

ADSORPTION OF BIOACTIVE MOLECULES OF MEDICINAL PLANTS

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Abstract

The adsorption of some herbal bioactive polyphenolics (anthraquinones, flavons) from solutions of different polarity onto a surface of highly dispersed silica (entherosorbent Silics) is studied. It is found that the structure of adsorption complexes and the mechanism of their formation depend essentially on the solvent nature. Preliminary clinical researches have shown high medicinal activity of Phytosil composites regarding children's herpes stomatitis. The effectiveness of Phytosil composite corresponds to the level of thymic immunostimulators, but its application makes the process of treatment simpler and cheaper.

In the last few years scientific workers of the Institute of Surface Chemistry of the NAS of Ukraine conducted comprehensive physicochemical and medico-biological studies directed at developing a new generation of composite medicinal preparations of the Phytosil series incorporating native compounds and entherosorbent. The therapeutic activity of biomolecules and their complexes in similar composites is combined with a broad gamut of detoxicating action of entherosorbent Silics based on a highly dispersed silica [1].

It is natural to assume that therapeutic properties of medicinal plants are determined not by a single substance (even if it is the most active one) but by the whole complex of curative substances and its natural environment. For this reason, when forming a phytosorptive composite one should not use, as far as possible, traditional galenicals. It is much more expedient to use medicinal herbs in the highly dispersed form. When applying this approach, the bioactive complex of a medicinal plant that has not been subjected to damaging effect of temperature and extractant or solvent preserves its natural composition, molecular weight and ratio of components, conformation and environment, and, as a consequence, it will exhibit its maximum therapeutic activity. Besides, the pharmacological action of natural compounds in composites of this type will be accompanied by the detoxicating effect of an entherosorbent. In this way it is possible to rule out completely or to decrease considerably the probability of blocking the curative substances of a phytocomplex by endo- or exotoxins, which results in a substantial increase in the bioavailability and efficiency of these substances.

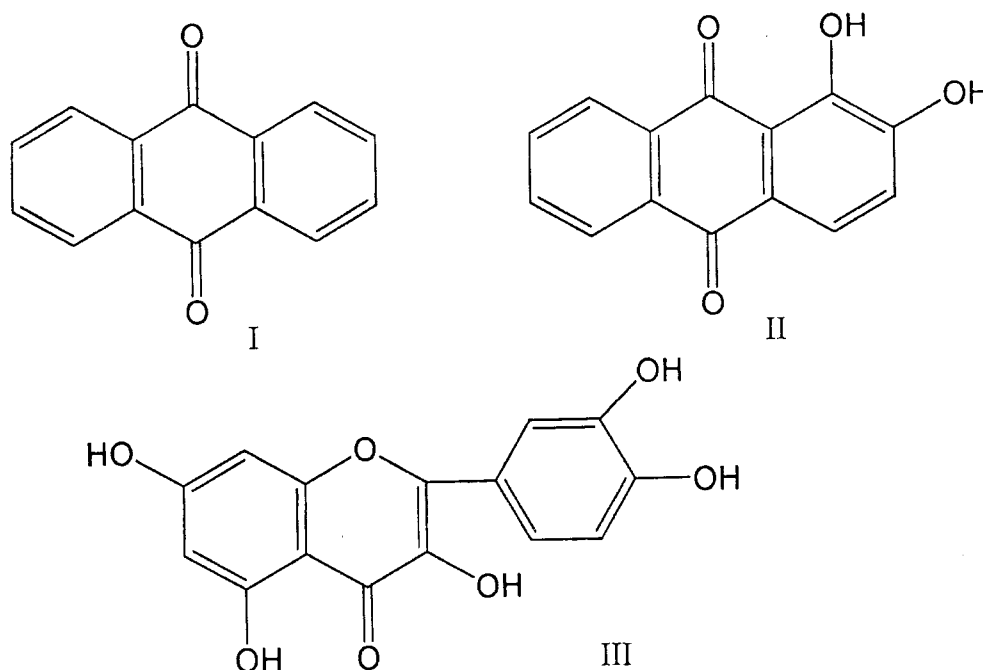
The above-mentioned gives ground to believe that the developed medicaments of the Phytosil series, in comparison with the familiar forms (extracts, tinctures, etc.), will offer a number of advantages: a) a more broad gamut of curative properties, b) enhanced intensity of action, c) prolongation and directionality, d) comparable therapeutic effect at a lower dose, e) shortening of terms of medical treatment, f) economical and technological effectiveness of production, g) simple methods of application.

