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LONG-ACTING COMPOSITE SYSTEMS BASED ON POWDERED MEDICINAL PLANTS AND NANOSILICA

The state of water in the powdered plant materials (calendula, hibiscus) and their composite systems with A-300 nanosilicas having different bulk density has been studied by low-temperature ^{1}H NMR spectroscopy method. The change in bulk density has been found to significantly affect the radius of inner cavities in interfibrillar space of plant components. The composite systems based on wetting-drying compaction of nanosilica and plant powder have been showed to form a mix with high interaction energy of heterogeneous particles. This results in the effective retention of plant bioactive complex by composite, which enables the development of long-acting plant drugs.

Keywords: low-temperature ^{1}H NMR spectroscopy, plant materials, calendula, hibiscus, nanosilica, and composite systems.

As of today, several types of composite systems based on plant components and amorphous silica have been developed and are promoted to the market under the general name of *Phytosil* [1—3], in particular, *Hepatonorm* developed by joint efforts of the Rubets Institute of Genetics and Animal Breeding and the Bohomolets National Medical University [4]. Studies have showed that choleretic effect of active substances immobilized on silica surface almost 40 times exceeds that of individual administration of the same bioactive complex. *Inulan* LTD has been producing medical drugs under the brand name *Phytosil*, which have hypocholesterolemic, hepatoprotective, anti-inflammatory, and anticoagulating properties.

A series of dietary supplements of *Phytosil* type [3, 5], which are intended for the prevention and treatment of disorders of the gastrointestinal tract, infections of open wounds, liver diseases, and psycho-neurological disorders (TU 10.8-03291669-018 2013) have been developed at Chuiko Institute of Surface Chemistry (ISC). The medications are made on the basis of milled collection of medical plants and nanosilica A-300 which are mixed and additionally activated in a ball mill for obtaining a homogeneous powdered mixture. It is assumed that the mechanism of action of these drugs is rapid desorption of bioactive plant complex in parallel with detoxification effect caused by nanosilica. Measurements have showed that adsorption of biologically active substances on nanosilica in these compositions is insignificant and does not exceed 10% of the total amount desorbed to biological media [5].

In order to raise the efficiency of *Phytosil* type drugs it is necessary to design a composite system where silica particles are strongly bound with particles of milled plant raw materials and are able to influence bonding and release of bioactive complex substances. In this case, depending on type of pretreatment, preconditions for controllable release of active substances in patient's stomach or intestine are formed. Besides, nanosilica will perform a function of delivering active substances to mucosa and activating their absorption. The product is based on previously discovered phenomenon of nanosilica effect on the bulk water-filled interfibrillar gaps in microcrystalline cellulose and starch particles [6—8].

Two model medical plants, *Hibiscus sabdariffa* and *Calendula officinalis* having different mechanical characteristics and amount of active substances, have been studied using nanosilica A-300 from pilot plant at Chuiko Institute of Surface Chemistry (Kalush, Ukraine 99.8% purity). The initial (SiO_2in) one with a bulk density of 50 mg/ml and the compacted by wetting-drying up to a bulk density of 250 mg/ml (SiO_2d) [9].

Microphotographing of powders was taken using *Primo Star* microscope (*Zeiss*, Germany) at ×100 magnification. The micropictures of powdered flowers *Calendula officinalis* (*a*) and composite systems based on the initial (*b*) and the wetting-drying compacted silica (*c*) in reflected light are showed in Fig. 1. Plant particles have a size of 5–100 μm (Fig. 1, *a*) and easily distinguished particles of components in the composite system with initial silica (Fig. 1, *b*). For wetting-drying compacted silica (Fig. 1, *c*) various particles are barely distinguishable in the mixture. This indicates a close contact between plant and mineral components.

