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Interaction of ascorbic acid with hydrophilic-hydrophobic silicas

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Hydrophilic-hydrophobic silicas with different degrees of silanol groups substitution by trimethylsilyl groups were obtained via gas phase modification of fumed silica with trimethylchlorosilane. The stability of ascorbic acid in ethanol solution in the presence of hydrophilic-hydrophobic silicas and reducing ability of adsorbed ascorbic acid were examined. Interaction of ascorbic acid with the initial and modified silicas was studied by means of UV and IR spectroscopy. The structure of adsorption complexes of ascorbic acid on the silica surface was modelled by the methods of quantum chemistry, as well. It has been found that interaction with the silica surface leads to formation of adsorption complexes providing an increase in ascorbic acid stability against oxidation.

1. INTRODUCTION

Improvement in drugs oxidation stability is the important task for the preparation of novel effective formulations for medicine, cosmetology and veterinary. One of the possible ways to enhance the drugs stability is to disperse the active substances on the surface of inorganic, e.g. highly dispersed silica, nanoparticles [1]. The immobilization of drugs onto nanoparticles surface may have special significance for the preparation of complex formulations including such relatively unstable compounds as ascorbic acid.

Ascorbic acid (AA) is a very important bioactive compound having a variety of biological and pharmaceutical functions [2-4]. However, low stability of ascorbic acid in relation to air, moisture, heat, light, oxygen restricts its application in pharmaceutical and dermatological formulations. To enhance ascorbic acid stability in the formulation, many different approaches were used, including synthesis of more stable AA derivatives or AA encapsulation with microemulsions or inorganic layered matrices [4-6]. In our work we tried to stabilize ascorbic acid by its adsorption on highly dispersed silica and to study the interaction of AA with the silica particles having hydrophilic or hydrophilic and hydrophobic surface groups.

2. EXPERIMENTAL

Fumed silica A200 with a specific surface area of 200 m^2/g was used as the initial adsorbent. The surface modification was accomplished by the chemisorption of trimethylchlorosilane from the gas phase; the degree of substitution of silanol groups by trimethylsilyl groups was regulated by varying the amount of the modifying agent and monitoring by IR spectroscopy. The A200, AMT10, AMT40, AMT70 samples with a degree of surface modification of 0, 10, 40 and 70% respectively were used in the adsorption experiments. Since our preliminary experiments showed rapid oxidation of ascorbic acid in the aqueous media and some deceleration of the process in ethanol solution (see also [7, 8]), AA adsorption on silica particles was performed from concentrated solutions in 96%-ethanol; immediately after centrifugation of the suspension the sediment was dried under vacuum for several hours. The total amount of ascorbic acid in a dried sample was determined taking into account the changes in concentration of solution after adsorption and the volume of the remaining solution in the moist sediment. Concentrations of ascorbic acid in the solutions were determined from the UV absorption band at $\lambda = 247$ nm using literature data as to its molar extinction coefficient [9]. Interaction of ascorbic acid with the silica surface was studied by means of IR and UV spectroscopy. The fractions of silanol groups involved in AA adsorption were estimated from the change in the intensity of 3750 cm^{-1} band in the IR spectra of the samples before and after adsorption [10]. Antioxidant activity of dissolved and adsorbed ascorbic acid was characterized by using the total polyphenolic index and DPPH-assay [11, 6]. To determine the total polyphenolic index 11.5 ml of distilled water, 5 ml of 20% solution of sodium carbonate, 1.25 ml of Folin–Chiocalteu reagent (Merc) and 6.25 ml of distilled water were gradually added to the suspension of silica with adsorbed ascorbic acid in 1 ml of water or to 1 ml of ascorbic acid solution. Then the suspension or solution was stirred for 30 min at room temperature and centrifuged; then the absorbance of the supernatant at 750 nm was measured and the total polyphenolic index was calculated by multiplying the value of absorbance by 5 [11]. The free radical scavenging activity of the dissolved and adsorbed ascorbic acid was assayed using a relatively stable free radical, DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl, Sigma), according to the technique described in [6]. Solution of ascorbic acid and the suspension of silica with adsorbed AA in 70% ethanol was prepared, then 8 ml of 0.15 mM DPPH solution in 70% ethanol were added to 12 ml of solution or suspension. The mixtures were stirred for 30 min and centrifuged, after that the absorbance of the supernatant at 517 nm was measured and the free radical scavenging activity was quantified by comparing the change of absorbance [6]. Structural and electronic characteristics of model clusters of silica, their complexes with ascorbic acid, and free energy of adsorption (ΔG_{ads}) have been calculated using the solvation model SM5.42 with the 6-31G(d) basis set (GAMESOL program package, Version 3.1 [12]).

