

# THE STATE OF THE WATER IN BRAIN TISSUE IN PRESENCE OF TS-100 SILICA NANOPARTICLES

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By the method of low-temperature <sup>1</sup>H NMR spectroscopy the structure of the hydrate layers of water associated with brain cells, the changes of these parameters during necrotic lesions (stroke) and in the presence of trifluoroacetic acid, which suggest to differentiate intracellular water clusters according to their ability to dissolve the acid, were studied. Also the impact of silica TS-100 nanoparticles on the state of water in brain tissue, namely on the water binding parameters in the air and in the presence of a weakly polar solvent was considered.

The distributions by the radii and change of Gibbs free energy for clusters of strongly bound interfacial water were obtained. It was shown that the hydration properties of the native brain tissue differ from the hydration properties of necrotic damaged tissue by the structure of weakly bound water clusters. In intact tissue all the water is associated and is a part of clusters and domains, most of which have a radii R = 2 and 20 nm. The media with chloroform stabilizes water polyassociates with the radius up to R = 100 nm and trifluoroacetic acid stabilizes water polyassociates with radii R = 7–20 nm. It was found that the partial dehydration of the investigated tissue samples is accompanied by decreasing of weakly bound water amount and some increasing of strongly bound water that indicates a change of molecular interactions between the components of cells-nanoparticles composite system. The ischemic necrosis area presence leads to decrease of water binding due to the average size water polyassociates increasing. This effect is observed both in air and in a weakly polar organic solvent medium (deuteriochloroform).

**Key words:** ischemic stroke, the strongly and weakly bound water, <sup>1</sup>H NMR spectroscopy.

There are great achievements in the study of electrophysical characteristics of the different parts of brain at biotechnology development [1, 2], which can be compared with details of its morphological structure determined by magnetic resonance tomography (MRT) [3, 4]. It is known, there is 84% of intracellular water in brain (in neurons and glia). The presence of so much water amount in brain suggests the possibility of its active participation in the brain function not only as a medium providing delivery of nutrients to the cells and removing decomposition products (it is carried out by neuroglia cells) but also in specific functions performing.

The water in brain tissue cells is studied by NMR spectroscopy for more than 50 years [6–9] which served largely as the basis for creation

and wide spreading of MRT methods. As a basic research method the low-temperature <sup>1</sup>H NMR spectroscopy was chosen [10–13], whereby we can determine the amount of strongly and weakly bound water by changes in the intensity of the NMR signal during the thawing process of samples, and using Gibbs-Thomson equations — the nonfreezing water clusters radii distribution [14, 15]. The magnitude of water chemical shift made it possible to calculate the average association degree of water molecules in polyassociates. This takes into account the fact that protons of not associated (weakly associated) water have a chemical shift  $\delta_H = 1-1.5$  ppm, ice-like structures characteristic for hexagonal ice have  $\delta_H = 7$  ppm [16], and liquid water has  $\delta_H = 4.5-5$  ppm.

Decreasing of the brain tissue cells interaction may be realized by silica nanoparticles administration into intercellular space. This allows determining the contribution of intercellular interactions in the thermodynamic properties of water associated with brain cells.

The aim of the work was to determine water parameters associated with brain cells, their parameters change in the case of necrotic lesions (stroke) and in the presence of an acidic agent (trifluoroacetic acid — TFA) allowing differentiation of intracellular water clusters by their capability to solve the acid [17, 18].

### Materials and Methods

*Materials.* Samples of brain tissue were obtained by dissection of 50 years man's cadaver with the postmortem diagnosis: cerebrovascular disease, ischemic cerebral stroke (death occurred within 10 days after the onset of the disease). An autopsy of the body of the deceased was carried out in 6 hours followed by the detection of biological death. Sampling was carried out directly from the section of ischemic necrosis and distant part of the brain (macroscopically — the zone of intact tissue). Macroscopic description of the brain, photography in macro mode on Digital Cameras Canon Pover Shot A510 (Canon, China), selection of brain tissue samples for histological and NMR spectroscopic studies were carried out.

For the manufacture of histological preparations the fragments of the tissue after fixation with 10% neutral formalin solution according to a standardized method were dehydrated in increasing concentrations of ethanol and then embedded in paraffin blocks. Block sections with thickness of 3–5 microns were prepared using a sledge microtome, stained with hematoxylin-eosin by standard procedure.

Histological preparations (slices) were photographed by the camera Canon Power Shot A510 (Canon, China) using the separation capacity of 5 megapixels on the microscope Leica DM LS2, a lens 10×, 20×, ocular 10× and sim out adapter Leica DM LS2 (Leica Microsystems Wetzlar GmbH, Germany) using the program Remote Capture (Canon, China).

For NMR studies the tissue slice of about 2 cm thick was used. Tissues were frozen and stored at a temperature of 258 K. For the initial sample (Norm) obtaining the substance from the inside of frozen brain slice was taken by the puncture with 4 mm glass tube and

placed in a 5 mm NMR ampoule and then in a pre-cooled to 210 K NMR spectrometer sensor. The intensity of the water signal was measured during the thawing of the sample up to a final temperature of  $T = 300$  K.

To study the effect of the hydrophobic liquid media (deuteriochloroform —  $\text{CDCl}_3$ ) 0.5 g of frozen tissue was ground up to pieces of 1–3 mm<sup>3</sup> size, pieces were thawed and placed in  $\text{CDCl}_3$  medium and maintained for 1 h. Then the tissue was placed in a 5-mm measuring ampoule and cooled up to 210 K, and then the measurements of <sup>1</sup>H NMR spectra during defrosting were carried out.

Effect of the solid medium was studied by administering the brain tissue in a matrix of silica TS-100 (manufactured by Cabot Corporation, USA). For this purpose 0.5 g of tissue was mixed with 100 mg of silica. Five minutes after the mixing of tissue with silica a homogeneous powder mass was formed in which the pieces of tissue up to 100 microns, and possibly a small group of individual cells, were coated with silica particles. Then a composite powder was placed in a 5-mm NMR ampoule in which in one and the same sample the state of the water in the initial sample and chloroform and (or) other organic substances additive containing sample could be studied. Partial dehydration of tissues containing particles of TS-100 silica was carried out at 313 K with an electronic scales provided with a heating device (a halogen lamp). In this way, the water content in the samples was adjusted to 50% of the original.

