

COMPOSITE SYSTEMS FOR MEDICAL APPLICATIONS BASED ON METHYL METHYLSILICA AND BIOLOGICALLY ACTIVE SUBSTANCES

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Abstract. The possibility of creating nanocomposites for medical applications based on hydrophobic silica biologically active substances of plant origin is considered. A simple method for wetting with water of hydrophobic silica powder or its mixtures with hydrophilic substances is described. It has been shown that for the composite system based on methylsilica (MS) and grape seed extract (Enoxil) the solid hydrophobic component reduces the binding of water to a very hydrophilic biomaterial due to the transition of a part of water to interparticle gaps MS, with which the water interaction is significantly less. Herewith a liquid hydrophobic medium (chloroform) increases the energy of water binding due to reducing the radius of the adsorbed water clusters. For the supplement Lymphosilica, created on the basis of milled medicinal plants and hydrophilic silica, the amount of bound water is several times higher than water binding energy with both hydrophilic and hydrophobic silica. This is probably due to the retention of a significant part of the water in the space formed by cellulose fibrils. It was concluded that by incorporating additives of hydrophobic silica or a liquid hydrophobic medium into the composition of composites, one can control the free energy of water binding in a wide range. This can be used to create oral and transdermal medicinal preparations.

Keywords: ¹H NMR spectra, silica, methylsilicas, Enoxil, nanocomposites, Lymphosilica, medicinal herbs.

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1. Introduction

Organosilicon polymeric substances (Blunder *et al.*, 2011; Moretto *et al.*, 2005; Silicone Polymers 2, 2003) are classified as biologically inert, therefore in the form of silicones [R₂SiO]_n they are widely used in medicine as the basis for implants, hydrophobic liquids, polymers, elastomers, and also as parts of medical devices that have been contacting with tissues of living organisms for a long time. The hydrophobic properties of the surface of such materials can be controlled by changing the synthesis conditions of polymer products (Hood *et al.*, 2017). One of the ways to create highly dispersed organosilicon substances is chemical modification of pyrogenic silica (Bergna, 2005; Tertykh & Belyakova, 1991). As a result, non-porous highly dispersed materials with a hydrophobic surface are obtained. They are not moistened with water, but getting on the water surface they do not sink and can be on its surface indefinitely due to air adsorption in interparticle gaps. Meanwhile, methylsilicas (MS) are promising carriers in complex biocomposite systems, allowing delivering biologically active substances to the mucous membrane of the intestinal tract by oral supplementation.

Possessing lipophilic properties, MS particles have a high affinity for phospholipid structures of the mucous membrane, which allows significantly increasing the time of their contact, and, consequently, the bioavailability of bioactive substances immobilized on their surface.

For effective usage, composite systems containing MS should be transferred to the aquatic medium. Earlier, a simple and technologically easy feasible method was developed for partially or complete wetting out of hydrophobic powders with water, both initial MS and composite systems based on it (Gun'ko *et al.*, 2018a; Gun'ko *et al.*, 2018b; Turov *et al.*, 2018). It has been found that under the influence of mechanical loads (for example, grinding in a mortar, center former, processing in a ball mill). It has been found that under the influence of mechanical loads (for example, grinding in a mortar, roll milling, processing in a ball mill) with water addition, the volume of which is comparable to the volume of internal voids in the aggregates of the hydrophobic material, then after some time the air is replaced by water in the interparticle gaps. The composite looks like a dense, moist powder and upon further wetting with water completely passes into the aqueous medium, where it is in the form of a precipitate or suspension. If the composite is created on the basis of biologically active substances, it might be used as a medicinal agent for oral supplementation.

Thus, the aim of this work was juxtaposition energies of binding water to the surface of the MS and hydrophilic silica A-300, and as well as composites containing hydrophilic biologically active substances immobilized on the surface of the MS, that are included as a compound of a mixture of ground up medicinal plants (composite "Lymphosilica") or grape seed extract (preparation "Enoxil", Moldova). The principal difference between these composites was that in the case of the MS/Enoxil system, biologically active substances are in direct contact with MS particles, while in the MS/Lymphosilica composite, the active substances interact with the surface of solid particles during their desorption from the cellulose matrix.

2. Materials

Methylsilica AM1-300 was chosen as the highly dispersible hydrophobic component, with specific surface area $S_{\text{BET}} = 285 \text{ m}^2/\text{g}$, produced on research experimental plant of Chuiko Institute of Surface Chemistry, Kalush, Ukraine. It is produced by replacing the surface hydroxyl groups of hydrophilic silica A-300 with methyl ones as a result of chemical modification (Tertykh & Belyakova, 1991). The composition of the supplement "Lymphosilica" (LimfSil) includes wetting-drying highly dispersed silica A-300 (Hydrosil, TU U 20.1-3291669-015:2016) and ground up medicinal plants – *Hibiscus sabdariffa*, *Calendula officinalis*, *Calluna vulgaris*, *Trifoliumpratense*, *Echinacea purpurea*, *Taraxacum officinale*, *Elytrigia répens*. Silica A-300 was compacted to the bulk density $\rho_d = 300 \text{ mg}/\text{cm}^3$ in a dry state in accordance with the methodology, described in (Krupska *et al.*, 2016; Krupskaya *et al.*, 2018). After an addition to the dry composite 1200 mg/g distilled water, its bulk density increased to $\rho_d = 470 \text{ mg}/\text{cm}^3$. To prepare the composite system, containing hydrophobic component (AM1 with bulk density $\rho_d = 50 \text{ mg}/\text{cm}^3$) equal or half the weight of MS was added to the weighed amount of dry composite of Lymphosilica. After that the mixture was thoroughly mixed in a porcelain mortar and distilled water was added in an amount of 1200 mg/g of dry mixture. The wet powder was subjected to high mechanical loads (intensive rubbing with a porcelain pestle) until a homogeneous compact mass with a

