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SYNTHESIS AND BIOPROTECTIVE PROPERTIES OF HYBRID MONTMORILLONITE-POLYSACCHARIDE COMPOSITES

Abstarct. Polysaccharide-silicate composites based on pectin (PC) and Tagansorbent (TS) - montmorillonite of the Tagansky deposit – have been synthesized. The calculated amount of pectin in the composites was 5, 10, 20, and 40%. Viscosimetric method showed the degree of fixation of pectin on the surface of the inorganic sorbent Samples of the prepared pectin-containing composites have been studied by various physicochemical methods (IR-spectroscopy, scanning electron microscopy, X-ray diffraction). The data of IR – spectroscopy and scanning electron microscopy showed the presence of polysaccharide in the prepared composites. The IRS method has confirmed the shifting absorption bands of the pectin functional groups in the polymer fixed to Tagansorbent. SEM images has demonstrated modification of the surface of inorganic sorbent after treatment with pectin.

The adsorption of lead ions (Pb^{2+}) on the developed polysaccharide-silicate composites has been studied for further their testing as enterosorbents. The optimum adsorption activity was observed in the presence of the composite with a pectin content of 9.4%. The polysaccharide-silicate composites have shown bioprotective properties at the study of the total proteolytic activity (TPA) of the intestinal walls of lead-intoxicated rats.

Keywords: Pectin, polysaccharides, tagansorbent, organo-inorganic composite, sorption activity, bioprotective properties.

Introduction

Recently a wide variety of new technologies on syntheses of polymer-inorganic nanocomposites is being developed. One of the most potentially complementary applications of the hybride composites is the creation of new materials with improved properties. Polysaccharides as the components of the composites are in the focus of the researchers [1-6] due to biodegradability, environmental friendliness and renewability of ssuch kind of polymers [7-14]. Hybrid sorbents consisting of biocompatible components can be used in medicine (as enterosorbents) for removing toxic substances from living organisms [15-18].

In the present work, pectin and montmorillonite of the Taganskii deposit are used to develop polysaccharide-silicate composites. It is known [19] that Tagansorbent is an effective detoxicant, which is used as a preparation for removing toxic substances from the human body. Pectin is also one of the most important and recognized sorbents. It is known [20] that pectin substances are extremely effective and absolutely harmless natural detoxicants. Pectin is very important for the stabilization of metabolism, It reduces the cholesterol in the body, improves peripheral circulation, as well as intestinal peristalsis. But, nevertheless, its most valuable property is the ability to purify living organisms from harmful substances: heavy metals, radionuclides, nitrates, pesticides and other toxins [21, 22].

The object of the paper is to determine the sorption capacity of lead ions on pectin-montmorillonite composites for further their testing as enterosorbents.

Experimental part

Montmorillonite of the Tagansky deposit produced by LLP "Sorbent" (Tagansorbent), pectin (PC, Mw = 15000, the content of the uronide components is 91.3%, the degree of esterification is 64.3%, pure

for analysis), lead nitrate (II), pyridylazo-resorcinol (PAR, pure for analysis.) were used without further purification.

Synthesis of pectin-containing hybrid materials based on Tagansorbent (TS) was carried out by adsorption of pectin on this inorganic sorbent at room temperature and constant stirring for 2 hours. The precipitate was then kept in the mother liquor within 24 hours. The amount of polymer injected to 1.0 g of Tagansorbent was ranged from 0.05 to 0.67 g.

The completeness of the fixing of polymers to Tagansorbent was evaluated by the method of a calibration curve by measuring the viscosity of the mother liquor before and after pectin sorption. The viscosity of solutions was determined in a cryostatic cell KRIVOVIST-01 at a temperature of 20±0.1°C using Ubellode viscometer ($k = 0.001077\ mm^2/s^2$).

IR spectra were obtained using a Karl Zeiss Specord-IR-75 with a resolution of 3 cm^{-1} in the 4,000-400 cm^{-1} region. Pellets for infrared analysis were prepared by grinding a mixture of 1 mg sample with 100 mg dry KBr, followed by pressing the mixture into a mold. Instrumental errors at frequency determining were: 4000-2500 $cm^{-1} \pm 3\ cm^{-1}$; 2000-400 $cm^{-1} \pm 1\ cm^{-1}$.

