

WATER BINDING IN COMPOSITE SYSTEMS BASED ON MILLED MEDICINAL PLANTS AND NANOSILICA

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The aim of the study was identifying the influence of wetting-drying compacted nanosilica particles on the binding of water by milled plant raw materials in neutral and acidic media. Flowers of *Hibiscus sabdariffa* and *Calendula officinalis* were used as the model materials. According to the microphotographs and low temperature ^1H NMR spectroscopy data, the silica film forms on the surface of the milled plant particles, and it can significantly influence their hydration. According to the suggested scheme, some of the water from the inner cavities of plant raw materials moves (as evidenced by the decreasing radius of water-filled pores) to the zone of contact of the composite components (the radius of clusters of adsorbed water increases). In studies of desorption of active substances from milled medicinal plants and their composites by the initial and wetting-drying compacted nanosilica, it has been shown that the formation of a composite significantly reduces the rate of desorption. Minimal desorption is observed in composites containing wetting-drying compacted nanosilica. The studied composite systems are promising for biomedical researches.

Key words: ^1H NMR spectroscopy, wetting-drying compacted silica, *Hibiscus sabdariffa*, *Calendula officinalis*.

Currently, there are several types of composite systems based on components of medicinal plants and amorphous silica and promoted under the general name Phytosil. One of the first is the Hepatonorm product line, jointly developed by the Zubets Institute of the Development and Genetics of Animals and the Bogomolets National Medical University (A. A. Begma and others). Studies show that the cholagogue effect of the active substances immobilized on the silica surface is almost 40 times greater than the effect of the individual administration of the same bioactive complex. Inulan Ltd under the brand Phytosil produces preparations with hypocholesterolemic, hepatoprotective, anti-inflammatory, anticoagulant properties [1–4].

The researchers of Chuiko Institute of Surface Chemistry (V. K. Pogoreliy, V. N. Barvinchenko, etc.) developed several Phytosil-like dietary supplements for prophylactics and treatment of wound infections, gastrointestinal and neuropsychiatric disorders (TC U 10.8-03291669-018:2013)

[5, 6]. Preparations are based on milled medicinal plants and A-300 nanosilica, mixed and additionally activated in a ball mill into homogenous pulverulent mass. The mechanism of their action is assumed to consist of rapid desorption of bioactive complex of the medicinal plants and simultaneous detoxication, caused by nanosilica. The measurements show that in these formulations the adsorption of biologically active substances on nanosilica is not high and does not exceed 10% of their total amount desorbed into the bio medium.

In order to increase the effectiveness of preparations of the Phytosil type, we set the task of creating a composite system in which silica particles firmly bind to particles of milled plant raw materials and are able to influence the binding-release processes of bioactive complex substances. In order to increase the effectiveness of Phytosil-like preparations, we aimed to create a composite system in which silica particles would firmly bind to particles of milled plant matter and can

influence the processes of binding-releasing the substances of the bioactive complex. Then, depending on the type of pretreatment, the prerequisites for programmable release of active substances in the patients' stomach or intestine will be created. In addition, nanosilica will perform the function of delivering the medicinal substances to the mucosa and activate the process of their absorption. The basis for the development was the previously discovered effect of nanosilica on the capacity of interfibrillar gaps in microcrystalline cellulose and starch particles, filled with water [7].

The method of low-temperature ^1H NMR spectroscopy is an effective method for studying the state of bound water in heterogeneous systems [7–9]. By the changes in the signal intensity at the 200–273 K temperature range, it allows one to determine the concentration of non-freezing water and the magnitude of its chemical shift, to assess the degree of its association. Assuming that according to the Gibbs-Thomson equation [10, 11], the freezing (melting) of the intracellular water polyassociates is determined by their linear dimensions, the distributions of the cluster (nanodroplets, domains) radii of the intracellular water can be calculated by the dependence of the changing concentration of not frozen water on the freezing-point depression.

The aim of the present work was to study the nanosilica influence in the neutral and acidic media on the water binding by the milled plant matter, modeled by flowers of *Hibiscus sabdariffa* and *Calendula officinalis*.

Materials and Methods

Materials

Hibiscus (Hibiscus sabdariffa) is a plant of the Malvaceae family. Flowers 5–7 cm in diameter with a strong aroma, and contain 13 organic acids, among them citric, malic and tartaric acids which give the tea a sour taste and thoroughly quench the thirst. Vitamins, trace elements, biologically active substances contribute to strengthening the immunity being an excellent prophylaxis against cold and flu, increase physical endurance, reduce nervous tension. Natural dried flowers of *Hibiscus sabdariffa* and *Calendula officinalis* were used, with initial moisture content below 5% by weight. They were milled using *Laboratory mill 3100* hammer mill (Sweden) to a powdered state with the particle size from 1 to 50 μm .

Nanosilica A-300 (Pilot plant at the Chuiko Institute of Surface Chemistry, Kalush, Ukraine, 99.8% purity) was wetting-drying compacted [12] to the bulk density of about 200 mg/ml. Before the preparation of the composite system, the moisture content of the plant raw materials and silica was adjusted to 250 mg/g by adding a certain amount of distilled water. The samples were then kept at room temperature for 7 days, until the adsorption equilibrium was completely established. Composite systems were prepared in a porcelain mortar by intensive grinding of the mixture of components for 10 min.

Two samples of A-300 nanosilica, differing in bulk density (ρ_d), were selected for the preparation of the composite. The initial nanosilica (A-300_{in}) had $\rho_d = 50$ mg/ml, and the wetting-drying compacted nanosilica (A-300_d) had $\rho_d = 250$ mg/ml. Both silica were kept at 160 °C before use, after which their moisture content did not exceed 5% wt. The composite systems were prepared by grinding silica and plant raw materials in a china mortar in equal amounts. For the A-300_{in} sample, mixing was carried out without a high mechanical load (careful mixing for 5 minutes) in order to prevent a significant change in its bulk density. The second sample was intensely ground in the mortar for 5 min. An equal amount of water was added to both composite samples and their constituents, so the total water content was 250 mg/g.

NMR spectroscopy

^1H NMR spectra were recorded using a Varian 400 Mercury spectrometer of high resolution with an operating frequency of 400 MHz. Eight probing 600 impulses of 1 ms duration were used with a bandwidth of 20 kHz. The temperature in the sensor was controlled by the Bruker VT-1000 thermal attachment with an accuracy of ± 1 deg. Signal intensities were determined by measuring the peaks area using the procedure of decomposing the signal into its components under the Gaussian shape assumption and optimizing the zero line and phase with an accuracy no less than 5% for the well-resolved signals, and $\pm 10\%$ for the overlapping. To prevent supercooling of water in the studied objects, the non-freezing water concentrations were measured by heating the samples previously cooled to a temperature of 210 K.

The value of the chemical shift of protons (δ_{H}) was used as the main parameter determining the grid structure of hydrogen bonds of water. It was assumed that water

in which each molecule participates in the formation of four hydrogen bonds (two due to protons and two due to unshared electron pairs of oxygen atoms) has a chemical shift $\delta_H = 7$ ppm (realized for hexagonal ice), and weakly associated water (not involved in the formation of hydrogen bonds as a proton donor) has a chemical shift δ_H of 1–1.5 ppm [7–9]. To determine the geometric dimensions of the clusters of adsorbed water, the Gibbs-Thomson equation was used, relating the radius of a spherical or cylindrical water cluster or domain (R) to the value of the freezing point depression [10, 11]:

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

where $T_m(R)$ is the melting temperature of ice localized in pores of radius R , $T_{m,\infty}$ the bulk melting temperature, ρ is the density of the solid, σ_s the energy of solid-liquid interaction ΔH_f the bulk enthalpy of fusion.

For practical use, equation (1) can be applied in the form $\Delta T_m = (k/R)$, in which the constant k for many heterogeneous systems containing water is close to 50 deg·nm [11]. The technique of NMR measurements and methods for determining the radii of clusters of interphase water is described in detail in [7–9]. Clusters can be considered polyassociates, with a radius $R < 2$ nm. The polyassociates of a larger size can be considered domains or nanodroplets, since they contain several thousand water molecules [7].

The process of freezing (melting) of bound water corresponds to changes in the Gibbs free energy, caused by the effects of limited space and the natural interface of the phases. The difference from the bulk process lessens inversely to the distance of water layer from the surface. The water that freezes at $T = 273$ K has properties which correspond to bulk water, and as the temperature decreases (without taking into account the supercooling effect), the layers of water that are closer to the surface freeze. Changes in the free energy of bound water (ice) follow the ratio:

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

where the numerical coefficient is a parameter related to the temperature coefficient of the Gibbs free energy variation for ice [12, 13]. Determining the temperature dependence of the unfrozen water concentration $C_{uw}(T)$ by the value of the signal intensity in accordance with the procedure detailed in [7–9], the amount

of strongly and weakly bound water and the thermodynamic characteristics of these layers can be calculated.

The water interphase energy at the boundary with solid particles or in its aqueous solutions was determined as the modulus of the total decrease in the free energy of water due to the presence of a phase interface [7–9] according to the formula:

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

where C_{uw}^{max} is total amount of non-freezing water at $T = 273$ K.

Microscopy

Microphotos of powders and emulsions was performed with a Primo Star microscope (Zeiss, Germany) in reflected and transmitted light, with magnification of $\times 40$ and $\times 100$.

The electron absorption spectra of solutions of desorbed biologically active substances were recorded on the Specord M-40 UV spectrometer (Carl Zeiss, Jena, Germany). For the measurements, 0.5 g samples of composite systems or corresponding powder of the initial plant material (0.05 g) were weighed. The samples were transferred to a beaker and filled with 50 ml of distilled water, stirred vigorously for 30 minutes, and then 5 ml of the solutions were taken and centrifuged for 20 min at 3000 rpm. The length of the optical path was 10 mm.