Thus, with the view of creating a new generation of composite medicinal preparations the purposeful researches were conducted in the following three directions:

1. ascertainment of regular patterns of sorptive interfacial interactions between molecules of natural bioactive compounds and enterosorbent in various media including physiologic ones;
2. designing of qualitative and quantitative compositions of a curative composite; developing of methods for blending its constituents into a single medicamentous form; working out of processing technology for such a form;
3. carrying out of clinical trials and, on the basis of the outcome of the trials, optimization of the composition and methods of application of a curative composite.

The indispensable condition which defines the possibility of creating curative composites of sorptive action is reversibility of adsorption of bioactive molecules and their complexes on the surface of an enterosorbent in a physiological salt solution. Hence, practical developments of medicinal preparations require knowledge of fundamental laws of adsorption interactions.

It is known [2] that the most active biocompounds obtained from medicinal plants include flavonoids, cardiosteroids, alkaloids as well as derivatives of anthraquinone, coumarin, etc. We have studied adsorption of 9,10-anthraquinone (I), 1,2-dihydroxy-9,10-anthraquinone (alizarin, II), 3,5,7,3',4'-pentahydroxyflavone (quercetin, III) used in the capacity of model molecules or bioactive compounds of



the anthraquinone and flavonoid series. The adsorbent has been pyrogenic highly dispersed silica with particle sizes of $d < 0.9 \mu\text{m}$ and specific surface area of $380 \text{ m}^2 \text{ g}^{-1}$. The adsorption has been effected from various media, namely inert aprotic solvents (hexane, toluene), polar protic solvents (water, alcohol, and their mixtures).

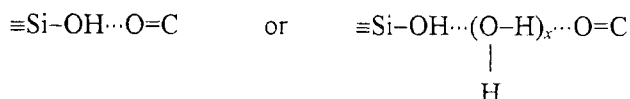
Reactive sites of sorbate molecules include isolated carbonyl groups (electron donors), species with an intramolecular hydrogen bond ($\text{OH} \cdots \text{O}=\text{C}$), and hydroxyls in *o*- and *m*-positions capable of acting as donors and acceptors of protons in intermolecular interactions. These features of the sorbate chemical structure imply variety of mechanisms of specific sorption on hydroxylated surface of SiO_2 mainly through formation of hydrogen bonds with groups OH on the surface or of coordination bonds of silicon atoms with

mono- or bidentate ligands [3]. The experimental procedures applied have been described in Ref. [4].

It has been found that compounds I – III in their molecular form are not sorbed by silica from aqueous, alcoholic, or water-alcohol solutions. This fact provides evidence for competition among protic solvent molecules and adsorbate molecules for reactive sites on the surface. Besides, it also gives evidence for predominance of solvation effect over adsorption one. Anthraquinone is not sorbed even from toluene. The molecular adsorption is observed only in the case of inert solvent *n*-hexane. The linear character of the recorded adsorption isotherms that have no parts of saturation is attributed to the fact that the highest possible adsorption value considerably exceeds the solubility value of compounds I–III in *n*-hexane [4].

In going from I to II the adsorbability substantially increases. Consequently, hydroxy groups of alizarin act as additional reactive sites that seem to partake in the interaction with the surface through formation of hydrogen bonds of the O–H...O–H type with hydroxyls on the surface or in its hydrated layer. The important role of water molecules on the interface in the process of adsorption of protophilic molecules (I) or protogenic molecules (II) is evidenced for by the results achieved by comparing maximum adsorption values recorded in the case of the starting sorbent and dehydrated sorbent obtained after the heat treatment in air for 2 h at 190°C. It turns out that even a partial removal of water from a solvation cover of silica markedly decreases the adsorption value, which provides evidence for participation of water molecules of a near-surface layer in binding of protoactive adsorbate molecules.