Diffractograms of TS-containing samples were examined on a PANalytical X'Pert MPD PRO diffractometer in copper filtered radiation with a wavelength of 0.154 nm. Preparation of the samples for analysis was carried out by pipetting the aqueous suspension of the sample onto a glass plate, followed by drying in air until the water was completely removed.

The morphology and structure of the initial Tagansorbent and the pectin-containing composites were examined by a scanning electron microscope JEOL JSM-6610 LV (Japan).

The sorption properties of polysaccharide-silicate composites were studied by the following procedure: 10 ml of water were added to 0.1 g of the sorbent and mixed until a homogeneous suspension has been formed. A hydrochloric acid solution (0.01 N) was added dropwise to the resulting suspension to reach pH of 2.5 and then 10 ml of a lead nitrate solution (100 mg Pb^{2+}/l) were added. Adsorption was carried out for 4 hours. After filtration the precipitate was analyzed by SF-2000 spectrophotometer using a PAR reagent according to the procedure [23].

The detoxification properties of the developed polysaccharide-silicate composites were evaluated on the lead intoxicated rats (190-220 g) by testing changes in total proteolytic activity (TPA). The TPA index was determined in the acute experiments under Nembutal anesthesia (4 mg/100g body weight, intramuscularly). An abdominal cavity of rats was opened over the white line, blood was taken from the venous vessel and intestinal samples were prepared for further experiments. The tissue (200 mg) were taken, then tissue samples were homogenized with an Ultra-TurraxT8 dispersant. The level of total proteolytic activity of homogenates of the small intestine wall was determined by precipitation of proteins with ethyl alcohol. The calibration curve was plotted using amino acid phenylalanine and the obtained data were presented in μg of phenylalanine (Fen) per 1 mg tissue (for homogenates) for 1 hour of incubation. The control was samples without incubation.

Results and discussion

The amount of pectin adsorbed on Tagansorbent was determined using a calibration plot of the viscosity of the polysaccharide solution versus its concentration (Fig. 1).

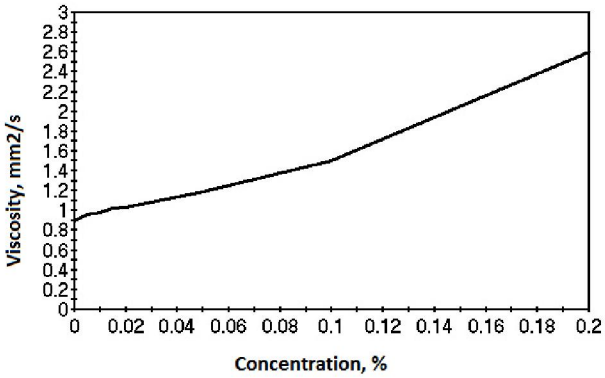


Figure 1 - Calibration chart for determination of pectin concentrations in solutions

Using the graph, the amount of adsorbed polymer was determined based on the difference in the values concentrations. The results of calculations on the content of polysaccharide in composites are presented in Table 1

Table 1 - Adsorption of pectin on Tagansorbent

The amount of pectin injected to 1 g of TS		ν (sol-n) after sorption, мм^2/s	m(PC) in sol-n after sorption, g	m(PC) adsorbed., g	Degree of adsorb., %	Comp-n of PC, %
m(PC), g	w(PC)*, %					
0.0526	5	0.9750	0.0025	0.0501	95.2	4.8
0.1111	10	1.0498	0.0078	0.1033	93.0	9.4
0.2500	20	1.2560	0.0247	0.2253	90.1	18.4
0.6666	40	2.5980	0.1047	0.5619	84.3	36.0
Note: * - the mass fraction of the injected polymer according the sum of the masses of all injected components of the composite (PC + TS), %						

It has been established that the degree of adsorption of the polysaccharide on Tagansorbent decreased with increasing amount of the biopolymer introduced. As a result, PC/TS composites were obtained, the content of pectin in which was slightly different from the calculated data (5, 10, 20% and 40%) and were 4.8, 9.4, 18.4% and 36.0%, respectively (Table 1).

