

ADSORPTION OF SOME VITAMINS ONTO HIGHLY DISPERSE SILICA SURFACE

N.N. Vlasova, L.P. Golovkova, A.A. Svezhentsova, and A.A. Chuiko

*Institute of Surface Chemistry, National Academy of Sciences
Gen. Naumov str. 17, Kyiv-164, 03164, UKRAINE, e-mail: vlabars@i.com.ua*

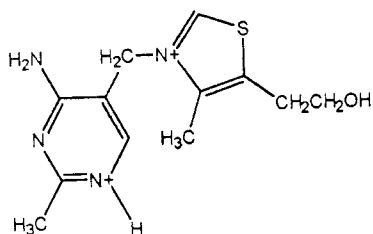
Abstract

Adsorption of thiamine (vitamin B₁), riboflavin (B₂), and pyridoxine (B₆) from aqueous solution onto amorphous silica surface was studied. The investigation of adsorption of tocopherol (vitamin E) and its short-chained analogues from hexane and aqueous micellar solution was performed. Some data on mechanism of vitamin adsorption and structure of adsorption layer were obtained by electron spectroscopy and mass-spectrometry.

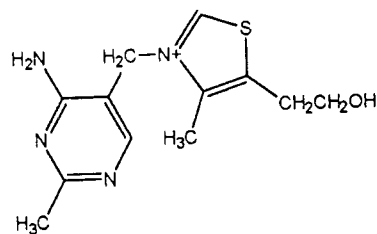
Practical application of siliceous sorbents in medicine in the capacity of enterosorbents poses a number of questions, with one of the most relevant and serious being the question concerning adsorption of vitamins on disperse silicas. Vitamins necessary for cells to perform their normal functions are not synthesized in a human organism and must be supplied with food. Therefore, if a given vitamin is efficiently bound to silica, prescription of silica-containing enterosorbents may lead to deficiency of the vitamin in an organism, and the organism by itself is not able to make up for the deficiency. Thus, when taking such a sorbent over a long period of time, one should exert control over the level of the vitamin in the organism and in case of need fill for a deficiency.

On the other hand, weak bioassimilation of fat - soluble vitamins makes actual problem of creation new medical form of such substances. Using the adsorbents as carrier for bad-soluble vitamins it's possible to create the new forms of drugs. Such forms of drugs, in addition to adsorption of toxic substances from organism, may be used as a reserve fat soluble and poor assimilate vitamins. One of the methods of increasing of biological accessibility of fat-soluble vitamins is application of surfactants providing solubilization of poor soluble substances in water, and carries with developed surface for following adsorption of formed micelles.

First, by way of example, consider adsorption of thiamine (B₁). Thiamine is a component of thiamine diphosphate. It is a coenzyme of a series of enzymes that catalyze decarboxylation of α -keto acids and decomposition of α -oxyketones. Besides, it exerts an effect on protein, fat, and carbohydrate metabolism [1]. A low content of vitamin B₁ in an organism may cause a number of diseases, first of all diseases of nervous system. A complete deficiency of vitamin B₁ in food leads to a severe avitaminosis and to such a serious disease as beri-beri.



HT²⁺



T⁺

We carried out the detailed studies of the thiamine adsorption on highly disperse silica from aqueous solutions. [2-4]. Thiamine dibromide (official preparation) in an aqueous solution undergoes electrolytic dissociation and, depending on pH, exists as single- and double-charged cations.

The dependence of the percentage of these forms on pH of solutions as well as adsorption of thiamine as a function of pH are illustrated in Fig. 1. In acidic solutions at pH 2-3 the adsorption of thiamine is insignificant, but with increasing pH it rapidly increases. The absence of adsorption of thiamine cations in acidic solutions, i.e. under conditions close to the zero-charge point of the silica surface, provides evidence for the presence of a substantial contribution of ion-ion interactions to the adsorption. This assumption is corroborated by the data collected through experiments on desorption of the adsorbed thiamine into an electrolyte solution (0.1 M NaCl). The availability of sodium ions to make the desorption more deep proved such mechanism of adsorption.