The comparative analysis of the infrared and electronic spectra recorded for molecules in solution and on the silica surface made it possible to form some judgements on the mechanism of adsorption from an inert solvent. When compound (I) passes from the solution onto the surface, one can observe (see Table 1) a shift of the valence vibration band of the carbonyl group ($\Delta\nu = 10 \text{ cm}^{-1}$) into the low frequency region, as well as a broadening and bathochromic shift of the band of transition $\pi \rightarrow \pi^*$ ($\Delta\lambda = 10 \text{ nm}$). The changes in the infrared and electronic spectra observed during adsorption are due to formation of hydrogen bonds between groups C=O of compound (I) and hydroxyl groups of the surface or its hydrated cover:



Analogous changes are also detected in the electronic spectra of alizarin. The two bands of transition $\pi \rightarrow \pi^*$ observed in the case of solution (see Table 1) and related to groups C=O bound with intramolecular hydrogen bonds or to free groups C=O in the spectrum recorded for adsorbed compound (II) manifest themselves in the form of a diffuse line with $\lambda_{\text{max}} = 334 \text{ nm}$. Two bands of transition $\pi \rightarrow \pi^*$ characteristic of the molecular form of compound (II) in the spectrum for the adsorbed state manifest themselves as a broad line with $\lambda_{\text{max}} = 437 \text{ nm}$. Therefore, alizarin is also adsorbed on the surface of SiO_2 through formation of hydrogen bonds between C=O and O–H groups and hydroxyls of the surface, with participation of intermediate molecules of water being possible. It should be noted that in the case of similar systems the participation of water molecules bound to the surface in the formation of adsorption complexes is especially effective. The point is that groups O–H both on the surface and in molecules of compound (II) in virtue of their acidic character possess a more profound protogenic capacity, while water molecules exhibit identical proton-donating and proton-accepting characteristics with reference to hydrogen bonds.

Since adsorption of compounds (I) and (II) on the silica surface from solutions of inert solvents is reversible and caused by formation of hydrogen bonds whose strength does not exceed intensity of thermal effects of solvation of protoactive molecules in physiological media, one may assume that adsorption interactions in systems of a similar type will not exert a negative influence on the efficacy of a curative composite.

As it has already been mentioned above, compounds (I) – (III) in their molecular form are not sorbed on silica from water or from water-alcohol solutions. Since alizarin ($pK_a = 5.47$) and quercetin ($pK_a = 7.47$) in polar solvents exist in two forms (molecular and deprotonated) whose reactivities with respect to the surface sites differs substantially, it was of interest for us to investigate the adsorbability of compounds (II) and (III) depending on pH of water-alcohol solution (1:1). As the pH values for the onset of adsorption and dissociation of silanol groups coincide, one may assume that deprotonated silanol groups ($\equiv\text{SiO}^- \cdots \text{H-OAr}$) act in the capacity of primary adsorption sites for bonding of polyphenols. However, the adsorbabilities of the polyphenols studied are low ($a_{\max} = 4 \times 10^{-7}$ and 1×10^{-7} mol g⁻¹ for compounds (II) and (III) respectively) even in the case of relatively concentrated solutions ($C_0 = 10^{-3}$ mol l⁻¹). The maximum adsorption value for quercetin is considerably lower than that for alizarin in spite of the presence of a large number of groups OH in the molecule of the former. It is probable that with increasing number of hydroxy groups in the adsorbate molecule the total thermal effect of solvation in protoactive solvents (water, alcohol) increases, which, naturally, hinders the passage of a polyphenol from solution onto the surface. And, finally, in the case of quercetin (weaker acid) at a fixed pH value the concentration of phenolate ions that seem to be the most reactive form with respect to the surface is minimal.