The presence of pectin in the composites obtained was confirmed by IR spectroscopy (Table 2). The wide band in the area of 2800-3000 cm^{-1} is characteristic of the asymmetric and symmetric stretching vibrations of -CH- groups of pectin. The shifts of stretching (3000-3700 cm^{-1}) and bending (1200-1400 cm^{-1}) of the -OH as well as C = O groups of pectin indicated their interaction with the surface hydroxyl groups of Tagansorbent. A slight shift of some bands characteristic of Al-O and Si-O of TS was also observed confirming bonding of the polymer to TS.

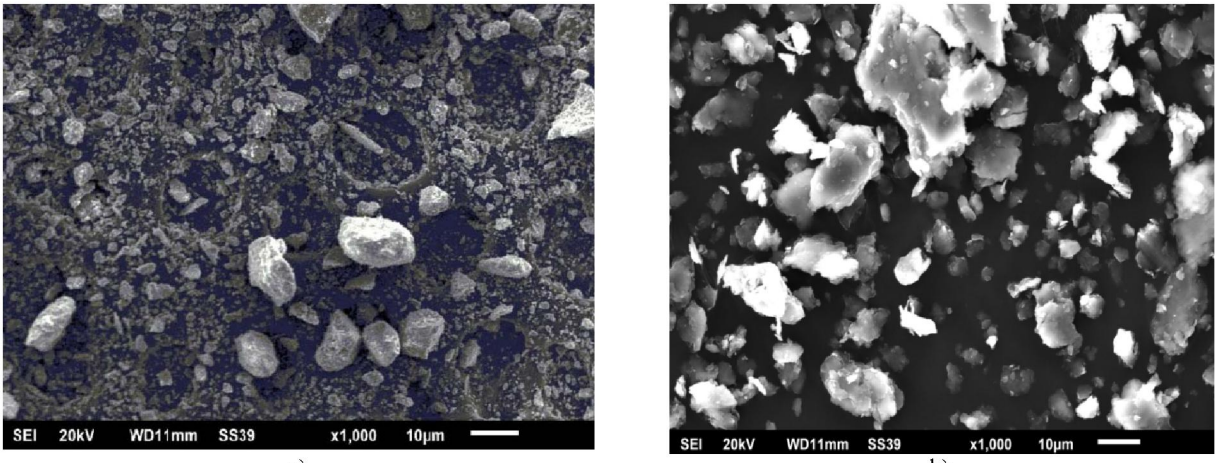
Table 2 - IRS data of the examined samples

Sample	νOH	νCH	νC=O	δCH δOH	νC-O-C νC-C νC-O	δOH(TC) νSi-O νAl-O
Pectin	3429	2933 2857	1762 1638	1445 1348 1248	1123 1076 1060	
TS	3630 3430	-	-		-	1030 914 527 472
9.4%PC/TS	3631 3408 3169	2925 2847	1758 1635	1408 1340	Overlapped by a PC signal	1039 925 529 468

Thus, the conducted studies indicate the possibility of creation of hybrid materials by adsorption of the polysaccharide on Tagansorbent. The results of IR spectroscopy have indicated the formation of polysaccharide-silicate composites through chemisorption of the polymer on the surface of the aluminosilicate.

The study of synthesized composites by scanning electron microscopy (SEM) showed that modification of the clay mineral with pectin promoted the aggregation of the aluminosilicate into larger particles to compare with initial TS (Figure 2).

It is known that the X-ray diffraction method is widely used in the study and identification of clay minerals and their composites by determining the position of basal reflection (001).



a) b)

Figure 2 - Micrographs of SEM of Tagansorbent (a) and 9.4% of PC/TS composite (b)

The diffraction pattern is represented by a wide reflex at 2θ = 7.06 ° (d001 = 12.55 Å) and with two second order reflexes at 2θ = 12.10 ° (d002 = 7.31 Å) and 14.20 ° (d002 = 6.26 Å), which indicate that the pectin-modified sodium montmorillonite is in a partially intercalated state (d001 = 14.62 Å) (Figure 3) [17]

