

Adsorption of cholic acid on silica modified with benzyltrimethylammonium groups

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Chemical fixation of benzyltrimethylammonium groups on vinyl silica surface has been realized using a copolymerization reaction. It has been found that cholic acid is better adsorbed on the modified silica than on cholestyramine. Main parameters of adsorption — constants of ion exchange, aggregation, and stability — have been calculated, and conclusions about mechanism of cholic acid adsorption on modified silica have been made.

С помощью реакции сополимеризации осуществлено химическое закрепление бензилтриметиламмониевых групп на поверхности винилкремнезема. Установлено, что холевая кислота адсорбируется на модифицированном кремнеземе лучше, чем на холестыраме. Рассчитаны основные параметры адсорбции — константы ионного обмена, агрегирования и стабильности. Сделаны выводы относительно механизма адсорбции холевой кислоты на модифицированном кремнеземе.

Bile acids are the most significant products of cholesterol transformation [1]. The increased content of bile acids in a living organism related with disorder of cholesterol biosynthesis may cause various diseases [2, 3]. Adsorbents-sequestrants, for example cholestyramine, are employed in medicinal practice to regulate the level of bile acids in organism. However, the efficiency of medicinal adsorbents-sequestrants is insufficient. Therefore, in order to attain a pronounced therapeutic effect, it large doses of adsorbents-sequestrants are to be administered (up to 30 g per day) [4, 5].

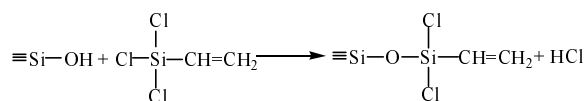
Highly dispersed silica is the most promising initial material to prepare adsorbents for bile acids. Silica has a high chemical and thermal stability, does not swell in contact with various solvents and biologic liquids; as a consequence, silica has good kinetic parameters of adsorption [6–8]. In this work, chemical immobilization of vinylbenzyltrimethylammonium chloride on silica surface is described. The cholic acid adsorption on silica modified with quaternary am-

monium groups and also on cholestyramine is fulfilled.

The amorphous silica Aerosil A-300 (from Lukor) with specific surface area 300 m²/g, particle size 5–12 nm, and concentration of free silanol groups 3.33 μmol/m² was used as the starting material. Air-dry cholestyramine (from Polymer) was used in Cl- and OH-forms. Vinylbenzyltrimethylammonium chloride (VBTMAC), chlorodimethylsilane, cholic acid (HCA), and silver nitrate (all from Fluka, ≥99.5 % purity), acetone (from Sinbias, analytical purity grade) were used without additional purification. Acetonitrile (from Sinbias) was purified by distillation before use. 2,2'-Azo-bis-isobutyronitrile (from Reakhim, pure grade) was purified by recrystallization. The run of chemical reactions on the silica surface was monitored using IR spectroscopy (IKS-29 spectrophotometer). The specific surface area of silicas was determined using nitrogen adsorption. The content of free hydroxyl groups on the silica surface was determined by chemisorption of chlorodimethylsilane

[9]. The content of quaternary ammonium groups was determined by Volgard method [10] and gravimetric quantitative analysis. The concentration of functional groups was calculated as the ratio between the content of functional groups ($\mu\text{mol/g}$) and the specific surface area of starting silica (m^2/g). Adsorption of cholic acid was studied by the multibatch method [11]. The content of HCA in solutions was determined from the optical density value in the absorption band at 389 nm of colored HCA complex with concentrated sulfuric acid [12] using a Specord M-40 spectrophotometer. The HCA adsorption values on the surface of adsorbents were calculated as the concentration difference between the initial and equilibrium solutions.

Copolymerization reaction between vinyl groups of modified silica and unsaturated bonds of vinylbenzyltrimethylammonium chloride already containing quaternary ammonium groups in the molecule was used to obtain quaternary ammonium groups immobilized on the silica surface. Vinylsilica was prepared in a quartz reactor by interacting Aerosil (preliminary treated in vacuum at 400°C) with saturated vapor of vinyltrichlorosilane at 400°C for 1 h:



The excess reagents and reaction products were removed by evacuation at 300°C for 1 h. The modified silica was kept in saturated water vapor at room temperature for 12 h and then at 100°C for 1 h and evacuated at the same temperature for 1 h in order to remove physically adsorbed water and hydrolysis products. The vinylsilica does not contain chlorine:

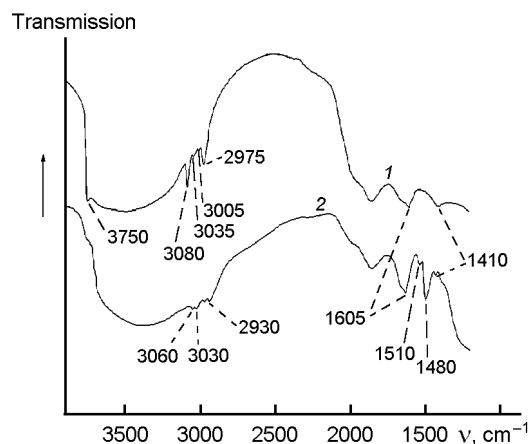
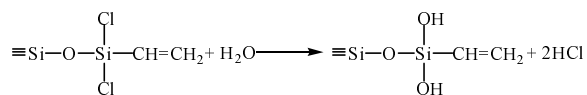


Fig. 1. IR spectra for vinylsilica prior to (1) and after (2) copolymerization reaction with VBTMAC at 90°C (silica vinyl groups/VBTMAC/initiator = 1/2/0.12).

Hydrolysis of Si-Cl groups of vinylsilica was realized to exclude any possibility of addition of hydrochloric acid to vinyl groups of vinylsilica and vinylbenzyltrimethylammonium chloride during the copolymerization reaction. The concentration of vinyl groups is equal to 3.33 $\mu\text{mol}/\text{m}^2$.

In the IR spectrum of vinylsilica (Fig. 1, curve 1), there are absorption bands at 3080, 3035, 3005, 2975, and 1410 cm^{-1} ascribed to the stretching and bending vibrations of the C-H bonds in grafted vinyl groups as well as absorption band at 1605 cm^{-1} attributed to the stretching vibrations of the C=C bonds [13].

To carry out the copolymerization reaction, 0.95 g of vinylsilica and 0.4 g of VBTMAC dissolved in 10 ml of water were placed into a quartz tube and the resulting suspension was bubbled with nitrogen within 10 min to remove atmospheric oxygen. Then, the copolymerization initiator (2,2'-azo-bis-isobutyronitrile) was added. The tube was put into an autoclave filled with

Table 1. Conditions of copolymerization and content of grafted VBTMAC (C) in a silica surface layer.

Content of components in reaction medium (mmol)			Reaction temperature (°C)	C ($\mu\text{mol}/\text{m}^2$)
Silica vinyl groups	VBTMAC	Initiator		
1	2	0.12	65	1.01
1	2	0.12	90	0.98
1	2	0.06	65	0.57
1	2	0.24	90	0.79
1	2	0.36	90	0.65

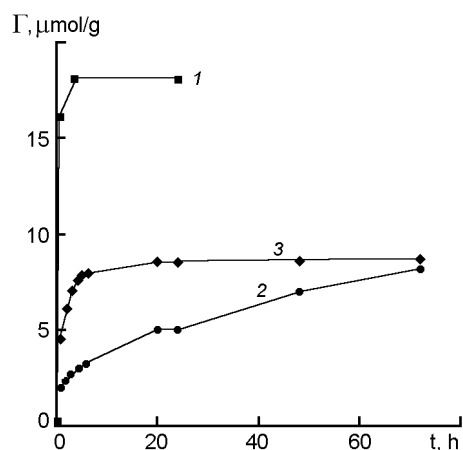


Fig. 2. Kinetic curves for HCA adsorption on BTMA-Aerosil (1), air-dry (2) and swollen (3) cholestyramines in Cl-form.

nitrogen and held in an electric furnace at a pre-specified temperature for 24 h.

We have studied the influence of two main factors (temperature and amount of initiator) on the copolymerization process of vinylsilica and vinylbenzyltrimethylammonium chloride. The initiator amount in the reaction mixture exerts substantial effect on immobilization of VBTMAC (Table 1). The highest content of grafted organic phase in the surface layer of vinylsilica is observed at following ratio: (silica vinyl groups)/(VBTMAC)/(reaction initiator) = 1/2/0.12. Lowering of the initiator amount to 0.06 mmol leads to decrease in the chemical attachment degree of VBTMAC in the silica surface layer. When the initiator content in the reaction mixture exceeds 0.12 mmol, one can also observe a decrease in organic phase content. This seems to be caused by dimerization of radicals formed due to homolytic decomposition of 2,2'-azobis-isobutyronitrile into stable compounds. As to the reaction temperature, it has been found that at the same initiator amount its elevation does not lead to any increase in grafted organic phase amount (Table 1).