Results and Discussion

Hibiscus flowers and resulting composites

Fig. 1 shows optical microphotos of powders of a composite hibiscus system with wetting-drying compacted silica (SiO_2 , bulk density 250 mg/ml) at a ratio of 1:1 component concentrations obtained in the “reflection” (*a, b*) and “transmission” modes (*c, d*). Particles of hibiscus and silica are easily identified, images are obtained in the reflection mode due to the difference in the color of the particles. The photographs show that they are crushed to a size of 5–300 μm during mechanical treatment. Most of the particles have dimensions of 50–100 μm . Silica has a high affinity for the plant material surface, so that it forms an almost continuous film on the surface, consisting of particles with sizes not exceeding 10–20 μm . An excess of silica

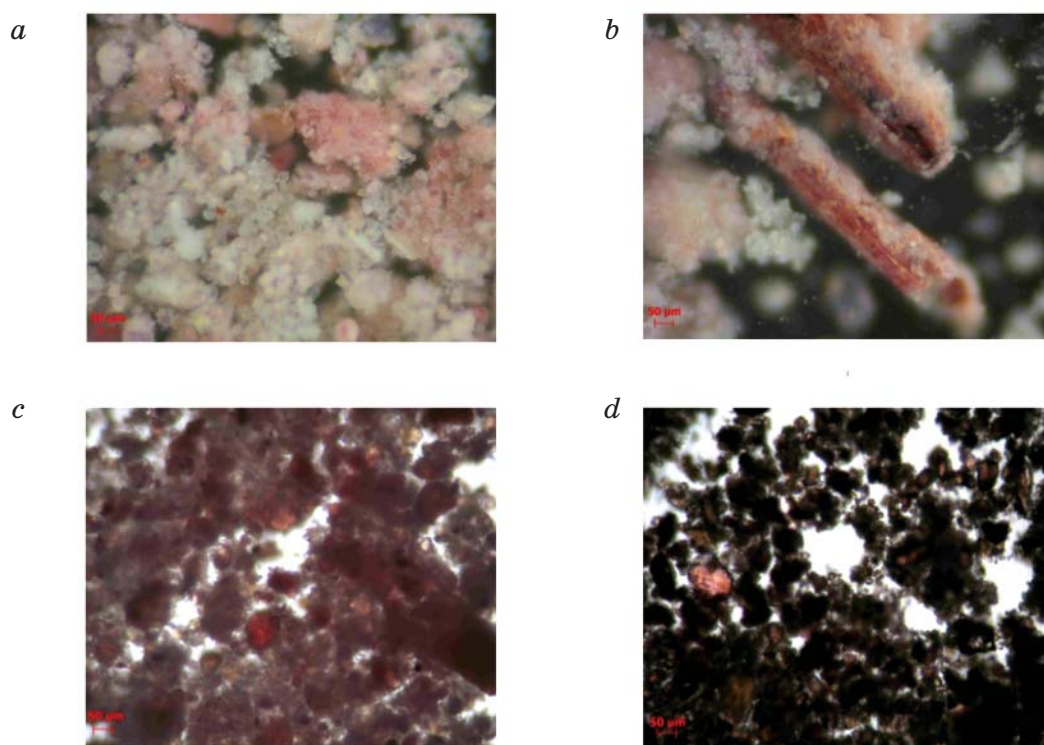


Fig. 1. Microphotographs of powders of a composite hibiscus system with wetting-drying compacted silica at a ratio of 1: 1 component concentrations, taken in the “reflection” (*a, b*) and “transmission” (*c, d*) modes

(not bound to the surface) is in the form of agglomerates, with sizes up to 50 μm .

Fig. 2 shows the spectra of ^1H NMR of water adsorbed on powders of hibiscus (*a*), SiO_2d , (*b*) and 4 to 1 hibiscus/ SiO_2d composite (*c*), taken at different temperatures. For other composite systems (hibiscus/silica = 1/1 and 2/1), the spectra are similar to those shown in Fig. 2, *c*.

For all studied systems, water is determined as a single signal with chemical shift δ_{H} of

4.5–7.5 ppm. With decreasing temperature, the intensity of the signal decreases due to the partial freezing of the interphase water, and the chemical shift increases because of the increase in the orderliness of the unfrozen part of the water. Large chemical shift values are characteristic for water bound by the plant component at low temperatures, indicating a greater ordering of water localized between cellulose fibrils compared to water in interparticle gaps of silica.

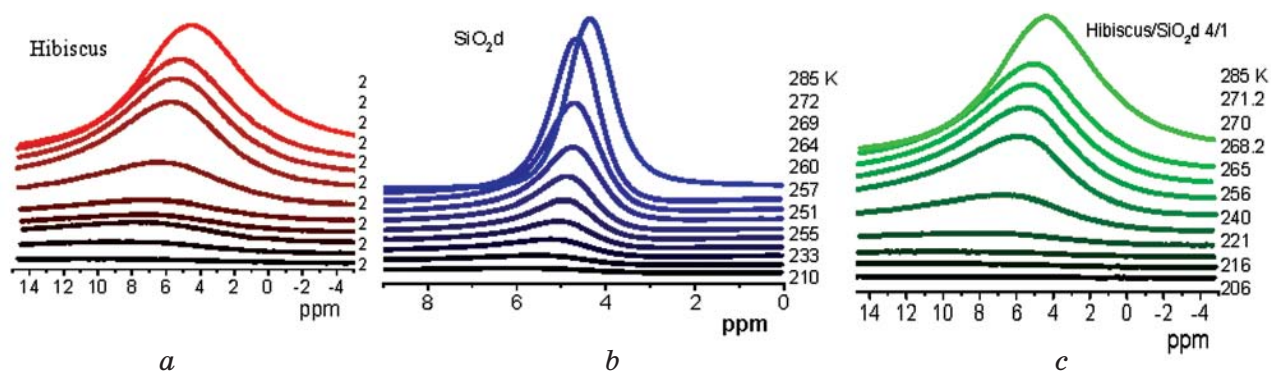


Fig. 2. ^1H NMR-spectra of water adsorbed at powders: hibiscus (*a*), nanosilica (*b*) and hibiscus/silica 4:1 composite (*c*) at different temperatures

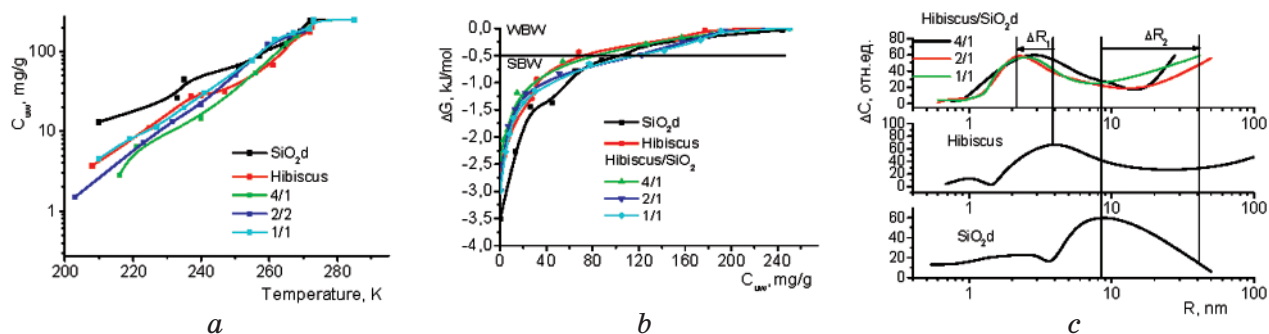


Fig. 3. Temperature dependences of the concentration of unfrozen water (a), the dependence of the change in the Gibbs free energy on the concentration of unfrozen water (b) and the distribution along the radii of clusters of adsorbed water (c) for powders of milled hibiscus, nanosilica and hibiscus silica composite systems

Since the amount of water (C_{H_2O}) in all studied systems was the same and equal to 250 mg/g, the amount of unfrozen water (C_{uw}) can be calculated from the change in signal intensity during heating of the samples for each temperature. The distributions of the unfreezing water clusters' radii can be estimated according to formulas (1) and (2) by the dependence of the change in the Gibbs free energy on the concentration of non-freezing water bound by solid particles (Fig. 3). The more the free energy of water is reduced by adsorption interactions, the lower the temperature at which the water turns into a solid state.

As follows from the data in Fig. 3, the $C_{uw}(T)$ dependence curve over a wide range of temperature changes is located lower for water adsorbed by hibiscus particles. Consequently, the binding of water to cellulose fibrils is more effective than the surface of silica particles. This is also evidenced by the relatively large width of the water signal in hibiscus powders and their composites with silica in comparison with pure silica (Fig. 2). Since the width of the NMR signal is largely determined by the transverse relaxation time of protons in molecules, which is associated with molecular mobility [14], the wider the signal, the less the mobility of the water molecules responsible for it.

The dependences of the change in the Gibbs free energy (ΔG) on the concentration of unfrozen water, obtained in accordance with formula (2), can be used to calculate such thermodynamic characteristics of bound water layers as the concentration of strongly and weakly bound water (C_{uw}^S and C_{uw}^W , respectively), the maximum decrease in free energy in the layer of strongly bound water (ΔG^S), the value of the interfacial energy of water, which determines the total decrease in

the free energy of water due to the presence of a phase boundary with solid particles (γ_S) and the radius distribution of the clusters of adsorbed water (Table 1, Fig. 3, c).

From the data in Table 1 results that wetting-drying compacted silica binds water more effectively than hibiscus particles. For example, the amount of strongly bound water in hydrated silica is 30% higher. However, in the formation of the composite, a redistribution of the ratio of the concentrations of strongly and loosely bound water occurs, manifesting in a change in the interfacial energy. In a composite system containing 20% silica, the total water binding (γ_S) decreases due to a relative decrease in the amount of strongly bound water. Large amounts of silica in the composite lead to a certain increase in γ_S . Binding of water in hibiscus particles is less effective than in silica. In composite systems containing at least one third of the plant component, the overall effect of the change in the γ_S value becomes positive, indicating the possibility of a significant effect of silica on the fibrillar structure of the cellulose component.