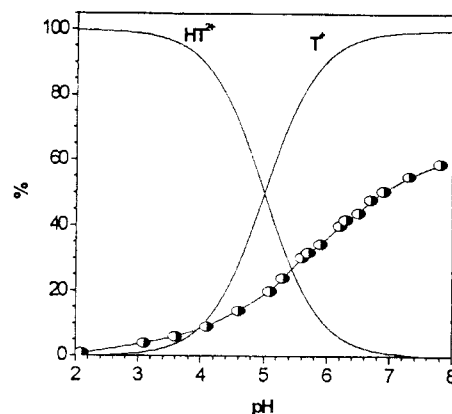
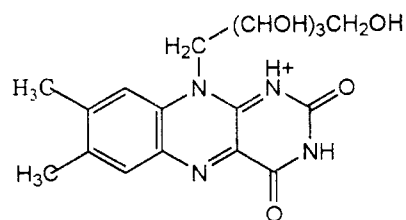


Fig.1 Distribution diagram of thiamine in solution and adsorption of thiamine onto silica A-300 as a function of pH ($C_T = 1 \text{ mM}$, $C_{\text{SiO}_2} = 8 \text{ g/l}$).

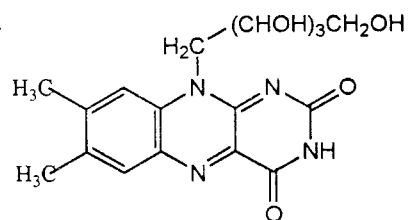
The adsorption isotherm at pH 7.2 (under these conditions all the thiamine is in the form of single-charged cation) is satisfactorily described by the Langmuir equation. The calculated constant of adsorption equilibrium, K_p , is equal to $(1.97 \pm 0.30) \times 10^4 \text{ l/mol}$, the maximum adsorption value, a_∞ , amounts to $54 \pm 3 \text{ } \mu\text{mol/g}$.

Some data about the mechanism of the thiamine adsorption and about the structure of adsorption layers have been gathered through comparative studies of electronic spectra of thiamine in crystals, in solutions, and in the case of its adsorption on SiO_2 [3] as well as through mass spectroscopic investigations of the thermolysis of free and adsorbed thiamine [4]. The mass spectroscopy has shown that immobilization of thiamine involves participation of end hydroxyl groups of its molecules and is accompanied with formation of ionic hydrogen bonds between cations of thiamine and dissociated silanol groups of the silica surface. The studies of the electronic spectra have revealed changes in the conformation of a thiamine molecule upon its adsorption, namely: as a result of the action of adsorption forces the folded conformation of a thiamine molecule in solutions [5] changes into a planar configuration on the SiO_2 surface, which seems to be facilitated by bonding of end hydroxyl groups of thiamine to the surface.

Riboflavin (RF) is a component of a large number of flavoproteins i.e. enzymes which catalyze numerous reactions in an organism [6]. Bidzilya *et al.* [7] have studied adsorption of riboflavin on a highly disperse silica from aqueous solutions under static conditions. The concentration of riboflavin in initial and equilibrium solutions was determined by a spectrophotometric technique. It has been found that the adsorption value of RF, in contrast to thiamine, decreases with increasing pH (1 - 10) (Fig.2). This fact is explained in terms of the acid-base properties of RF and silanol groups of the sorbent surface and reactions that may proceed on this surface.

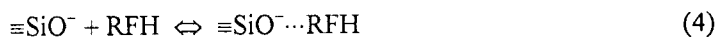
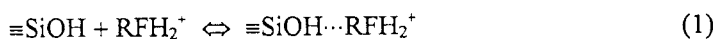


RFH₂⁺



RFH

From the dependence of the percent content of acidic-basic forms of RF on pH (Fig.2) it is seen that in the interval $1 < \text{pH} < 3$ the predominant form of riboflavin in the solution is its protonated positively charged form RFH₂⁺, while at $\text{pH} > 5$ its neutral form RFH. It follows that the reactions which may proceed on the adsorbent surface in an acidic medium can be represented by equations (1) and (2), whereas in subacidic and neutral media by equations (3) and (4).



Reactions (1), (3), and (4) imply formation of hydrogen bonds between silanol groups of the surface and molecules of RF. The contribution of reaction (2) to the adsorption seems to be insignificant because after addition of riboflavin to the silica suspension that was allowed to stay for several hours any decrease in pH of the solution was not observed. It is also evident that the decrease in the adsorption value with increasing pH of the solution from 4 to 7 (i.e. in the range where the concentration of dissociated silanol groups on the surface increases) permits us to assume that the contribution of reaction (4) to the adsorption should also be relatively small.