The solid-phase products obtained by interaction between vinylsilica and VBTMAC have been studied by IR spectroscopy. After interaction between VBTMAC and vinylsilica at 90°C in the case of molar ratio (silica vinyl groups)/(VBTMAC)/(reaction initiator) = 1/2/0.12, absorption bands at 3060 and 3030 cm^{-1} appear in the IR spectrum ascribed to the stretching vibrations of C–H bonds in benzene rings as well as an intense band at 1480 cm^{-1} attributed to the stretching vibrations of C–H bonds in

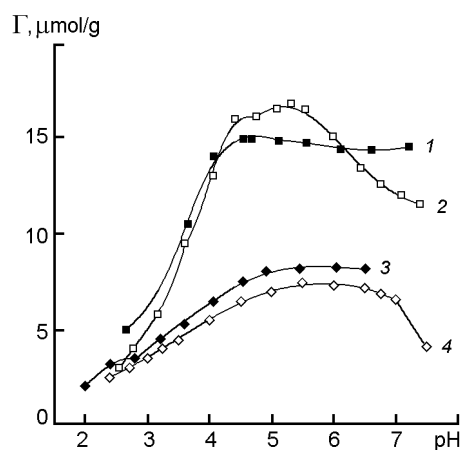
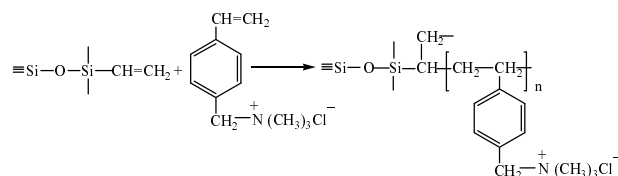


Fig. 3. HCA adsorption on BTMA-Aerosil in Cl- (1) and OH-forms (2) and on swollen cholestyramine in Cl- (3) and OH-forms (4) as a function of solution pH.

methyl groups, as well as a band at 1510 cm^{-1} related to the stretching vibrations of the C=C bonds in benzene rings. The absorption band at 3090 cm^{-1} attributed to the stretching vibrations of C–H bonds in vinyl groups of VBTMAC is absent. Besides, an increased intensity of the absorption band at 2930 cm^{-1} is observed ascribed to the stretching vibrations of C–H bonds (Fig. 1, curve 2). The IR spectrum may provide evidence to the fact that the copolymerization reaction on the vinylsilica surface proceeds in the following way:

As a result of copolymerization reaction



between vinylbenzyltrimethylammonium chloride and vinylsilica, an organosilica with chemically attached quaternary ammonium groups (the concentration of benzyltrimethylammonium groups is equal to 0.93 $\mu\text{mol}/\text{m}^2$) was synthesized (Table 2). The structure of immobilized benzyltrimethylammonium groups is similar to that of cholestyramine functional groups.

Adsorption of cholic acid from aqueous and buffer solutions on BTMA-Aerosil and air-dry cholestyramine has been studied under identical experimental conditions to compare the efficiency of these adsorbents for extraction of bile acids.

The adsorption equilibrium for BTMA-Aerosil is attained within 4 h (Fig. 2). In

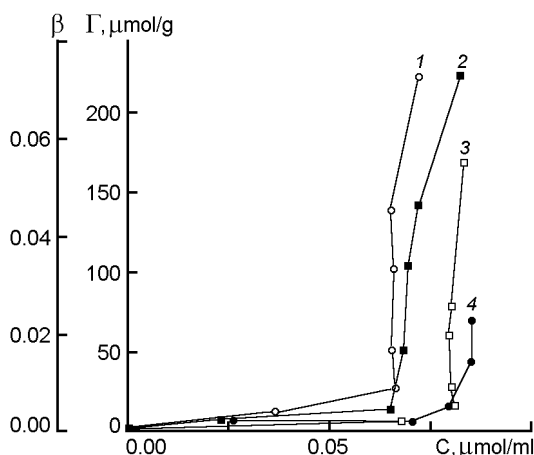


Fig. 4. Dependences of cholic acid adsorption (Γ) and binding degree (β) on equilibrium concentration of solution for air-dry cholestyramine in Cl-form at 20°C: 1, aqueous solution; 2, phosphate buffer; 3, phosphate buffer with 0.0001 M NaCl; 4, phosphate buffer with 0.1 M NaCl.