The described regularities can be related to the silica influence on the radius of water clusters adsorbed by the composite particles (Fig. 3, c). Two maxima are fixed at $R = 2$ and 8.5 nm on the distribution along the radii of clusters of bound water for hydrated silica (SiO_2d). In hibiscus particles, the main maximum is located at $R = 4$ nm. In addition, distributions peak at $R = 1$ and 100 nm. In composites, the form of the distributions varies significantly. The maximum at $R = 9$ nm (main for pure silica) decreases sharply, instead of it a significant amount of water appears in large water domains with $R > 10$ nm.

Also the main maximum, characteristic for the initial hibiscus powder, is shifted to the

Table 1. Characteristics of unfrozen water layers in raw materials and composite systems based on milled hibiscus flowers and A-300 wetting-drying compacted nanosilica

Sample	C_{uw}^S , mg/g	C_{uw}^W , mg/g	ΔG^S , kJ/mol	γ_S , J/g
SiO_2d	100	150	-3.5	8.9
Hibiscus	70	180	-2.75	6.1
Hibiscus/ SiO_2d 4/1	80	170	-2.4	5.9 (-0.7)
Hibiscus/ SiO_2d 2/1	120	130	-2.8	7.4 (+0.4)
Hibiscus/ SiO_2d 1/1	120	130	-3.0	7.8 (+0.3)

region of smaller values of R . The observed changes are shown in Fig. 3, in the ΔR_1 and ΔR_2 displacements which reflect the influence of nanosilica particles on the binding of water in the cellulose matrix of finely dispersed hibiscus. Reduced radius of water clusters in composites (ΔR_1) can lead to an increase in water binding and, accordingly, prolongation of the desorption time of active substances. The increased number of large water clusters (ΔR_2) can be associated with water in the gaps between the particles of nanosilica and hibiscus.

Schematically, the effect of silica nanoparticles on the structure of water aggregates in a cellulose matrix can be described by the scheme shown in Fig. 4. The main structural element of cellulose is fibrils in packets of closely located polysaccharide chains [15, 16]. These chains form crystalline areas, shown by a system of vertical lines. The length of the crystalline regions is relatively small and they are separated by much less ordered amorphous regions. A significant amount of water can penetrate into the gaps between the fibrils [17, 18] (shown in blue), which changes the geometry of crystalline polysaccharide structures due to the disjoining pressure (most natural cellulose materials are known to swell in water or damp environments). For composite systems containing hydrated cellulose particles coated with a hydrated nanosilica film, the geometric parameters of the composite particles depend on the ratio of the mechanical forces acting on the microfibrils through the water absorbed by them and silica particles forming the hydrogen-bonded complexes with the cellulose surface. The decrease in the radius of water clusters inside the cellulose fiber (ΔR_1) can be interpreted as a decrease in the disjoining water pressure in the cellulose capillaries due to its partial displacement to the boundary with the silica particles and the formation of water clusters with a radius (ΔR_2) greater

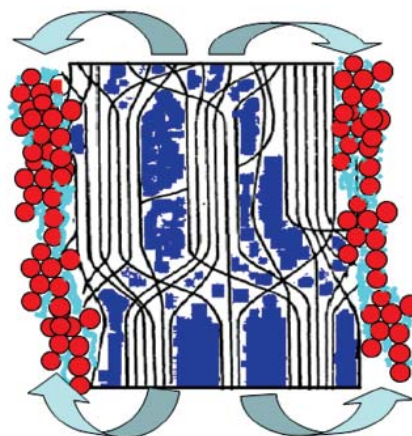


Fig. 4. Effect of nanosilica on the hydration of dispersed cellulose

than the radius of water clusters in silica interparticle gaps. However, with positive values of the change in the interfacial energy, one can expect an increase in the hydration of the cellulose component, i.e., the transfer of part of the water from interparticle gaps of silica to the cellulose matrix.

Composite systems based on crushed medicinal plants and nanosilica can be used in the composition of medicinal dietary additives. At the same time, for optimal absorption of bioactive substances, the particles of medicinal plants must contact with the intestinal mucosa, which includes a significant number of hydrophobic regions formed by fatty acid derivatives [19]. The interaction of the composite particles with such regions can be modeled by the use of a hydrophobic medium, in particular the deuteriochloroform, a weakly polar organic solvent. The 1H NMR spectra of water adsorbed by particles of hibiscus and its composite system 1/1 with silica SiO_2d obtained at different temperatures are shown in Fig. 5, and the dependences of $C_{uw}(T)$, $\Delta G(C_{uw})$, $\Delta C(R)$, $C_{uw}(T)$, $\Delta G(C_{uw})$, $\Delta C(R)$ are given in Fig. 6.

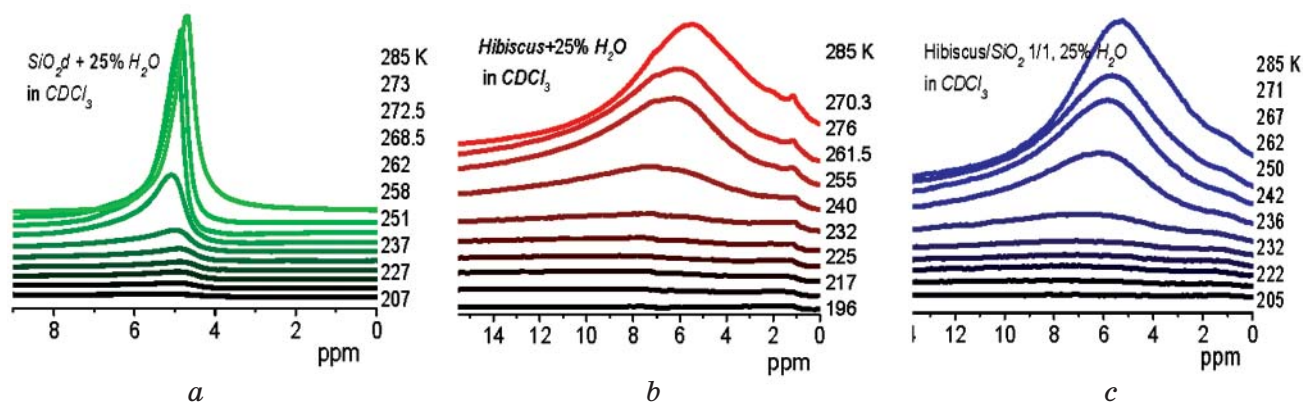


Fig. 5. ^1H NMR-spectra of water adsorbed at powders of hibiscus, nanosilica, and hibiscus/nanosilica 1/1 composite in CDCl_3 medium at different temperatures

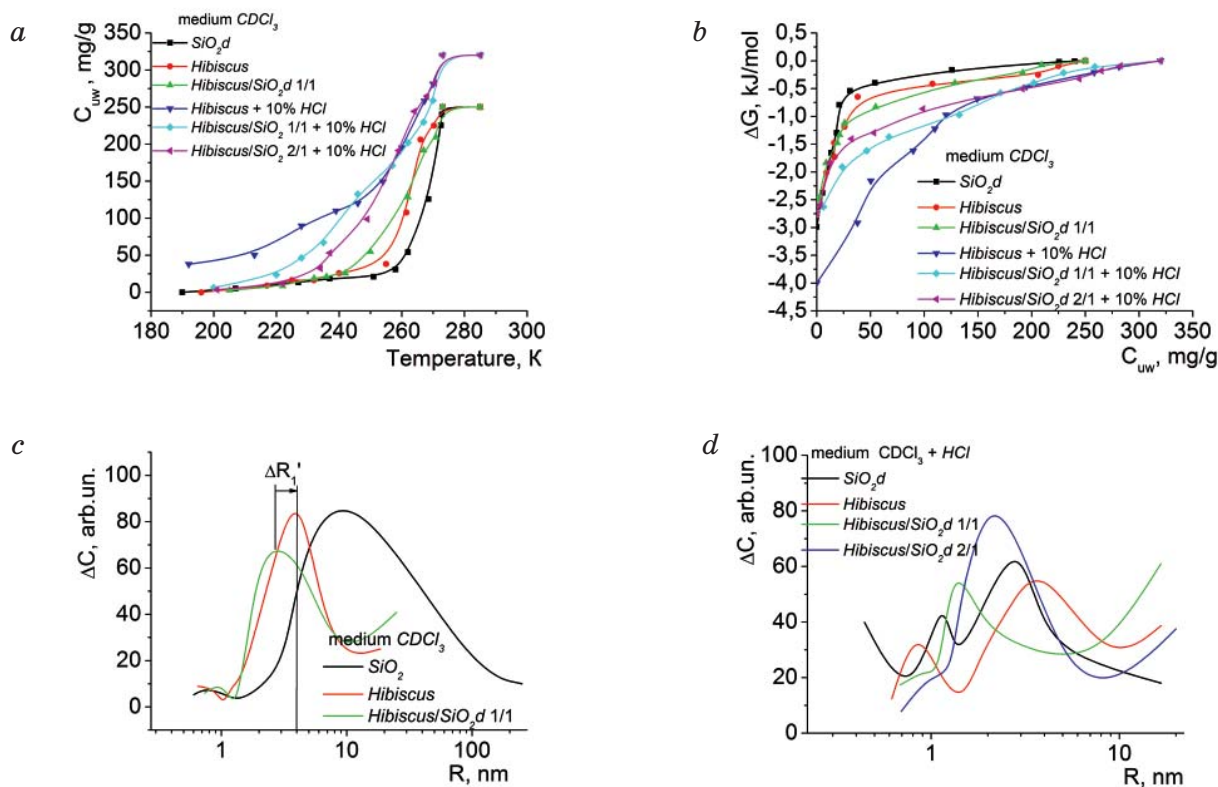


Fig. 6. Dependence of the concentration of unfrozen water on temperature (a), changes in Gibbs free energy on the concentration of unfrozen water (b), and radius distribution of the bound water clusters (c, d) for hibiscus powder and its composites with A-300 nanosilica in CDCl_3 and CDCl_3 media, adding 10% by weight (based on the solid phase mass) of 36% HCl

The spectral parameters of the main signal of adsorbed water in the chloroform medium hardly differ from the signal in the air medium (Fig. 2). In addition to it, a small signal of weakly associated water with a chemical shift $\delta_{\text{H}} = 1.5$ ppm is recorded in the spectra, with intensity increasing with temperature. This signal is associated with water forming a

water-chloroform solution at the boundary with nanoscale particles [7–9].