3. RESULTS AND DISCUSSION

Figure 1 gives the concentration of ascorbic acid versus the storage time for a fresh ethanol solution and for the same solution containing initial or modified silica.

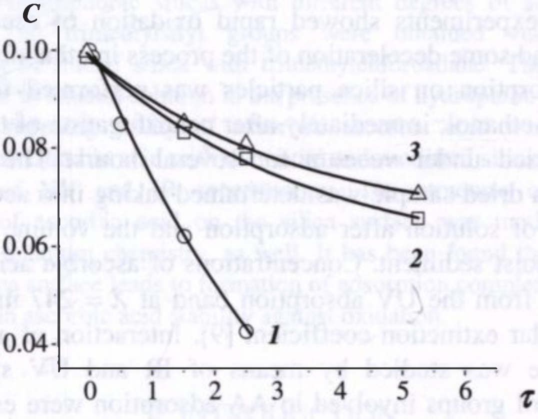
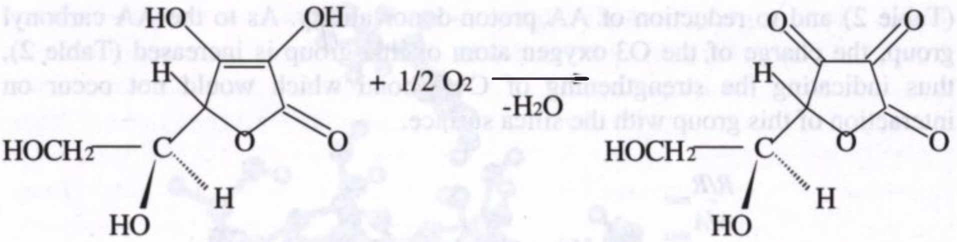


Fig. 1. Concentration of ascorbic acid in solutions versus time of storage:
1 – ascorbic acid solution without adsorbent,
2 – ascorbic acid solution in the presence of hydrophilic A200 silica,
3 – ascorbic acid solution in the presence of hydrophilic-hydrophobic AMT10 silica
τ – time, hours; C – concentration, mmole/l.

As one can see from Figure 1, the concentration of ascorbic acid in the solution without adsorbent decreases sharply; according to the data [2, 3, 9] which is caused by interaction of AA with dissolved oxygen resulting in its transformation into dehydroascorbic acid:



In the presence of both initial and modified silica the stability of ascorbic acid in solutions is enhanced significantly (Figure 1, curves 2, 3). This stabilization may be caused by the adsorption of ascorbic acid on the silica surface and the formation of surface complexes providing the improvement of AA stability. Indeed, the data on adsorption of ascorbic acid on the silica surface show that the significant amount (~0.1 mmole/g) of AA may be extracted from the solution by silica particles.

Being adsorbed on the silica surface, ascorbic acid appears to retain the monomer form which is characteristic of the dissolved ascorbic acid. Indeed, as one can see from Figure 2, the UV spectrum of adsorbed ascorbic acid (curves 2, 3) differs sharply from the spectrum of initial crystalline AA (curve 1) and is very similar to the spectrum of AA in the freshly prepared solution (curve 4).

At the same time, the stability of adsorbed ascorbic acid against oxidation arises significantly. Table 1 gives the data of antioxidant activity tests showing the reducing ability of dissolved and adsorbed ascorbic acid after different storage time.

As can be seen from Table 1, adsorbed ascorbic acid retains ~80–100% of its activity after one-year storage while the dissolved one loses its antioxidant properties during several hours.

The improved stability of the adsorbed form of ascorbic acid seems to be due to the peculiarities of the ascorbic acid–particle interaction. There are evidences in literature [6], that ascorbic acid may be adsorbed on silica due to the interaction of the C=O group of the acid with the silanol group of silica surface. On the contrary, the results of our quantum chemical calculations (Figures 3 and 4, Table 2) show that it is the hydroxyl group of ascorbic acid which takes part in the formation of adsorption complexes.

