueous solutions reflect the intermolecular dynamics of water; therefore, a change in the THz spectra of biomolecule solutions is usually interpreted as a change in the dynamics of water in hydration shells. It has been shown that the intramolecular dynamics of the protein correlates with the dynamics of its hydration shells (Born et al. 2009). The mutual correlation of the dynamics of proteins a kinesin catalytic domain and a tubulin dimer separated by a hydrate layer up to 2 nm thick is also described at frequencies less than 2 THz (Kuffel and Zielkiewicz 2015).

It has been repeatedly shown that the dynamics of hydration shells changes during protein denaturation, and the stability of proteins itself is related to the dynamics of hydration shells. For example, there are significant changes in the dynamics of hydration shells of human serum albumin during denaturation, which is associated with the influence of hydrophobic protein sites exposed to water (Luong et al. 2011). In many ways, the mechanism of action of denaturing agents is due to the acceleration

of water dynamics under their influence, rather than a direct effect on proteins (Samanta et al. 2014). Similar conclusions were made when studying BSA solutions with alkylammonium chloride salt additives (Das Mahanta et al. 2017). The dependence of water rotational dynamics on the denaturation temperature of ribonuclease A in a solution with additives of 15 osmolytes was considered. Osmolytes that stabilize proteins are accompanied by bound hydration water with slow dynamics, while the collective dynamics of water is accelerated in the case of denaturant osmolytes (Hishida et al. 2022). A change in protein stability as a result of mutations is also associated with a change in the dynamics of the hydration shell (Adams et al. 2021). From these examples, it follows that not only the primary hydration shell but also the dynamics of water in remote areas of hydration are a significant factor in the stability of proteins.

There are many examples of changes in the thickness and molecular dynamics of hydration shells of proteins during their structural rearrangements, which is associated with the participation of hydration shells in the realization of biological functions of proteins. For example, the hydration thickness of the protein λ^*_{6-85} more than 1 nm is realized only for wild-type, whereas for mutants replacing a single polar glutamine side chain with aromatic residues, the hydration thickness is significantly reduced. Also, the extended hydration shell disappears during denaturation of this protein (Ebbinghaus et al. 2008). Ubiquitin mutations also lead to a decrease in the hydration thickness (Born et al. 2009).

In winter flounder antifreeze peptide, hydration shells up to 2 nm thick are observed, and in four mutants of this protein with greatly reduced antifreeze activity, the hydration thickness is noticeably less. This direct correlation may mean that extended hydration shells play an essential role in the realization of the antifreeze function of this protein (Ebbinghaus et al. 2012). Similar conclusions were made in the analysis of beetle *Dendroides canadensis* proteins and their mutant forms (Meister et al. 2013). The formation of glycoprotein, which leads to a sharp decrease in the antifreeze activity of some proteins, leads to a decrease in the differences in the dynamics of the hydration shell and undisturbed water (Ebbinghaus et al. 2010). At the same time, the primary hydration of antifreeze proteins, which causes the binding of ice crystal nuclei, is also important for cryoprotection. In the hydration shell, a gradient of retardation of water motions towards functional sites of proteins is detected, where ice crystals bind. This gradient has been called a hydration funnel and may be one of the mechanisms of remote molecular recognition of proteins (Nibali and Havenith 2014).

Significant differences have been shown in the dynamics of the hydration shell of β-lactoglobulin at pH 6 and 8. At the same time, a change in pH from 6 to 8 reversibly opens or closes the binding cavity by a transition of the E–F loop and promotes protein dimerization (Vondracek et al. 2014).