Thermodynamic characteristics of interfacial water are given in Table 2. As follows from the data, a significant decrease in the energy of interaction of water with the surface occurs in the chloroform medium, the γ_{S} value decreases almost twice. Therefore, the

hydrophobic medium is capable of displacing water from the contact zone with the surface of the silica. In this case, the maximum at $R = 2$ nm disappears completely in the radii distribution of the clusters of adsorbed water (Fig. 3, *c* and 6, *c*). Earlier, a similar effect was observed on a large number of silica materials [7]. A significant increase in the binding energy of water (γ_S increases by 1.3 J/g) is observed for a hibiscus powder in a hydrophobic medium, due to some increase in the amount of strongly bound water. However, the radius of bound water clusters remains practically unchanged (Fig. 6, *c*). Probably, penetrating into the interfibril gaps, the chloroform molecules go to the widest cavities, changing the geometry of the internal interfibril space somewhat.

For a composite system containing equal amounts of hibiscus and silica powder, the change in hydration is within the experimental error. The radius of clusters of adsorbed water is also practically independent of the presence of a hydrophobic medium (Table 2, Fig. 6, *c*). Perhaps chloroform, penetrating into the interfibrillar space, increases the interaction of the plant component with water and this effect prevails over the reduction of water binding in the surface layer of silica.

Since in the stomach food is digested under conditions of high acidity, the effect of concentrated hydrochloric acid on water binding by the components and the composite system itself has been studied. Fig. 7 shows the ^1H NMR spectra of an aqueous solution of hydrochloric acid adsorbed on silica, milled hibiscus, and their 1/1 composite at different temperatures.

In the presence of an acid, the form of the spectra becomes much more complicated. For nanosilica, a signal is recorded in the spectra, with a chemical shift increased from

6 to 7.5 ppm in the conditions of decreased temperature from 285 to 208 K (Fig. 7, *a*). The signal shift to the region of weak magnetic fields (large chemical shifts) is associated with a rapid proton exchange between H_2O and HCl molecules, which is characterized by a chemical shift $\delta_{\text{H}} = 9\text{--}10$ ppm at a concentration of 36% [20]. The appearance of a fine signal structure can be associated with the formation of clusters of water, differently dissolving acids, on the silica particles surface. Then signals with a large chemical shift correspond to clusters of a more concentrated water-acid solution [8].

For the plant component, three signals are observed in the spectra, differing in magnitude of the chemical shift and intensity (1–3, Fig. 7, *b*). The main signal for the hibiscus sample, depending on the temperature, has a chemical shift $\delta_{\text{H}} = 5\text{--}6.5$ ppm, which is noticeably less than for the acid solution adsorbed by nanosilica. In addition, the spectra record signals of water with a partially destroyed grid of hydrogen bonds with $\delta_{\text{H}} = 4$ ppm (2) and a signal of weakly associated water (3, $\delta_{\text{H}} = 1.5$ ppm). The values of the chemical shift, relatively less in comparison with silica, can be associated with the formation in the bulk of hibiscus particles of salt-like products that belong to the solid phase and do not participate in metabolic processes with adsorbed water. In composite systems containing a different component concentrations ratio (Fig. 7, *c*), the spectra are similar to those in Fig. 7, *b*.

When the hydrochloric acid system is added to the system, the freezing temperature of the adsorbed water decreases not only due to adsorption interactions, but also due to the solvation effect of HCl by bound water molecules. The energy of solvation can be judged from the growth of interfacial energy as a result of the addition of fixed amounts of

Table 2. Characteristics of unfrozen water layers in the initial material of milled hibiscus flowers and their composite systems with SiO_2d nanosilica, in CDCl_3 medium, without and with 36% HCl

Sample	HCl , %	C_{uw}^S , mg/g	C_{uw}^W , mg/g	ΔG^S , kJ/mol	γ_S , J/g
SiO_2d	0	50	200	−3.0	4.8 (−4.1)
Hibiscus	0	80	170	−2.75	7.4 (+1.3)
Hibiscus/ SiO_2d 1/1	0	120	130	−2.75	7.9 (+0.1)
SiO_2d	10	250	80	−3.5	18.7
Hibiscus	10	180	140	−4.0	19.7
Hibiscus/ SiO_2d 1/1	10	180	140	−2.75	14.8
Hibiscus/ SiO_2d 2/1	10	180	140	−2.75	12.9

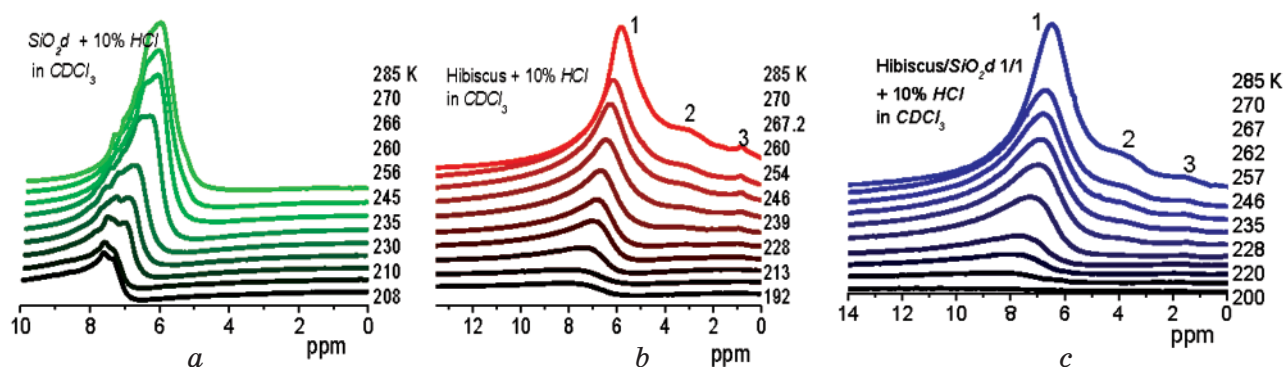


Fig. 7. ^1H NMR spectra of an aqueous solution of hydrochloric acid adsorbed on silica (a), milled hibiscus powder (b), and their 1/1 composite (c) at different temperatures

acid to the system (Table 2). For instance, for silica, an increase in g_s from 4.8 to 18.7 J/g is observed. Then the solvation energy $E_c = 13.9$ J/g. In composite systems, the solvation energy of HCl is much lower and is 5–7 J/g. This may be associated with an increased likelihood of salt products formation due to the interaction of acid with the basic chemical groups of the plant component molecules or the adsorption of dissociated acid ions on the surface of nanosilica.

Assuming that water crystallizes in the form of hexagonal ice from the solutions of hydrochloric acid during their freezing, in the same way as pure water, distributions along the radii of clusters of frozen water can be calculated using the equation (1), (Fig. 6, d). For silica, the curve of the distribution $\Delta C(R)$ contains 3 maxima at $R = 0.3, 1$ and 3 nm, and for the particles of the dispersed hibiscus $R = 0.8$ and 4 nm. For composite systems, the shape of the distributions varies markedly, which indicates the possibility of redistribution of the aqueous acid solution between the inner cavities of the hibiscus particles, interparticle gaps of silica, and the silica-hibiscus contact zone.

UV absorption spectra of desorbed biologically active substances of the initial hibiscus powder (a) and calendula (b) (1) and composite systems based on them using two kinds of nanosilica, the initial SiO_2in (obtained by grinding in a mortar for 30 min under load) (2) and hydrodensified SiO_2d (3) are shown in Fig. 8. It can be seen that the absorption spectra of the biologically active substances of the initial plant material and the SiO_2in composite system have peaks characteristic of hibiscus and calendula, respectively, whereas in the system of condensed silica they are absent. The absence of peaks indicates the formation of a composite and the presence of

a close contact between the plant and mineral components, which leads to the fixation of the bioactive complex on the surface of the wetting-drying compacted silica, and can contribute to programmable prolongation of the release of active substances.

Calendula flowers and derivative composites

Microphotographs of milled flowers of calendula (a) and composite systems prepared on the basis of the initial (b) and wetting-drying compacted silicas (c) in reflected (a-c) and transmitted (d) light are shown in Fig. 9. Microphotographs of milled calendula flowers show that the particles of the plant component have a size of 5 to 100 μm (Fig. 9, a). In the composite system with the initial silica (Fig. 1, b), both types of particles are easily distinguishable. When using wetting-drying compacted silica (Fig. 9, c), a mixture is formed in which dissimilar particles are difficult to distinguish. This indicates a close contact between the plant and mineral components.

The spectra of water adsorbed in the amount of 250 mg/g at the surface of SiO_2in (a) and SiO_2d (b) nanosilica particles, crushed calendula flowers (c) and composite systems based on the initial (d) and compacted (e) silicas are shown in Fig. 10.