bulk density $\rho_d = 760 \text{ mg/cm}^3$ was formed. Then the samples were isolated from the air and placed in 5 mm ampoules for NMR spectroscopic studies.

Mechanical activation of hydrophobic AM1 and hydrophilic silica A-300 was also carried out. The transfer of initial MS to the aquatic medium was carried out by chafing equal quantities of AM1 and water in a porcelain mortar using a large mechanical load for 20-30 minutes. As a result, a layer of water was formed in the gap between the pestle and the mortar, into which the MS powder was getting. The air was pressed out of it, which was modified by water. After 10-15 minutes powder density increased, and after 20-30 minutes it, together with water, formed a dense compact mass on the walls of the mortar, which was removed from the surface with a spatula. The initial density of the compacted composite AM1/water was about 1000 mg/cm^3 , but with light grinding and putting in the 5 mm ampoule of NMR it slightly decreased and amounted to 700 mg/cm^3 . Thus, the characteristics of water filling the interparticle space of hydrophobic and hydrophilic silica, the bulk densities of which in the wet state were $\rho_d = 700$ and 600 mg/cm^3 , water content $h = 1000$ and 1125 mg/g , correspondingly were compared.

SEM images of hydrophobic silica AM1 initial (*a,b*) and MS, compacted by water up to the bulk density $\rho_d = 1000 \text{ mg/cm}^3$ (*c,d*) are shown in Fig. 1.