Polyphenols (II) and (III) are not desorbed by water (pH 1-6), alcohol, acetone, and chloroform, which provides evidence for strong bonding of the adsorbate. The changes observed in the electronic spectra allow one to make some judgements on the structure of adsorption complexes. It turns out that the reflection spectra for compounds on the surface and absorption spectra in the case of solutions differ considerably (Table 1). The spectra for compounds (II) and (III) display an analogous bathochromic shift of the maximum as a result of adsorption in comparison with the molecular form in solution, which gives evidence for the fact that the mechanism of bonding of polyphenols with the silica surface is similar.

Table 1
Spectral characteristics of adsorbate and adsorption complexes

Compound	In solution	On surface
<i>Inert solvent</i>		
Anthraquinone	$\nu_{\text{C=O}} 1680 \text{ cm}^{-1}$	$\Delta \nu_{\text{C=O}} 10 \text{ cm}^{-1}$
	$\lambda_{\pi-\pi^*} 324 \text{ nm}$	$\Delta \lambda_{\pi-\pi^*} 10 \text{ nm}$
Alizarin	$\lambda_{\pi-\pi^*} 324 \text{ nm}, 334 \text{ nm}$	$\lambda_{\max} 334 \text{ nm}$
	$\lambda_{n-\pi^*} 406 \text{ nm}, 424 \text{ nm}$	$\lambda_{\max} 437 \text{ nm}$
<i>Water-ethanol mixture (1:1)</i>		
Alizarin ($pK_a 5.5$)	$\lambda_{\max} 435 \text{ nm}$ (molecule)	$\lambda_{\max} 500 \text{ nm}$
	$\lambda_{\max} 530 \text{ nm}$ (anion)	
Quercetin ($pK_a 7.5$)	$\lambda_{\max} 336 \text{ nm}$	$\lambda_{\max} 385 \text{ nm}$

The irreversibility of adsorption and substantial changes in the electronic spectra for the adsorbed states give evidence for chemisorption of polyphenols on silica which seems to proceed through formation of chelate rings. When the coordination number of silicon increases up to six, the ability for chelating becomes rather remarkable and may be considered a specific property of *o*-diphenols [5] in whose molecules the oxygen atoms are at a distance exactly equivalent to that required by the structure with octahedral coordination of silicon atoms [6]. It is this possibility of forming strong chelates that is responsible for the irreversible character of the chemisorption of compounds (II) and (III) whose molecules have the necessary reactive sites. One more argument for the validity of this inference is the order in which the λ_{max} values increase in the case of transition $n \rightarrow \pi^*$ displayed in the spectra for alizarin in solution at different pH and on the SiO_2 surface:

solvent, pH < 3	surface	solvent, pH > 3
$\lambda_{max} = 435 \text{ nm}$	$\lambda_{max} = 500 \text{ nm}$	$\lambda_{max} = 535 \text{ nm}$

It is in this sequence of the structures that the energy of the orbital of n -electrons for groups OH of phenols may increase, with the same being also true for the bathochromic shift of the corresponding transition. It should be emphasized, however, that the structure of the adsorption complex with octahedral coordination of silicon atoms may be considered only as a limit structure. With allowance for steric hindrances, formation of surface compounds of polyphenols whose silicon coordination number does not exceed five seems to be more probable.

Thus, it has been established that the nature of a solvent exerts a profound effect on the mechanism of formation and on the structure of adsorption complexes on hydrated surfaces of dispersed silicas. In the case of inert solvents the compounds studied are reversibly adsorbed through formation of hydrogen bonds directly with OH groups or through the intermediary of water molecules in the layer of solvates on the surface. In the situation with polar protoactive solvents (water, alcohol) the adsorption of molecular forms of natural polyphenols on silica is negligible because solvation effects predominate. The adsorption is observed only for deprotonated molecules of polyphenols in the range of pH > 3. It is an irreversible process, which can be explained by formation of surface structures with strong coordination bonds among silicon atoms and adsorbate anions. In practice it means that formation of curative composites on the basis of silica enterosorbent and bioactive polyphenols should be effected with obligatory and due account of the nature of extractants.