In the NMR spectra of the studied samples, one broadened signal is observed with a chemical shift $\delta_{\text{H}} = 4.5\text{--}6.0$ ppm for silica, and $\delta_{\text{H}} = 4.5\text{--}7.5$ ppm for crushed medicinal raw materials. On the basis of the classification given in [21], it can be concluded that all adsorbed water is strongly associated, that is, each molecule takes part in the formation of more than 2 hydrogen bonds. With decreasing temperature, the intensity of the signal of unfrozen water decreases due to partial

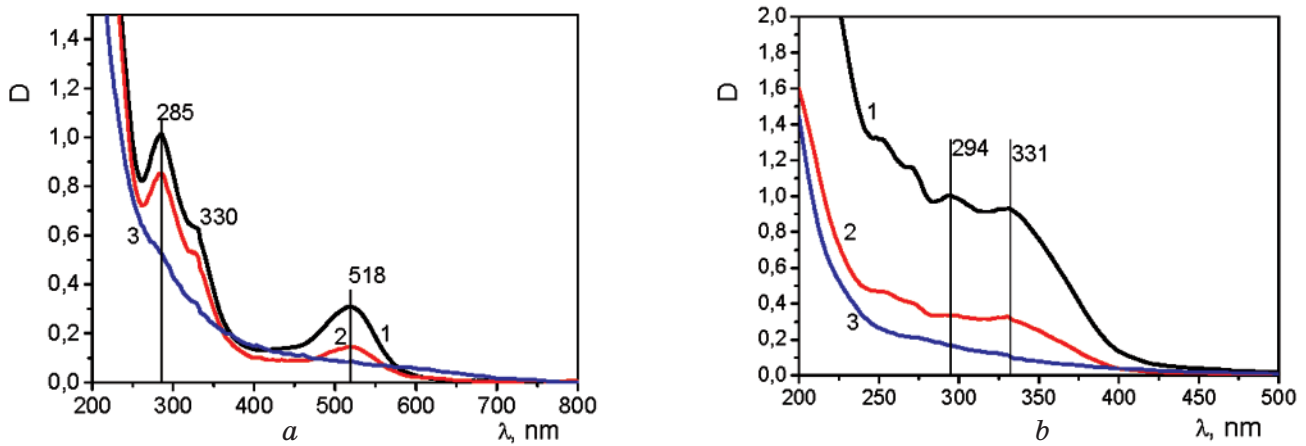


Fig. 8. The absorption spectra of aqueous extracts of hibiscus (a) and calendula (b) (1) powders, and corresponding composite systems with SiO_2in (2) and SiO_2d (3)

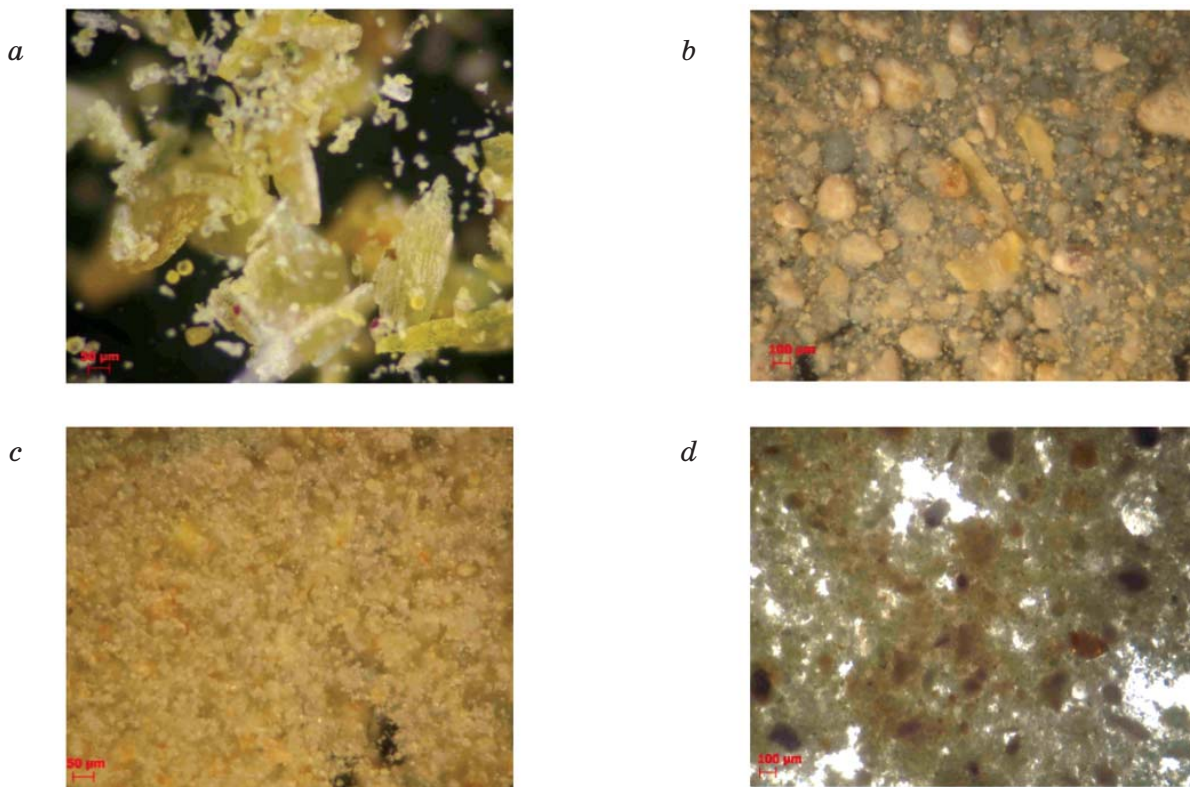


Fig. 9. Microphotographs of crushed flowers of calendula (a) and composite systems prepared on the basis of the initial (b) and wetting-drying compacted silicas (c) in reflected (a-c) and transmitted (d) light

freezing of bound water, and the magnitude of the chemical shift increases, indicating the growing network of hydrogen bonds in the part of the water that freezes at lower temperatures.

Based on the temperature dependences of the intensity of the unfrozen water signal, the temperature dependences of the concentration of unfrozen water can be

constructed (Fig. 11, a), and in accordance with formulas (1 and 2), the dependences of the change $\Delta G(C_{uw})$ (Fig. 11, b) and the radius distribution of clusters of adsorbed water (Fig. 11, c). In the studied systems, the amount of water was chosen to be the same and equal to 250 mg/g. Since no free aqueous phase is formed, we can assume that there is no bulk water. In accordance with the data of Table 3,

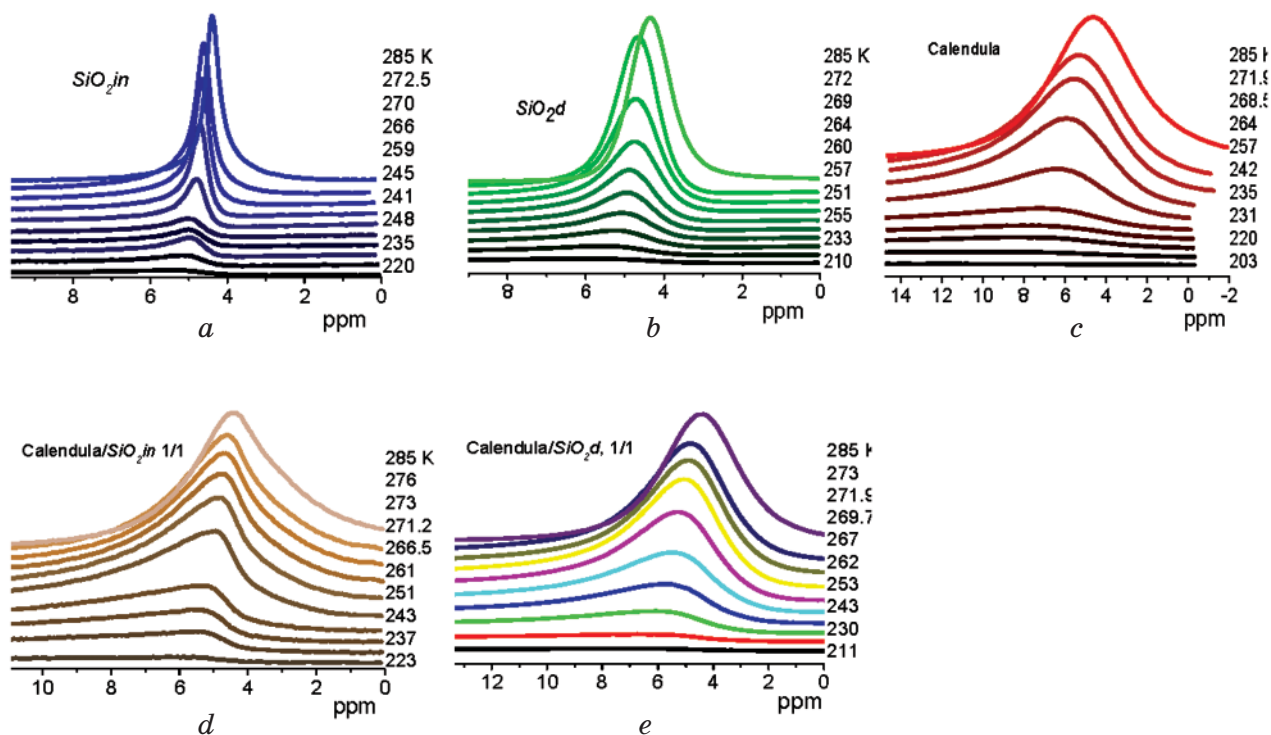


Fig. 10. ^1H NMR spectra of water adsorbed at samples of SiO_2 (a, b), calendula (c), SiO_2 in/calendula (d) and SiO_2d /calendula (e) at different temperatures