As one can see in Figure 3, it is thermodynamically favourable for the ascorbic acid molecule in ethanol solution to form the hydrogen bond between its hydroxyl group and the silanol group of silica surface. The adsorption complex of similar structure is formed on interaction of ascorbic acid with modified silica as well (Figure 4), with the free energy of adsorption being practically the same as for the initial adsorbent. In turn, such interaction leads to decrease in the charges of hydrogen atoms of hydroxyl groups of ascorbic acid

(Table 2) and to reduction of AA proton-donor ability. As to the AA carbonyl group, the charge of the O3 oxygen atom of this group is increased (Table 2), thus indicating the strengthening of C=O bond which would not occur on interaction of this group with the silica surface.

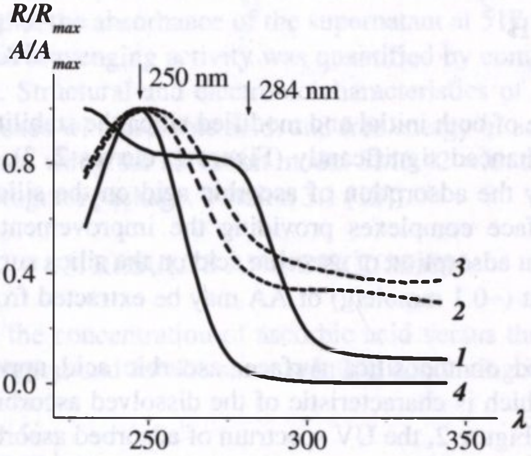
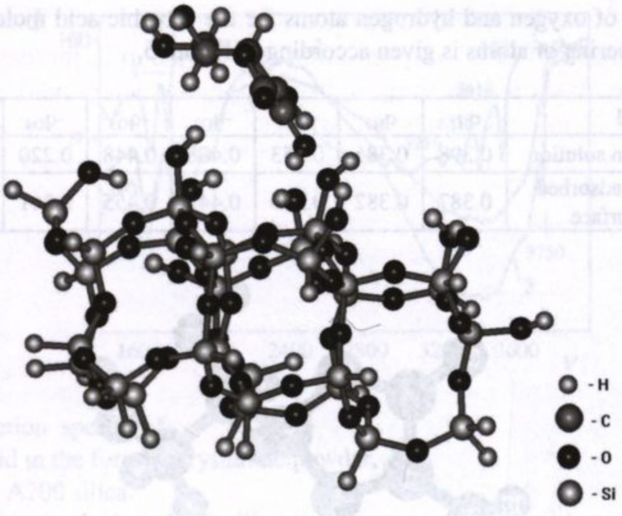


Fig. 2. Normalized UV diffusive reflection (1-3) and UV absorption (4) spectra of ascorbic acid:
1 – ascorbic acid in the form of crystalline powder,
2 – ascorbic acid adsorbed on hydrophilic A200 silica,
3 – ascorbic acid adsorbed on hydrophilic-hydrophobic AMT40 silica,
4 – ascorbic acid dissolved in ethanol.
λ – wavelength, nm; $R/R_{\max}$, $A/A_{\max}$ – normalized reflection/absorption intensity.

Tab. 1. Antioxidant activity of ascorbic acid in the adsorbed state and in the solution after different storage time.

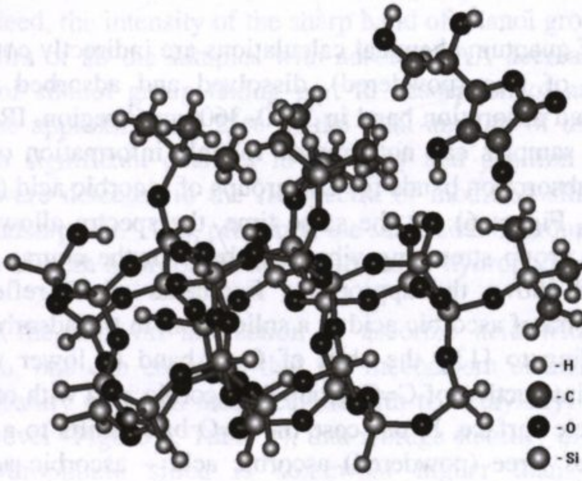
Sample*	Storage time	Total polyphenolic index	Quantity of DPPH radicals inactivated, %
Ascorbic acid in solution	-	1.0	95
Ascorbic acid in solution	~30 min	0.3	n.d.
Ascorbic acid on A200 silica	~1 year	0.9	80
Ascorbic acid on AMT40 silica	~1 year	1.0	96

* – Content of ascorbic acid in all the samples is ~ 0.5 μmol.