As is known [7], properties of a curative composite depend not only on its composition but also on the type of its dosage form. Composites Phytosil can be administered in the form of powders, suspensions, tablets, ointments, etc. In stomatology, for instance, treatment for various diseases of oral cavity with Phytosil is most effective when this composite is in the form of slow-disintegrating tablets. Our experiments have shown that tableting of mixtures of comminuted vegetal resources and highly dispersed silica makes it possible to create composites whose healing properties can be even higher than those of simple mechanical mixtures of powders of both substances. This synergistic effect is probably due to the fact that during the course of pressing there arise interactions among particles of silica and vegetal biopolymers. Formation of such useful adducts may vary the preparation adsorption time or affect the permeability of cell membranes thereby increasing the bioaccessibility of active compounds. The interactions among particles of adsorbents and biopolymers of plant tissues should affect first of all the characteristics of layers of water bound to the surface, as it has been observed in the case of interactions between the disperse silica surface and protein molecules dissolved in water [8]. Such interactions can be studied by techniques of high resolution proton magnetic resonance

spectroscopy in conjunction with the procedure that involves freezing of a liquid phase [9]. This approach permits one not only to determine the amount of bound water but also to make estimation of a hydrate cover in terms of the interactions with the surface as well as to calculate values of free surface energy of adsorbents on the basis of the total decrease in the energy of a system caused by the presence of the phase interface [10].

Applying the H^1 NMR method we have investigated hydration processes on the surface of dispersed silicas, comminuted medicinal plants (matricary, sage), and their pressed mixtures. Dried plants were comminuted to particle sizes of 1–15 μm , mixed in a ratio of 1:5 with dispersed silica ($d < 0.01$ mm), and tableted at a pressure of 15×10^6 Pa. The experimental and computational procedures are described in Refs. [8–10]. The results of our investigation of the hydration processes are summarized in Table 2, where ΔG is the free energy change, C_{H_2O} is the amount of bound water, Sil, M, and S stand for silica, matricary, and sage respectively. Upper indices w and s denote weakly and strongly bound water; lower index p is used to denote pressed samples.

From the data listed in Table 2, it follows that in the case of pressed composites one can observe an increase in the free surface energy. In the system with matricary this increase (almost by a factor of two) is attained owing to a rise in the concentration of both strongly and weakly bound water, while in the situation with sage a rise is only observed for the concentration of weakly bound water. On the basis of the results achieved one can infer that the process of pressing the Phytosil composites does bring about interactions among particles of silica and plants and that these interactions lead to some changes in the structure of hydrate covers of reacting surfaces. Furthermore, the interactions between the silica surface and protein molecules is accompanied with dehydration of the surface [8], while in the case of the interactions among solid particles the opposite is true, namely the degree of hydration of the surface increases. In all probability, adducts in such systems are formed due to the action of electrostatic forces because the presence of weakly bound water can also lead to an increase in the degree of hydration, i.e. the effect is a long-range one.

Earlier it was shown [10] that the main cause of increases in the degree of hydration of surface in water suspensions was formation of unlikely charged portions of the surface and their interaction with dipoles of water molecules. The interaction of this type is a long-range one because it is effective at distances comparable with the basis of surface dipoles (interval between charged portions of the surface). In systems under consideration the appearance of charges on the surface of solid particles can be caused either by reactions of proton transfer from hydroxyl groups on the silica surface to proton-accepting (electron-donating) sites on molecules of biopolymers or by polarization of the surface portions at points of close contact between particles of silica and curative plants. In any case, such a close contact should lead to deformations of cell membranes, to increase in their permeability, and, subsequently, to extension of bioaccessibility of active molecules [11].

