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Water structure in fungi *Amanita muscaria* and their composite system with hydrocompacted nanosilica A-300

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The state of water in the fruit body of the *Amanita muscaria* fungus and its composite with “wetting-drying” nanosilica A-300 was studied by low-temperature ¹H NMR spectroscopy. It was found that even in the initial biomaterial, part of the water is in a weakly associated state. After drying, the portion of weakly bound water decreases, but in the medium of CDCl₃, almost all water becomes weakly bound. This effect is accompanied by a threefold increase (from 3.4 to 10.3 J/g) of the total binding energy of water which is probably due to the transition of water from the system of spherical (cylindrical) clusters to a two-dimensional film, uniformly distributed over the surface of the biomaterial. It is suggested that, due to the binding by the surface of the silica from the composite, the toxic substances present in the mushroom in the form of complexes with enzymes will not be desorbed.

1. INTRODUCTION

The fungi *Amanita muscaria* (red *Amanita*) are classified as poisonous fungi. They are toxic because a large number of toxic

substances bound by enzymes are present in them, such as chlorocroto glycine and 2-hexadien-4,5-oic acid. Both are derivatives of glycine which is capable of inducing an inhibitory effect on the activity of certain enzymes [1, 2]. Previously *Amanita muscaria* was used for ritual purposes to push consciousness to the border state [3], which is due to the presence of such psychotropic substances in mushrooms as ibotenic acid and muscimol, isolated from different types of *amanitas* more than half a century ago [4, 5]. A detailed analysis of the literature on biologically active substances present in *Amanita muscaria* is given in [6]. Currently it is used not only in folk but also in traditional medicine for the treatment of such serious diseases as epilepsy, atherosclerosis, varicose veins, etc.

A promising trend in the use of *Amanita muscaria* for both internal and external applications can be its inclusion in the composition of nanocomposite systems based on amorphous, highly dispersed silica dioxide [7]. This type of silica dioxide is non-toxic, practically insoluble in biological fluids, and in some cases promotes the assimilation of medication immobilized on its surface, interacting with it through adsorption interactions [8, 9]. At the same time, it can be expected that toxic substances that are bound with *Amanita muscaria* enzymes will not be desorbed from the nanocomposite system due to the high protein-sorbing ability of nanosilica dioxide [7, 8] while bioactive substances of low and medium molecular weight will be delivered to the intestinal mucosa. Previously it was shown that when nanosilica gets in contact with hydrophobic phospholipid fragments of the intestine, a part of the water adsorbed in the interphase gap can be transformed into a weakly associated state [10] which contributes to the increase of solubility of weakly polar substances in the aquatic environment, and, consequently, the bioavailability of substances adsorbed on the surface of silica dioxide nanoparticles.

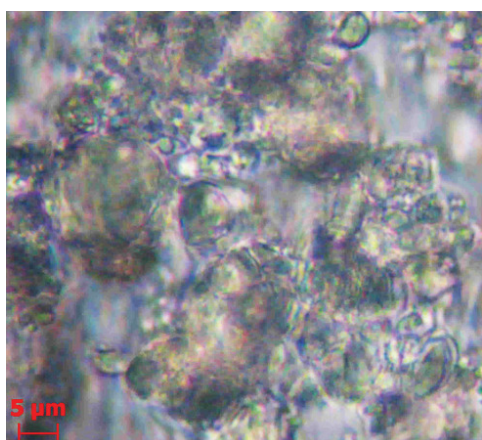
2. EXPERIMENTAL

The purpose of this work was to study the water binding energy and the structure of water in the fungi *Amanita muscaria*, the initial ones dried to a residual moisture of 120 mg/g and contained in the composition of the nanocomposite with silica dioxide A-300 after its hydro-compaction [11] to a bulk density of $P_d = 200$ mg/ml. As the main method of testing, low-temperature ^1H NMR spectroscopy [8, 12, 13] was chosen, and it allows to draw conclusions about the structure of the interphase water

