

DERIVATION OF ORGANOMODIFIED ALUMOSILICATES FOR THE CLEARING OF BIOSOLUTIONS

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Abstract - The organomodified aluminosilicates were got on a basis of natural zeolite for the clearing of biosolutions. The synthesizing was made by a chemical deposition of chitosan on the zeolite surface with further cupric sulphate aqua treatment, potassium ferricyanide aqua gradually. Sorbents composition was calibrated by elemental analyses. Physicochemical characteristics of initial zeolite and modifications were studied by using positron annihilation spectroscopy (PAS), infrared spectroscopy, X-ray phase analysis, raster electron microscopy, adsorption properties – by persorption of brilliant green. The adsorption capacity of zeolite to endotoxins was studied. It is indicated that the sorbing agent containing ferricyanide and chitosan complex has the highest persorption capacity for lipopolysaccharides (endotoxins).

Keywords – organomodified aluminosilicates, natural zeolite, chitosan, physicochemical characteristics, lipopolysaccharides.

INTRODUCTION

Natural zeolites are widely used as filtering materials for the clearing of manufacturing enterprises sewage disposals and also as cation exchangers (Brek, 1976). It is well known that zeolites have high capacity towards heavy metal ions (Khorounzhina, 1988), in particular clinoptilolite is a sorbing agent which has high affinity to cadmium (Ceramic, 1989) and thorium (Dyer, 1992) ions. Acidization of mordeniteiferous tuffs allows to increase effective diameter of channels due to decationization of crystalline grid what increases the speed of sorption processes and dynamic capacity (Lukac, 1992; Hlozek, 1992; Ovcharenko, 1987; Bobonich, 1983; Klopp, 1989). Acid activation of natural zeolites leads to restructurings and to appearance of supernegative charge which is compensated by formation of new silanol groups. At that clinoptilolite is collapsed more than mordenite in particular with chlorohydric acid reinforcement from 2 N to 3 N.

The modification of natural zeolites by metals ferricyanides leads to getting of new ion exchangers (Nikashena, 1994; Komarov, 1994). On the base of potentiometric data (Komarov, 1994) it was showed that as a result of modification the surface of zeolite becomes more acidulous than the surface of original zeolite because of the appearance of protons of hydroferricyanic acid.

The modification of natural zeolites by organic compounds leads to getting of specific sorbents particularly the modification of clinoptilolite by polyguanidine processing (Zentgraf, 1992) results sorbent realization having the main surface and the interreacting of zeolite with galaksidaz brings to appearance of thermally stable sorbent with controlled hydrophobic nature (Cody, 1990; Shapkin, 2001). Also zeolite modified by chitosan (Davydova, 2008) which approved as effective enterosorbent was got. It is

known (Ermak, 1994) that chitosan effectively fixes endotoxins (lipopolysaccharides - LPS) of G-ve bacteria which exist in biological fluids. At that a water soluble LPS-chitosan complex forms and consequently a removal of endotoxins (LPS) from biological fluids in this case turns out to be difficult (Shapkin, 2002). Therefore development of a way of getting of high efficiency sorbents on the basis of solids is the most correct decision for the clearing of biosolutions from endotoxins (LPS). In work (Brandt, 1960) a way of getting of organo-mineral sorbents is described; it lies in processing of a surface of natural zeolite by chitosan and an opportunity of its usage for endotoxins persorption.

EXPERIMENTAL PROCEDURE

Zeolite from Chuguevskiy occurrence was used as original, it contains 68% of clinoptilolite. The ultimate composition of zeolite is given in table 1.