$\Delta G_{ads} = -30$ kJ/mole

Fig. 3. Ascorbic acid molecule adsorbed on the surface of A200 silica. Silica is modelled as a cluster of 12 tetrahedrons $SiO_{4/2}$.



$\Delta G_{ads} = -31$ kJ/mole

Fig. 4. Ascorbic acid molecule adsorbed on the surface of AMT40 silica. Silica is modelled as a cluster of 12 tetrahedrons $SiO_{4/2}$.

Tab. 2. Charge of oxygen and hydrogen atoms for the ascorbic acid molecule in ethanol solution. Numbering of atoms is given according to Figure 5.

Model	q_{H1}	q_{H2}	$-q_{O1}$	$-q_{O2}$	$-q_{O3}$	$-q_{O4}$	$-q_{O5}$	$-q_{O6}$
Ascorbic acid in solution	0.398	0.385	0.353	0.408	0.448	0.220	0.481	0.492
Ascorbic acid adsorbed on silica surface	0.387	0.382	0.390	0.442	0.455	0.261	0.476	0.491

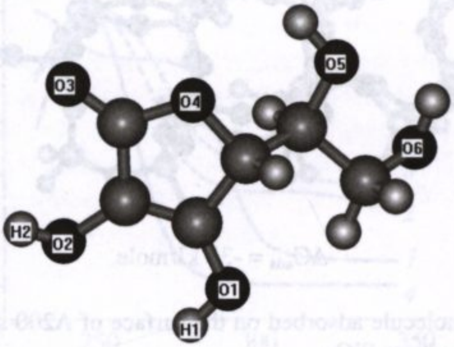


Fig. 5. Ascorbic acid molecule with the numbered oxygen and hydrogen atoms.

The results of quantum chemical calculations are indirectly confirmed by the IR investigation of free (powdered), dissolved and adsorbed ascorbic acid. Because of a broad absorption band in 3200–3600 cm^{-1} region, IR spectra of the silica-containing samples can not provide reliable information on the position and intensity of absorption bands for OH groups of ascorbic acid (at 3216, 3315, 3410, 3525 cm^{-1}, Figure 6). At the same time, the spectra allow to follow the changes in C=O group stretching vibrations band in the course of adsorption. Figures 7 and 8 shows the appropriate fragments of IR reflection and IR transmission spectra of ascorbic acid in a solid form, in the adsorbed state and in solution. According to [13], the shift of C=O band to lower wave numbers should occur on interaction of C=O group of ascorbic acid with other molecules or with the particle surface. In our case the C=O band shifts to a higher energy region in a series "free (powdered) ascorbic acid – ascorbic acid in ethanol solution / ascorbic acid on the modified silica surface – ascorbic acid on the initial silica surface" (Figures 7 and 8), thus indicating the decrease of C=O group contribution to any interaction.

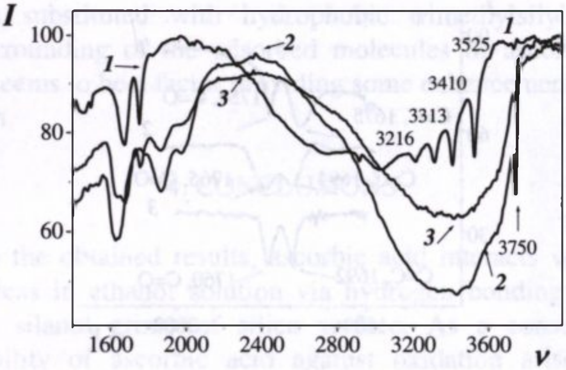


Fig. 6. IR reflection spectra of:
1 – ascorbic acid in the form of crystalline powder,
2 – hydrophilic A200 silica,
3 – ascorbic acid adsorbed on A200 silica.
ν – wavenumber, cm^{-1}; I – reflection intensity.