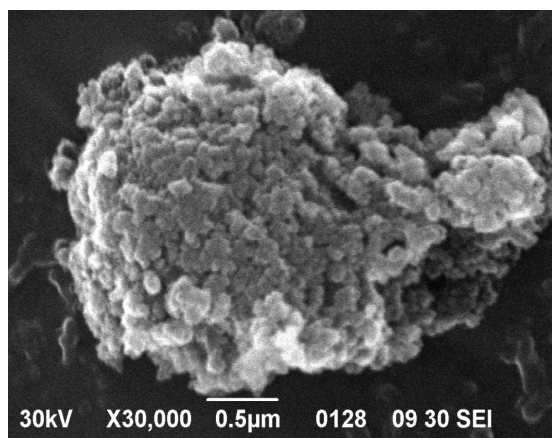
based on the magnitude of the chemical shift of the interphase water (δ_H) protons (the average number of hydrogen bonds to which each water molecule contributes), and from the temperature dependence of the concentration of non-freezing water ($C_{uw}(\Delta T)$), the change in the free Gibbs energy (ΔG) [13] and the distribution along the radii of clusters of adsorbed water $R(\Delta C)$ in accordance with the Gibbs-Thomson equation ($\Delta T = (k/R)$) [14] can be calculated.

3. RESULTS AND DISCUSSION

In Fig. 1a a microphotography image ($\times 100$, transmittance, Primo Star (Zeiss, Germany)) of the nanocomposite, 1:9 hydrocompacted silica dioxide A-300 and of the dried material of fungus *Amanita muscaria* is presented. The composite was prepared by homogenizing the mixture of mineral and bio-components in a ball mill for 4 hours. As it can be seen from the figure, fungal particles, 5-10 μm in size, are coated with much smaller silica dioxide particles. The type of obtained particles indicates a high affinity of the biomaterial for the silica dioxide surface. Electronic microphotography images (SEM JSM-6490-LV, JEOL, Japan, Img. 1c-d) show that the outer surface of the composite particles is formed mainly by nanosilica particles that completely cover the fungal particles. In some case periodicity of the structure can be observed (Figures 1c, d), which is probably due to the structure of the biological component of the composite.



a)



b)