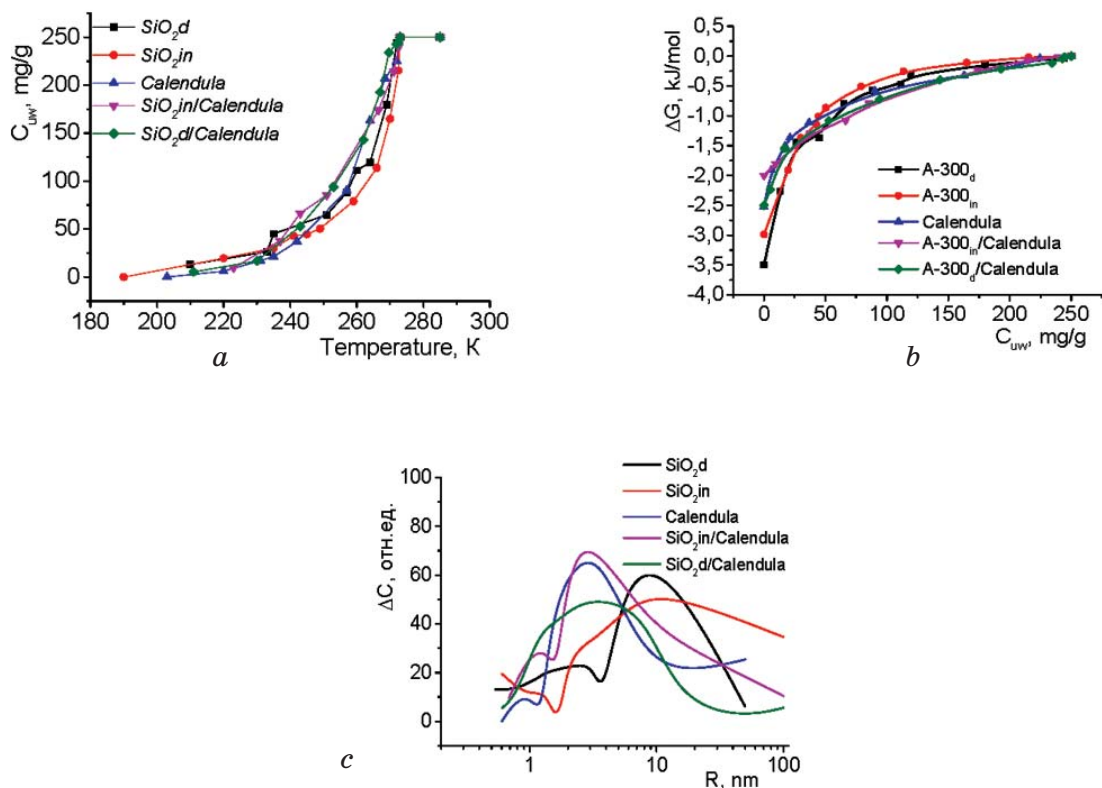


Fig. 11. Temperature dependences and distributions of clusters radii of adsorbed water for SiO_2d nanosilica, plant component and composite systems of SiO_2in /calendula and SiO_2d /calendula in samples containing 250 mg/g H_2O

the hydro-saturation of silica is accompanied by an increase in the binding of water due to the growth of the amount of strongly bound water and the value of ΔG^S . The amount of strongly bound water in the particles of plant raw materials turned out to be somewhat larger than for SiO_2d nanosilica, and the ΔG^S value for SiO_2d is much larger. As a result, large values of γ_S are recorded for the wetting-drying compacted nanosilica, and smaller for the initial silica. These differences are due to the different structure of the internal cavities in which the bound water is concentrated. For silica, these are interparticle gaps formed by primary particles whose radius does not exceed 20 nm [21], while for plants these are the gaps between cellulose fibrils. As can be seen from the obtained distributions of the radii of clusters of adsorbed water (Fig. 11, c), two maxima in the wetting-drying compacted silica correspond to the $R = 2$ and 10 nm clusters, and for the plant component and initial silica there is one maximum at $R = 2.5$ nm.

For composite systems containing mineral and vegetal components, there is a tendency to increase the total water binding, which is more clearly manifested for the initial silica (Table 3), which may be due to the partial destruction of silica aggregates and the formation of strong adducts of plant and mineral components, and the influence of silica particles on the interaction between cellulose fibrils. Previously, a similar effect was observed in the study of the interaction of nanosilica with microcrystalline cellulose particles [6]. The change in the size of clusters of bound water for composite systems can serve as evidence of the significant effect of silica nanoparticles on the binding of water in a dispersed plant component. As can be seen from Fig. 10, c, the amount of water contained

in clusters with a radius greater than 20 nm decreases substantially in the wetting-drying compacted silica. At the same time, in the milled calendula flowers, almost all the bound water is included in the polyassociates with $R < 10$ nm. Therefore, one can expect that with the transition of a portion of the adsorbed water from the hydrated shells of the nanosilica particles to the interfibrillar cellulose space, the free binding energy will increase.

Fig. 12 shows the spectra of 1H NMR of water adsorbed by crushed calendula flowers and its composites with nanosilicae obtained at different temperatures. In contrast to the water spectra adsorbed on hibiscus powders in $CDCl_3$ medium (Fig. 5), in the case of calendula, several signals of water with different chemical shifts are observed in the spectra. In addition to the main signal ($\delta_H = 5-6$ ppm), a signal is recorded at $\delta_H = 3.5$ ppm, caused by clusters of water with a partially destroyed grid of hydrogen bonds. A signal of weakly associated water ($\delta_H = 1.5$ ppm), probably formed at the boundary of hydrated cellulose particles with a hydrophobic medium, is also observed in the spectra. Since the width of the water signal decreases noticeably in the chloroform medium (Fig. 10 and 12), it can be assumed that chloroform penetrates into the interfibrillar space of the cellulose matrix.

Fig. 13 gives the calculated from the data in Fig. 12 temperature dependences of the concentration of non-freezing water (a), changes in the Gibbs free energy from C_{uw} (b), and the distribution of the clusters radii of adsorbed water for dispersed colors of calendula and its composites with nanosilica.

The uniformity of the obtained dependences allows us to conclude that the

Table 3. Characteristics of unfrozen water layers in the raw materials and composite systems created on the basis of milled calendula flowers and A-300 nanosilica in air

Sample	C_{uw}^S , mg/g	C_{uw}^W , mg/g	ΔG^S , kJ/mol	γ_S , J/g
SiO_2d	100	150	-3.8	8.9
SiO_2in	80	150	-3.0	7.4
Calendula	113	137	-2.5	8.2
SiO_2d /Calendula	127	123	-2.0	9.2 (+0.3)
SiO_2in /Calendula	125	125	-2.5	9.4 (+2)
Calendula, medium $CDCl_3$	150	100	-2.5	10 (+1.8)
SiO_2d /Calendula, medium $CDCl_3$	175	75	-2.5	10.7 (+1.5)
SiO_2in /Calendula, medium $CDCl_3$	150	100	-2.5	9.4 (0)

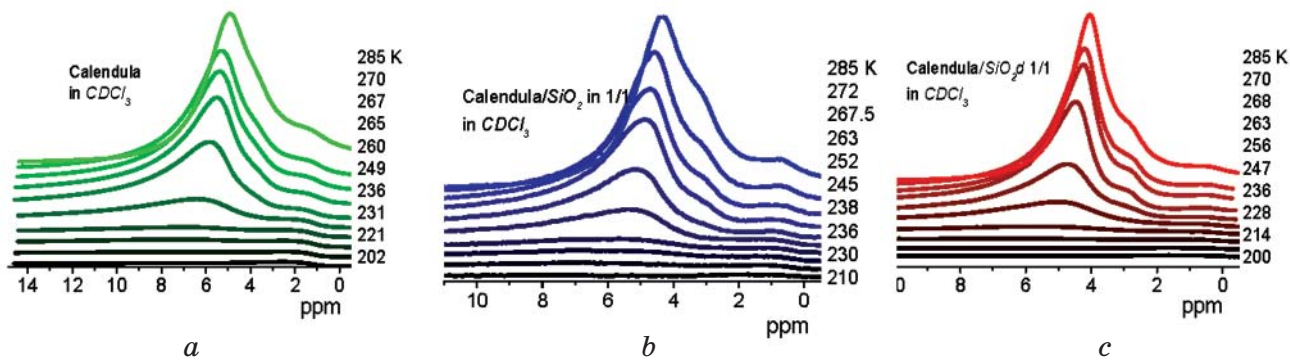


Fig. 12. ^1H NMR spectra of water adsorbed on dispersed calendula flowers (a) and its composites with the initial (b) and the wetting-drying compacted (c) silicas at different temperatures