*Low-temperature <sup>1</sup>H NMR spectroscopy.* NMR spectra were recorded on the NMR spectrometer of high resolution (Varian “Mercury”) with an operating frequency of 400 MHz. Eight 60°- sounding pulses of duration 1 μs at a bandwidth of 20 kHz were used. The temperature in the sensor was regulated by the Bruker VT-1000 temperature controller with an accuracy of ± 1 degree. The signal intensity was determined by measuring the peak areas using the procedure of signal decomposition into its components, assuming a Gaussian waveform and optimizing the zero line and phase with an accuracy that for well-resolved signals did not fall below 5% and for overlapping signals of ±10%. In order to prevent water super-cooling in the studied objects, the concentration of non-freezing water was measured at heating the samples, pre-cooled to a temperature of 210 K. The temperature dependences of NMR signals intensity were obtained in an automated cycle, when incubation of samples at a

constant temperature was 9 minutes, and the measurement time was 1 minute.

As the main parameter that determines the structure of water hydrogen bond network, the chemical shift of the protons ( $\delta_H$ ) was used. It was assumed that the water in which each molecule is involved in four hydrogen bonds forming (two due to protons and two due to unshared electron pairs of oxygen atoms) has a chemical shift  $\delta_H = 7$  ppm (for hexagonal ice), and weakly associated water (not involved in the formation of hydrogen bonds as proton-donor) has the chemical shift  $\delta_H = 1-1.5$  ppm [10–13]. For the geometric dimensions of the adsorbed water clusters determination, the Gibbs-Thomson equation, relating the radius of the spherical or cylindrical water cluster or domain ( $R$ ) with the depression value of the freezing temperature [14,15], was used:

$$\Delta T_m = T_m(R) - T_{m,\infty} = \frac{2\sigma_{sl}T_{m,\infty}}{\Delta H_f \rho R}, \quad (1)$$

where  $T_m(R)$  is the melting temperature of the ice localized in the pores of radius  $R$ ,  $T_{m,\infty}$  is the bulk ice melting temperature,  $r$  is the solid phase density,  $s_{sl}$  is the solid-liquid interface energy and  $\Delta H_f$  is the bulk enthalpy of melting. For practical use, equation (1) can be used in the form  $\Delta T_m = (k/R)$ , where the constant  $k$  for many heterogeneous water containing systems is close to 50 degrees·nm [13]. The methodology of NMR measurements and methods of determining the radii of interfacial water clusters has been described in detail in [10–13]. At that poly-associates with a radius  $R < 2$  nm may be considered as clusters, while larger polyassociates — as domains or nanodrops as they contain several thousand molecules of water [11].

The changes in the Gibbs free energy due to the effects of the limited space and the native interface correspond to the process of freezing (melting) of bound water. The smaller are differences because freezing in

the volume; farther away from the surface is a layer of water. At  $T = 273$  K water freezes, the properties of which correspond to the bulk water, and with temperature decreasing (excluding the effect of hypothermia) the water layers, located closer to surface, freeze. For the free energy of bound water (ice) change the following relation is valid

$$\Delta G_{ice} = -0.036(273.15 - T), \quad (2)$$

where the numerical coefficient is a parameter, associated with the temperature coefficient of Gibbs free energy for ice change [19]. Determining by the magnitude of signal intensity the temperature dependence of nonfreezing water  $C_{uw}(T)$  concentration, in accordance with the procedure described in detail in [10–13], the quantities of strongly and weakly bound water and thermodynamic characteristics of these layers can be calculated.

Water interfacial energy at the interface with solid particles, or in aqueous solution was determined as the modulus of total lowering of water free energy due to the presence of the phase boundary [10–13] by the formula:

$$\gamma_s = -K \int_0^{C_{uw}^{max}} \Delta G(C_{uw}) dC_{uw}, \quad (3)$$

where  $C_{uw}^{max}$  is the total quantity of nonfreezing water at  $T = 273$  K.

## Results and Discussion

Signs of a moderate edema-swelling of encephalon are marked macroscopically (furrows smoothness, tissue flabbiness, effusion on the cut surface of a transparent liquid drops), the area of ischemic necrosis (stroke) is determined in the left parietal-temporal zone as a structureless necrotic brain tissue about 4×5 cm in size, without clear borders (Fig. 1, a, b).

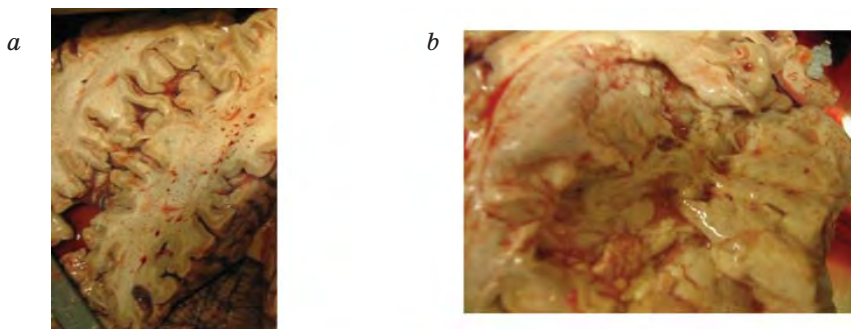


Fig. 1. Brain:

a — in health; b — an ischemic necrosis (stroke) zone without clear borders

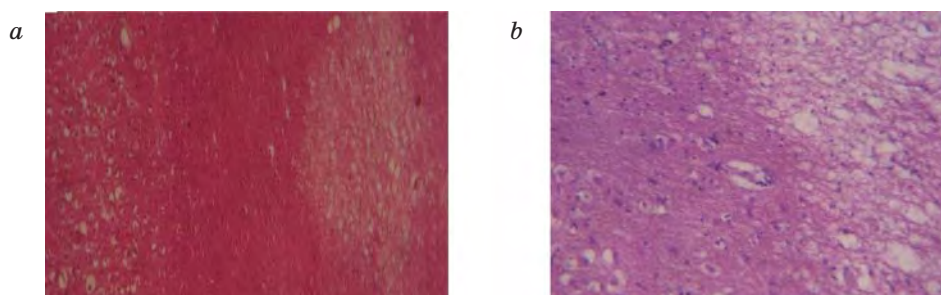
The irregular blood filling of microvasculature vessels is noted on the light-optical level at pathomorphological investigation. There are blood redistribution into plasma and blood cells and intravascular erythrocytes aggregation in some part of vessels. The moderate sclerosis and plasmatic impregnation are observed in the vessel walls. Marked perivascular and pericellular edema, moderate net edema of varying prevalence are noticed. The degenerative changes of neurocytes with varying severity dominate: a significant portion of neurons are in swollen state, with karyorhexis, as a “melting” neurons and cells — “shadows”. Extensive necrosis regions with karyolysis and neurocytolysis signs are registered. There is expressed glial cell response along the periphery of necrosis area (Fig. 2, *a, b*).