The ability of heterogeneous solid particles to form composite systems and their specified adsorption-desorption properties in aqueous medium strongly depends on hydration parameters. One of few methods enabling both to measure the amount of water bound with fine particles and to determine thermodynamic parameters of bound water is low temperature ^{1}H NMR-spectroscopy [8, 10–11]. Water that forms hydrated shells of the particles is perturbed by surface which results in lower temperature of water freezing (below 273 K). The stronger is adsorption interaction, the lower is temperature of freezing water bound with surface. Then, determining temperature dependence of unfrozen water concentration, the total change in water free energy caused by interfacial interactions can be estimated. The value is called interfacial energy (γ_S) and measured in J/g [8, 11].

To compare the interfacial energy in the systems based on dispersed plant particles and nanosilica, the amount of water added to the system will be the same: hydration of samples was equal C_{H_2O} = 250 mg/g. Fig. 2 shows a diagram of variation in the interfacial energy for the studied systems. Water is bound more effectively in wetting-drying compacted nanosilica than in the initial one, with difference in the interfacial energy being equal to 1.5 J/g. Energy of water interaction with particles of milled calendula flowers has an intermediate γ_S value for both of initial and wetting-drying compacted silica.

It should be noted that nanosilica and milled plant particles are hydrated in different ways. Water is located in nanosilica interparticle gaps. In the case of plant material, water fills cavities between the cellulose microfibrils. According to the Gibbs-Thomson equation [10 11], by lowering the freezing point of adsorbed water, the size distribution of water clusters that are located in interparticle or interfibrile gaps can be obtained. Fig.3 shows that in wetting-drying compacted nanosilica, the amount of water in clusters having radius larger than 20 nm significantly decreases. At the same time, in the milled calendula flowers almost all bound water is a part of poly-associates with R< 10 nm. Therefore, an increase in free energy of binding with transmission of a part of adsorbed water from hydrated shells of nanosilica particles into the interfibrillar cellulose space can be expected.

An increase in interfacial energy (Fig. 2) of composite systems in comparison with the initial components can be explained by an increase in energy of water binding due to the formation of strong bound water clusters in interparticle gaps of silica and milled plants or by nanosilica influence on microfibrilll interaction in cellulose particles [7]. In particular, increase in S can be explained by better cellulose affinity to water, which leads to redistribution of water in gaps between silica particles and fibrous structure of plant material. Also, this is testified by changes in distributions of adsorbed water clusters. For the composite systems the amount of water located in large clusters is reduced by increasing the number of small clusters. Mainly the fibrous structure of cellulose of milled plants is responsible for these small clusters.

The composite systems based on nanosilica and medical plants are administered orally. Entering the human organism, they contact with tissues of

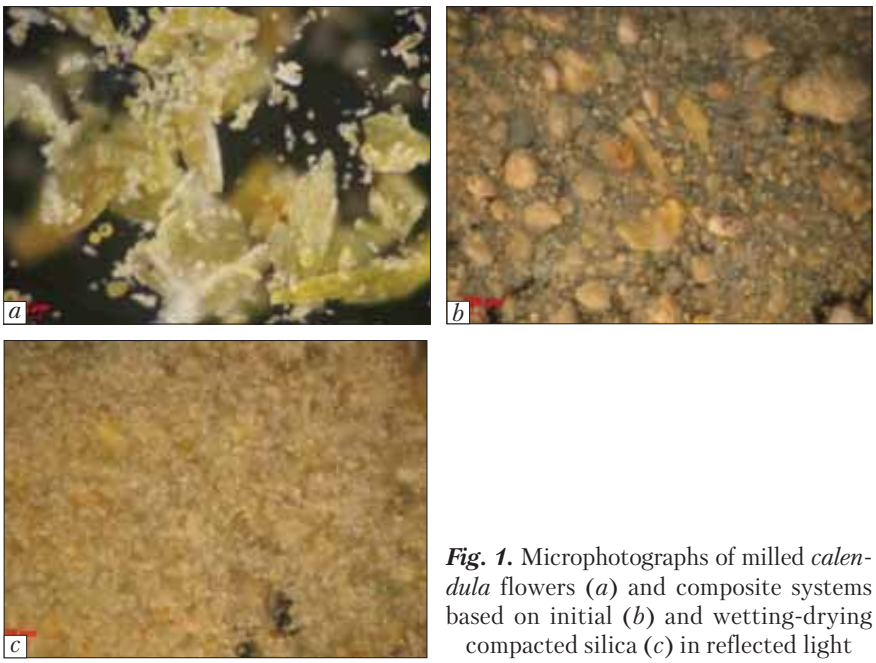

Fig. 1. Microphotographs of milled *calendula* flowers (*a*) and composite systems based on initial (*b*) and wetting-drying compacted silica (*c*) in reflected light