Derivation of modified zeolite. Zeolite weighting 100g is warmed up at 150 °C during one hour. Chitosan is spread on zeolite according to the following method: in preparation of 1,5% chitosan in 2% preparation of acetic acid zeolite of a grain's diameter equal to 0.25 mm is added while correlation zeolite : chitosan is 98:3. This suspension is mixed for an hour then 1 M NH₄OH solution is added till pH 9-10. The suspension is filtered, flushed by distilled water. 20 ml of concentrated solution of copper sulphate is added to the suspension and it is mixed for one hour. The suspension is filtered and flushed from cuprum ions excess. Then they stick a solution of potassium ferricyanide of average concentration to the suspension till a formation of dense sludge. The sludge is flushed by distilled water till the discoloration of an ion of trivalent ferrum.

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Zeolite is modified in the same manner using ferric chloride instead of copper sulphate. The data of the elemental analyses of the modified zeolite which was made on an energy-dispersive roentgenofluorescive diffractometer “EDX-800-HS” are given in table 1. A complex on the surface containing 2.8 % of copper rises to the empirical formula $Cu(chit \cdot NH_3)_{2.3}[Fe(CN)_6]_{1.8} \cdot K_{5.5}$.

Table 1. Data of elemental analyses of original and modified zeolites

№	Sample	Content, %						
		SiO ₂	Al ₂ O ₃	CaO	Na ₂ O	K ₂ O	Fe ₂ O ₃	MgO
1	natural zeolite	72.3	17.1	3.61	1.14	3.79	1.60	0.20
2	zeolite + chitosan (3 %)	70.9	14.7	3.56	0.90	3.60	4.32	0.35
3	zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	63.6	14.3	4.29	0.50	2.23	4.30	1.85
№	Sample	Content, %						
		C	Cu	Fe				
2	zeolite + chitosan	3.11-3.25	–	–				
3	zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	2.61- 2.74	0.51	2.23				
4	zeolite + chitosan + Fe _x [Fe(CN) ₆] _y	2.58-2.85	–	2.80				

Infrared spectra were written down by a spectrometer “Perkin Elmer Spectrum-1000” in tablets KBr. XRF-spectra were written down on a diffractometer “Bruker Advance-D8” (Cu-K_α-rays) with 0.02 (θ) with a resolution in a bracket 2° < 2θ° < 90. Adsorption isotherms by sorption of brilliant green were got at 25 °C from 0.01% water solution of colourant with determination of optical density on a spectrophotometr “Unico-1200”. The data of a positron annihilative spectrography shown on “ORTEC PAL System spectrometer” are given in table 2. N_{PS}, V_{PS}, R_{PS}, N_{e⁺}, V_{e⁺}, R_{e⁺} are calculated according to the free volume model (Brandt, 1960; Grafutin, 2002).

A way of endotoxin fixation zeolite.

A solution containing lipopolysaccharides was added to zeolites and kept for 15 minutes, 3 hours and 48 hours. An actual of LPS in the solution before and after processing was appointed by the reaction with 2,2'- dimethylmethylene blue (Taylor blue) and also by dint of high performance liquid chromatography (HPLC) on a chromatograph “Agilent 1100” with a chase “Shodex GS-620” and “Shodex GS-7B” and refractometer “RID G 136A”. The chase was eluated by 0.9% solution of NaCl at temperature 25 °C and flow rate 0.5 ml/min.

The efficiency of sorption was calculated according to the following formula:

$$Sorption\ efficiency\ (\%) = \frac{C_{original\ LPS} - C_{LPS\ aftersorption}}{C_{original\ LPS}} \cdot 100\%$$

where C – concentration of LPS.