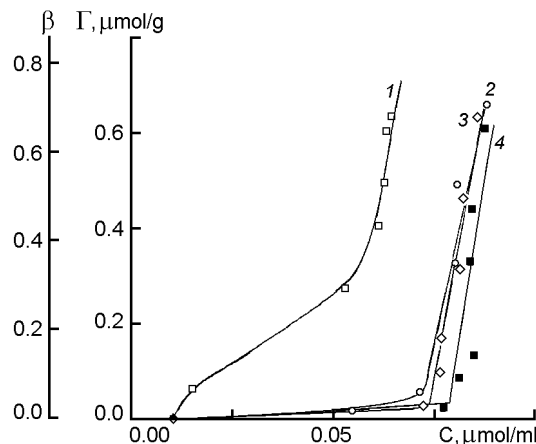


Fig. 5. Dependences of cholic acid adsorption (Γ) and binding degree (β) on equilibrium concentration of solution for BTMA-Aerosil in Cl-form at 20°C: 1, aqueous solution, 2, phosphate buffer; 3, phosphate buffer with 0.0001 M NaCl; 4, phosphate buffer with 0.1 M NaCl.

the case of swollen cholestyramine, the adsorption equilibrium is achieved within 24 h, while for air-dry cholestyramine such equilibrium is not attained even after 72 h of contact with an aqueous HCA solution (Fig. 2). Besides, as it is seen from Fig. 2, the adsorption capacity of BTMA-silica is higher than that of cholestyramine.

Dependences of HCA adsorption values on pH of equilibrium solutions are presented in Fig. 3. With increasing pH of solutions, HCA adsorption values increase and reach maxima at pH 4–5, which gives a conclusive evidence for the ion-exchange

type of HCA adsorption. Cl^- or OH^- anions situated near positively charged nitrogen atoms of quaternary ammonium groups of adsorbents are substituted by cholic acid anions. Adsorption of HCA increases as the concentration of cholic acid anions in the solution rises. At $\text{pH} > 6$, a decrease in HCA adsorption values is observed, caused by the concurrent anion-exchange sorption of hydroxyl ions from the solution. Besides, lowering of HCA adsorption values observed with increasing pH is more pronounced for adsorbents in OH-form. The adsorption capacity of silica adsorbents with chemically at-

Table 2. Adsorption parameters for silica modified with benzyltrimethylammonium groups and cholestyramine.

Adsorbent	S_{sp} (m^2/g)	QAG content (mmol/g)	Sorption medium	T ($^{\circ}\text{C}$)	Binding parameters		
					K_i (L/mmol)	K_s (L/mmol)	K_{ag}
BTMA-Aerosil	265	0.28	Aq. solution, $\text{pH} = 4.13\text{--}4.55$	20	0.71 ± 0.09	20 ± 1	28 ± 2
			0.025 M phosphate buffer, $\text{pH} = 6.86$		0.38 ± 0.06	14 ± 1	38 ± 3
			0.025 M phosphate buffer, containing 0.0001 M NaCl, $\text{pH} = 6.86$		0.27 ± 0.04	14 ± 1	52 ± 4
			0.025 M phosphate buffer, containing 0.1 M NaCl, $\text{pH} = 6.86$		0.18 ± 0.02	13.5 ± 0.6	77 ± 5
Cholestyramine*	500	3.15	0.01 M phosphate buffer, $\text{pH} = 7.4$	25	0.0245	0.429	17.7
			0.01 M phosphate buffer, containing 0.12 M NaCl, $\text{pH} = 7.4$		0.0107	0.198	18.5

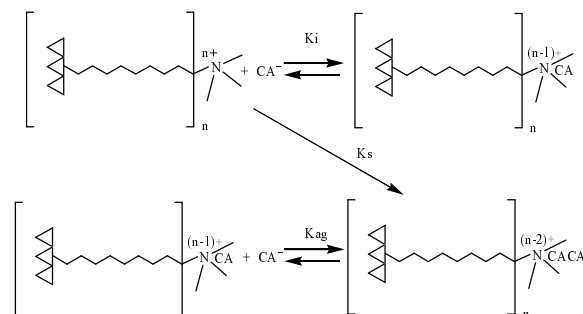
* from [7]

tached benzyl-trimethylammonium groups is higher than that for cholestyramine (Fig. 3).