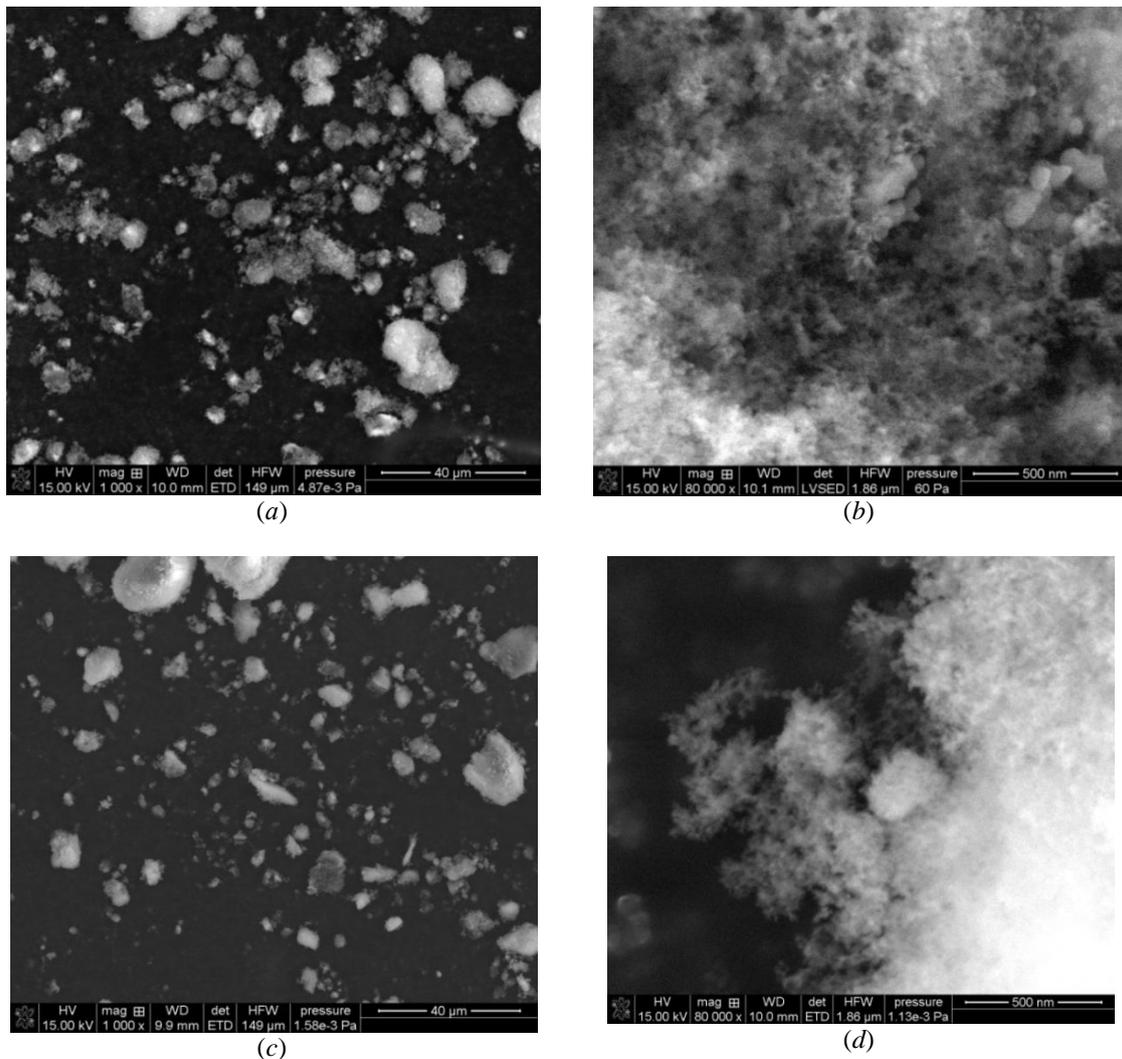


Figure 1. SEM images of initial (*a,b*) and wetting-drying (*c,d*) methylosilica AM1

Despite a significant (from 50 to 1000 mg/ml) change in bulk density, the type of aggregates of MS and their surface changed slightly.

Optical microphotos Lymphosilica obtained by intensive chafing with an equal by weight amount of methylsilica (AM1) and 1200 mg/gH₂O are shown in Fig. 2. As a result of mechanical activation, homogeneous composite systems are formed, in which the particles of plant material and silica aggregates constitute a compact mass and they are in a state of close physical contact. Herewith a homogeneous composite system is formed, the color of which is mainly determined by the presence of *Hibiscus sabdariffa* flowers.

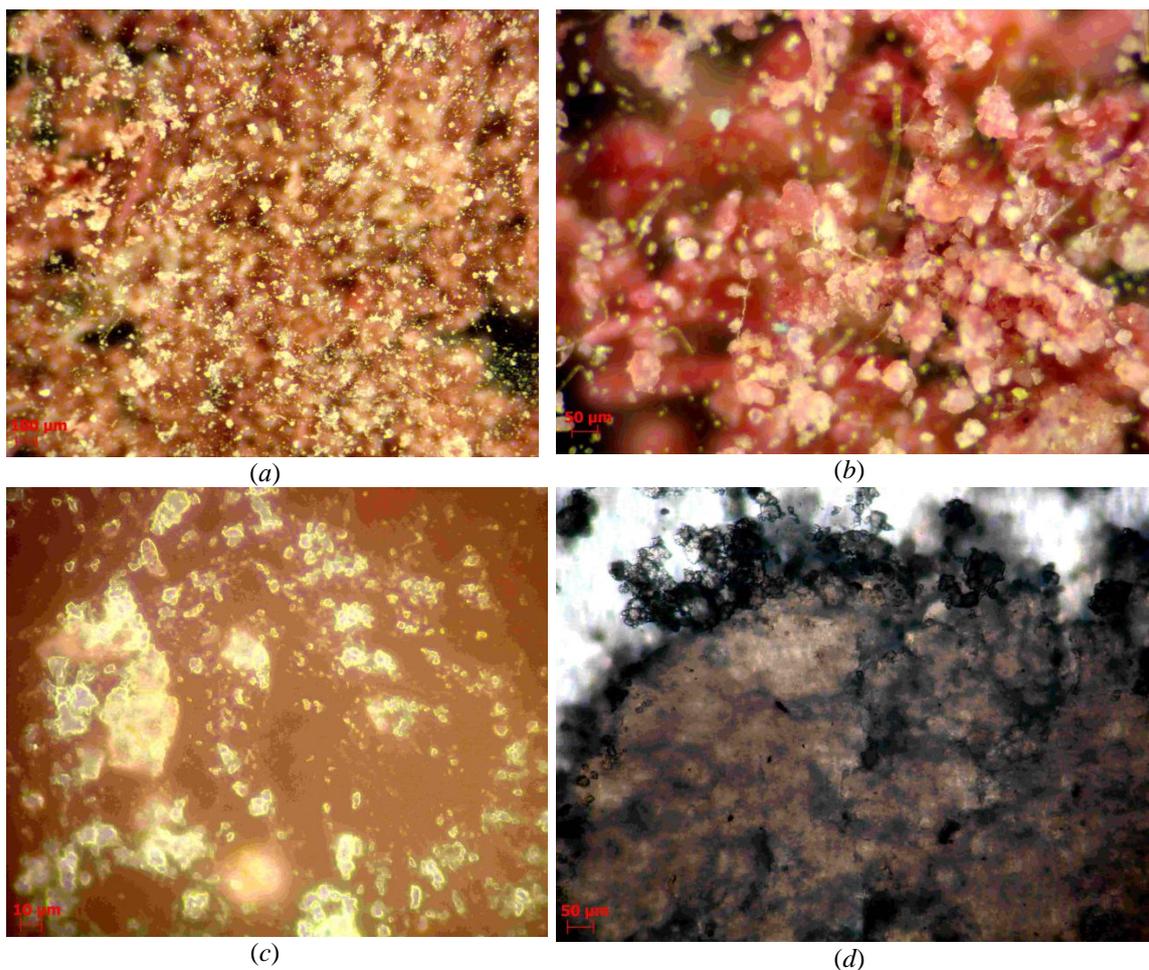


Figure 2. Optical microphotos of composite system at a ratio of component concentrations 1:1, obtained by mechanical activation of the mixture of composite “Lymphosilica” with AM1 taken in modes of reflection (a-c) and transmission (d)

The micrographs of composite system MS/Enoxil, taken with modes of transmitted (a) and reflected (b) light are demonstrated in Fig.3. The composite particles are present in the form of large aggregates with the size reaching to several tens of microns. In addition, relatively small aggregates of MS, with the size of 5-20 microns are visible on images.