On the basis of the data available in literature it is possible to draw some tentative inferences about correlation between the chemical structure of biomolecules and their state in solution, on the one hand, and the mechanism of their adsorption, on the other hand. A considerable contribution to adsorption on silica from aqueous solutions of biosubstances (particularly of low-molecular substances) is probably made by electrostatic interactions. At physiological pH values of solutions when the silica surface is charged negatively and low-molecular biosubstances in these solutions are in the form of anions, their molecules are not adsorbed or are adsorbed only slightly. Electrostatic interactions make also a considerable contribution to adsorption of biopolymers (proteins, nucleic acids). Polar biomolecules (for example glucosamine) can be sorbed on silica through formation of

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hydrogen bonds with silanol groups on the silica surface. The adsorption is markedly enhanced due to the fact that on a biopolymer molecule there are many sites capable of forming hydrogen bonds with the SiO_2 surface (this seems to be the case for glucosamine glycanes). Biomolecules that exist in solution in the form of zwitterions (lecithin, pyrodoxine) can be sorbed at the expense of interactions between negative sites on the silica surface and positive sites on the biomolecules.

The mechanism of adsorption of proteins is still under discussion. The best-reasoned seems to be the viewpoint according to which there are two ultimate types of proteins differing in the mechanism of their adsorption on a solid surface, namely 'hard' and 'soft' proteins. Adsorption of 'hard' proteins whose molecules are distinguished for their rigid adherence (for example ribonuclease) is governed by electrostatic and hydrophobic interactions as well as by dehydration of the surface and protein molecules. During adsorption of 'soft' proteins there appears an additional driving force bound up with changes in the structure of protein molecules, and the contribution made by this force may exceed unfavourable contributions made by electrostatic repulsion. As a result, in contrast to 'hard' proteins, 'soft' proteins can be adsorbed by a hydrophilic similarly charged surface.

Table 2
 Effect of pressing on hydration characteristics of composite Phytosil*

System	$C_{\text{H}_2\text{O}}^s$ mg g ⁻¹	$C_{\text{H}_2\text{O}}^w$ Mg g ⁻¹	ΔG_{max}^s kJ mol ⁻¹	ΔG_{max}^w kJ mol ⁻¹	ΔG_x kJ mol ⁻¹
Sil	500	-	3.2	-	14.40
Sil _p	400	400	3.0	0.8	13.40
M	250	250	4.5	1.2	12.80
S	270	130	2.8	1.5	8.58
(Sil+M) _p	700	600	4.0	1.0	30.60
(Sil+S) _p	320	580	4.0	1.2	17.70

*As regards the notation, see the text.

THE RESULTS OF CLINICAL TRIALS OF PHYTOSIL

We constructed Phytosil tablets of medicinal plants richest on components, which have anti-inflammatory and immune modulative activity. The ethony was added to tablets for the prophylaxis of the development of bacterial complications. As the application of tablets is convenient at therapy of diseases of mouth cavity first of all we tested Phytosil in the stomatology. The tests were carried out on Faculty of therapeutical stomatology of National medical university named by O.O.Bogomoletc (doctor of medical sciences E.S.Yavorska, candidate of medical sciences A.V.Viderska) and Faculty of children stomatology of Kiev medical academy of postgraduate education (candidate of medical sciences H.O.Savitchuk, aspirant S.V.Nedashkovska).

It is established, [11] that composite Phytosil is effective at local application as tablets wick slowly resolve in a cavity of a mouth. The preparation has increased pathogenetic properties. It was marked the removal of pain, significant decrease of symptoms of the mucous shell Inflammation (oedema, hyporemia) and fast epithelization

of erosions and excoriations. At the patients, which suffer glossitis the reduction of oedema of soft fabrics and mucous shell of a cavity of a mouth, reduction of fur and, main, elimination of paresthesia were observed. Especially effective there were Phytosil tablets in the treatment of children having acute herpes stomatitis (Table 3).

The analysis of these results has revealed higher clinical efficiency of Phytosil tablets rather of Etiotropic treatment and Levamizol by all investigated criteria. The clinical efficiency of Phytosil composite corresponds to a level of thymic immunomodulators, but the application of phytocomposite simplifies and makes cheaper medical process.

Naturally, in the given time not all pharmacological properties of a new class of medicinal preparations of family Phytosil are known for us. The further physico-chemical and medical-biological researches will be directed on expansion of a spectrum of action of preparations have been developed already and creation of new composites, effective in therapy and preventive maintenance of cardio-vascular, gastric-intestinal and other diseases.