HCA adsorption isotherms for BTMA-Aerosil and cholestyramine are of a sigmoid shape (Figs. 4, 5). This may be caused by proceeding of several processes on the surface of adsorbents. The ion exchange between cholic acid anions and counter-ions connected with positively charged centers (quaternary ammonium groups) of adsorbents takes place already at low HCA concentrations in solution (0.025–0.050 mmol/L). This process can be described by a constant K_i , which is a measure of electrostatic attraction between positively charged centers of adsorbent and cholic acid anions in a solution. Adsorption of HCA occurs also due to dispersion attraction between hydrophobic sections of adsorbent and steroid segments of cholic acid molecules. Hydrophobic sections are: 1) benzyl radicals providing the fixation of quaternary ammonium groups on the adsorbent surface; 2) surface ionic complexes "QAG⁺ – CA⁻". Contribution of hydrophobic interactions to the total HCA adsorption can be described by the aggregation constant K_{ag} between cholic acid anions in solution and cooperative surface adsorption centers [14], which include hydrophobic sections of grafted functional groups and surface complexes of bound quaternary ammonium groups with cholic acid anions. Hence, the equilibrium constant of total adsorption (cholic acid binding) that is, the stability constant K_s , is the product of ion exchange constant by aggregation constant [14]:

$$K_s = K_i \cdot K_{ag}$$

The whole adsorption process for the low-soluble cholic acid at the binding extent $\beta = 0.5$ can be presented as the following scheme:



The stability constant is the inverse of the cholic acid equilibrium concentration in solution at binding extent $\beta = 0.5$:

$$K_s = 1/C_{eq}, \text{ and } d\beta/d\ln C_{eq} = \sqrt{K_{ag}/4}.$$

The calculated adsorption parameters K_s , K_i and K_{ag} for BTMA-Aerosil are presented in Table 2. The similar constants for cholestyramine cannot be calculated from isotherms of cholic acid adsorption, because the majority of adsorption centers of air-dry cholestyramine does not adsorb HCA (Fig. 4). Therefore, Table 2 presents the constants for cholestyramine calculated from the sodium cholate adsorption isotherm [14]. It is to note that the cholic acid solubility is lower than that of sodium cholate.

Constants of stability K_s , ion exchange K_i and aggregation K_{ag} for silica adsorbent exceed considerably the same parameters for cholestyramine (Table 2). The increase of solution pH and ionic strength results in decrease of K_s and K_i for cholestyramine and BTMA-Aerosil (Fig. 3). Perhaps this is caused by competing exchange with OH⁻ anions or anions of buffer solution. At the same time, the aggregation constant K_{ag} increases.

Thus, immobilization of quaternary ammonium groups on silica surface by the copolymerization reaction between vinyl-silica and vinylbenzyltrimethylammonium chloride has been realized. Adsorption of cholic acid has been investigated as a function of the contact duration, equilibrium concentration, pH and ionic strength of solution for silica modified with benzyl-trimethylammonium groups and for an adsorbent-sequestrant (cholestyramine) having a similar structure of functional groups. The main adsorption parameters K_i , K_{ag} , K_s have been calculated from the cholic acid adsorption isotherm on the surface of BTMA-Aerosil. An essential contribution of hydrophobic interactions to the total adsorption of cholic acid on BTMA-Aerosil has been revealed.

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Адсорбція холевої кислоти на кремнеземі, модифікованому бензилтриметиламонієвими групами

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За допомогою реакції кополімеризації здійснено хімічне закріплення бензилтриметиламонієвих груп на поверхні вінілкременезему. Встановлено, що холева кислота адсорбується на модифікованому кремнеземі краще, ніж на холестираміні. Розраховано основні параметри адсорбції — константи іонного обміну, агрегування і стабільності. Зроблено висновки щодо механізму адсорбції холевої кислоти на модифікованому кремнеземі.