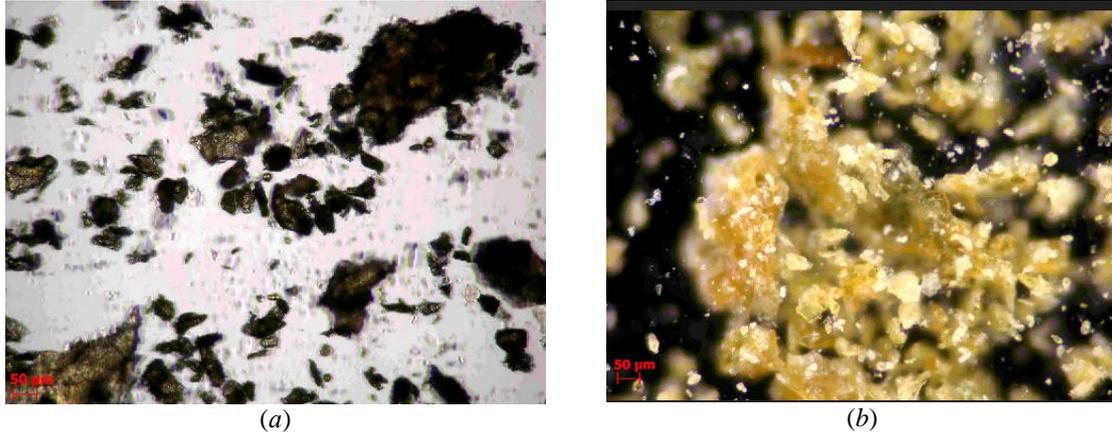


Figure 3. Optical micro photos of composite system MS/Enoxil, taken in transmitted (a) and reflected (b) light.

Technique of measurement

The ^1H NMR spectra were recorded using a Varian 400 Mercury spectrometer of high resolution with an operating frequency of 400 MHz. Eight probing 60° impulses of $1\ \mu\text{s}$ duration were used with a bandwidth of 20 kHz. The temperature was controlled by means of a Bruker VT-1000 device with an accuracy of ± 1 deg. The signal intensities were determined by measuring the area of the peaks using the procedure for decomposing the signal into its components under the assumption of a Gaussian form of signal and optimizing of the zero line and phase with an accuracy that was not less than 5% for well-resolved signals and $\pm 10\%$ for overlapping signals. To prevent super cooling of the studied systems, the measurements of the amounts of unfrozen water were carried out on heating of samples preliminarily cooled to 210 K. The method for determining of thermodynamic characteristics and calculating the radius of interfacial water clusters by means of ^1H NMR spectroscopy has been described in detail elsewhere (Gun'ko & Turov, 2013; Gun'ko *et al.*, 2005; Gunko *et al.*, 2009).

Here with, to determine the geometrical dimensions of nanoscale aggregates of liquid limited by solid surface, the Gibbs – Thomson equation (Thermodynamic properties of individual substances, 1978) can be used, connecting radius of spherical or cylindrical pores (R) with the value of the depression of freezing temperature :

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

where $T_m(R)$ is melting temperature of ice in the pores (voids) of radius R , $T_{m,\infty}$ – melting temperature of bulk ice, ρ – density of the solid phase, σ_{sl} – the energy of the solid-liquid interaction (for example, via hydrogen bonds), ΔH_f – the bulk enthalpy of melting.

The process of freezing (melting) of interfacial water, localized in a solid porous matrix, takes place in accordance with changes in the Gibbs free energy due to the influence of the surface. The farther the studying layer of water is from the surface, the less the influence is. At $T = 273\ \text{K}$ water freezes, the properties of which do not differ from ones of bulk water, and as the temperature decreasing (without taking into account the effect of super cooling), the water layers, that are located closer to the surface, freeze. Thus, for interface water the ratio is valid:

$$\Delta G_{\text{ice}} = -0,036(273,15-T), \quad (2)$$

where the numerical coefficient is a parameter, associated with the temperature coefficient of variation of the Gibbs free energy for ice (Petrov & Furó, 2009). Determining the concentration of unfrozen water as a function of temperature $C_{\text{uw}}(T)$ by the signal intensity, in accordance with the technique detailed in (Gun'ko & Turov, 2013; Gun'ko *et al.*, 2005; Gunko *et al.*, 2009), the amount of strongly and weakly bound water and the thermodynamic characteristics of these layers can be calculated.

The interfacial energy of solids or biopolymers was determined as a modulus of the total decrease in the free energy of adsorbed water, due to the presence of an internal water-polymer interface by the formula:

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

where $C_{\text{uw}}^{\text{max}}$ – total amount of unfrozen water at $T = 273$ K.

The magnitude of the interfacial energy is a convenient parameter that allows you to compare the energy of water binding in different systems, especially if the amount of water in them is the same.