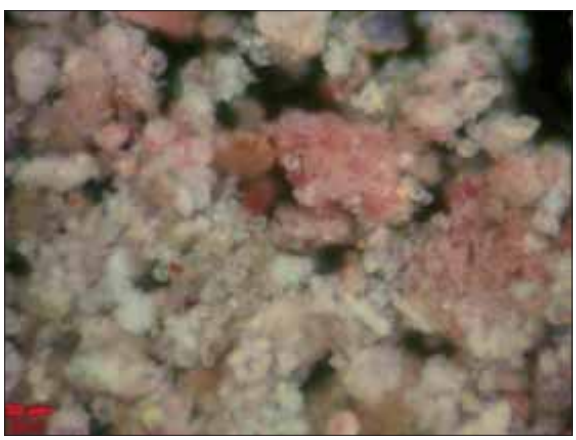

Fig. 5. Microphotographs of composite system (1/1) based on milled *hibiscus* flowers and wetting-drying compacted nanosilica

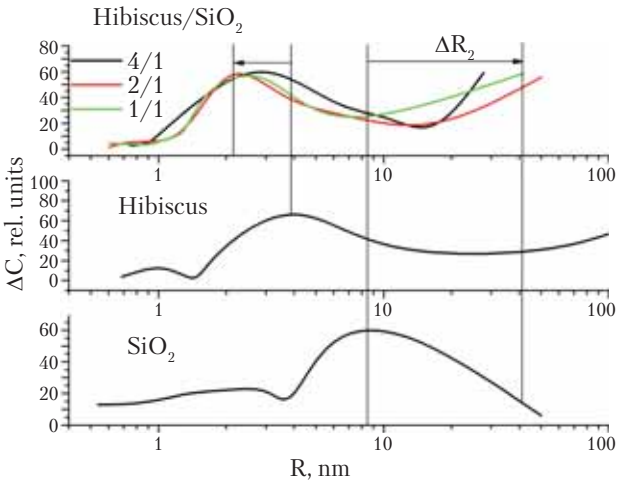

Fig. 7. Size distribution of adsorbed water clusters in composites based on milled flowers of *hibiscus* and wetting-drying compacted nanosilica at C_{H_2O} = 250 mg/g