Fig. 3, *a, b* shows  $^1\text{H}$  NMR spectra of the initial brain tissue sample registered at different temperature and device sensitivity, respectively. One broad signal is observed which chemical shift changes from  $\delta_H = 4.5$  ppm at  $T = 300$  K till  $\delta_H = 5.8$  ppm at  $T = 220$  K. It may be related to the strongly associated water [8–11]. Protons of lipid structures, as of biopolymers, do not appear in the spectra due to the short time of proton relaxation. Splitting into two signals with different intensities is observed in the spectra after thawing. This can be caused as by spatial inhomogeneity of the sample as by the existence of two forms of strongly associated water with different ordering which are the highest for the signal corresponding to larger value of the chemical shift. The signal intensity decreases with temperature reduction as a result of partial water freezing.

The width of the spectrum is significantly reduced after holding the tissue pieces in chloroform medium which also could fill the gaps between the individual pieces of tissue

and penetrate into the bilipid layer of cell membranes (Fig. 3, *c, d*). Besides strongly associated water the group of signals lying in the range of  $\delta_H = 1\div 4$  ppm appear at the spectra. The most intense signal has a chemical shift  $\delta_H = 1.25$  ppm which can be caused by the lipid component. The intensity ratio of water and lipids signals shows that their quantity is 5–6 wt% relative to the total water quantity. This is consistent with the amount of lipids in the brain determined by biochemical methods [5]. The appearance of lipids' signal in chloroform presence indicates that  $\text{CDCl}_3$ , dissolving in the substance of cell membranes, transfers them from the liquid crystal state (characterized by small time of nuclear magnetic relaxation) to more mobile — liquid state. It should be noted that the signal of weakly associated water may be located in the same spectral range [8–11]. However, we can assume that this form of water is absent or its NMR signal is very low at the chosen experimental conditions.

The cell structures in the brain are associated with intercellular interactions, which are implemented by neurotransmitters, and also by hydrogen, van der Waals and other types of interactions. In previous works on studying of the hydrated hydrophilic nanosilica interaction with hydrophobic organic substances [19, 20] or lactic zoogloea cell cultures with nanosilica TS-100 [21] it was found that the stable heterogeneous structures of micron size may be formed in a wide range of component concentrations where hydrophobic and hydrophilic sites coexist without phase stratification. This method can be used for brain tissue components encapsulating by silica. Nanoparticles reduce intercellular interactions penetrating into the intercellular gaps. The changes in the characteristics of intracellular water enable to estimate the influence of intercellular interactions on the



**Fig. 2. Brain fragments:**

*a* — necrosis zone with marked pericellular edema ( $\times 100$ );  
*b* — “melting” neurons and cells — “shadows”, necrosis zone with the neurons decomposition ( $\times 200$ ).  
 Hematoxylin-eosin

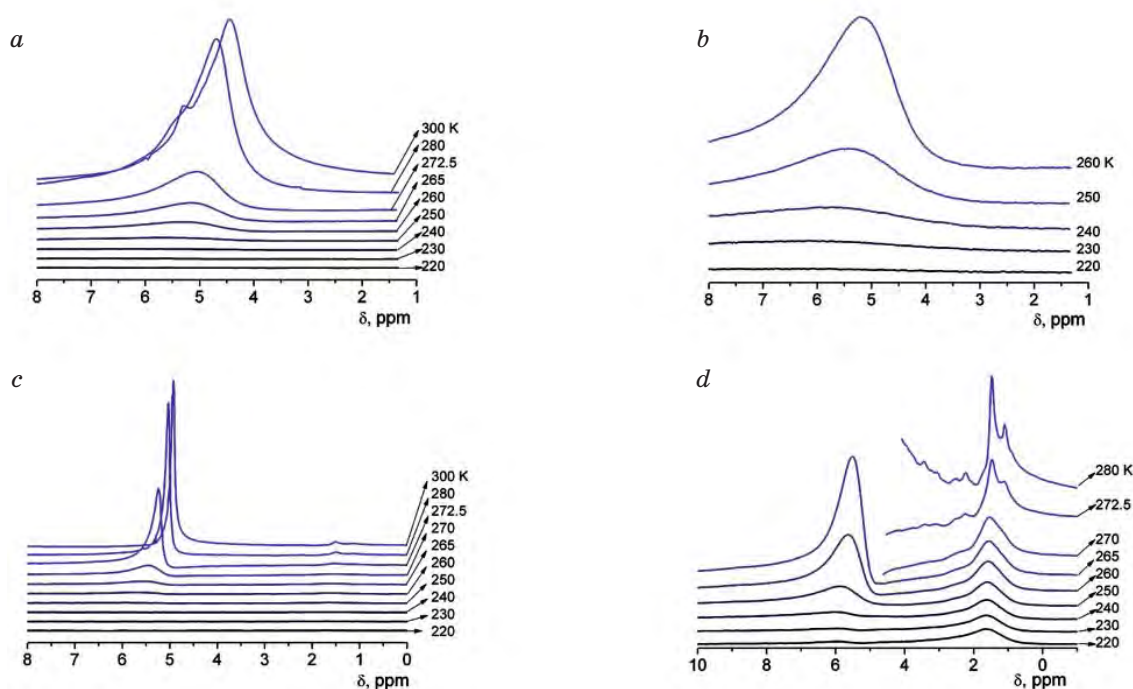


Fig. 3.  $^1\text{H}$  NMR spectra of the tissue:

at different temperature (*a, b*) and higher sensitivity of NMR spectrometer (*b, d*), native tissue sample (*a, b*) and exposed for 1 h in  $\text{CDCl}_3$  medium (*c, d*)

Note: hereinafter there are the typical experiment results.

water mostly located within cells (glia and neurons).

Fig. 4 shows registered  $^1\text{H}$  NMR spectra of brain tissue samples containing silica TS-100 in the air (*a, b*) and in  $\text{CDCl}_3$  medium (*c, d*) at different temperatures. In comparison with the similar spectra on Fig. 3, the spectra feature on Fig. 4, *a–d* is a little bit larger signals width. This complicates the registration and separation of the signals of proton containing groups belonging to the lipid component. Significant reduction of intracellular water freezing temperature indicates the clusters ( $R \leq 2$  nm) or domains ( $R > 2$  nm) formation in cells, which size is determined according to the equation 1.