The ^1H NMR spectra were recorded in air and deuteriochloroform media, initial and with addition of small amount of acetone $\text{C}_2\text{H}_6\text{CO}$, as a standard for chemical shift ($\delta_H = 2$ ppm). There are instances where some amount of deuterotrifluoroacetic acid was added to the deuteriochloroform. The use of deuterium preparations was carried out in order to avoid the presence of extraneous intense signals in the spectra.

Optical microphotos were obtained by means of a Primo Star microscope (Zeiss, Germany) in reflected and transmitted light, with magnification of $\times 100$.

A FEI Nova NanoSEM (5 kV) scanning electron microscope (SEM) was used to study silica samples. The morphology was also analyzed using a DualBeam Quanta 3D FEG FEI apparatus under conditions of low vacuum at an accelerating voltage of 2–10 kV.

3. Results and discussion

In air medium (Fig. 4,*a,b*) the adsorbed water spectra in the form of single signals are observed, which intensity decreases with temperature reducing caused by the partial freezing of interfacial water. For the sample with greater bulk density signal width is significantly larger due to the lower mobility of adsorbed water molecules (Abragam, 1961) or the presence of several signals with similar values of chemical shift. Range of chemical shifts change – from $\delta_H = 4.5$ ppm at $T = 285$ K to $\delta_H = 7.5$ ppm at $T = 205$ – 216 K indicates that water is in a strongly associated state, when each molecule simultaneously takes part in the formation of 2-3 hydrogen bonds (Gun'ko & Turov, 2013).

In a weakly polar organic CDCl_3 medium (Fig. 4,*c*), a group of signals appears in the spectra in the region $\delta_H = 0$ – 1.5 ppm. They can be caused by the appearance at the interface of MS or Enoxil with chloroform some amount of weakly bound water (Gun'ko & Turov, 2013), which practically does not participate in the formation of hydrogen bonds or the dissolution of organic substances in chloroform including in Enoxyl composition and containing aliphatic groups.

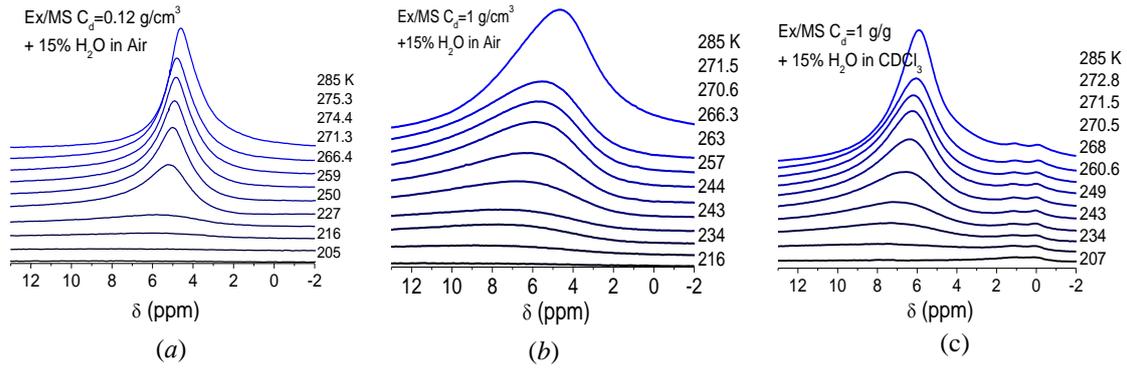


Figure 4. ^1H NMR spectra (recorded at different temperatures) of water in composite system Enoxil/MS/ H_2O with bulk density $\rho_d = 0.12$ and 1 g/cm^3 (a) and (b), correspondently, in air and CDCl_3 media $\rho_d = 1 \text{ g/cm}^3$ (c)

Assuming, that water content in all samples is the same and equal $C_{\text{H}_2\text{O}} = 150 \text{ mg/g}$, by change in the SAW signal intensity during the heating of the samples the temperature dependences of nonfreezing water concentration ($C_{uw}(T)$) and the changes in the Gibbs free energy ($\Delta G(C_{uw})$) can be calculated in accordance with formulas (1) and (2) that are demonstrated in Fig. 5,a in coordinates $\Delta G(T)(C_{uw})$ and the size distributions of water clusters ($\Delta C(R)$, Fig. 5,b). Concentration of strongly and weakly bound water (C_{uw}^S and C_{uw}^W , correspondently), and maximum reduction of Gibbs energy in a layer of strongly bound water (ΔG^S) can be calculated, using the data of Fig. 5,a (Gun'ko & Turov, 2013). In this case, weakly bound water (WBW) is considered that part of the water that freezes at $T > 265 \text{ K}$ ($\Delta G > -0.5 \text{ kJ/mol}$) (Krupska *et al.*, 2016). The interfacial energy (γ_S), is defined as the area limited by the dependency graph ($\Delta G(C_{uw})$). This is a total weight characteristic that determines the change in the free energy of water due to the presence of the interface with a solid substance. Juxtaposition of interface energies for the studied systems is shown in the diagram in Fig. 5,c. For comparison, the γ_S value of Enoxil containing 500 mg/g of water taken from work (Turov *et al.*, 2016) is also given.