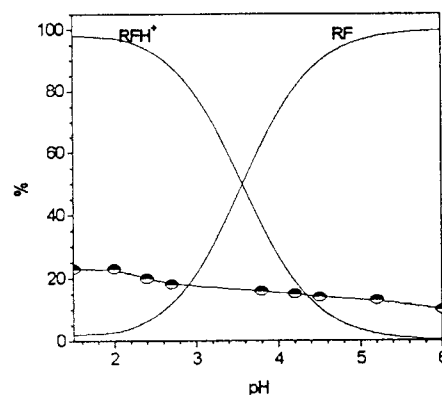


Fig 2. Distribution diagram of riboflavin in solution and pH-dependence of riboflavin adsorption onto silica A-300 ($C_R = 0.7 \text{ mM}$, $C_{\text{SiO}_2} = 10 \text{ g/l}$).

Vitamin B₆ is a member of the family of three interconvertible compounds, namely pyridoxine, pyridoxal, and pyridoxamine [8]. Tissues of plants contain mainly pyridoxine. Vitamin B₆ present in tissues of animals and in animal products is predominantly in the form of pyridoxal, pyridoxamine, and their phosphates. Phosphoric ester of the aldehyde form of B₆ (pyridoxal, P4) is an essential component of a number of enzymes that catalyze transformations of amino acids and amines. Pyridoxine is a standard form of vitamin B₆ offered for sale. In aqueous solutions pyridoxine may exist in several forms according to the scheme of acid-base equilibria :

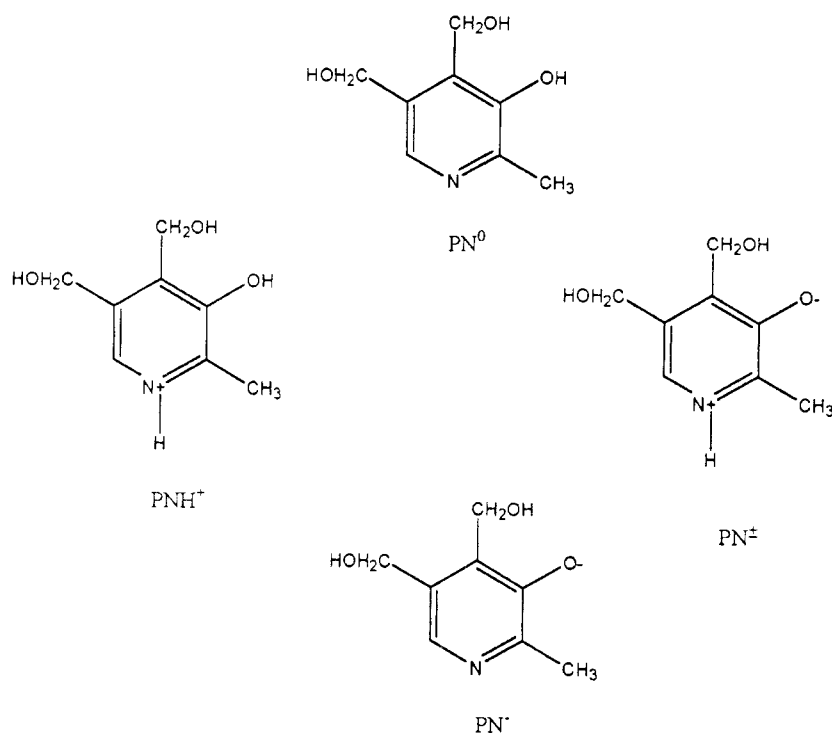


Figure 3 illustrates percentage of acidic and basic forms of pyridoxine as a function of pH. In acidic solutions pyridoxine is in its protonated form PNH^+ . As pH increases, there appears a deprotonated form that exists as two tautomers, namely bipolar ion PN^{\pm} and neutral molecule PN^0 . In aqueous solutions the equilibrium is shifted towards the bipolar ions, while in alcoholic solutions towards the neutral molecule [8]. In alkaline solutions pyridoxine is present in the form of anion PN^- .

We collected relevant information about the adsorption of pyridoxine from aqueous solutions on a highly disperse silica [9]. The adsorption in static conditions, with the concentration of various acidic and basic forms of pyridoxine being determined by a spectrophotometric technique was studied. Our preliminary studies allowed us to find that tautomer PN^0 practically was not adsorbed from aqueous solutions. Dependence of the pyridoxine adsorption value in the case of solutions with pyridoxine concentration of 0.2 and 0.8 mM is displayed in Fig. 3. As a result of the consideration of possible reactions on the silica surface with allowance for the corresponding acid-base equilibria we advanced the following scheme of adsorption interaction at various pH.