It is known that acids are dissolved well in the bulk water but its solubility drops sharply at its transition into nanostructured state (when water polyassociates are less than 20 nm of size) [17, 18]. This effect could be caused by reducing of hydration energy of acid molecules in strongly associated water clusters (domains) due to the necessity for significant hydrogen bonds restructuring. Inasmuch as the proton chemical shift in acids is significantly more (11 ppm in acid) than in strongly associated water (4–5 ppm), so in the presence in cells of water polyassociates differently dissolving

TFA, several signals with different chemical shift value could be observed in  $^1\text{H}$  NMR spectra.

The addition of 20 wt% TFA into the cell mass (Fig. 4, *e, f*) results in three signals (1–3 at Fig. 4, *e, f*) of water-acid solution with different component concentrations. There is the maximal acid concentration in strongly associated water clusters, corresponding to the signal 1, a bit smaller concentration is for the clusters manifested as the signal 2. The chemical shift values for the signal 3 are the same as in the samples without TFA. As its intensity is maximal it could be concluded that a significant portion of intracellular water practically does not dissolve TFA. A redistribution of signals intensity and their displacement in the region of larger chemical shift values take place in the spectrum with temperature decreasing. Mainly that water freezes which does not contain TFA. The signal intensity of  $\text{H}_2\text{O}$ -TFA solution increases again ( $T = 210$  K) after reaching some minimal value at  $T = 220$  K. This can be related with the existence of metabolic processes (with water molecules, acid or protons participation) that occur among solution clusters located inside a cell mass and adsorbed on the surface of the solid silica particles.

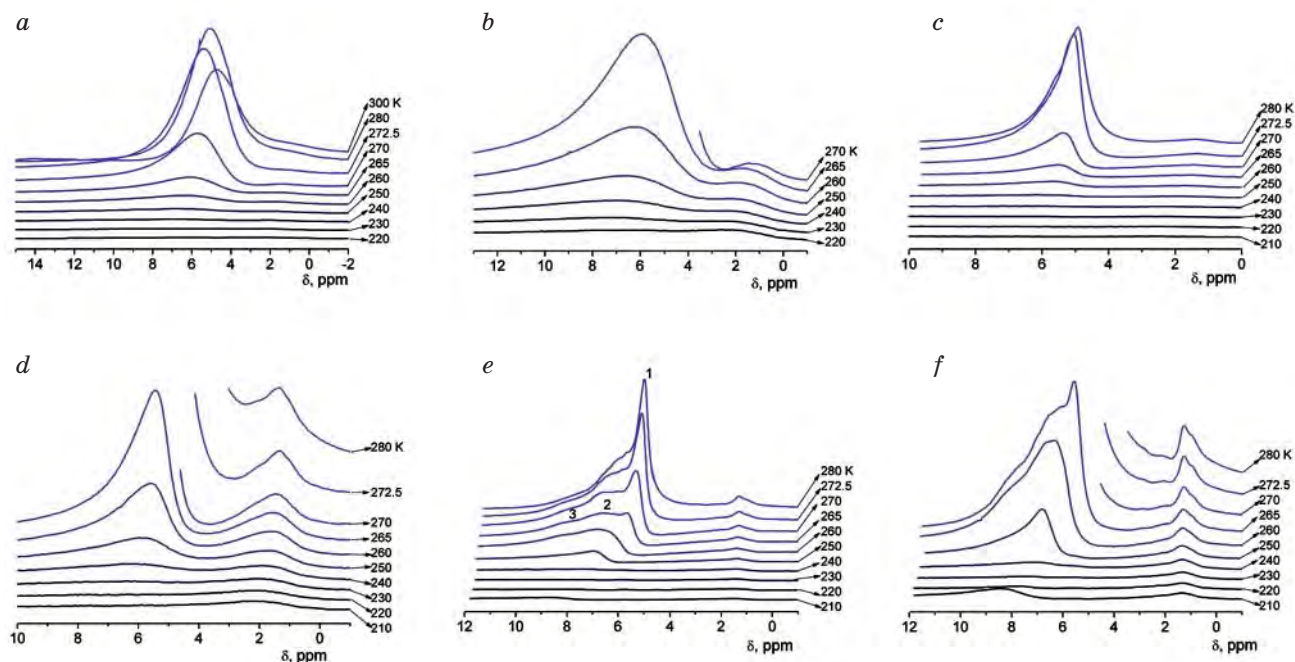


Fig. 4.  $^1\text{H}$  NMR spectra of the tissue:

at different temperatures (a, c, e) and spectrometer sensitivity (b, d, f); containing silica TS-100 in the air (a, b);  $\text{CDCl}_3$  medium (c, d) and  $\text{CDCl}_3$  medium with 20% TFA (e, f)

The layer characteristics of different forms of water ( $C_{uw}^s$  and  $C_{uw}^w$  for strongly and weakly bound water, respectively) and interfacial energy value ( $\gamma_S$ ) which characterizes the total decrease in the free energy of water, caused by the presence of phase boundaries, and calculated on the basis of changes in the concentration of nonfreezing water depending on the temperature (Fig. 5, a) and Gibbs free energy changes depending on the concentration of nonfreezing water (Fig. 5, b) are shown in the table. Fig 5, c shows the bound water clusters distribution by radii.

According to the table data, the maximum value of the free energy reduction in the layer of strongly associated water ( $\Delta G^S$ ) is practically independent on the medium, in which measurements were accomplished. The exposure of the sample in chloroform medium leads to some reduction in the contribution of strongly associated water. Respectively,  $\gamma_S$  value decreases from 36 to 28.5 J/g. Addition of TFA to organic medium results in significant  $\gamma_S$  value increasing (2 times). Probably, it is related to salvation effect of water and acid interaction, which contributes

to lowering the freezing temperature of water in aqueous acidic solution.

Water in the initial sample is presented as a system of clusters and domains, for which the maximum of the distribution responds to domains with  $R = 20$  nm. Except this there is a small maximum with  $R = 2$  nm. The exchange of the air on chloroform medium leads to decreasing of the main maximum and to increasing the total volume of domains with  $R = 100$  nm. Thus, weakly polar organic medium reduces the energy of the water interaction with the internal interfaces. Acid addition results in relative increasing of the influence of domains and clusters of smaller radius (Fig. 5, c). This is due to the ice crystallization from an aqueous TFA solution when strong intermolecular water-acid interactions hinder the formation of hexagonal ice bulk crystals.