The IR investigations of silica adsorbents before and after adsorption of ascorbic acid confirm the contribution of silanol groups to the interaction as well (Figure 6). Indeed, the intensity of the sharp band of silanol group (at 3750 cm^{-1}) in the IR spectra of all the samples with adsorbed AA decreased significantly. The fraction of silanol group taking part in adsorption of ascorbic acid was estimated to be approximately 25% of the total amount of the groups. At the same time, no significant changes in intensity and position of trimethylsilyl groups band were detected in the IR spectra of modified silica as a result of ascorbic acid adsorption. Thus, probably, the same silanol groups are responsible for interaction of both hydrophilic and hydrophilic-hydrophobic adsorbents with ascorbic acid.

Comparing the data on interaction of ascorbic acid with the initial and modified silica, one can conclude that the mechanism of adsorption does not change significantly on silica modification with trimethylsilyl groups. It should be noted, however (Figure 1, Table 1), that storage stability of AA adsorbed on hydrophilic-hydrophobic silica is somewhat higher than in the case of hydrophilic adsorbent.

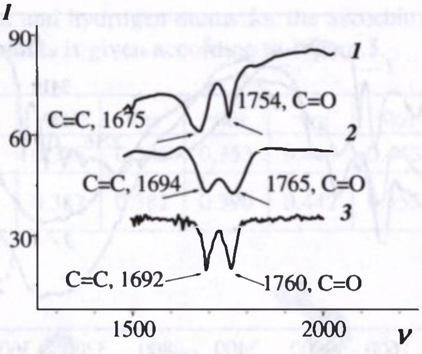


Fig. 7. Fragments of IR reflection (1, 2) and IR transmission (3) spectra of ascorbic acid:
1 – ascorbic acid in the form of crystalline powder,
2 – ascorbic acid adsorbed on hydrophilic A200 silica,
3 – ascorbic acid dissolved in ethanol.
ν – wavenumber, cm^{-1}; I – reflection/transmission intensity.

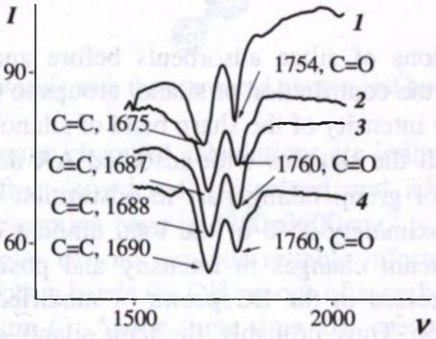


Fig. 8. Fragments of IR reflection spectra of ascorbic acid:
1 – ascorbic acid in the form of crystalline powder,
2 – ascorbic acid on AMT10 silica,
2 – ascorbic acid on AMT40 silica,
3 – ascorbic acid on AMT70 silica.
ν – wavenumber, cm^{-1}; I – reflection intensity.

Perhaps, the stability of ascorbic acid in a dried state may be affected by the quantity of water in the close vicinity of the AA molecule in the adsorption layer. As mentioned above, only ¼ part of the surface silanol groups of hydrophilic silica took part in the interaction with ascorbic acid. When exposed to air after vacuum drying, the silica particle with adsorbed AA can additionally adsorb significant amounts of water due to high affinity of the remaining group for the water molecules. In the case of modified silica, the noticeable fraction of free silanols is substituted with hydrophobic trimethylsilyl groups. More hydrophobic surrounding of the adsorbed molecules of ascorbic acid on the modified silica seems to be a factor providing some enhancement of AA stability against oxidation.

4. CONCLUSIONS

According to the obtained results, ascorbic acid interacts with hydrophilic-hydrophobic silicas in ethanol solution via hydrogen bonding of its hydroxyl group with the silanol group of silica surface. As a consequence of this interaction, stability of ascorbic acid against oxidation arises significantly, indicating the opportunity to stabilize AA by its adsorption on highly dispersed silica. Partial modification of silica surface with hydrophobic trimethylsilyl groups does not change the mechanism of adsorption but seemingly provides some additional improvement of ascorbic acid storage stability.

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