For 5 different stable conformations of BSA, determined by pH, there are also differences in hydration shells, mainly in the parameters of orientation relaxation of water molecules. Interestingly, the smallest differences between hydration shells and undisturbed water are recorded for the native BSA conformation. For a protein at pH 2.5, when it takes the form of a molten globule, there is a pronounced destruction of the water structure, which is explained by the contact of the internal hydrophobic groups of the protein with water. Hydration shells of BSA in some conformations show both increased connectivity and increased destruction of the water structure. This is explained by the "two-layer" structure of the hydration shell, consisting of a strongly bound primary layer and a partially destroyed secondary one. The latter is formed due to the impossibility of having an undisturbed water structure immediately behind the structurally distorted primary layer (Penkov et al. 2018). Similar conclusions were made for f-actin containing two layers of hydration, a strongly inhibited primary and a hypermobile secondary (Kabir et al. 2003).

When using THz spectroscopy in conjunction with X-ray absorption analyses and molecular modeling, changes in the molecular dynamics of water between the enzyme and the substrate are shown, which imply the participation of hydration shells in the mechanism of molecular recognition (Grossman et al. 2011).

Solutions of flavoenzyme ferredoxin-$NADP^+$-reductase (FNR), electron transfer protein ferredoxin-1 (PetF), and the transient complex that results from their interaction were analyzed. According to THz spectra, a slowdown in the dynamics of water in hydration shells FNR, PetF, and the FNR:PetF was detected. The sensitivity of hydration dynamics to the surface charge of proteins is shown. Complex formation between the positively charged FNR:$NADP^+$ pre-complex and the negatively charged PetF returns the dynamics of water in hydration shells to the characteristic times of undisturbed water. It is concluded that this process is not only entropically favored, but is also associated with the process of molecular recognition. This also indicates the involvement of hydration shells in electron transfer processes between proteins (Adams et al. 2020).

For human lysozyme protein, there is a relationship between the dynamics of hydration shells and the distance between protein molecules, from which it is concluded that hydration shells play a role in protein–protein interaction and the formation of fibrils (Novelli et al. 2017). Changes in the dynamics of water in hydration shells were also observed for BSA during the fibrillation process (Pyne et al. 2021).

For ubiquitin, a change in the molecular dynamics of the hydration shell during protein folding has been shown. This process was recorded at millisecond times using the kinetic THz absorption method (Kim et al. 2008). This method was called promising by the authors at the time of the work, but later, for some reason, it did not gain popularity.

For BSA solutions with glucose, changes in the dielectric parameters of water in the THz range were recorded for several days, which were explained by a decrease in the spectral contribution of glucose hydration shells due to its binding to protein (Nazarov et al. 2016).

Hydration shells of phospholipids

For stacked bilayers of DOPC phospholipids, it is shown that the hydration is accompanied by the appearance of two fractions of water: strongly bound and free water molecules. Free water molecules are understood to be connected by a maximum of one hydrogen bond and exhibit accelerated dynamics. The number of strongly bound and free water molecules increases with an increase in the volume fraction of phospholipid. Interestingly, with increasing temperature, the proportion of strongly bound water molecules increases, while the proportion of free ones almost does not change (Tielrooij et al. 2009).

For suspensions of liposomes of various compositions based on DOPC, DOPG, and DMPC, nonmonotonic concentration dependences of the molecular relaxation time of water are observed, which depend on the charge of hydrophilic heads. Both delayed and accelerated dynamics of water in hydration shells are shown (Pal et al. 2018). However, the authors compared the hydration of liposomes of different compositions at a temperature of about 20 °C without taking into account possible differences in phase states.

However, for DMPC multilamellar vesicles, it is shown that the volume of hydration shells increases sharply during the temperature phase transition of the gel-liquid crystal. With an increase in temperature for phospholipid in the gel phase, the usual decrease in the Debye relaxation time occurs for water, while after the gel-liquid crystal transition, the relaxation time begins to increase, which indicates an increase in the binding of water in hydration shells (Choi et al. 2012).