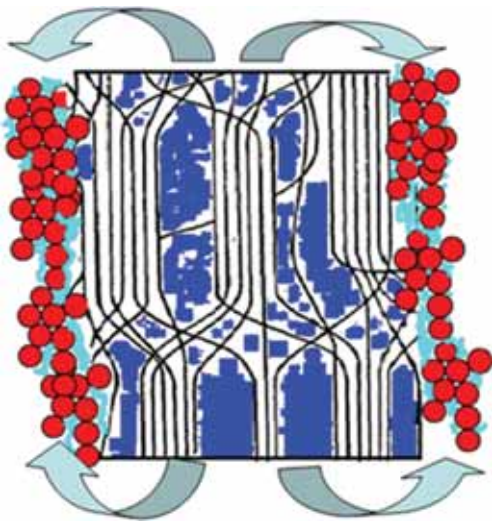

Fig. 8. Effect of nanosilica on hydration of dispersed cellulose

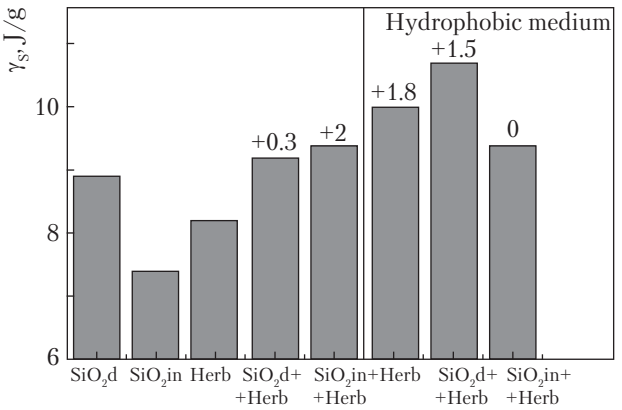

Fig. 2. Diagram of change in interfacial energy in composite systems based on milled calendula flowers and nanosilica and influence of hydrophobic deuterated chloroform (C_{H_2O} = 250 mg/g)

mucous membrane of stomach and intestines [12–13]. A large part of the mucous membrane is formed by hydrophobic sites of lipoproteins and proteins. Deuterated chloroform with a dielectric permittivity ε = 4 that is approximate to local dielectric constant for many aliphatic compounds was used for modelling of phytocomposite contact with hydrophobic medium.

Fig. 2 shows that contact with hydrophobic medium leads to a significant increase in free energy of water interaction with composite system components. Maximum effect is observed for the composite based on wetting-drying compacted nanosilica. An increase in water bonding in fibrous structure of medical plant materials has to be accompanied with a simultaneous increase in energy of biologically active substances that are a part of bioactive complex and an increase in time of their desorption.

The phytocomposite system gets into stomach. Therefore, it is interesting to study condition of interfacial water in phytocomposite system at the presence of both hydrophobic medium and certain amount of hydrochloric acid. As it has been showed for other nanostructured systems [10–11], the strong acids are hardly soluble in clustered water, which enables to record water clusters with different solvent power with respect to acid in ^{1}H NMR spectra.

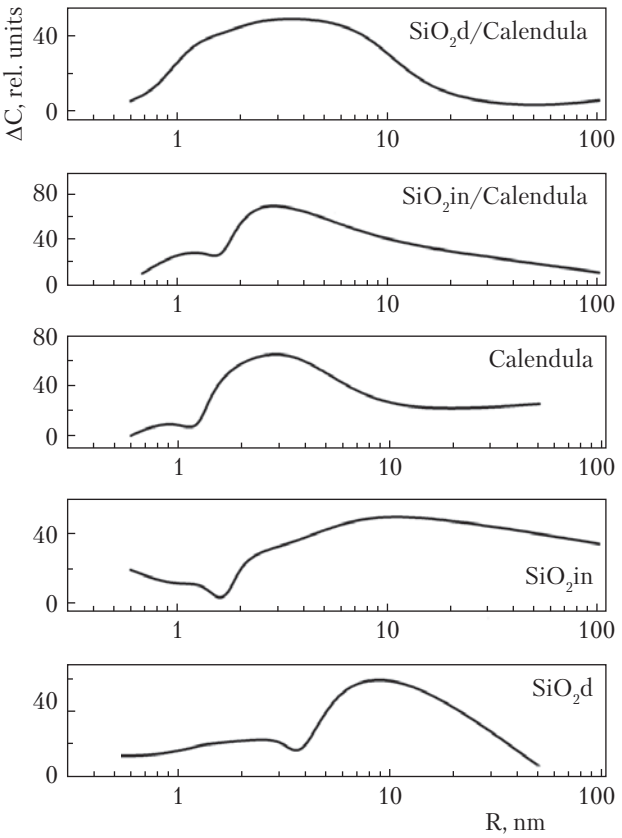

Fig. 3. Size distribution of adsorbed water clusters in milled calendula flower, nanosilica, and their composite systems at C_{H_2O} = 250 mg/g