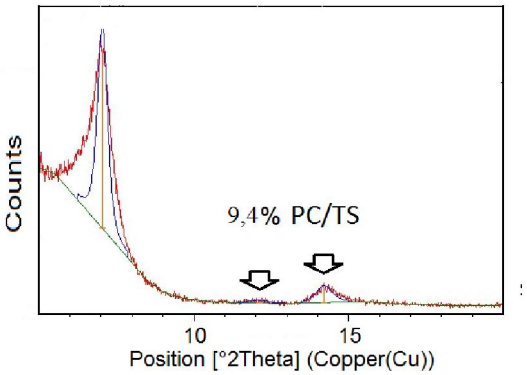


Figure 3 - Diffractogram 9.4% of PC/TS

The sorption properties of the developed polysaccharide-silicate composites towards lead ions (Pd^{2+}) were studied by spectrophotometry.

It was shown (Table 3) that the modification of Tagansorbent with pectin led to an increase in the sorption capacity of composites. The sorption capacity of PC/TS with a pectin content of 4.8% was 9.2 mg/g versus 8.4 mg/g for the original TS. A further increase in the content of biopolymer (up to 9.4%) in PC/TS led to a slight increase in sorption capacity (Table 3). In the presence of 36.0% PC/TS composite, the amount of the adsorbed lead was reduced to a level corresponding to the unmodified montmorillonite. Thus, the maximum adsorption of lead (93%) was achieved on the hybrid PC/TS composite with 9.4% polysaccharide content (Table 3).

Table 3 - Sorption of lead on PC/TS composites as a function of polymer content

PC comp-n, %	Mass of sorb., мg	$C(Pb^{2+})$ in sol-n before sorpt-n, мg/l	$C(Pb^{2+})$ in sol-n before sorpt-n, мg/l	Degree of sorption, %	Sorbent capacity, мg/g
0	102.3	50	7.0	86.0	8.4
4.8	101.6	50	3.7	92.6	9.1
9.4	101.0	50	3.5	93.0	9.2
18.4	101.4	50	4.9	90.2	8.9
36.0	102.0	50	7.1	85.8	8.4

To study the bioprotective effect of the developed polysaccharide-silicate composites, a control test was conducted. The activity of proteolytic enzymes in the intestinal part of experimental rats has been compared for the lead-intoxicated, PC/TS taken and the control group of animals (Table 4).

Table 4 - Data of total proteolytic activity of the intestinal wall after lead intoxication against the background of pectin-containing composites

№	Index	Guts μgFen/mg * hr
1	Control	465.50 ±15.72
2	$Pb(NO_3)_2$	1213.88±18.60
3	4.8% PC/TS+ $Pb(NO_3)_2$	787.45 ± 23.21*
4	9.4% PC/TS+ $Pb(NO_3)_2$	747.91 ± 12.80*
5	18.4% PC/TS+ $Pb(NO_3)_2$	714.99 ± 14.62*
Note: * - p≤0,001; compared with the data of a group of rats subjected to lead intoxication.		

The results of the experiment showed a 2.6-fold increase in the activity of proteolytic enzymes in the intestines of rats subjected to lead intoxication, as compared to control animals. The increase in proteolysis demonstrates the increased process of digestion and deep purification of body from conformationally altered lead-intoxicated proteins. It reflects the significant aggressiveness of the xenobiotic damaged intestinal wall. The enzymatic activity decreased by 1.5-1.7 times with the injection of PC/TS hybrid composites, indicating protective function of the obtained materials. A more pronounced protective effect is achieved in the presence of sorbents with a pectin content of 9.4% and 18.4%.

Thus, PC/TS composites protected the wall of the small intestine of rats from the toxic effect of lead nitrate. With an increase in the content of pectin from 4.8% to 18.4%, the activity of proteolysis decreased from 70% to 50% compared to the data for the group subjected to lead nitrate poisoning. The active removal of toxic substances from the body in the presence of composites is probably due to the synergistic effect of combining the complexing properties of pectin with the sorption ability of Tagansorbent.

Conclusions

The results of this work have shown the possibility to synthize polysaccharide-silicate composites by a simple and environmentally friendly approach through adsorption of pectin on Tagansorbent (Kazakhstan natural montmorillonite). Fixation of pectin to the surface of the inorganic sorbent with partial formation of intercalated structures has been confirmed by physico-chemical methods. The results of the study showed high sorption and bioprotective properties of the developed hybrid enterosorbents based on natural components such as pectin and Tagansorbent.