Even more subtle hydration effects are described for DPPC liposomes. During the polymorphic transition from the gel to the ripple phase, the effect of phospholipid on the hydration shell changes from weakening intermolecular hydrogen binding to the opposite, strengthening. Interestingly, the thermodynamically main transition (ripple phase-liquid crystal) leads only to a slight increase in hydrogen bonding. The thickness of liposome hydration shells in the ripple phase was estimated at more than 5 nm (Penkov et al. 2021). For DMPC liposomes in the liquid crystal phase, the thickness of hydration shells was estimated to be about 1 nm, but in this case, a direct structural correlation of water molecules was meant, and not water with altered molecular dynamics (Hishida and Tanaka 2011).

As in the case of proteins, two layers, primary and secondary, can be distinguished in the hydration shells of phospholipid membranes. For PC lipid membranes surface, the water dynamics in the primary and secondary hydration layers are slowed, and for PE membrane primary hydration layer is slowed, whereas secondary hydration layer is accelerated. It is concluded that a less hydrophilic hydration layer will slow the water dynamics, while a better hydrophilic hydration layer accelerates the dynamics. The hydration thickness of PC lipid membranes is much larger than that of PE lipid (Hishida et al. 2014).

For membranes from DOPC and DOPG, a slowing in the dynamics of hydration shells was also detected, but for charged DOPG, the slowing is greater. However, when cholesterol is added to the composition of phospholipids, the dynamics of water accelerates, that is, the binding of water in hydration shells weakens (Pyne et al. 2022).

A special method was developed to study the binding of cations to phospholipids based on the analysis of THz spectra of reverse micelles emulsion in hexadecane. There is a change in the collective dynamics of water in the range of 0.3–2 THz, depending on the specific binding of phospholipids to Cu^{2+} cations, which is not observed for Mg^{2+} ions. The influence of Cu^{2+} on the dynamics of water in the hydration shells of DOPG is more pronounced than for DOPC, which is obviously related to the charge structure of the phospholipid heads (Yang et al. 2019).

Hydration shells of nucleic acids

Analysis of THz dielectric permittivity spectra of solutions of four nucleotides with concentrations up to 1 mM showed that dGMP increases, and dCMP reduces the Debye relaxation time of water. dTMP and dAMP do not show convincing trends (Glancy and Beyermann 2008).

For DNA, a slowing in the relaxation dynamics of water in hydration shells has been shown (Son et al. 2012). Consistent results were obtained for DNA from salmon testes, for which enhanced water binding in hydration shells was detected. The thickness of the hydration shell is estimated at 1.6–1.7 nm (Glancy 2015).

In a similar study of DNA solutions from calf thymus and salmon sperm, it is stated that there is no significant effect of DNA on hydration shells (Polley et al. 2013). The authors refer to data describing the opposite effect of purine

and pyrimidine nucleotides on water binding (Glancy and Beyermann 2008). However, most likely, the lack of effect is due to the too low concentration of DNA in the solution, about 1 mg/ml. For example, solutions with concentrations of about 25 mg/ml were analyzed to reliably detect the effects of DNA hydration (Penkova et al. 2021).

The study of DNA solutions by THz spectroscopy with an extension of the spectral range to MHz revealed the presence of four fractions of water molecules with different mobility (with characteristic times from 8 ps to 1 ns). These fractions belong to different areas of DNA hydration. The average thickness of the hydration shell is 1.8 nm (Singh et al. 2021).

For pET-11c plasmid DNA in the circular (relaxed) form, the presence of three fractions of water molecules in the hydration shell was revealed, distinguishing it from undisturbed water: more strongly bound molecules, a region with a large number of free molecules and a region with a large number of hydrogen bonds. It has also been shown that Mg^{2+} ions have a weak effect on the hydration shell of DNA, whereas K^{+} ions in a concentration close to intracellular (150 mM) partially destroy it. The latter fact may have biological significance (Penkova et al. 2021).