^{1}H NMR spectra of HCl aqueous solutions adsorbed by milled calendula flowers (*a*) and composite systems 1/1 based on the initial (*b*) and the wetting-drying compacted (*c*) nanosilica at various temperature are given in Fig. 4. For the plant component there are three signals having different chemical shift and intensity (signals 1–3, Fig. 4, *b*) in the spectra. The main signal for calendula sample has a chemical shift δ_H=5–6.5 ppm depending on temperature, that is much less than for the acid solution adsorbed by nanosilica (about 9 ppm). In addition, there are signals of water with partially destroyed hydrogen bonds net (δ_H=4 ppm, signal 2) and weakly associated water (signal 3, δ_H = =1.5 ppm) in the spectra. Relatively less chemical shifts (as compared with silica) can be caused by the formation of salt-like products in the bulk of

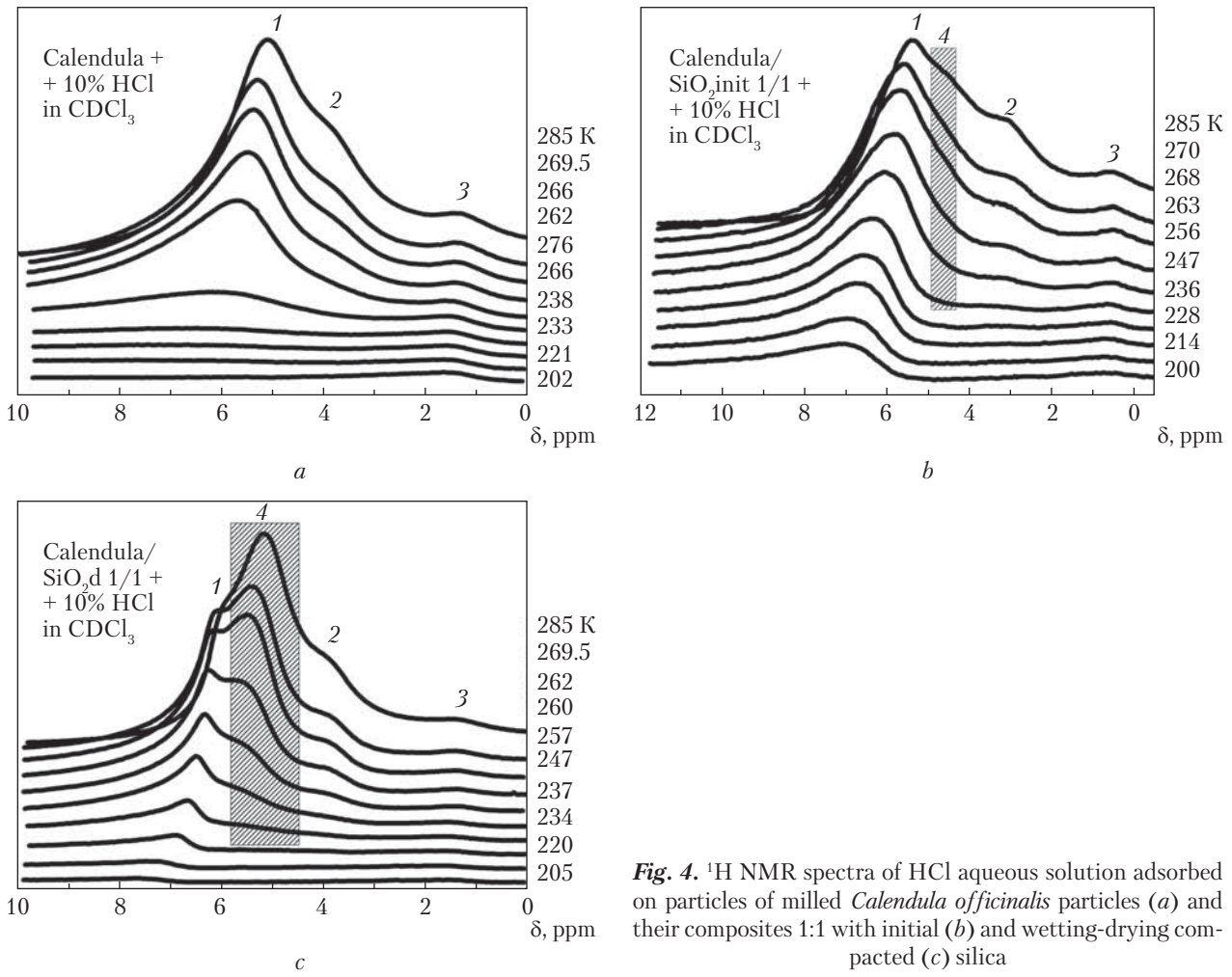

Fig. 4. ^{1}H NMR spectra of HCl aqueous solution adsorbed on particles of milled *Calendula officinalis* particles (*a*) and their composites 1:1 with initial (*b*) and wetting-drying compacted (*c*) silica

solid milled plant particles that don't take part in exchange processes with adsorbed water.

For the composite systems, signal 4 (Fig. 4, *b*, *c*) appears, which intensity sharply increases as the initial silica is replaced by wetting-drying compacted one. This signal can be assigned to the formation of adducts of plant and mineral components. Perhaps, the clusters of water that is hardly soluble in acid are responsible for signal 4 and located in the contact zone of silica particles and phytomaterial. Respectively, the creation of phytocomposite system is associated with the formation of water clusters that weakly dissolve the acid and their concentration strongly depends on method of composite material preparation. Optimal conditions are ensured by wetting-drying compacted silica. For this composite, maximum effect of the mineral component on desorption of active substances from the particles of plant component is expected.

For comparison, both hydration and formation of composite systems based on other plant (*Hibiscuss abdariffa*) containing a large amount of organic acids and antioxidants in its flowers have been studied. Vitamins, microelements, and biologically active substances prevent cold and influenza, stimulate reinforcement of immune system, enhance physical endurance, and alleviate emotional stress. Natural dried *Hibiscuss abdariffa* flowers with initial wet content less than 5 % wt. have