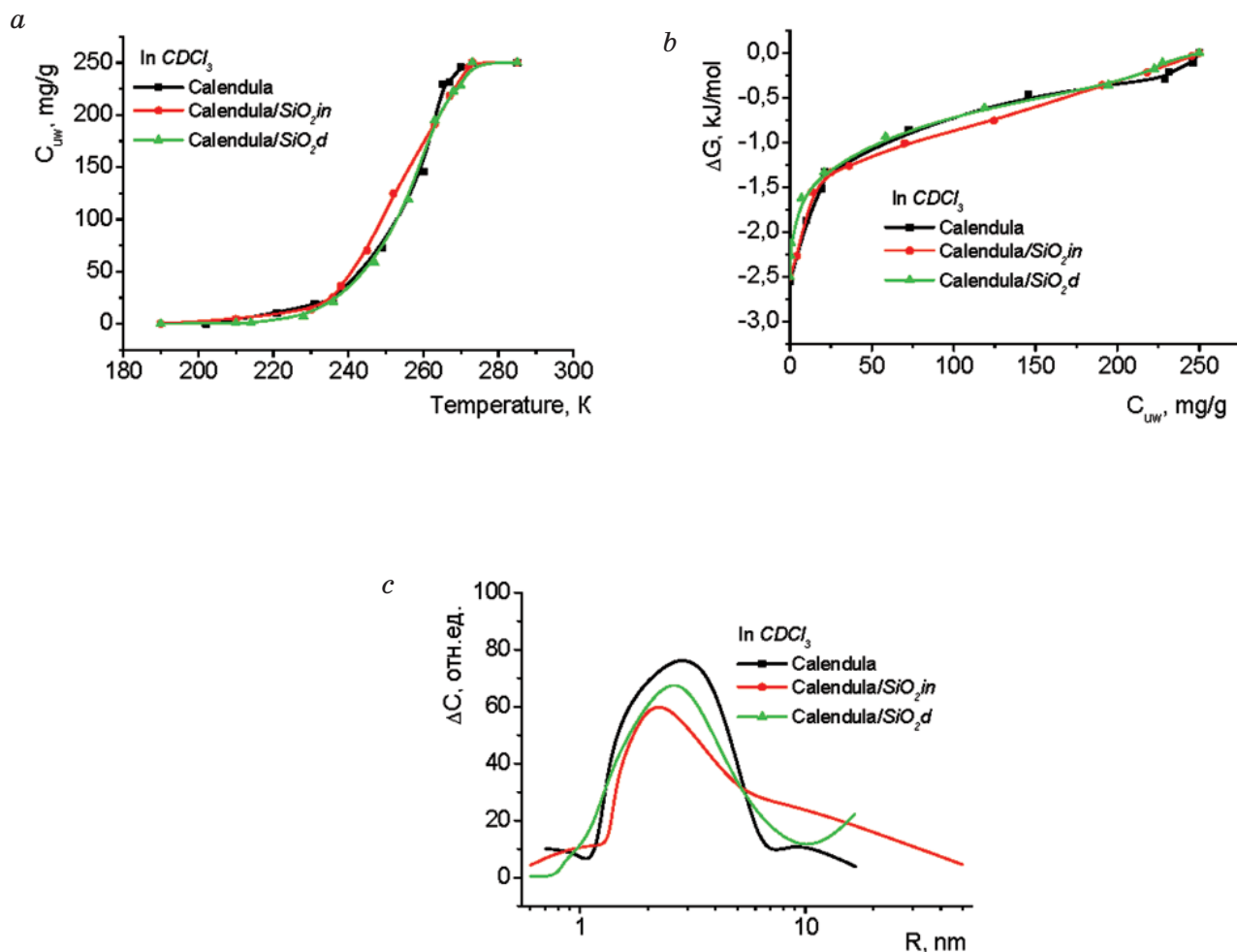


Fig. 13. The temperature dependence of the concentration of unfrozen water (a), the change in free Gibbs energy from C_{uw} (b) and the distribution of the clusters radii of adsorbed water for dispersed colors of calendula and its composites with nanosilica

thermodynamic characteristics of the adsorbed water in the studied systems are close (Table 3). In comparison with the air medium, there is a tendency to increase the total binding of water, which is most clearly manifested for calendula powder and is not observed for a composite system with initial (uncompacted) silica. The distributions of the radii of clusters of adsorbed water contain the main maximum at $R = 2-3$ nm.

The shape of the spectra shown in Fig. 10 and 14 for the initial and composite systems is similar. The difference consists in the changed shape of the NMR lines, which can be due to the presence of several types of water clusters whose chemical shifts practically coincide. As was shown in [20, 22], the clustered water is characterized by a reduced, and depending on the structure of the clusters, solubility of mineral acids that mix with water in any proportions. For the experiments, hydrochloric acid was chosen, which plays a significant role in the process of digestion of food. Its concentration in the stomach is 0.1n.

The ^1H NMR spectra of water adsorbed on samples of initial materials and their nanosilica composites containing an additive of 10% wt. (relative to the solid component) of concentrated HCl in a weakly polar organic solvent, CDCl_3 , obtained at different temperatures are shown in Fig. 14.

In the presence of an acid, the form of the spectra becomes much more complicated. The signal shift to weak magnetic fields (large chemical shifts) is associated with a rapid proton exchange between H_2O and HCl molecules, which is characterized by a chemical shift $\delta_{\text{H}} = 9-10$ ppm at a concentration of 36% [22]. The appearance of a fine signal structure can be associated with the formation of clusters of water on the surface of silica particles that dissolve the acid in different ways. Then signals with a large value of the chemical shift correspond to clusters of a more concentrated water-acid solution [22].

For the plant component, 3 signals are observed in the spectra, differing in magnitude of the chemical shift and intensity (1-3, Fig. 3, b). The main signal for the *Herb* sample,

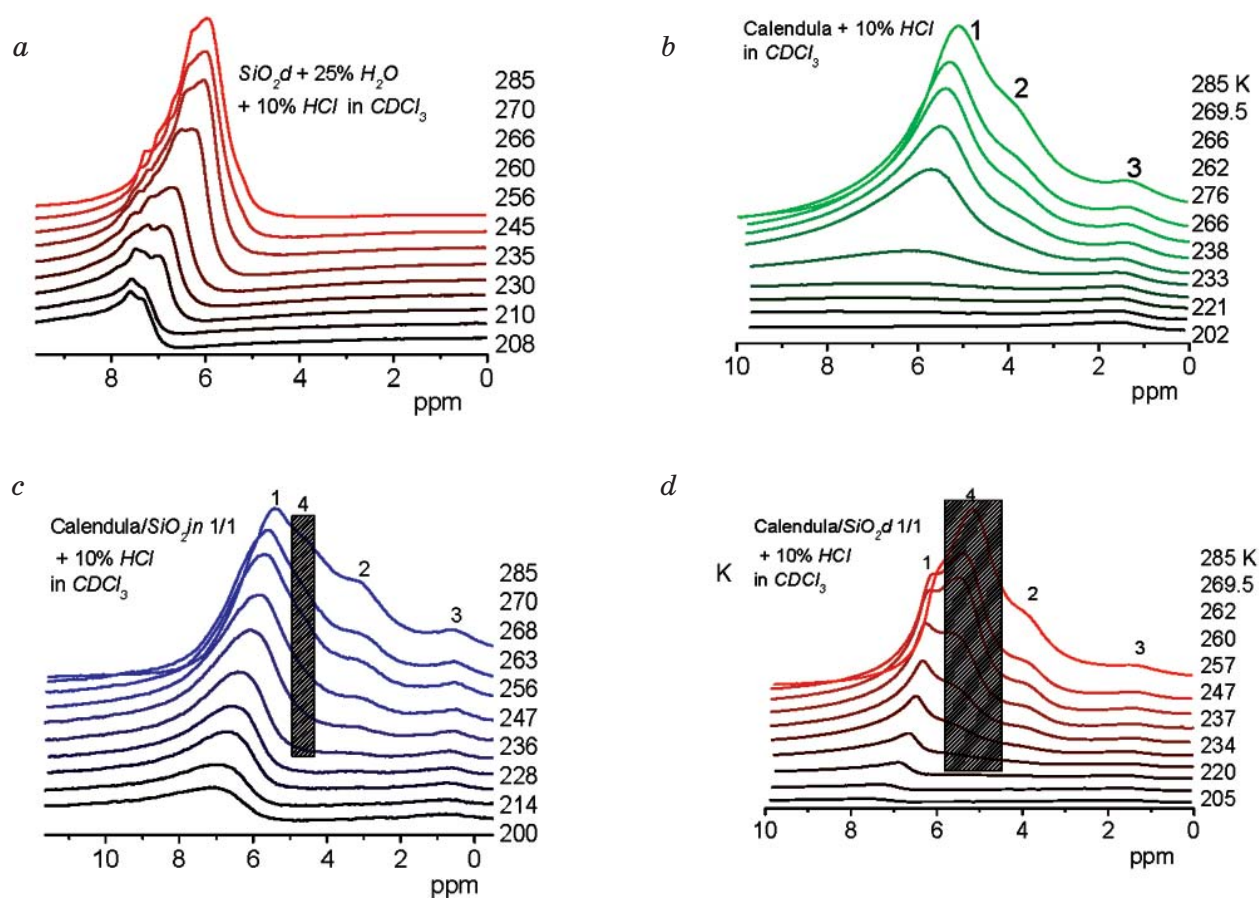


Fig. 14. ^1H NMR spectra of aqueous solutions of HCl adsorbed on samples of SiO_2d (a), calendula (b), $\text{SiO}_2\text{in}/\text{calendula}$ (c), and $\text{SiO}_2\text{d}/\text{calendula}$ (d) at different temperatures

depending on the temperature, has a chemical shift $\delta_H = 5\text{--}6.5$ ppm, which is noticeably less than for the acid solution adsorbed by nanosilica. In addition, spectra record water signals with a partially destroyed grid of hydrogen bonds with $\delta_H = 4$ ppm (2) and a signal of weakly associated water (3, $\delta_H = 1.5$ ppm). Relatively lower values of chemical shift compared with silica can be associated with the formation of salty products in the volume of *Herb* particles, which belong to the solid phase and do not participate in metabolic processes with adsorbed water.

For composite systems, the signal 4 appears in the spectra (Fig. 14, *b, c*), the intensity of which increases noticeably when the original silica is replaced by a the wetting-drying compacted one. It can be attributed to the formation of adducts of plant and mineral components. Consequently, the formation of a composite system manifests in the formation of water clusters in concentrations depending on the method of preparation of the composite material, and which weakly dissolve acid. Ensuring optimum conditions for the formation of the composite, which are realized using the wetting-drying compacted silica, causes the properties of the composite system to differ from those of the original components. For this composite, the maximum effect of the mineral component on the processes of desorption of active substances from particles of plant material can be expected.

The absence of a similar signal in a composite system prepared on the basis of the wetting-drying compacted silica and crushed hibiscus flowers may be due to the different structure of the plant material. In hibiscus flowers, a significant part of the inflorescences is formed by a female cellulose matrix, upon contact with which the silica particles have less influence on the state of water localized in the interface layers.