Despite the fact that silica nanoparticles penetrate into neuroglia intercellular space and into the gaps between neurons, thereby reducing intercellular interactions, the significant increasing in the interfacial energy of associated water is observed in the

investigated brain tissue samples (table). It can be assumed, that destruction of the gel glia structure is accompanied by formation of composite silica-cells particles with low content of weakly bound water. The difference in the values of interfacial energy of the initial brain tissue and its encapsulated form should be attributed to the energy predominance in the composite formation. At the same time there

are significant changes in the characteristics of intermolecular interactions in the intercellular gaps. Accordingly, the main maximum on the distribution curve  $\Delta C(R)$  shifts toward lower values and corresponds to domains with  $R = 7$  nm (table, Fig. 5, c). The chloroform medium in the encapsulated sample stabilizes domains of larger radius. This process is accompanied by a significant decrease in  $\gamma_S$  value.

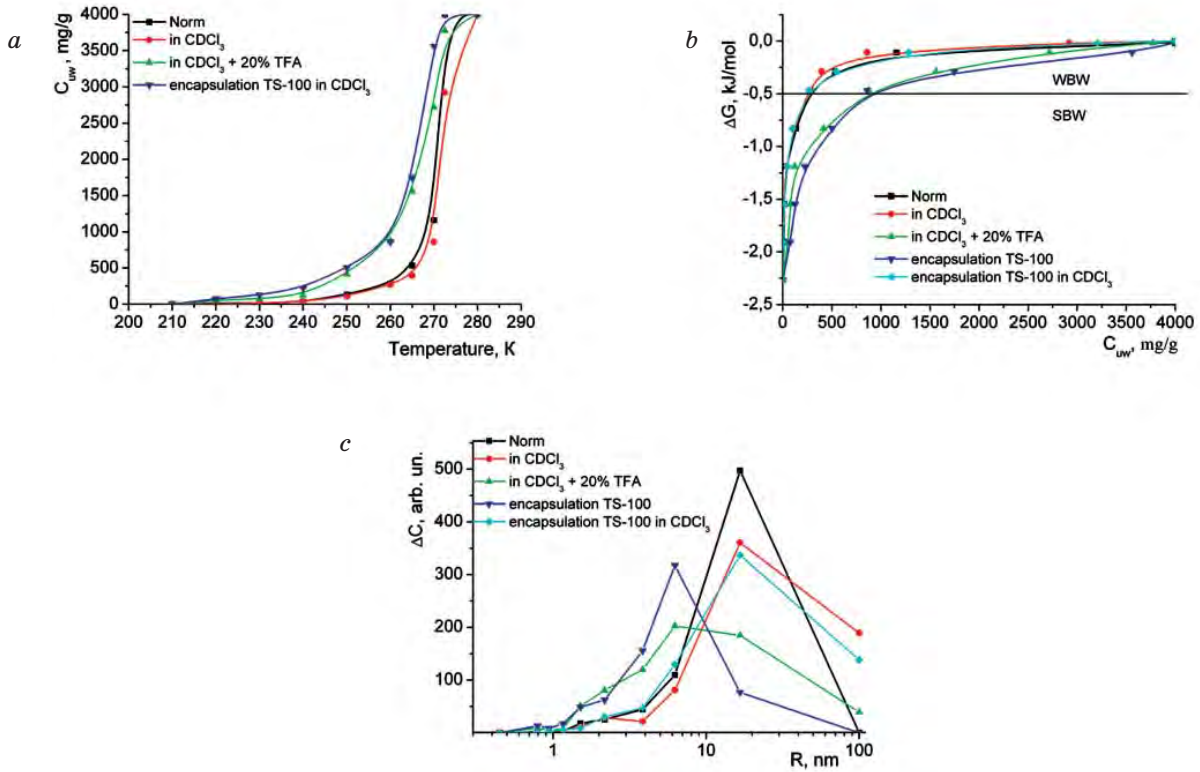


Fig. 5. The temperature dependences: a — nonfreezing water concentration; b — changes of Gibbs free energy; c — distribution of water clusters associated with brain tissue by radii

The characteristics of the water layers in the brain tissue samples in different media

Sample	Medium	$C_{H_2O}$ (mg/g)	$\Delta G^s$ (kJ/mol)	$C_{uw}^s$ (mg/g)	$C_{uw}^w$ (mg/g)	$\gamma_S$ (J/g)	
*Norm	Air	4000	-2.25	250	3750	36	
*Norm	$CDCl_3$	4000	-2.25	225	3775	28.5	
*Norm	$CDCl_3 + 20\% TFA$	4000	-2.25	900	3100	76	
Brain tissue + silica TS-100	*Norm	Air	4000	900	3100	89.5	
	*Norm	$CDCl_3$	4000	400	3600	34	
	*Norm	Air	2000	-2.8	1000	1000	66
	**Lesion	Air	2000	-2.25	500	1500	39.5
	*Norm	$CDCl_3$	2000	-2.25	1200	800	69.4
	**Lesion	$CDCl_3$	2000	-2.25	300	1700	23.5

Notes: \* — normal brain tissue; \*\* — brain tissue from the region of ischemic stroke.

Fig. 6 shows  $^1\text{H}$  NMR spectra obtained at different temperatures for the investigated brain tissue samples containing 2 g/g of bound water for samples of initial tissue and of the tissue located in the ischemic stroke zone. Measurements were performed in air (Fig. 6, *a, b*) and  $\text{CDCl}_3$  medium (Fig. 6, *c, d*). The temperature dependences of nonfreezing water concentration, changes in Gibbs free energy and clusters (domains) distribution by radii, calculated according to the equation of Gibbs-Thomson, are shown in Fig. 7, *a-c*.

For both samples, in air in  $^1\text{H}$  NMR spectra one signal is registered with the chemical shift of 5–6 ppm which corresponds to better ordering of hydrogen bonds structure in the brain in comparison with liquid water, which usually has  $\delta_H = 4,5\text{--}5$  ppm [8]. The signal of lipid component appears in the spectra in chloroform medium at  $\delta_H = 1\text{--}3$  ppm. When temperature is lowering the signals intensity decreases due to water and lipid transition to the solid state.

A comparison of the  $C_{uw}(T)$  dependences and strongly and weakly bound water amounts calculated on their basis (table) demonstrates that the dehydration leads to reduction of

weakly bound water amount while strongly bound water amount increases significantly. For the brain tissue sample obtained from the area of ischemic stroke the contribution of weakly bound water is much greater as evidenced by a comparison of the respective  $C_{uw}^s$ ,  $C_{uw}^w$  and  $\gamma_S$  values.