In the study of ATP hydration using THz-TDS, it was shown that ATP strongly binds and distorts the structure of water, obviously due to the hydration of phosphate groups, whereas the Mg•ATP complex, on the contrary, regularizes the structure of water, contributing to the formation of additional hydrogen bonds in hydration shells (Penkov and Penkova 2021a). Later studies have clarified that this is not a purely charging effect, since the binding of ATP to Ca^{2+} does not contribute to the formation of additional hydrogen bonds. This may make biological sense, due to the fact that ATP participates in the vast majority of biologically significant reactions in the Mg^{2+}-bound state (Penkov et al. 2022). Comparison of ATP and DNA solutions for all analyzed parameters of water dielectric permittivity demonstrates a similar type of their hydration. However, all manifestations of hydration are more pronounced for DNA, which indicates the cooperative hydration effects of nucleic acids (Penkova et al. 2021).

The study of RNA hydration in solutions by THz spectroscopy has not been described in the literature, apparently for reasons of difficulties in obtaining sufficient concentrations, achieving conformational identity, and problems with stability of RNA.

Hydration shells of carbohydrates

Along with proteins, nucleic acids, and lipids, carbohydrates were the last to be included in a number of main types of biomolecules (Rademacher et al. 1988), but their hydration has been studied for more than 100 years (Scatchard 1921). Sugars have the simplest structure compared to other biomolecules, but even their hydration shells are still poorly understood. A clear confirmation of this is the lack of consensus on a seemingly simple question: are sugars a water-structure maker or breaker? This question is even raised in modern scientific research (Sato and Miyawaki 2016; Shiraga et al. 2017; Singh et al. 2018)! THz spectroscopy has significantly expanded the knowledge about the carbohydrates hydration.

In one of the first studies of aqueous solutions of sugars by the THz-TDS, it was shown that the concentration dependence of the dielectric permittivity of sucrose solutions is not linear. It was concluded that there are hydration shells of sugars with a thickness noticeably greater than 1 layer of water (Nagai et al. 2006), which was subsequently repeatedly confirmed (Arikawa et al. 2008; Shiraga et al. 2013, 2017; Sajadi et al. 2014). For example, the thickness of the lactose hydration shell was estimated to be more than 5 Å (Heugen et al. 2006). Similar values were obtained for several mono- and disaccharides (Shiraga et al. 2015a, b). According to another study, the thicknesses of the hydration shells of lactose, trehalose, and glucose are 6, 7, and 4 Å, respectively. It is stated that the effectiveness of the formation of the hydration shell of sugar is determined by the number of surface hydrogen bonds (Heyden et al. 2008).

Using the THz-TDS ATR method, it was calculated that the hydration shells of monosaccharides contain 10–15 water molecules, and the hydration shells of disaccharides contain more than 20. The hydration of sugars, which includes, in addition to the primary strongly bound water molecules, also more distant areas of hydration, has been called "global hydration." With an increase in the concentration of sugars, the volume of hydrated water per sugar molecule decreases, which can be explained by the partial overlap of the hydration shells. Global hydration is more pronounced the more hydrophilic groups (hydroxyl groups and acetal oxygens) a sugar molecule has. The authors even suggest that understanding the global hydration of sugars may contribute to a better understanding of the mechanism for sweetness reception (Shiraga et al. 2013).

However, even with an equal number of hydrophilic groups, glucose exhibits more pronounced hydration than galactose, mannose, fructose, and trehalose exhibits more pronounced hydration than sucrose. This is associated with the number of equatorial OH groups of sugar molecules. The more of them, the more structured the hydration shell is (Shiraga et al. 2013, 2015b). This conclusion is consistent with the conclusions made on the basis of other methods (Galema et al. 1990; Sato and Miyawaki 2000; Penkov 2021) and is explained by the degree to which the arrangement of the OH groups of the sugar molecule corresponds to the structure of undisturbed water.