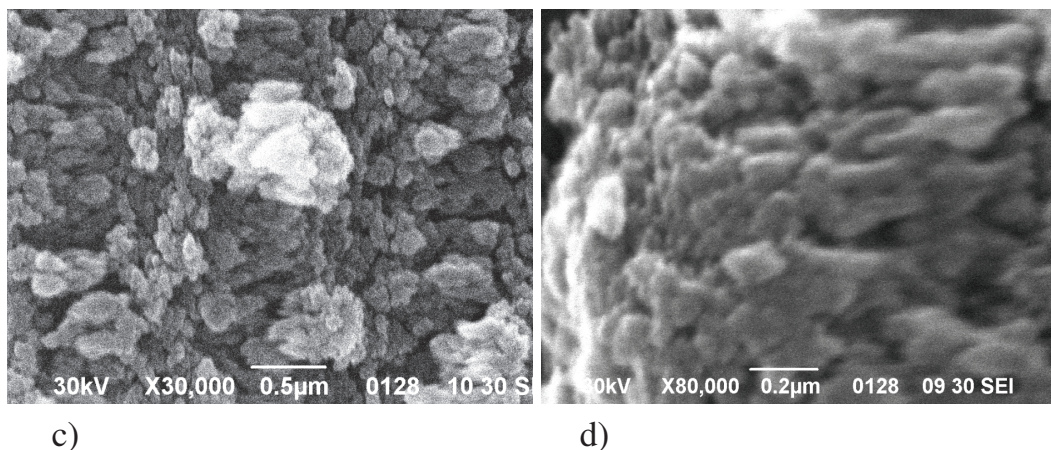
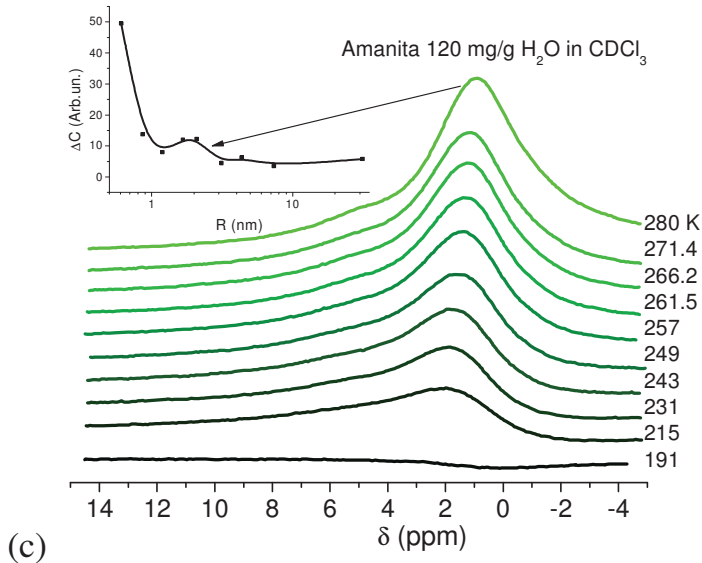
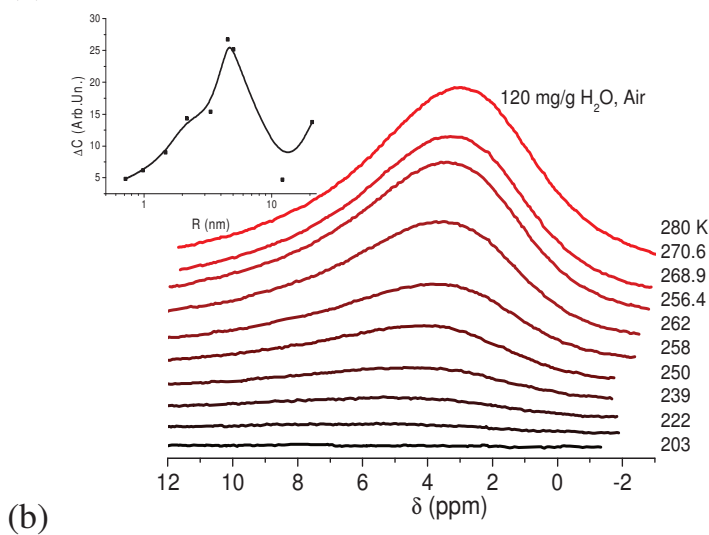
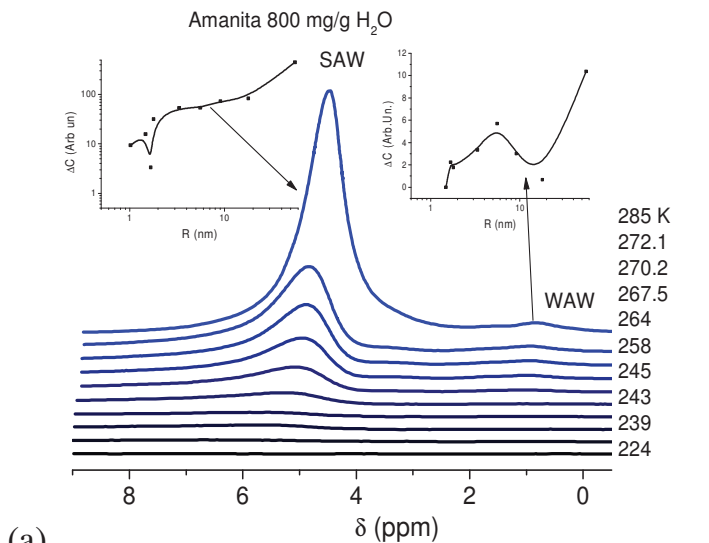
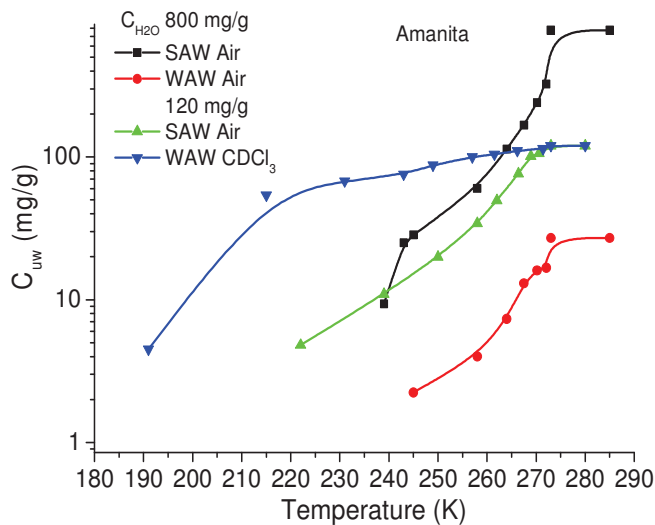