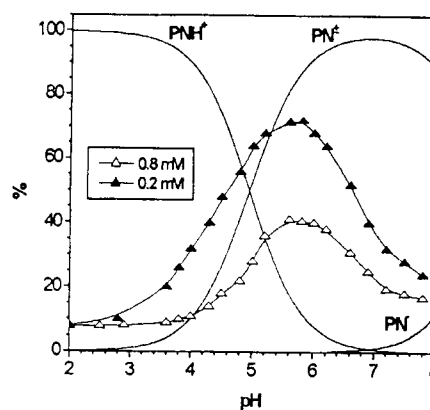
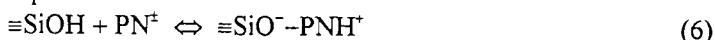


Fig. 3 Distribution diagram of pyridoxine in solution and pH-dependence of pyridoxine adsorption onto silica A-300 ($C_P = 0.2$ and 0.8 mM, $C_{\text{SiO}_2} = 8$ g/l).

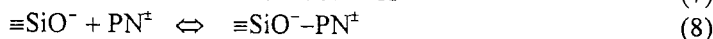
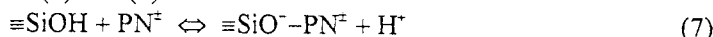
The adsorption interaction at pH < 3.5 was attributed to linking of pyridoxine cations with nondissociated silanol groups through formation of hydrogen bonds:



The increase in the adsorption value observed in the range of pH from 3.5 to 5.0 was ascribed to the appearance of bipolar ions of pyridoxine in the solution and formation of complexes according to the equation



In compliance with this equation the process involves transfer of a proton from silanol groups of silica to oxygen atom of pyridoxine. The complex formed is stabilized owing to ion-ion interactions among its species. In spite of the further increasing in the concentration of bipolar ions in solutions, in the range of pH from 5 to 7 the adsorption value does not increase, which may be related to proceeding of reactions according to equations (7) and (8):

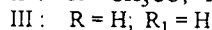
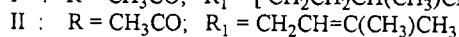
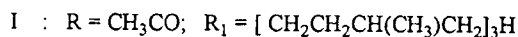
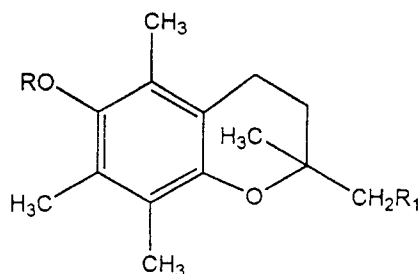


The complex formed in conformity to these equations seems to be characterized by a low stability because of the mutual repulsion of its two negative charges. At higher values of pH in the solution there appear anions of pyridoxine, which hinders its interaction with the surface whose number of negatively charged sites becomes more large.

The adsorption isotherms constructed for cations and bipolar ions of pyridoxine are obtained. The maximum adsorption value for bipolar ions calculated from the Langmuir equation (35 mmol/g) is approximately by an order of magnitude greater than for cations of pyridoxine (1.2 mmol/g).

The reflection spectra recorded for pyridoxine adsorbed from acidic and neutral solutions provide an indirect corroboration of the above-mentioned assumptions about the character of the adsorption processes proceeding in this system. In the spectra for both samples there is an intense band attributed to protonated pyridoxine, PNH^+ , and there is practically no band that could be assigned to bipolar ions, PN^+ . Consequently, pyridoxine in both samples is in the protonated form.

Fat-soluble vitamins, including α -tocopherol, are known to possess antioxidant properties so that vitamins of this type show the capability for inhibiting oxidation of lipids. These characteristics make it possible to use them in medicine as radioprotectors and as agents for treatment for some tumoral diseases. But, unfortunately, such vitamins are inconvenient for application and storage (they are readily oxidized under the action of UV radiation). With a view to put powderlike fat-soluble vitamins into a more manageable loose form immune to oxidation, a study [10] was made of adsorption of vitamin E and of its two short-chained analogues (II) and (III) which also possessed antioxidant properties.



The content of the vitamin and its analogues on the sorbent surface was determined by a spectrophotometric technique in terms of the difference in their concentrations in

solutions before and after the adsorption. The spectral characteristics of the preparations are summarized in Table.