The partially dehydrated sample of normal tissue (Norm) has greater contribution of water clusters with a radius  $R = 2$  nm in comparison with initial sample (if compare Fig. 5, *c* and Fig. 7, *c*). The domains contribution with  $R = 20$  nm increases when placing this sample in a weakly polar medium. The presence of ischemic necrosis area leads to stabilizing of water polyassociates with radius  $R = 20$  nm, and in  $\text{CDCl}_3$  medium — with radius  $R = 100$  nm, which are characteristic for weakly bound water.

The measurements of an ability of strongly associated water polyassociates (clusters, domains) to dissolve TFA were performed for tissue samples in  $\text{CDCl}_3$  medium (Fig. 8). Addition of 20% TFA, that can be dissolved in cell water, into  $\text{CDCl}_3$  medium results in three signals (1–3) with chemical shifts in the range of  $\delta_H = 5\text{--}10.5$  ppm (Fig. 8). Taking into

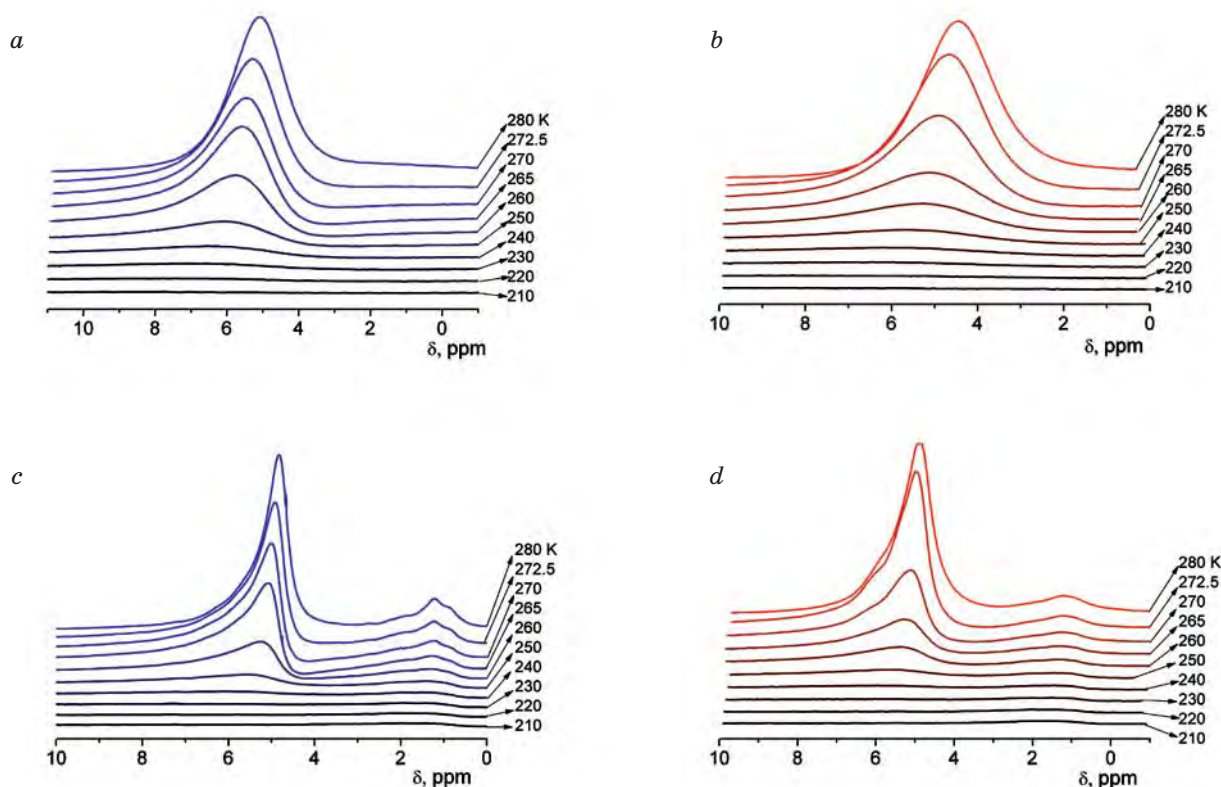


Fig. 6.  $^1\text{H}$  NMR spectra of brain tissue samples containing silica nanoparticles TS-100: for normal tissue at different temperatures (*a, c*); from the area of ischemic stroke (*b, d*) in the air (*a, b*) and in  $\text{CDCl}_3$  medium (*c, d*) at  $C_{\text{H}_2\text{O}} = 2$  g/g



account that the chemical shift of strongly bound water in brain tissue is  $\delta_H = 4.5\text{--}6$  ppm and  $\delta_H = 11$  ppm for pure TFA, it may be concluded that the signal 3 was caused by the water clusters (domains), which practically do not dissolve TFA (the TFA concentration is less than 10 wt% there); signal 2 ( $\delta_H = 7\text{--}8$  ppm) — by polyassociates, which are able to dissolve up to 30 wt% of TFA; and signal 1 — by concentrated solution of water–TFA. With temperature lowering the intensity of all signals decreases due to water freezing (likely, in the form of hexagonal ice), but at low temperatures so does — TFA.