Fig. 1. Optical (a) and SEM-micro photography (b-d) of a 9:1 composite of hydrocompacted nanosilica A-300 and crushed material of the fungus *Amanita muscaria*.

The composition of the most species of lamellar fungi, which includes *Amanita muscaria*, is similar to one another. Freshly picked mushrooms contain about 90% of water and after slight drying in the air this amount decreases to 80% of weight. The dried biomaterial contains 20-30% of polysaccharide and about the same amount of protein components. The amount of fats does not exceed 5% of weight. Fungi can also contain a few percent of sugars (lures). Dry material is very hygroscopic and easily absorbs water from the air. If the fungus material retains its elasticity, it contains 20-25% of residual water.

Fig. 2a shows the ^1H NMR spectra of water in the fungus pulp, containing 80% of the water, taken at different temperatures (during the thawing of samples preliminarily cooled to $T = 200\text{ K}$). (Varian "Mercury" with an operating frequency of 400 MHz and a bandwidth of 100 kHz when a 90° probe pulse of $3\ \mu\text{s}$ duration is used). Spectra record two main signals of which the more intense one can be identified as a signal of strongly associated water (SAW), its molecules take part in the formation of 2-3 hydrogen bonds, and less intense one as a weakly associated water that practically does not participate in the formation of hydrogen bonds [8, 13]. The temperature dependencies of the concentration of non-freezing water, calculated from the intensities of the water signal, assuming that at $T > 273\text{ K}$ $C_{uw} = 800\text{ mg/g}$, are presented in Fig. 2d. With the decrease of temperature, the intensity of signals of both types of water (and consequently, C_{uw}) decreases due to its partial freezing.





(d)

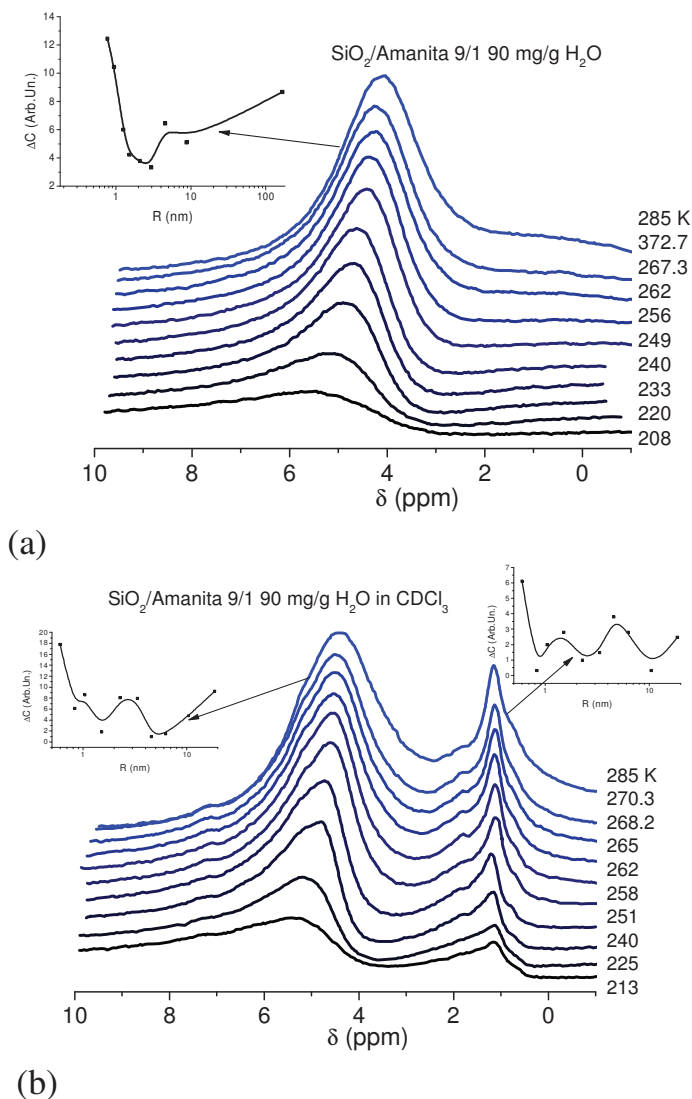
Fig. 2. The ^1H NMR spectra of water adsorbed in the tissues of the fungus *Amanita muscaria*, containing 800 mg/g water (a), 120 mg/g water (b, c) in the aerial medium (b) and in a medium of a weakly polar organic solvent – deuteriochloroform (c), recorded at different temperatures, as well as temperature dependencies of the concentration of different forms of non-freezing water (d).