Composite systems Enoxil/MS/ H_2O , differing in bulk density have a different structure. So, with joint chafing of equal amounts of Enoxil and MS powders in a mortar, a mixture is formed, in which micron-sized particles of a biological product are separated by methyl silica particles. Then, with addition 150 mg/g of water to the system, it is distributed between the hygroscopic Enoxil particles, and the hydrophobic particles of methyl silica do not allow them to stick together and form the bulk phase of the hydrophilic component. On the contrary, in the compacted (up to 1000 mg/cm^3) composite under the influence of high mechanical loads, the penetration of hydrophobic particles occurs in the aqueous phase of Enoxil solution (Turov *et al.*, 2016). Herewith a homogeneous composite is formed in which a concentrated solution of Enoxil is located in the gaps between the hydrophobic particles of the MS and interacts with them by forming Van der Waals bonds.

As a result of the formation of the compacted Ex/MS/ H_2O composite, the γ_S value decreases, despite a significant increase in its bulk density. This is due to the redistribution in cluster sizes of adsorbed water. In a compacted sample, the contribution from water clusters having the radius of $R > 20 \text{ nm}$ increases (Fig. 5,b). Comparing the interfacial energies for the initial Enoxil/ H_2O and the Ex/MS/ H_2O

composite system with a bulk density of $\rho_d = 120 \text{ mg/cm}^3$ (Fig. 5,c), it can be seen that the composite γ_s is almost twice smaller, due to primarily the different amount of adsorbed water.

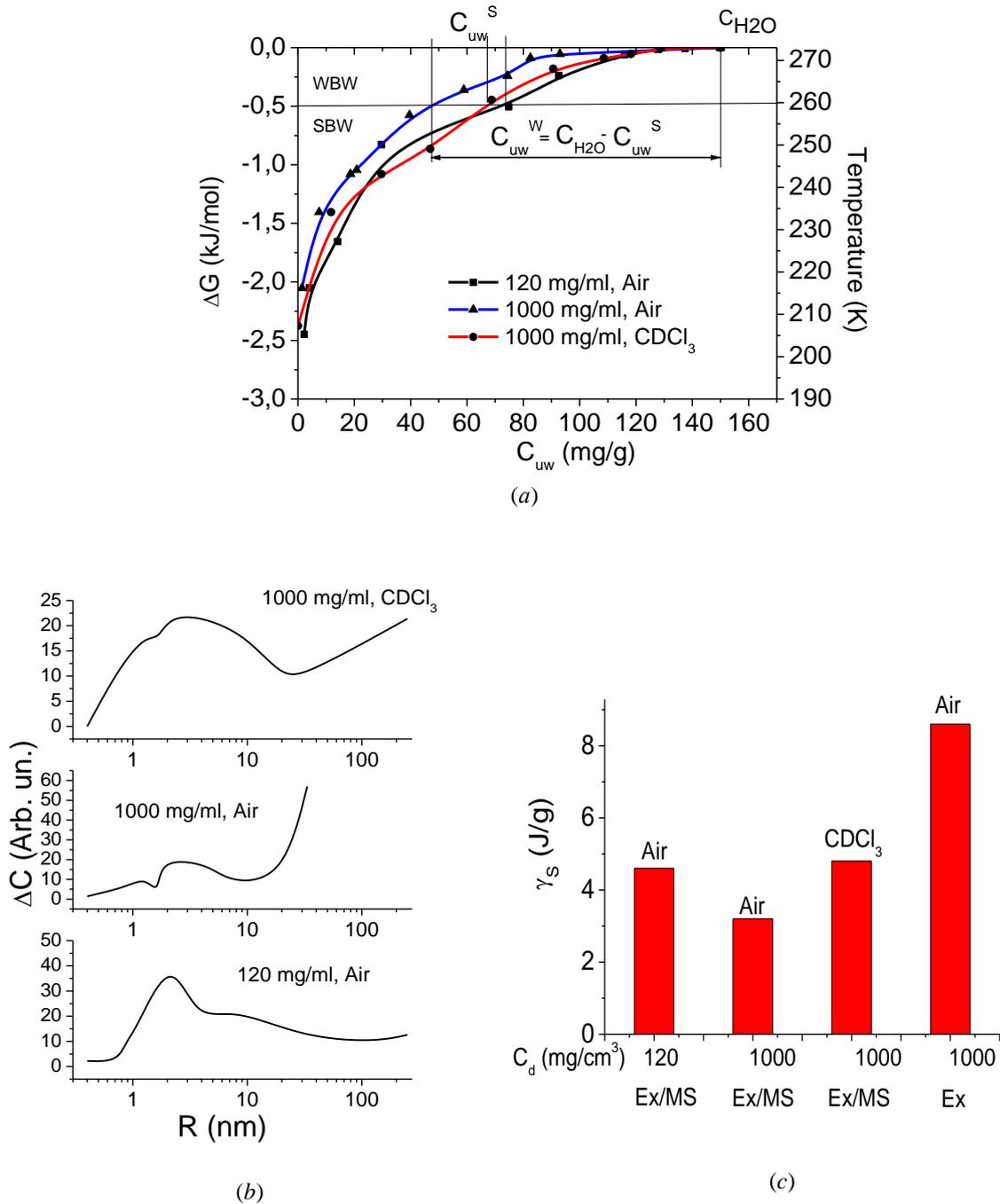


Figure 5. Dependences of the concentration of nonfreezing water C_{uw} on temperature T and dependences of change in the free Gibbs energy ΔG on C_{uw} (a), distribution of adsorbed water structures over the size R (b) and diagram of changes in interfacial energy (c) for systems based on Enoxil and MS.