been used. As compared with calendula, *hibiscus* flowers consist of harder cellulose formations.

Microphotographs of hibiscus-wetting-drying compacted silica composite powder (component ratio 1:1) in reflection mode are showed in Fig. 5. Both *hibiscus* flowers and silica particles are easily identified in microphotographs by different colors of particles. The mechanical treatment results in the fact that the particles of plant material have a size within 5–300 μm. The majority of particles has a size within 50–100 μm. Silica has a high affinity to the surface of plant material, which leads to the formation of continuous film consisting of semitransparent particles with a size of 10–20 μm, on the surface. The excessive part of silica (not bound to the surface) forms agglomerates with a size of 50 μm.

Integral characteristics of bound water in composite systems are showed in Fig. 6. As compared with calendula, bonding of water in *hibiscus* particles is less effective than in silica. However, in the composite systems containing, at least, one third plant component, the total effect of γ_S variation is positive, which indicates possible significant silica effect on the fibrous structure of cellulose component.

The size distribution of bound water clusters for hydrated silica has two maxima at R = 2 and 9 nm (Fig. 7). In the *hibiscus* particles, the main maximum is at R = 4 nm. In addition, there are maxima at R = 1 and 100 nm. In the composites, cluster size distribution changes significantly. A sharp decrease in maximum at R = 9 nm (the main one for pure silica) is reported. A significant amount of water constituting large aqueous domains with R >10 nm appears. In addition, the main maximum typical for initial *hibiscus* powder shifts towards lesser R value. These changes are showed in Fig. 7 by shifts ΔR_1 and ΔR_2, which reflect impact of nanosilica particles on bonding of water in cellulose matrix of dispersed *hibiscus*. Like in the case of calendula, a decrease in radius of water clusters in the composites (ΔR_1) can lead to intensification of water bonding and, consequently, to prolonged desorption time of active substances. An increase in the number of