Table

Spectral characteristics of α -tocopherol and its analogues and parameters of their adsorption from hexane on a highly disperse silica

Compound	λ Nm	ϵ	K_p	Γ_∞ $\mu\text{mol g}^{-1}$	ρ	S nm^2	Surface coverage %
I	284.4	2230	3.15	271	0.996	0.90-1.22	50 – 66
II	287.7	2690	2.98	424	0.992	0.90-1.10	76 – 100
III	297.6	3500	2.71	597	0.997	0.80	100
IV	284.4	2350	3.16	86	0.994	0.90-1.22	15 – 21

Notation. λ : absorption band maximum; ϵ : extinction coefficient; K_p : equilibrium constant of adsorption; Γ_∞ : maximum adsorption; ρ : correlation coefficient; S : area occupied by a molecule.

The isotherms of the adsorption of preparations I – III and pharmaceutical preparation of vitamin E (5 % solution of α -tocopherol acetate in oil) from hexane fall into a class of the Langmuir isotherms. The maximum adsorption values and adsorption equilibrium constants are listed in Table . From the collected data it is seen that with decreasing of the side chain length the maximum adsorption value increases. The maximum adsorption value of the official preparation is substantially lower than that of pure α -tocopherol, which may be related to the competing adsorption of the oil (component of the preparation) on the adsorbent surface. The more high adsorption values observed for the short-chained analogues of vitamins E are due to their lower hydrophobic properties in comparison with tocopherol and, subsequently, to their lower affinity for the solvent (hexane) and to their higher affinity for the hydrophilic silica surface. The highest affinity for the silica surface is characteristic of preparation III whose chroman ring contains a hydroxyl group and not an acetate group. On the basis of these data we made an assumption that the adsorption of α -tocopherol and its analogues on the silica surface proceeds mainly through hydrogen bonds between silanol groups of the surface and oxygen atoms of chroman rings and acetate or hydroxyl groups [10].

The maximum adsorption values made it also possible to calculate areas occupied by adsorbate molecules on the silica surface (see Table). The comparative study of α -tocopherol and its analogues involved construction of models of molecules for the compounds in question, as well as estimation of the area of their projection on a horizontal plane at various orientations of the molecules with respect to this plane (chroman ring lies flat on the surface and hydroxylic radicals of compounds I and II either lie flat on the surface or are oriented outwards). The projection area estimates made in this way also are listed in Table. The surface coverage equal to 100 % is attained only upon the adsorption of short-chained analogue III. As regards adsorption of compound II, the hundred-per-cent surface coverage would be possible only if both chroman rings and hydroxylic radicals lay flat on the silica surface. In the case of adsorption of α -tocopherol from hexane the maximum surface coverage depends on the orientation of its molecules and amounts to only 50 %. And, finally, upon adsorption of the official preparation only 15 – 20 % of the surface can be covered with molecules of α -tocopherol.

Further, it has been shown that the adsorption-desorption curves recorded for tocopherol acetate completely coincide, i.e. the adsorption is reversible. The spectra taken for the solutions obtained after the desorption of compounds I – III completely preserve the shape and positions of absorption bands. Upon storing the preparations in the open air at room temperature for 3 months in the spectra for the desorbed compounds there appear no additional bands, which gives evidence for the fact that the adsorbed preparations are resistant to oxidation.

The assimilability of α -tocopherol as well as of other fat-soluble vitamins in the gastrointestinal tract is insufficient. One of the methods to make fat-soluble substances more readily available to a living organism consists in application of surface active substances (SAS) that increase water solubility of slightly soluble substances. We have studied solubilization of tocopherols I – III in aqueous solutions of nonionic SAS Tween 80 [11]. The examination of the absorption spectra for tocopherols in aqueous micellar solutions of Tween 80 made it possible to establish that compounds I and II are solubilized through insertion into micellae of Tween 80, with their loci being hydrophobic nuclei of micellae. Compound III is solubilized both through insertion into micellae and through formation of associates of molecules of compound III with molecules of Tween 80. The loci of molecules of compound III in the case of both the insertion and formation of associates is the polyoxyethylene sheath of molecules of Tween 80.