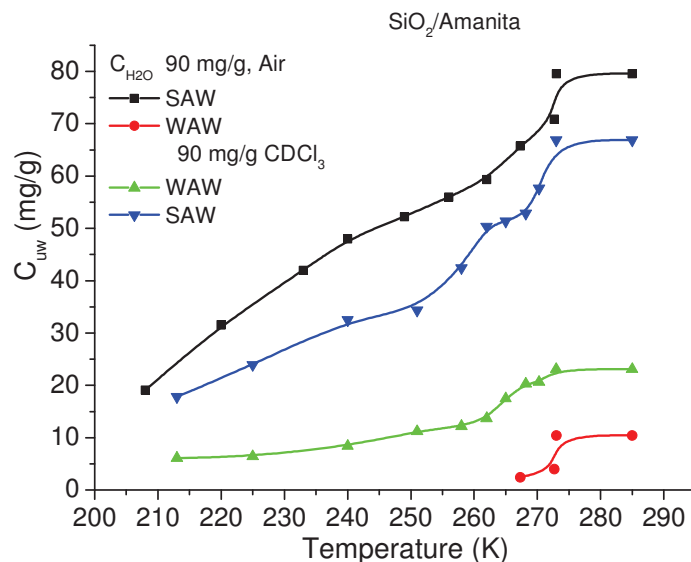
The figures are also shown for each signal of water distribution along the radii of clusters of water absorbed by the fungal tissues, calculated in accordance with Eq. of Gibbs Thomson. It can be seen that in the initial material, the bulk of the water (more than 70%) is included in clusters the radius of which exceeds 10 nm. During the distribution the maxima are observed at $R = 6$ and 14 nm for weakly associated water (Fig. 2a).

After drying the material (up to $c_{\text{H}_2\text{O}} - 120$ mg/g), the shape of the spectra changes (Fig. 2b). The width of the signal increases, probably because the mobility of the residual water molecules decreases, and the chemical shift slightly decreases which may be due to a decrease in the degree of association of the residual water [13], and the weakly associated water ceases to be detected in the NMR spectra. At the same time, water forms part of clusters, the main part of which has a radius $R = 5$ nm.

Upon ingestion into the gastrointestinal tract, the biomaterial particles may contact with both hydrophilic and hydrophobic areas of the mucosal surface. The latter are mainly represented by phospholipid structures and fats. To simulate contact with hydrophobic areas there can be used weakly polar organic solvents, in particular chloroform with the dielectric constant ($\epsilon = 4$) close to that of many weakly polar hydrocarbons.

Fig. 2c shows the spectra of partly dehydrated material of fungus *Amanita muscaria* in the medium of CDCl_3 , taken at different temperatures. It can be seen that a contact with a weakly polar medium results in a sharp decrease in the chemical shift of absorbed water. Thus its significant part is passed from a strongly to weakly associated state. This process is accompanied by a decrease of freezing temperature caused by a decrease in the radius of adsorbed water clusters (Fig. 3c, d).