Table 2. Data of positron annihilative spectroscopy

The second spectrum coupler									
№	Sample	K ₂	τ ₂	N _{e⁺} · 10 ²²	V _{e⁺}	J ₂	U _{e⁺} /sec.	R _{e⁺}	β
2	zeolite + chitosan	108.0	3.62	6.14	2.111	30.1	8.05	4.34	0.41
3	zeolite + chitosan + Fe _x [Fe(CN) ₆] _y	78.0	3.59	4.43	1.522	23.7	5.80	4.35	0.54
4	zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	55.3	3.53	3.20	1.111	19.5	4.21	4.36	0.72
The third spectrum coupler									
№	Sample	K ₃	τ ₃	N _{PS} · 10 ²²	V _{PS}	J ₃	U _{PS} /sec.	R _{PS}	β
2	zeolite + chitosan	33.7	1.26	2.67	1.337	6.1	3.97	4.92	0.58
3	zeolite + chitosan + Fe _x [Fe(CN) ₆] _y	29.3	1.11	2.28	1.055	5.7	3.30	4.80	0.67
4	zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	36.5	1.12	2.88	1.331	7.6	4.17	4.80	0.73

For studying probes by the method of temporal positron annihilative spectroscopy was used temporal scintillation spectrometer made by a “chop-chop” delayed coincidence circuit on a base of multichannel pulse analyser AI-1024 with a detecting part on a base of fast plastic detectors and a multiplier electron-phototube MEP 87 (2T₀ ≈ 310 ps). A positron source ⁴⁴Ti with activity 0.6 · 10⁶ Bq was used in measurements.

RESULTS AND THEIR DISCUSSION

Physical and chemical properties of sorbents containing chitosan, chitosan + complexes were studied by different methods. The pore volume was determined by two methods: comparison of specific gravity with apparent (Dyer, 1992) and from the data of positron annihilative spectroscopy (PAS) (Cody, 1990). The characteristics of natural and modified zeolites are given in table 3.

The volume was calculated from the PAS data (table 2) by formula:

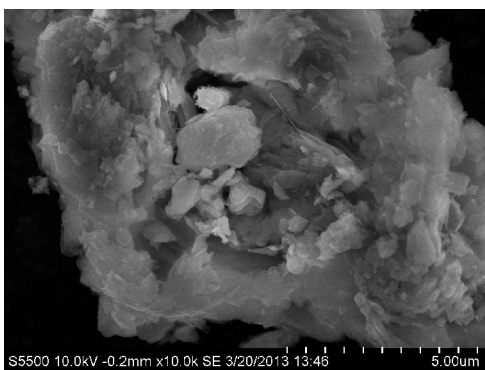
$$V_{ps} \text{ \AA}^3 = \frac{V_{ps} \text{ \AA} \cdot 10^{24} \text{ \AA}^3}{(V_{ps} + V_{e+}) \text{ \AA} \cdot N_{ps}}$$

where: $V_{ps} \text{ \AA}$ – volume of unordered intercrystalline space;
 $V_{e+} \text{ \AA}$ – volume of ordered intercrystalline space; N_{ps} – amount of annihilation in 1 sm^3 volume of unordered space.

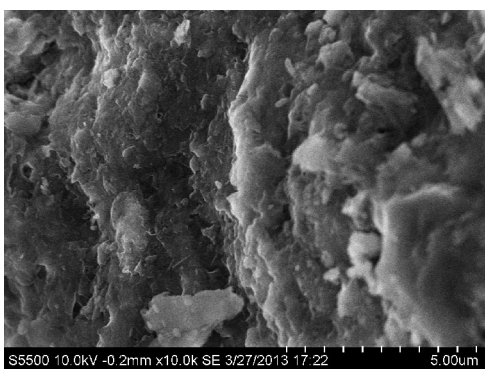
Table 3. Characteristics of natural and modified zeolites

№	Sorbent	$S_{spec.}, \text{m}^2/\text{g}$	$V_{ps} \text{ \AA}$	d, nm	$V_{pores}, \text{sm}^3/\text{g}$ (stand. method)	$V_{pores}, \text{sm}^3/\text{g}$ (PAS)
1	natural zeolite	10.0	191.0	0.897	0.270	0.275
2	zeolite + chitosan (3%)	14.1	112.6	0.898	0.570	0.680
3	zeolite + chitosan + $\text{Fe}_x[\text{Fe}(\text{CN})_6]_y$	13.2	179.0	0.896	0.560	0.650
4	zeolite + chitosan + $\text{Cu}_x[\text{Fe}(\text{CN})_6]_y$	9.9	189.0	0.898	0.560	0.690