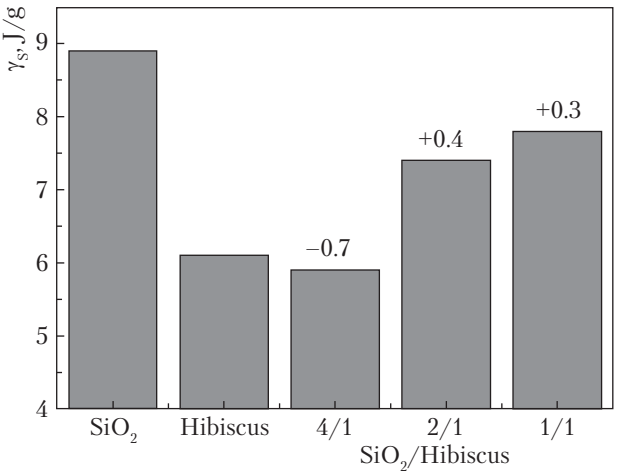

Fig. 6. Diagram of change in interfacial energy in composite systems based on milled *hibiscus* flowers and wetting-drying compacted nanosilica at C_{H_2O} = 250 mg/g

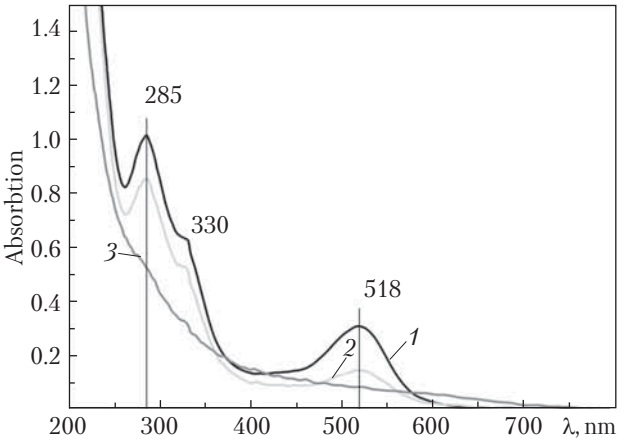

Fig. 9. Absorption spectra of aqueous extracts of *hibiscus* powder and composite systems based on it: initial *hibiscus* powder (*1*), composite systems with SiO_2in (*2*) and SiO_2d (*3*)

large clusters (ΔR_2) can be caused by water in interparticle gaps of nanosilica and *hibiscus*.

The influence of nanoparticles on the structure of aqueous aggregates in the cellulose matrix can be described by a diagram showed in Fig. 8. The main structural element of cellulose is fibrils consisting of packs of closely located polysaccharide chains. These chains form crystalline parts showed in the diagram as system of vertical lines. The length of crystalline parts is relatively small and they are separated from each other by amorphous

areas with much lesser ordering. A significant amount of water can penetrate into the interfibrillar gaps and change the geometry of crystalline polysaccharide structures by wedging pressure (the majority of natural cellulose materials are known to swell up in aqueous or wet medium [14]). For the composite systems containing hydrated particles of cellulose covered with film of hydrated nanosilica, the geometric parameters of composite particles depend on ratio of mechanical forces acting on microfibrils from adsorbed water and nanosilica particles that form hydrogen-bond complexes with superficial cellulose. A decrease in water cluster radius inside the cellulose fibers (ΔR_1) can be explained by a decrease in wedging pressure of water in cellulose capillaries due to its partial shift towards the interface with silica particles and formation of water clusters there having a radius (ΔR_2) larger than that of water clusters in the silica particles gaps. However, as it has been showed for the composites based on calendula particles, an inverse process such as displacement of absorbed water part from silica interparticle gaps into interfibrillar cellulose space takes place.

UV-absorption spectra of desorbed biologically active substances of initial *hibiscus* powder (1) and composite systems based on it and two types of nanosilica, the initial SiO_2in (obtained by milling in mortar for 30 min under load) (2) and the wetting-drying compacted SiO_2d (3) are showed in Fig. 9. The composite system samples with a weight of 0.5 g or respective sample of powdered initial plant material (0.05 g) were used for measurements. The samples were carried into a glass containing 50 ml distilled water and stirred vigorously for 30 minutes, then 5 ml of solution was centrifuged for 20 min at a rate of 3000 rpm. Finally, optical density of the resulting solution in 1cm thick cell was measured.