(c)

Fig. 3. The ^1H NMR spectra of water adsorbed in the composite system of $\text{SiO}_2/\text{Amanita}$, containing 90 mg/g water (a), and in a medium of a weakly polar organic solvent - deuteriochloroform (b), recorded at different temperatures, as well as temperature dependencies of the concentration of different forms of non-freezing water (c).

Since the layer of weakly-associated water is formed in the gap between the solid particles and the weakly polar medium and makes a surface stabilized concentrated solution of water in a weakly polar medium, the reduction of the linear dimensions of the water structures can be associated with the transition of water from a system of spherical clusters of different diameters into a thin film of a solution, the thickness of which varies depending on the relative position of the solid particles of the biomaterial. Then the radius R in Fig. 2c can refer to the thickness of the layer in the chloroform adjacent to the surface of the water solution.

Similar but less pronounced processes of transformation of the interphase structure water can be observed in the composite system consisting of 9 parts of nanosilica and one part of the biomaterial of the fungus *Amanita muscaria*. Figures 3a and b show the ^1H NMR spectra of the water in the composite $\text{SiO}_2/\text{Amanita}$, containing 90 mg/g of absorbed water in the air medium in Fig. 3a and in the medium of CDCl_3 (b) at different temperatures. In the aerial medium, the concentration of weakly associated water is low and can be recorded only at $T > 237$ K. Upon contact with a weakly polar medium, the amount of weakly associated water increases sharply (Fig. 3c). The linear dimensions of clusters of

absorbed (or adsorbed) water change as well (Figures 3a and b). If in the air the maximum radius of clusters of water structures can reach 100 nm, then in a chloroform medium it decreases to 10 nm. For the weakly associated water, the distribution along the radii of water structures has a complex form with several maxima in the range $0.5 < R < 12$ nm.

Fig. 4 shows the dependence of the free Gibbs energy change on the concentration of non-freezing water, obtained from the equation $\Delta G_{ice} = -0.036(273.15 - T)$, which relates the decrease in the freezing point of water in the adsorption layer to the adsorption interactions and the change in the free Gibbs energy for ice (ΔG_{ice}) [8,12,13].