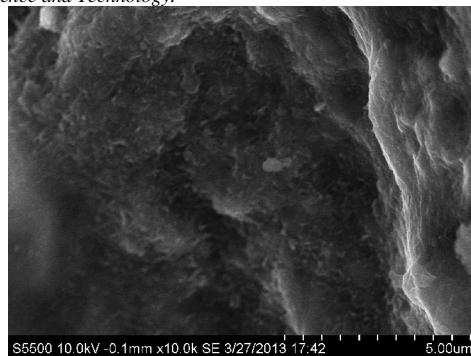
According to the data of X-ray phase analysis it was found out that steady-state conditions of clinoptilolite stayed unchanged while its modification. The results of scanning electron microscope investigation point at the fact that surface morphology rather changes especially formation of copper complex leads to determination of more bulk surface (Fig. 1).



a)



b)



c)

Fig. 1. Surface sorbents: zeolite (a), zeolite + chitosan (b), zeolite + chitosan + $\text{Cu}_x[\text{Fe}(\text{CN})_6]_y$ (c)

According to the data of infrared spectroscopy on the sorbent surface (zeolite + chitosan) some groups containing N-H, C-OH tieups appear, absorption lines $3400\text{-}3300 \text{ cm}^{-1}$, $1000\text{-}1200 \text{ cm}^{-1}$ refer to their natural oscillations, on the surface of sorbent (zeolite + chitosan + complex) one can find absorption lines $2030\text{-}2100 \text{ cm}^{-1}$. The internal volume is gradually increased by the modification of zeolite surface by chitosan, chitosan + complex (table 3).

Zeolites modified by chitosan have a higher ability to fix LPS. In the same conditions the efficiency of zeolites sorption of LPS *Y. Enterocolitica* is higher than LPS *E. coli*. Than we used HELC method for studying the influence of incubation period and temperature, and also amount of sorbent necessary for more whole fixing of LPS *Y. Enterocolitica*. The amount of LPS sorbed depends on incubation period: fixing of endotoxines by zeolites increases while period extends. As one can see in table 4 a sorbent № 4 (200 mg) which fixes LPS up to 96% has the highest activity. The sorption efficiency depends on the amount of modified forms of zeolite used. In case of decrease of sorbent amount up to 100 mg the efficiency of sorption falls greatly. However the increase of temperature up to 37°C and the extend of the incubation period up to forty eight hours allows to increase the fixing of LPS and achieve the effect which has been seen while an incubation of 200 mg sorbents solutions (96% in case of № 4 sorbent, table 4). LPS sorption on № 3 and № 4 sorbents in actual fact doesn't depend on temperature while endotoxin sorption on № 2 sorbent extends only a little in case of temperature increase up to 37°C .

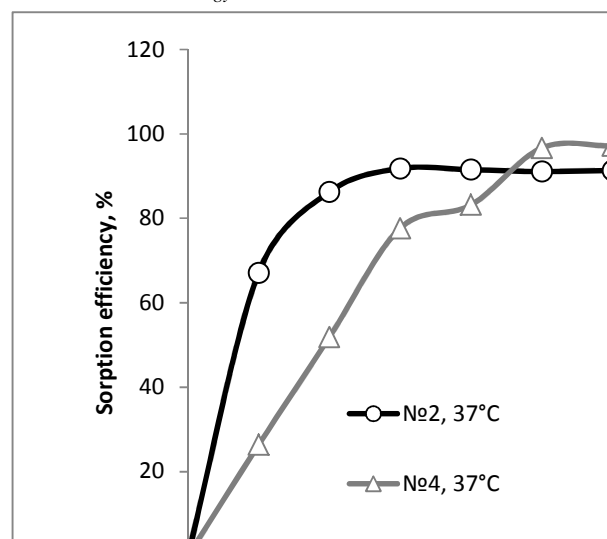
For sorbents № 2 and № 4 showing more effective LPS fixing optimum ratio of LPS were matched – a sorbent for quick (incubation takes not more than 15 minutes) and effective removal of endotoxin from the solution. For that purpose a standardized test solution containing LPS (in case of concentration equal to 2 mg/ml) was incubated for fifteen minutes with a certain quantity of sorbent and after that the efficiency of sorption was calculated. At 25°C a larger amount of sorbent is needed for the whole fixing of endotoxin. The rise of temperature brings to the increase of sorption efficiency what is typically for two types of sorbents.