Using THz-TDS, a decrease in the amplitude and an increase in the time of Debye relaxation of water in the presence of glucose were shown (Nazarov et al. 2016). This is obviously explained by the binding of water to the OH groups of sugar. Based on THz-TDS and dielectric spectroscopy in the GHz region data, a separate water molecule fraction of glucose solutions was isolated, slowed by 2–3 times relative to undisturbed water. The number of such delayed molecules is about 20 per glucose molecule. At the same time, a distortion of the water structure and the formation of more free water molecules under the influence of glucose were detected. Two types of hydration are distinguished: "δ hydration" and "hole hydration." The first refers to slowed molecules, the second to all structurally or dynamically altered water under the influence of sugar (Shiraga et al. 2015a, b).

The duality of sugar's influence on the structure of water—increased binding in the primary hydration shell and destruction of the structure in more distant areas of hydration—has been repeatedly noted (Shiraga et al. 2015b, 2017; Penkov 2021). This is probably the answer to the above question: "Are sugars a water-structure maker or breaker?".

Particular attention was paid to the study of the hydration of trehalose in comparison with other sugars (Heyden et al. 2008; Shiraga et al. 2015a, b) due to the fact that the mechanisms of the best bioprotective action of trehalose among sugars are still unclear (Crowe et al. 1984; Jain and Roy 2008). For example, it has been shown that trehalose, due to the symmetry of the molecule, enhances hydrogen bonding between water molecules and forms a uniform water structure, unlike maltose, although the hydrate numbers of both sugars are close (Hu et al. 2022). It was also noted that trehalose, in comparison with sucrose, slows the dynamics of water molecules in the hydration shell more strongly at close hydrate numbers, about 25 (Shiraga et al. 2017).

The analysis of the permittivity spectra of a mixture of glucose and water in the concentration range from crystalline glucose to highly dilute solutions was carried out. This made it possible to estimate the ratio of different phases and calculate the parameters of the dielectric permittivity of hydrated water using the effective medium model (Cherkasova et al. 2020).

Studies of hydration shells of polysaccharides in solutions using THz spectroscopy are practically absent. In the only work (Penkov 2021), the hydration parameters of monosaccharides (glucose, galactose, galacturonic acid) and polysaccharides (dextran, amylopectin, polygalacturonic acid) were compared. Much attention was paid to the correct use of efficient medium models to separate the contributions of carbohydrates and the aqueous phase. Many features of the hydration of sugars with different molecular structures have been established. For example, the axial orientation of the OH(4) group, in contrast to the equatorial one, contributes to the broadening of the energy distribution of hydrogen bonds in the hydration shell. The significant influence of carboxyl groups on the parameters of hydrogen bonding is shown. According to all analyzed parameters (strengthening of water binding, energy and number of hydrogen bonds, the number of free water molecules), the hydration of polysaccharides was less pronounced than the hydration of the equimolar amount of the constituent monosaccharides. This is explained by the fact that each glycoside bond of a polysaccharide is formed with the loss of two OH groups of binding monosaccharides. Obviously, for sugars, OH groups are the main factor of hydration, and with a decrease in their amount, hydration weakens. This fundamentally distinguishes carbohydrates from nucleic acids, for which cooperative hydration effects have been shown (Penkova et al. 2021). The water binding in the hydration shells of dextran and amylopectin is significantly different, despite the identical composition. This indicates the dependence of polysaccharide hydration on the type of glycosidic bonds or, more likely, on the presence of OH groups of monomeric units not used in glycoside bonds (Penkov 2021).

Conclusions and future perspectives

THz spectroscopy is the most sensitive method in the study of hydration shells. With its help, it was shown that the effect of a biomolecule on water is not limited to binding 1–2 layers of water molecules (about 3 Å thick), as follows from the data of other methods such as X-ray diffraction, neutron diffraction, NMR, DSC, IR spectroscopy, and Raman spectroscopy. Analysis of the totality of the data obtained shows that the hydration thicknesses of biomolecules are as follows: for sugars, 0.4–0.7 nm; for DNA, 1.5–2 nm; for proteins in most cases, 1–2 nm; for phospholipid membranes, up to 5 nm. There is a tendency to increase the thickness of hydration with an increase in the size of hydrated molecules.