The absorption spectra of initial plant material and composite system with SiO_2in have peaks typical for *hibiscus*, whereas the system composed of compacted silica does not have any peaks. The absence of peaks testifies to the composite formation and tight contact between plant and mineral components, which leads to sticking of bioactive complex on the surface of wetting-drying compacted silica, which can facilitate expected prolongation of active substance release.

CONCLUSIONS

The possibility of composite systems consisting of wetting-drying compacted and initial nanosilica with milled plants (*calendula* and *hibiscus* flowers) has been established. Based on NMR spectroscopy data silica film formed on the surface of milled plant particles can strongly affect their hydration. Varying component concentration impacts radius of inner cavities in the interfibrillar space of plant component.

A scheme according to which an in increase in the efficiency of binding water in interfibrillar space of plant component (radius of water filled pores decreases) is caused by nanosilica influence has been proposed. At the same time, adsorbed water clusters are formed in the gaps among silica and cellulose particles. These clusters have a radius larger than in silica for *hibiscus* and smaller than in silica, for *calendula*.

In the HCl presence, in particles of milled flowers and their composites with silica, there are several types of NMR signals of adsorbed solution. Thereat, a part of water is in the form of clusters that do not dissolve hydrochloric acid. For the calendula flowers, in the presence of HCl a new signal with a chemical shift $\delta_H = 5$ ppm appears in spectra. It can be explained by the presence of acid solution localized in interparticle gaps of cellulose and silica. Intensity of this signal is maximum for composites with wetting-drying compacted silica. This enables to assume that properties of these composites maximally differ from those of individual components. These composite systems are the most promising for medical and biological studies.

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КОМПОЗИТНІ СИСТЕМИ ПРОЛОНГОВАНОЇ ДІЇ НА ОСНОВІ ПОДРІБНЕНИХ ЛІКАРСЬКИХ РОСЛИН ТА НАНОКРЕМНЕЗЕМІВ

Методом низькотемпературної ^{1}H ЯМР-спектроскопії вивчено стан води в подрібненій рослинній сировині (календула, гібіскус) та її композитних системах з нанокремнеземами марки А-300, які відрізняються насипною густиною. Виявлено, що зміна величини насипної густини істотно впливає на радіус внутрішніх порожнин в міжфібрилярному просторі рослинної компоненти. Показано, що композитні системи на основі гідроущільненого кремнезему та порошку рослин формують суміш з високою енергією взаємодії між різнорідними частинками. Це приводить до ефективного утримання композитом біоактивного комплексу рослин, що дозволяє створювати фітопрепарати пролонгованої дії.

Ключові слова: низькотемпературна ^{1}H ЯМР-спектроскопія, рослинна сировина, календула, гібіскус, нанокремнезем, композитна система.

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КОМПОЗИТНЫЕ СИСТЕМЫ ПРОЛОНГИРОВАННОГО ДЕЙСТВИЯ НА ОСНОВЕ ИЗМЕЛЬЧЕННЫХ ЛЕКАРСТВЕННЫХ РАСТЕНИЙ И НАНОКРЕМНЕЗЕМОВ

Методом низкотемпературной ^{1}H ЯМР-спектроскопии изучено состояние воды в измельченном растительном сырье (календула, гибискус) и его композитных системах с нанокремнеземами марки А-300, различающимися насыпной плотностью. Выявлено, что изменение величины насыпной плотности существенно влияет на радиус внутренних полостей в межфибриллярном пространстве растительной компоненты. Показано, что композитные системы на основе гидроуплотненного кремнезема и порошку растений формируют смесь с высокой энергией взаимодействия между разнородными частицами. Это приводит к эффективному удерживанию композитом биоактивного комплекса растений, что позволяет создавать фитопрепараты пролонгированного действия.

Ключевые слова: низкотемпературная ^{1}H ЯМР-спектроскопия, растительное сырье, календула, гибискус, нанокремнезем, композитная система.