Thus, the possibility of creating composite systems with particles of the wetting-drying compacted silica and crushed plant raw materials is discovered. According to microphotographs, a silica film is formed on the surface of the particles of crushed plants, and according to NMR spectroscopy it can have a significant effect on their hydration. Changing the ratio of the concentrations of the

composite components affects the radius of the internal cavities in the interfibrillar space of the plant component. A scheme is proposed in which, under the influence of molecular forces, the interaction of silica with a surface moves some of the water from the internal cavities of the plant material (the radius of pores filled with water decreases) to the contact zone of the composite components (the radius of clusters of adsorbed water increases).

By studying desorption of active substances from crushed medicinal plants and their composites by the initial and the wetting-drying compacted silica, it is shown that the formation of a composite significantly reduces the rate of desorption. Minimum desorption is recorded from composites with the wetting-drying compacted silica, which can be used to create sustained-release medicinal preparations.

For milled hibiscus flowers it is established that a weakly polar organic medium ($CDCl_3$), which simulates interaction with the lipid structures of the mucosal surface, halves the energy of interaction of water with the surface of silica particles. This is associated with competitive adsorption of organic molecules (higher) and of water. For composite materials in a hydrophobic medium, the binding energy of water slightly increases, which is probably due to an increase in the interaction of the plant component with water. The effect predominates over the reduction of water binding in the surface layer of silica.

In the presence of HCl , several types of NMR signals of the adsorbed solution are observed in hibiscus crushed flower particles and their composites with silica. The water is partly present in the form of clusters that do not dissolve hydrochloric acid. The solvation energy of acid by interfacial water is significantly lower in composite systems than in individual components (silica and hibiscus). For calendula flowers in the presence of HCl , a new signal with a chemical shift $\delta_H = 5$ ppm appears in the spectra, probably due to an acid solution localized in interparticle gaps. The intensity of this signal is maximal for composites with the wetting-drying compacted silica, which suggests that the properties of such composites significantly differ from the properties of the constituent components. It is these composite systems that are most promising for biomedical testing.

REFERENCES

1. Medical Chemistry and Clinical Application of Silica. Eds. A. A. Chuiko. Kyiv: Naukova dumka. 2003. (In Russian).
2. Pogorelyy V. K. Patterns of adsorption of natural bioactive compounds on the surface of silica nanodispersed. *Poverkhnost*. 2009, 1 (16), 322–349. (In Russian).
3. Georgievsky V. P., Komissarenko N. F., Dmitruk S. E. Biologically active substances of medicinal plants. *Novosibirsk: Nauka*. 1990, 332 p. (In Russian).
4. Chuiko A. A., Pogorelyy V. K., Barvinchenko V. N., Lipkovskaya N. A., Kovtyuhova N. I., Turov V. V. Chemical and clinical verification of efficiency of drugs belonging to the phytosil family. *Chemistry, physic and technology of surface*. 1999, V. 3, P. 3–9. (In Russian).
5. Turov V. V., Gun'ko V. M., Barvinchenko V. N., Rugal A. A., Turova A. A., Fedyanina T. V. Hydration of cellulose in the presence of quercetin and organic solvents *Chemistry, Physics and Technology of Surface*. 2009, V. 15, P. 169–180. (In Russian).
6. Shulga O. V., Kerchhoff J., Turov V. V. The Influence of the Dispersed Oxides on the Interface Energy of the Microcrystalline Cellulose in Water Suspension. Methoporous and Microporous Materials. *Fiz. Khim. Tekhnol. Poverkhhn*. 2001, N 4–6, P. 267–274. (In Russian).
7. Gun'ko V. M., Turov V. V., Horbyk P. P. Water on the interface. *Kyiv: Naukova dumka*. 2009, 694 p. (In Ukrainian).
8. Turov V. V., Gun'ko V. M. Clustered water and its application. *Kyiv: Naukova dumka*. 2011, 313 p. (In Ukrainian).
9. Gun'ko V. M., Turov V. V. Nuclear Magnetic Resonance Studies of Interfacial Phenomena. *New York: Taylor & Francis*. 2013, 1070 p.
10. Strange J. H., Rahman M., Smith E. G. Characterisation of porous solids by NMR. *Phys. Rev. Lett*. 1993, V. 71, P. 3589–3591.
11. Petrov O. V., Furo I. NMR cryoporometry: Principles, application and potential. *Progr. in NMR*. 2009, 54 (2), 97–122.
12. Patent of Ukraine N 105151. Krupskaya T. V., Turov V. V., Barvinchenko V. M., Filatova K. O., Suvorova L. A., Kartel M. T. Method of nanosilica condensation. (In Ukrainian).
13. Thermodynamic properties of individual substances Eds. 1978, 495 p. (In Russian).
14. Abragam A. The Principles of Nuclear Magnetism. *Oxford University Press, Oxford, UK*. 1961.
15. Alyoshyna L. A., Gurtova V. A., Meleh N. V. (ed.) Structure and physico-chemical properties of cellulose and their nanocomposites. *Petrozavodsk: PetrGU*. 2014, 240 p. (In Russian).
16. Cellulose and cellulose derivatives. Ed. by N. M. Bikales, L. Segal. *New-York; London; Sydney; Toronto: Wiley-Interscience*. 1971, 510 p.
17. Hatakeyama T., Nakamura K., Hatakeyama H. Determination of Bound Water Content in Polymers by DTA, DSC and TG. *Termochemica Acta*. 1988, V. 123, P. 153–161.
18. Heiner A. P., Teleman O. Interaction Between Monoclinic Crystalline Cellulose and Water: Breacdown of the Odd/Even Duplicity. *Langmuir*. 1997, 13 (3), 513–518.
19. McMurtrie H., Rikel J. K. The Coloring Review Guide to Human Anatomy. 1990.
20. Turov V. V., Gun'ko V. M., Turova A. A., Morozova L. P., Voronin E. F. Interfacial behavior of concentrated HCl solution and water clustered at a surface of nanosilica in weakly polar solvents media. *Colloids Surf. A: Physicochem. Eng. Aspects*. 2011, 390 (1), 48–55.
21. Gun'ko V. M., Turov V. V., Bogatyrev V. M., Zarko V. I., Leboda R., Goncharuk E. V., Novza A. A., Turov A. V., Chuiko A. A. Unusual Properties of Water at Hydrophilic/Hydrophobic Interfaces. *Adv. Colloid Interface Sci*. 2005, V. 118, P. 125–172.
22. Gun'ko V. M., Morozova L. P., Turova A. A., Turov A. V., Gaishun V. E., Bogatyrev V. M., Turov V. V. Hydrated phosphorus oxyacids alone and adsorbed on nanosilica. *J. Coll. Interface Sci*. 2012, V. 368, P. 263–272.

ЗВ'ЯЗУВАННЯ ВОДИ В КОМПОЗИТНИХ СИСТЕМАХ НА ОСНОВІ ПОДРІБНЕНИХ ЛІКАРСЬКИХ РОСЛИН ТА НАНОКРЕМНЕЗЕМУ

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Метою роботи було визначити вплив частинок гідроупільненого нанокремнезему в нейтральному і кислому середовищах на зв'язування води подрібненою модельною рослинною сировиною, якою слугували квіти *Hibiscus sabdariffa* та *Calendula officinalis*. За даними мікрофотографій та ^1H ЯМР-спектроскопії на поверхні частинок подрібнених рослин формується плівка кремнезему, що здатна впливати на їхню гідратованість. Запропоновано схему, відповідно до якої за взаємодії кремнезему з поверхнею під впливом молекулярних сил частина води з внутрішніх порожнин рослинної сировини (радіус заповнених водою пор зменшується) переміщується в зону контакту компонентів композиту (радіус кластерів адсорбованої води зростає). Шляхом десорбції активних речовин з подрібнених лікарських рослин та їх композитів на основі вихідного та гідроупільненого кремнезему показано, що формування композиту істотно знижує швидкість десорбції. Мінімальна десорбція реєструється з композитних систем, утворених гідроупільненням кремнеземом. Вивчені композитні системи є перспективними для медико-біологічних досліджень.

Ключові слова: ^1H ЯМР-спектроскопія, гідроупільнений нанокремнезем, *Hibiscus sabdariffa*, *Calendula officinalis*.

СВЯЗЫВАНИЕ ВОДЫ В КОМПОЗИТНЫХ СИСТЕМАХ НА ОСНОВЕ ИЗМЕЛЬЧЕННЫХ ЛЕКАРСТВЕННЫХ РАСТЕНИЙ И НАНОКРЕМНЕЗЕМА

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Целью работы было определить влияние частиц гидроуплотненного нанокремнезема в нейтральной и кислой средах на связывание воды измельченным модельным растительным сырьем, в качестве которого использовали цветы *Hibiscus sabdariffa* и *Calendula officinalis*. По данным микрофотографий и ^1H ЯМР-спектроскопии на поверхности частиц измельченных растений формируется пленка кремнезема, способная оказывать влияние на их гидратированность. Предложена схема, в соответствии с которой при взаимодействии кремнезема с поверхностью под влиянием молекулярных сил часть воды из внутренних полостей растительного сырья (радиус заполненных водой пор уменьшается) перемещается в зону контакта компонентов композита (радиус кластеров адсорбированной воды возрастает). Путем десорбции активных веществ из измельченных лекарственных растений и их композитов исходным и гидроуплотненным нанокремнеземом показано, что формирование композита существенно снижает скорость десорбции. Минимальная десорбция регистрируется из композитов, образованных гидроуплотненным нанокремнеземом. Изученные композитные системы перспективны для медико-биологических исследований.

Ключевые слова: ^1H ЯМР-спектроскопия, гидроуплотненный нанокремнезем, *Hibiscus sabdariffa*, *Calendula officinalis*.