For all types of biomolecules, the hydration shell consists of two layers, i.e., primary, strongly bound, and secondary, in which water molecules are not strongly bound, but exhibit differences in intermolecular dynamics from undisturbed water. This extended secondary layer of hydration can only be detected by THz spectroscopy. The secondary hydration layer may have greater or lesser hydrogen bonding or, in another interpretation, delayed or accelerated dynamics, which is indirectly determined by the surface groups of the biomolecule.

Low molecular weight sugars have the simplest structure compared to other biomolecules, and a lot of data on hydration has been obtained for them. Using the example of sugars, it is possible to trace some dependencies of the structure of the molecule and the characteristics of its

hydration shell. One of the main factors, obviously, is the degree of correspondence of the location of the surface sites of hydrogen bonding to the structure of undisturbed water. The better this correspondence, the more ordered the structure and slowed dynamics of water in the hydration shell, and vice versa.

For biological macromolecules, the relationship of structure with hydration characteristics is much more complicated. And not only because they have a more complex structure, but also because they are not rigid, like small covalently bound sugar molecules. Because of this, their own intramolecular dynamics can introduce uncertainty into the location of surface binding sites of water molecules. To fully describe the hydration shells of biomolecules, it is necessary to construct a three-dimensional map of hydration, taking into account both the structural aspect and the dynamic one. Obviously, this is much more complicated than describing the structure of the biomolecule itself, which is usually considered static. Without a doubt, to solve such a problem, a comprehensive use of molecular modeling methods is required.

In the vast majority of cases, the study of hydration shells is carried out on selective examples of biomolecules, and as a result, a change in the thickness of the hydration shells or the dynamics of water in them is simply noted when the structure of the biomolecule changes. The physico-chemical foundations of the molecular organization of hydration shells of biomolecules are by and large not clear. At the same time, a large number of examples have been accumulated where, according to certain parameters, hydration shells turn out to be unique for biomolecules in a functionally active state, and when removed from this state, the hydration features disappear. These examples give reason to believe that extended hydration shells do not just accompany biomolecules, but participate in the performance of biomolecule biological functions. This phenomenon at the junction of physical chemistry and molecular biophysics is interesting and important, but it can hardly be studied without understanding the basics of the molecular organization of hydration shells.

It is worth noting some difficulties in this area of research that prevent the comparison and generalization of available data. Firstly, different methods are used: absorption and time-domain spectroscopy. There is an obvious advantage in THz-TDS, which allows recording spectra of dielectric permittivity. Secondly, there are still no generally accepted approaches to processing and interpreting THz spectra of biomolecule solutions. Apparently, the analysis based on the three-phase model (4) is the most informative for absorption spectra. For spectra of dielectric permittivity, the most complete analysis is seen based on the application of the water dielectric permittivity model (1) with preliminary subtraction of the dielectric contribution of biomolecules using effective medium models. Surprisingly, in such studies, the subtraction of the dielectric contribution of biomolecules is rarely carried out. This can lead to significant distortions of the results.

The THz-TDS method, although it is the most sensitive to hydration shells, is implemented in a fairly narrow spectral window. The THz range contains a high-frequency wing of the Debye relaxation band and a low-frequency wing of the intermolecular water vibration band. However, to obtain more accurate information about these types of water dynamics, in addition to THz-TDS, it would be useful to extend the spectral range to at least 0.01 THz using dielectric spectroscopy and at least to 10 THz using FTIR. Additional data from the method of Raman spectroscopy in the THz region may be useful. The spectroscopy of own radiation, which has not yet been tested in the THz range, seems interesting. This method has been developed for the mid-IR range (Penkov and Penkova 2020a, b), but there are no fundamental limitations of its application up to THz frequencies (Penkov and Penkova 2021c).

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Data Availability The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval Not applicable.

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