For the compacted composite Ex/MS/ H_2O , the hydrophobic medium of chloroform (Fig. 5) leads to a noticeable increase in the amount of water binding in the composite system. This is due to an increase in the contribution from water clusters with

$R < 10$ nm. The likely reason is the diffusion of chloroform over the surface of the hydrophobic particles of MS, as a result of which the solution of Enoxil is dispersed into smaller cluster formations.

Fig. 6 demonstrates the results of investigation of composite system Lymphosilica/H₂O and its mixture in the ratio 1/1 and 2/1 with methyl silica MS, and as well as hydrophilic (A-300) and hydrophobic (MS) silicas in air and CDCl₃ media by ¹H NMR spectroscopy.

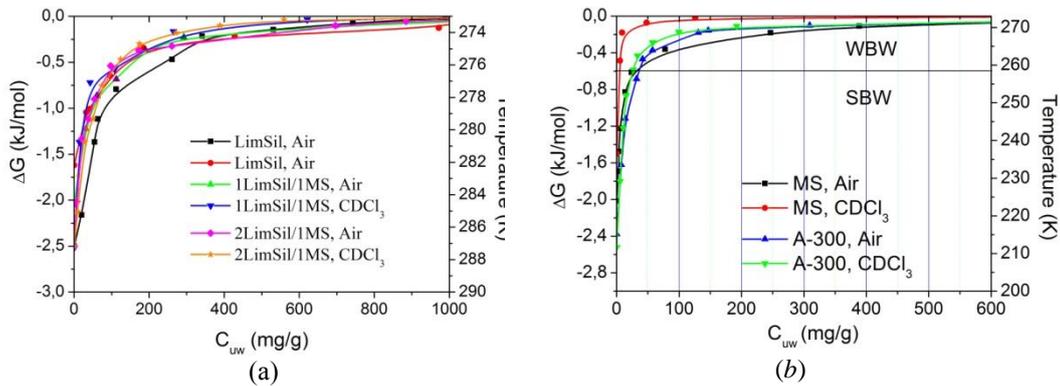


Figure 6. Dependences of the concentration of nonfreezing water C_{uw} on temperature T and dependences of change in the free Gibbs energy ΔG on C_{uw} in coordinates $\Delta G(T)(C_{H_2O})$ for composite systems based on Lymphosilica (a) in comparison with initial hydrophilic and hydrophobic silicas (b)

For a visual analysis of the effect of the composite composition on the water binding by it under the similar conditions of hydration of their surface, in fig. 6 shows a diagram of the change in γ_s and distribution over the radii of adsorbed water clusters.

The interfacial energy of water associated with hydrophobic or hydrophilic silica was significantly less than with Lymphosilica, and this is true for both the air and chloroform media, calculated in accordance with formulas 1-2. From this we can conclude that the plant component is responsible for the main part of water binding.

The maximum values of $\gamma_s = 19.9$ J/g are fixed for the composite Lymphosilica in air. In CDCl₃ medium it reduces to 17.5 J/g. Addition to the composite hydrophobic silica leads to decrease of interfacial energy by 3-4 J/g (Fig. 7,a). More stronger this effect is manifested in chloroform, where the decrease of γ_s reaches 7 J/g. Comparing the values of the interfacial energy with the size distributions of adsorbed water clusters, we can conclude that the chloroform medium or the presence in the composite of a solid hydrophobic component results in increasing of the probability of the formation of water clusters with smaller radius. Thus, for the Lymphosilica composite in air, a significant part of water is included in clusters with a radius of $R = 20-100$ nm, but for the same sample in a liquid hydrophobic medium, a maximum at $R = 9$ nm is observed in the $\Delta C(R)$ distribution (Fig. 7,b). An addition of a solid hydrophobic component (hydrated MS) into the composite leads to a similar result. In this case, one maximum is also observed in the $\Delta C(R)$ distribution, and there are practically no clusters with $R > 50$ nm.