Table 3
Clinical picture of efficiency of regional immunotherapy of children's acute herpes stomatitis*

Clinical parameters	Way of treatment	Time of treatment (days)	
		rather heavy form of disease	heavy form of disease
Epithelization (restoration) of elements of a damage	Phytosil	3.7	4.3
	Vilosin	3.7	5.0
	Thymalin	3.8	4.0
	Levamisol	5.0	5.8
	Etiotropic treatment	5.6	6.1
Disappearance of the Gingivitis (inflammation of the gum Tissues)	Phytosil	5.0	5.5
	Vilosin	5.1	5.8
	Thymalin	5.5	6.5
	Levamisol	6.6	7.4
	Etiotropic treatment	7.9	9.0
Disappearance of the Lymphadenitis (inflammation of lymph nodes)	Phytosil	6.9	7.9
	Vilosin	7.0	8.0
	Thymalin	9.6	10.7
	Levamisol	10.8	11.8
	Etiotropic treatment	11.8	15.1

* Amount of the patients (from them in the heavy form) and way of treatment:
29(13) - Phytosil, 24(13) - Vilosin, 24(10) - Thymalin, 24(10) - Levamisol,
33(17) - Etiotropic treatment

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References

1. Chuiko A.A., Pentyuk O.O., Scientific Principles of Development of Medicinal Preparations on the basis of Highly Disperse Silica// Proc. of the Scientific Session of the Department of Chemistry of the NAS of Ukraine Dedicated to the 80th Anniversary of the NAS of Ukraine, Kharkiv, June 9-11, 1998; Book Abstr. pp. 36-51. (in Russian).
2. Georgievskii V.P., Komisarenko N.F., Dmitruk S.E., Biologically Active Substances of Medicinal Plants. Novosibirsk: Nauka, 1990. - 332 p.
3. Chuiko A.A., Gorlov Yu.I. Surface Chemistry of Silica: Surface Structure, Active Sites, Sorption Mechanisms, Kyiv: Naukova Dumka, 1992. - 248 p. (in Russian).
4. Kovtyukhova N.I., Pogorelyi V.K. The adsorption of bioactive polyphenols on silica oxide from inert solvents // Ukr. Khim. Zh.- 1997. - V.63, N 1-2. - P.20-25. (in Russian).
5. Iler R.K., The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry. New York - Chichester - Brisbane - Toronto: A Wiley-Interscience Publication, 1979. - 1127 p.
6. Tentsova A.I. (Ed.), Pharmacist Reference Book, Meditsina, Moscow, 1981. - 354 p. (in Russian).
7. Georgievskii V.P., Konev F.A.(Eds), Technology and Standardization of Medicinal Substances. Khar'kov: "RIREG", 1996. - 784 p. (in Russian).
8. Turov V.V., Barvinchenko V.N. Structurally ordered surface layers of water at the SiO₂/ice interface and influence of adsorbed molecules of protein hydrolysate on them// Colloids and Surf. B. - 1997. - N. 8. -P. 125-132.
9. Study of interaction of albumine serum with dymethylsulfoxide on PMR spectra of the frozen water solutions / V.K. Pogorelyi, V.V. Turov, A.V. Turov, V.N.Barvinchenko// Teor.Eksp.Khim.- 1989.- V. 25,N 1.- P.104-108. (in Russian).
10. Turov V.V., Leboda R. Application of H¹ NMR spectroscopy method for determination of haracteristics of thin layer of water adsorbed on the surface of dispersed and porous adsorbents // Colloid and Interface Sci.- 1999. - V. 79. - P.173-211.
11. The investigation of physico-chemical and clinical properties of medicine composites belonging to the Phytosil family/ A.A. Chuiko, V.K. Pogorelyi, V.N. Barvinchenko, N.A.Lipkovska, N.I.Kovtyukhova, V.V. Turov // Visnyk Vinnyts'kogo Derzhavnogo Medychnogo Universytetu.- 1999. - N 3.- P. 253-256. (in Ukrainian).