Tocopherols I – III are effectively adsorbed on the surface of a highly disperse silica from aqueous micellar solutions of Tween 80 [12,13]. At concentrations over the critical micella concentration (c.m.c.) the adsorption of Tween 80 itself does not depend on the presence of tocopherols in solution. The adsorption isotherms recorded for Tween 80 and tocopherols are satisfactorily linearized in the Langmuir co-ordinates. The maximum adsorption values calculated for compounds I – III are practically identical (0.182 – 0.189 mmol/g). From the data gathered one can estimate the solubility values for tocopherols in an adsorption layer and in equilibrium solutions (at an initial ratio solubilizate:SAS of about 0.34). In the case of tocopherol I the solubility value in an equilibrium solution and on the surface are approximately identical and equal to the limit of solubilization of compound I in aqueous micellar solution. It implies that the tocopherol molecules found in a adsorption layer remain to be localized within micellae of Tween 80. As regards tocopherols II and III, the values of their solubility in an adsorption layer are higher than those in solution, which provides evidence for excess adsorption. The absence of any excess adsorption of tocopherol I is explained by the availability of long hydrocarbonic radicals in the molecules, which gives rise to a high affinity of compound I for the inner hydrophobic spheres of Tween 80 micellae and a lower affinity for the hydrophilic surface of silica. We assumed that the adsorption of tocopherol II may be caused by migration of some part of solubilized molecules from micellae to the adsorbent surface, while adsorption of tocopherol III may be thought of as adsorption both of free molecules that are not bound in micellae and of a fraction of formerly solubilized molecules that leave the micella sheath and pass to the adsorbent surface [12,13]. The validity of the assumptions is corroborated by the data gathered from the reflection spectra recorded for adsorbed samples.

References

1. Metzler D.E. Biochemistry. The Chemical Reactions of Living Cells. N.Y., San Francisco, London: Academic Press, 1977. - 1296 p.
2. Adsorption of thiamine onto highly disperse silica/ N.N. Vlasova, N.K. Davidenko, N.M. Shevchenko, V.I. Bogomaz, A.A. Chuiko // Ukr. Chim. Zh. – 1991. - V.57, N 12. - P.1277-1280.

3. Electronic spectra and conformation of thiamine adsorbed onto highly disperse silica/ N.N. Vlasova, N.K. Davidenko, V.I. Bogomaz A.A.Chuiko// Ukr. Chim. Zh.-1992- V.58, N 8.- P.621-623.
4. The mass-spectroscopic investigation of thermolysis of thiamine in free state and adsorbed on the highly disperse silica surface / V.A.Pokrovskii, N.N.Vlasova, N.K.Davidenko, T.V.Kulik // Ukr. Chim. Zh.-1993-V.59, N 12. - P.1257-1260.
5. Yu.M. Ostrovskii Active Sites and Groupings in the Thiamine Molecule. Minsk: Nauka i Tekhnika. 1975. 424 p.(in Russian).
6. Lehninger A.L. Biochemistry. N.-Y.: Worth Publishers Inc. 1972. 957 p.
7. Adsorption of Riboflavin and its Copper Complex onto Highly Dispersed Silica Surface/V.A. Bidzilya, L.P. Golovkova, N.N.Vlasova, V.I. Bogomaz// Zh. Fiz. Khim. - 1998. - V.72, N 3. - P.517-520.
8. Yu.V. Morozov, N.N. Bazhulina, Electronic Structure, Spectroscopy, and Reactivity of Molecules. M.:Nauka. 1988. 288 p. (in Russian).
9. Adsorption of vitamin B₆ onto highly disperse silica/ A.A. Svezhentsova, N.K. Davidenko, N.N. Vlasova, V.I. Bogomaz// Zh. Fiz. Khim.-1993 - V.67, N 6. -. P.1242-1245
- 10.Adsorption of α -tokophezol and its analogues onto silica surface/ V.A. Bidzilya, L.P. Golovkova, N.N. Vlasova, N.K. Davidenko// Ukr. Khim. Zhurn. -1994 - V.60, N 9. - P.616-619.
- 11.Solubilization of α -tokophezol and its analogues in aqueous solution of nonionic surfactant /V.A. Bidzilya, L.P. Golovkova, N.N. Vlasova, V.I. Bogomaz // Kolloidn. Zhurn. - 1995. - V.52, N 2. - P.173-175.
- 12.Adsorption of α -tokophezol and its analogues onto a highly disperse silica surface from aqueous micellar solution of nonionic surfactant /V.A. Bidzilya, L.P. Golovkova, N.N. Vlasova, V.I. Bogomaz // Zhurn. Fiz. Khim. -1996. - V.70, N 2. - P.290-301.
- 13.Solubilization and adsorption of short chain analogue of vitamin E in presence of nonionic s surfactant /L.P. Golovkova, V.A. Bidzilya, N.N. Vlasova, V.I. Bogomaz // Zhurn. Fiz. Khim. -1995. - V.69, N 6. - P.1061-1064.