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ГИБРИДТІ МОНМОРИЛЛОНИТ-ПОЛИСАХАРИДТІ КОМПОЗИТТЕРДІҢ СИНТЕЗІ ЖӘНЕ БИОПРОТЕКТРЛІК ҚАСИЕТТЕРІ

Аннотация. Пектин (ПК) және Таған кен орнының монтмориллониті - Тагансорбент (ТС) негізінде полисахарид-силикатты композиттер алынды. Композициядағы пектиннің есептелген көлемі 5, 10, 20 және 40% құрады. Вискозиметриялық әдісі арқылы бейорганикалық сорбенттің бетіндегі пектиннің бекітілу дәрежесі көрсетілді. Құрамында 4,8, 9,4, 18,4 және 36,0% пектині бар ПК/ТС жүйелер алынды. Алынған құрамында пектин бар композиттердің үлгілері әртүрлі физикалық-химиялық әдістері (ИКС, сканерден

өтетін электрондық микроскопия, рентген дифракциясы) арқылы зерттелген. ИК-спектроскопия, сканерлеу электрондық микроскопиясының деректері композит құрамында полисахаридтің болуын растады. ИКС әдісі арқылы пектинді Тагансерорбентке отырғызған кезде полимердің функционалдық топтарының жұтылу жолақтарының ығысуы байқалынады. Бейорганикалық сорбентті пектинмен модификациялағаннан кейін СЭМ микрофографияларында алюмосиликаттың бетінде өзгерістер байқалады.

Энтеросорбент ретінде әрі қарай қолдану мақсатында өндірілген полисахарид-силикатты композиттерде қорғасын иондарының (Pb^{2+}) адсорбциясы зерттелді. Оңтайлы сорбциялық кабілеттілікке ие болып құрамында 9,4% пектині бар композит екені анықталды.

Композиттерді қолдану аясында қорғасынның интоксикациясынан кейін егеуқұйрықтардың ішек қабырғасының жалпы протеолитикалық белсенділігін (ОРА) зерттеу барысында полисахарид-силикат сорбенттерінің қорғаныш қасиеттері бар екені көрсетілген.

Түйін сөздер. Пектин, полисахаридтер, тагансорбент, органикалық-бейорганикалық композит, сорбциялық белсенділік, биопротекторлық қасиеттер.

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СИНТЕЗ И БИОПРОТЕКТРНЫЕ СВОЙСТВА ГИБРИДНЫХ МОНМОРИЛЛОНИТ-ПОЛИСАХАРИДНЫХ КОМПОЗИТОВ

Аннотация. Синтезированы полисахарид-силикатные композиты на основе пектина (ПК) и Тагансорбента (ТС) - монтмориллонита Таганского месторождения. Расчетное количество пектина в составе композитов составляло 5, 10, 20 и 40%. Вискозиметрическим методом показана степень закрепления пектина на поверхности неорганического сорбента. Были получены ПК/ТС системы с содержанием пектина 4,8, 9,4, 18,4 и 36,0%. Образцы полученных пектин-содержащих композитов были изучены различными физико-химическими метдами (ИКС, сканирующая электронная микроскопия, рентгеновская дифракция). Данные ИК-спектроскопии, сканирующей электронной микроскопии подтвердили наличие полисахарида в составе композитов. Методом ИКС установлено, что при нанесении пектина на Тагансорбент происходит смещение полос поглощения функциональных групп полимера. На микрофотографиях СЭМ после модификации неорганического сорбента пектином наблюдается изменение поверхности алюмосиликата.

Исследована адсорбция ионов свинца (Pb^{2+}) на разработанных полисахарид-силикатных композитах с целью дальнейшего их тестирования в качества энтеросорбентов. Установлено, что оптимальной сорбционной способностью обладает композит с содержанием пектина 9,4%. При исследовании общей протеолитической активности (ОПА) стенки кишечника крыс после свинцовой интоксикации на фоне применения композитов показано, что полисахарид-силикатные сорбенты проявляют протекторные свойства.

Ключевые слова. Пектин, полисахариды, тагансорбент, органо-неорганический композит, сорбционная активность, биопротекторные свойства.

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