Table 4. Influence of temperature, incubation period and sorbent amount on LPS sorption

№	Sorbent	Sorption efficiency, %					
		25°C			37 °C		
		0.25 hour	3 hours	48 hours	0.25 hour	3 hours	48 hours
	Amount of sorbent	200 g			200 g		
2	Zeolite + chitosan	77.23 ± 1.36	91.28 ± 4.73	92.52 ± 2.03	-	-	-
3	Zeolite + chitosan + Fe _x [Fe(CN) ₆] _y	79.61 ± 6.98	86.17 ± 0.93	91.25 ± 3.00	-	-	-
4	Zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	82.49 ± 7.85	91.50 ± 3.02	96.55 ± 0.82	-	-	-
	Amount of sorbent	100 g			100 g		
1	Zeolite	5.38 ± 2.36	12.24 ± 3.35	15.28 ± 3.67	16.92 ± 0.38	15.20 ± 0.16	21.98 ± 0.81
2	Zeolite + chitosan	52.29 ± 9.03	78.56 ± 2.72	92.07 ± 1.44	61.50 ± 5.75	84.41 ± 1.57	92.12 ± 0.98
3	Zeolite + chitosan + Fe _x [Fe(CN) ₆] _y	59.76 ± 6.09	63.46 ± 2.78	80.03 ± 1.74	58.99 ± 5.09	69.72 ± 3.83	84.55 ± 0.59
4	Zeolite + chitosan + Cu _x [Fe(CN) ₆] _y	59.60 ± 9.93	71.37 ± 1.59	91.03 ± 0.59	55.08 ± 5.49	71.69 ± 2.61	96.67 ± 0.43

Table 5. LPS sorption efficiency by sorbents № 2 and № 4 depending on sorbent amount

Sorbent mass, mg	Sorption efficiency, %			
	Sorbent № 2		Sorbent № 4	
	25 °C	37 °C	25 °C	37 °C
50	37.47 ± 8.81	67.10 ± 3.41	24.51 ± 0.03	26.37 ± 4.86
100	51.43 ± 6.00	86.25 ± 1.75	40.76 ± 3.84	51.88 ± 4.75
150	59.45 ± 4.23	91.80 ± 0.78	57.60 ± 2.67	77.68 ± 2.35
200	69.66 ± 2.38	91.51 ± 1.27	62.78 ± 2.08	83.19 ± 2.99
250	70.92 ± 1.45	91.08 ± 0.43	88.89 ± 1.20	96.69 ± 0.21
300	77.69 ± 1.17	91.34 ± 0.39	94.45 ± 0.51	97.17 ± 0.55

**Fig. 2.** LPS adsorption efficiency depending on the amount of zeolite

According to the data resulting (Fig. 2, tab. 5), for the sorbent № 2 the maximum sorption (91%) is achieved at 37°C using 150 mg of sorbent however the further increase of sorbent amount doesn't brings to LPS fixing extend. For the sorbent № 4 the increase of sorption efficiency with a larger amount of sorbent is typical, it achieves maximum at 37°C in other words at temperature of a living being; 250 mg of sorbent are able to fix up to 97% of LPS.

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