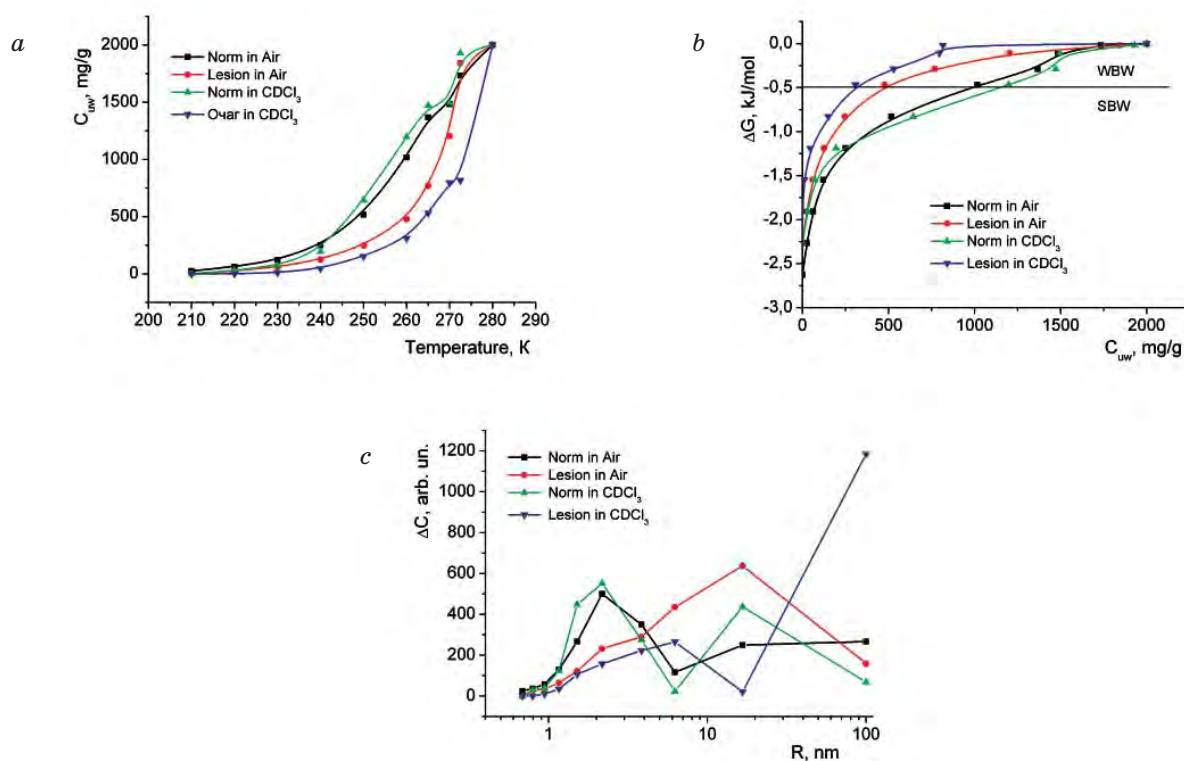
The ratio between different types of water–TFA polyassociates differs significantly for normal tissue and tissue from ischemic necrosis area (Fig. 9). In the normal tissue the largest amount of interfacial water is a part of the clusters (domains) responsible for signal 2 while signal 3 is recorded only at low temperatures. Exactly this signal is dominant in a wide temperature range in tissue damaged by necrosis. For the damaged tissue also the signal 1 has relatively lower intensity.

Thus, almost all the water, located in the brain tissue, is associated and is a part of the

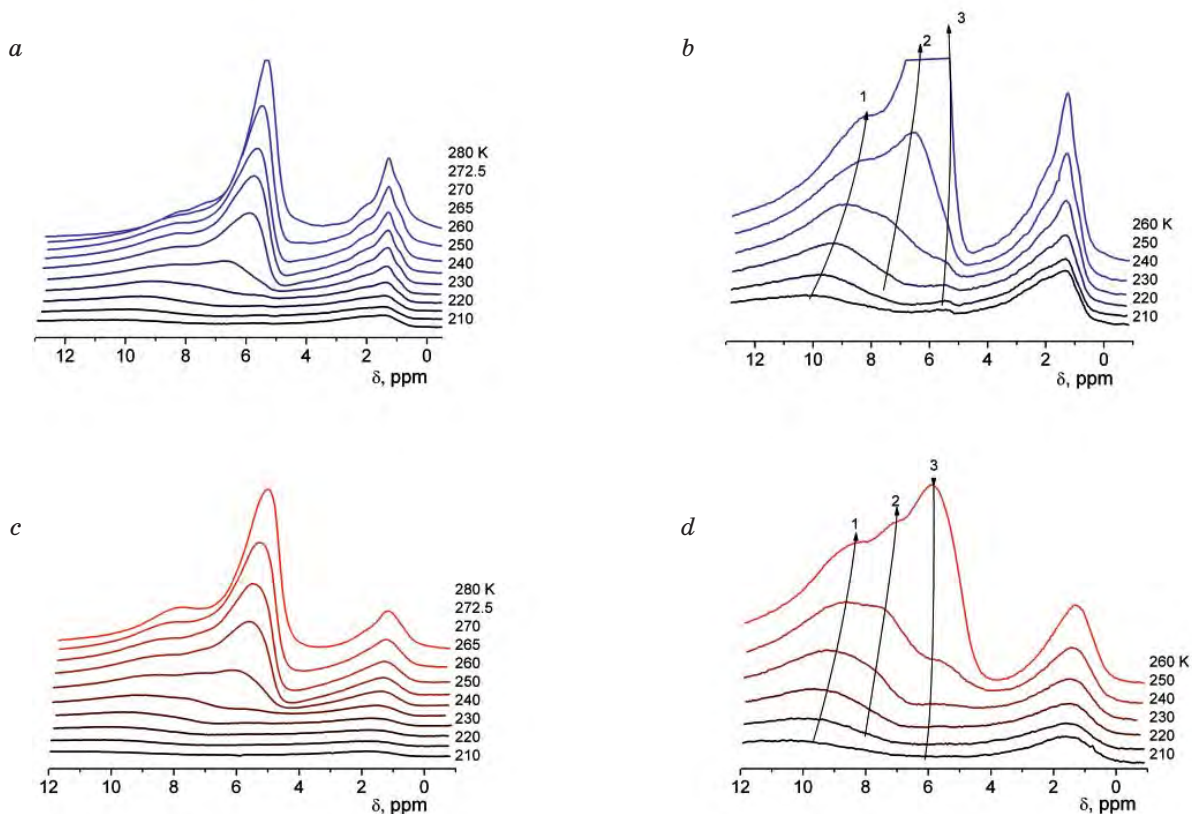
domain and clusters, significant part of which have radii  $R = 2$  and 20 nm. The chloroform medium stabilizes water polyassociates with radius less than  $R = 100$  nm, and TFA stabilizes water polyassociates with radii  $R = 7\text{--}20$  nm.

Addition of silica nanoparticles TS-100 into the brain tissue leads to formation of composite systems where parts of the tissue (and possibly separate cells) are surrounded by silica nanoparticles. This composite looks like a wet powder that facilitates to study the impact of the organic medium on the water polyassociates structure. In the composite system of brain tissue / silica the average size of water polyassociates is considerably less than in the initial tissue ( $R = 7$  nm). The average domain size increases up to 20 nm in  $\text{CDCl}_3$  medium. At least three types of domain differently dissolving acid are observed in the spectra in the presence of TFA.