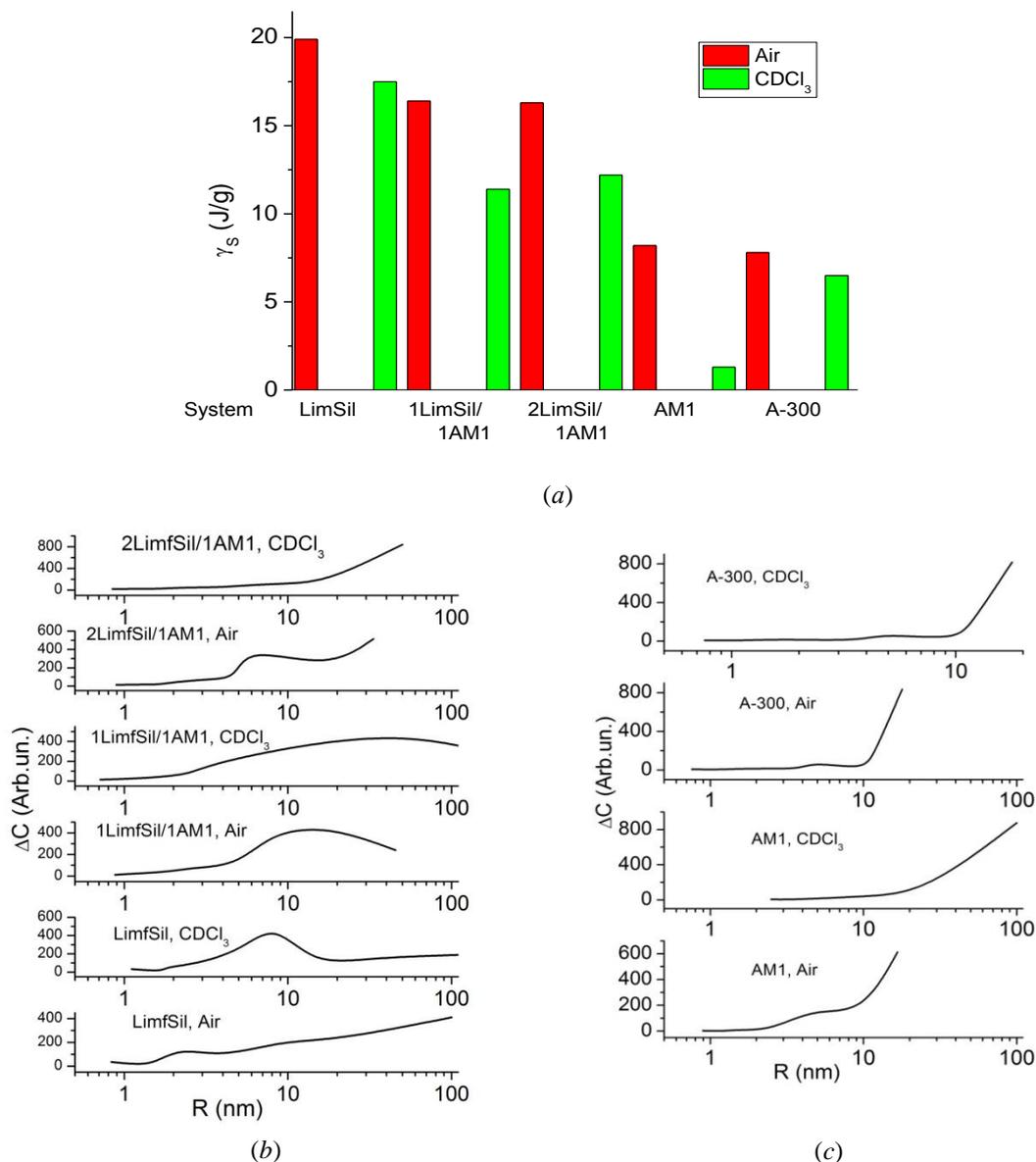


Figure 7. Diagram of changes in the interfacial energy of water vs the composition of the composite (a) and the distribution of adsorbed water clusters over the size R for composite systems based on the Lymphosilica (b), as well as hydrophilic and hydrophobic silica (c)

Liquid hydrophobic medium increases the contribution from small clusters of adsorbed water. Consequently, using solid and liquid hydrophobic additives allows us to regulate the energy of water binding in composite systems based on medicinal plants and mineral adsorbents in a wide range.

The mechanism of influence of hydrophobic agents on the binding of water in ground up medicinal plants may be different. Using many highly dispersed materials it has been shown that the medium of liquid weakly polar substances reduces the interaction of water with the surface due to the penetration of liquid into the gaps between the clusters of adsorbed water and the surface, which decreases the possibility of water clusters to interact with the primary centers of water adsorption (Turov &

Gun'ko, 2011). For a multi-component system such as Lymphsilica, this effect of hydrophobic liquids can be expected for its highly dispersed oxide components. The influence on the plant component may be more complex, since the water binding in the space between the cellulose fibers (Hatakeyama *et al.*, 1988) depends on the interaction of hydrophilic silica particles with them (Shulga *et al.*, 2001).

4. Conclusion

Depending on the type of composite system based on nanosilica and its hydrophobic analogs, the magnitude and mechanism of the influence of hydrophobic agents on the water binding are varied. So, replacement of compacted hydrophilic silica with methyl silica, which was transferred to the aquatic medium by mechanical loading, the water binding to a hydrophobic surface is almost the same as for hydrophilic one. However, for MS, a decrease in water binding under the influence of a liquid hydrophobic medium is several times greater due to probably the penetration of the liquid phase of chloroform in the interparticle gaps filled with water. In this case, the average radius of adsorbed water clusters decreases somewhat.

For the composite system MS/Enoxil, the solid hydrophobic component reduces the water binding to a very hydrophilic biomaterial due to the passage of part of the water to interparticle gaps of the MS, with which the water interaction is less. In this case, a liquid hydrophobic medium (chloroform) increases the energy of water binding by reducing the radii of the adsorbed water clusters.

For Lymphsilica composite based on milled medicinal plants and hydrophilic silica, the quantity of binding water is several times greater than its binding energy with both hydrophilic and hydrophobic silicas. This is probably due to the retention of a significant part of the water in the space formed by cellulose fibrils.

Using additives of hydrophobic silica or a liquid hydrophobic medium, it is possible to control the free energy of water binding over a wide range, which can be used to create oral and transdermal medicinal preparations.

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