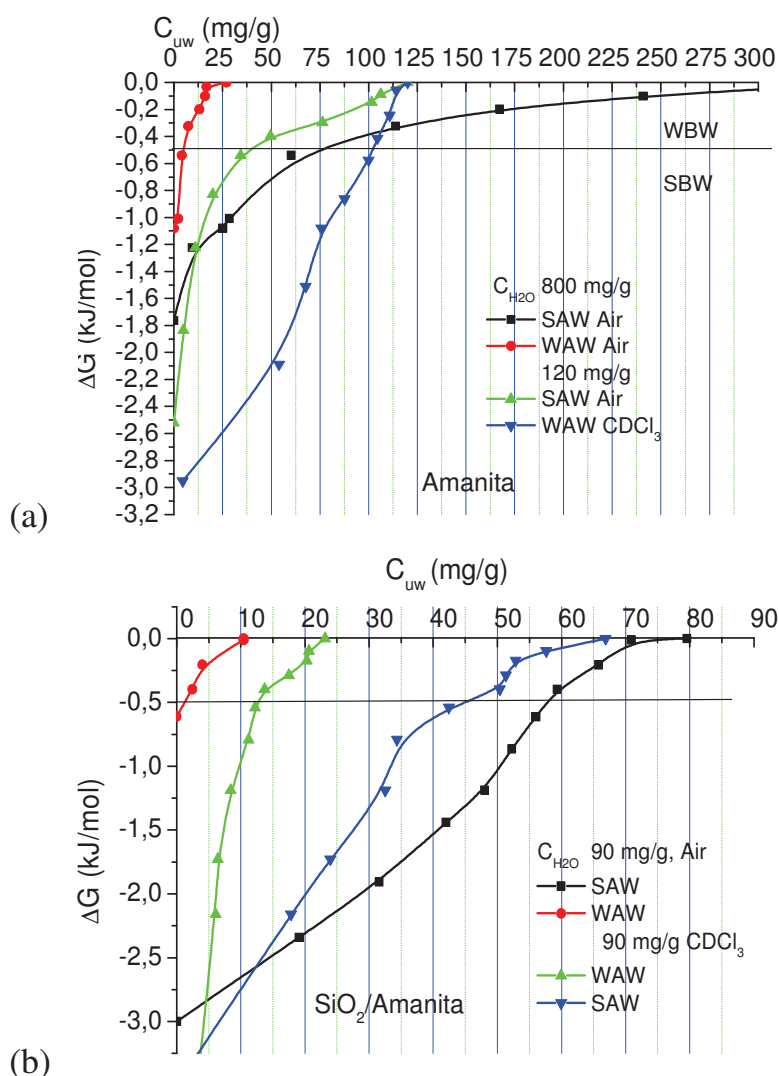


Fig. 4. Dependencies of change in the Gibbs free energy on the concentration of non-freezing water for the fungus *Amanita muscaria* with different hydration of biomaterial (a) and composite system $SiO_2/Amanita$ (b).

Assuming that the weakly bound water is that part of the interphase water that melts at $T > 265$ K ($\Delta G < -0.5$ kJ/mol), the amount of weakly bound (WBW) and strongly bound (SBW) water can be calculated for each type of water. The area limited by the graph of the dependence $\Delta G(C_{uw})$ and the abscissa axis determines the interphase energy (γ_s) as the total change in the free energy of water due to interphase interactions. The thermodynamic parameters of the interphase water layers in the studied systems are summarized in Table. 1.

Table 1. Characteristics of non-freezing water layers in the fungus *Amanita muscaria* with different hydration of the biomaterial and composite system $\text{SiO}_2/\text{Amanita}$.

Sample	C_{H_2O} mg/g	Medium	Type of water	C_{uw}^s (mg/g)	C_{uw}^w (mg/g)	G^s (kJ/mol)	γ_s (J/g)
<i>Amanita</i>	775	–	SAW	75	700	–1.7	1.2
	25	–	WAW	5	20	–1.1	0.4
	120	Air	SAW	37	83	–2.6	3.4
	120	CDCl_3	WAW	100	20	–3	10.3
$\text{SiO}_2/\text{Amanita}$	80	Air	SAW	57	23	–3	6.3
	10	Air	WAW	3	7	–0.75	0.13
	22	CDCl_3	WAW	12	10	–5	1.8
	68	CDCl_3	SAW	45	23	–3.5	4.8

In the initial biomaterial of the fungus *Amanita muscaria*, almost the whole water is in a bound state, however, only 12% of water present in the tissues of the fungus is strongly bound. The amount of weakly associated water is small and amounts to 25 mg/g, of which the majority (20 mg/g) also refers to loosely bound water. After the sample is dried, the fraction of strongly bound water increases to 30% in the aerial medium and up to 80% in the medium of CDCl_3 (table 1). In this case, the interphase energy increases almost three times – from 3.4 to 10.3 J/g. Such a high growth of water binding energy is probably due to its transition from the clustered state in air to the state of a nanoscale two-dimensional film in a medium of CDCl_3 . The difference in the value of γ_s determines the energy gain from such transformation.

During the formation of the composite SiO₂/*Amanita* this effect disappears because most of the interphase water interacts with the surface of the nanosilica dioxide particles, the amount of which substantially exceeds that of the biomaterial. However, in the composite system, contact with the hydrophobic medium increases the amount of weakly associated forms of water from 3 to 12 mg/g with the total amount of water in the composite C_{H₂O} = 90 mg/g as well.

4. CONCLUSIONS

It is proposed to use the fungus *Amanita muscaria* for the medical use as part of a nanocomposite system with amorphous, highly disperse silica dioxide. At the same time it is assumed that the main toxic components bound by the enzymes in the fruit bearing body of the fungus will be adsorbed by the surface of silica dioxide, which has a high affinity for the protein molecules and, together with it, is excreted from the body.

It is shown that the water contained in the fungus or its composite with nanosilica can be in strongly and weakly associated states. The latter is stabilized by contact with a weakly polar medium, which simulates the hydrophobic part of phospholipid structures and the intestinal mucosa. It is suggested that in the body this effect will increase the bioavailability of the active substances desorbed from the composite system.

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