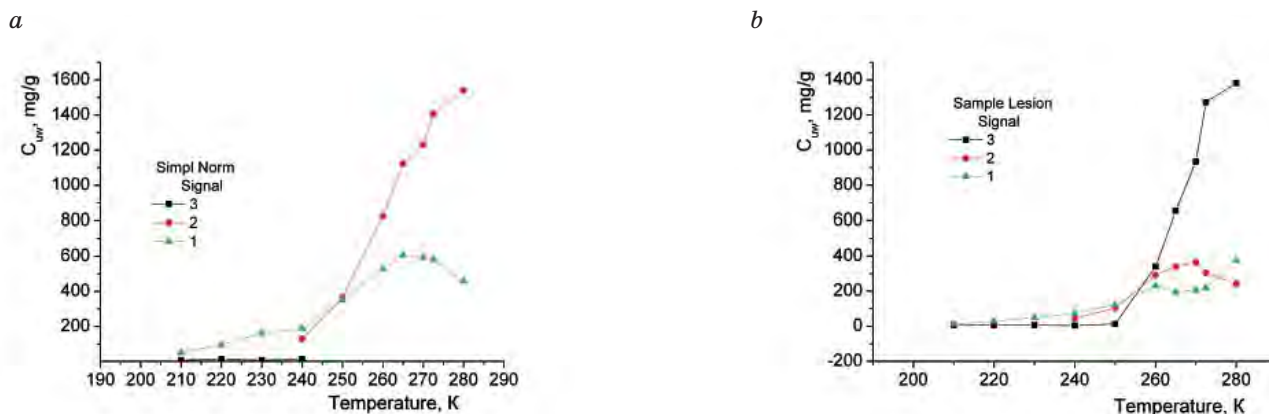
Partial dehydration of the investigated brain tissue samples is accompanied by reducing in the amount of weakly bound water and some increasing in the amount of strongly bound water, that indicates a change of molecular interactions between the components of cell-nanoparticle composite system.



**Fig. 7. The temperature dependences:** nonfreezing water concentration (a); changes in Gibbs free energy (b); and distribution by radii of water clusters (c); connected by normal brain tissue and by tissue from the ischemic necrosis area containing silica nanoparticles TS-100 (amount of residual water 2 g/g)



**Fig. 8. <sup>1</sup>H NMR spectra:**  
of brain tissue samples at different temperatures in CDCl<sub>3</sub> medium with addition of 20 wt% TFA for normal brain tissue (a, b) and for tissue from ischemic necrosis area (c, d)



**Fig. 9. The temperature dependences for water concentration, being a part of the different types of water-TFA polyassociates:**  
a — intact tissue, b — stroke

The presence of the ischemic necrosis area in the brain tissue increases the size of bound water polyassociates, which occurs as in air and in a weakly polar organic solvent (deuteriochloroform) media.

The addition of TFA in an organic medium enables to differentiate the clusters (domains)

of cell water with respect to their ability to dissolve the acid. In the normal brain tissue the bulk of the water is in a composition of polyassociates capable of dissolving up to 30% of acid. For the tissue obtained from ischemic necrosis area the bulk of the water is in the domains slightly dissolving the acid.

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## СТАН ВОДИ В ТКАНИНІ ГОЛОВНОГО МОЗКУ ЗА ПРИСУТНОСТІ НАНОЧАСТИНОК КРЕМНЕЗЕМУ TS-100

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Методом низькотемпературної <sup>1</sup>H ЯМР-спектроскопії вивчено будову гідратних шарів води, зв'язаної клітинами головного мозку, зміну цих параметрів за некротичних ушкоджень (інсульт) і за присутності трифтороцтової кислоти, що дає змогу диференціювати кластери внутрішньоклітинної води за їхньою здатністю розчиняти кислоту. Розглянуто також вплив наночастинок кремнезему TS-100 на стан води у тканині головного мозку, а саме на параметри зв'язування води на повітрі та за присутності слабополярного розчинника.

Для кластерів сильноасоційованої міжфазної води отримано розподіли за радіусами і змінами вільної енергії Гіббса. Показано, що гідратні властивості нативної тканини відрізняються від властивостей за некротичних ушкоджень будовою кластерів слабоасоційованої води. В інтактній тканині вся вода є зв'язаною і входить до складу кластерів і доменів, значна частина яких має радіуси  $R = 2$  і  $20$  нм. Середовище із хлороформом стабілізує водні поліасоціати з радіусом до  $R = 100$  нм, а трифтороцтова кислота — з радіусами  $R = 7-20$  нм. Встановлено, що часткова дегідратація досліджуваних зразків тканини супроводжується зменшенням кількості слабозв'язаної води і деяким зростанням кількості сильнзв'язаної води, що свідчить про зміну молекулярних взаємодій між компонентами композитної системи клітини–наночастинки. Присутність осередку ішемічного некрозу призводить до зменшення зв'язування води через збільшення водних поліасоціатів середніх розмірів. Цей ефект спостерігається як на повітрі, так і в середовищі слабополярного органічного розчинника — дейтерохлороформу.

**Ключові слова:** ішемічний інсульт, сильно- і слабозв'язана вода, <sup>1</sup>H ЯМР-спектроскопія.

## СОСТОЯНИЕ ВОДЫ В ТКАНИ ГОЛОВНОГО МОЗГА В ПРИСУТСТВИИ НАНОЧАСТИЦ КРЕМНЕЗЕМА TS-100

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Методом низкотемпературной <sup>1</sup>H ЯМР-спектроскопии изучено строение гидратных слоев воды, связанной клетками головного мозга, изменение этих параметров при некротических повреждениях (инсульт) и в присутствии трифтороуксусной кислоты, позволяющей дифференцировать кластеры внутриклеточной воды по их способности растворять кислоту. Рассмотрено также влияние наночастиц кремнезема TS-100 на состояние воды в ткани головного мозга, а именно на параметры связывания воды на воздухе и в присутствии слабополярного растворителя.

Для кластеров сильноассоциированной межфазной воды получены распределения по радиусам и изменениям свободной энергии Гиббса. Показано, что гидратные свойства нативной ткани отличаются от свойств при некротических повреждениях строением кластеров слабоассоциированной воды. В интактной ткани вся вода является связанной и входит в состав кластеров и доменов, значительная часть которых имеет радиусы  $R = 2$  и  $20$  нм. Среда с хлороформом стабилизирует водные полиассоциаты с радиусом до  $R = 100$  нм, а трифтороуксусная кислота — с  $R = 7-20$  нм. Установлено, что частичная дегидратация исследуемых образцов ткани сопровождается уменьшением количества слабосвязанной и некоторым ростом количества сильносвязанной воды, что свидетельствует об изменении молекулярных взаимодействий между компонентами композитной системы клетки–наночастицы. Присутствие очага ишемического некроза приводит к уменьшению связывания воды за счет роста водных полиассоциатов среднего размера. Этот эффект наблюдается как на воздухе, так и в среде слабополярного органического растворителя — дейтерохлороформа.

**Ключевые слова:** ишемический инсульт, сильно- и слабосвязанная вода, <sup>1</sup>H ЯМР-спектроскопия.