DETOXIFICATION OF BIOLOGICAL FLUIDS BY LIGNOCELLULOSE ION-EXCHANGERS

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Detoxification capabilities of N- *and* S-*containing phytosorbents based on grafted copolymers of wood and lignin for blood serum of patients with various ophthalmic pathologies, including diabetic retinopathy, were determined.*

Key words: wood, lignin, ion exchanger, hemosorbent, phytosorbent, detoxification, bilirubin, cholesterol.

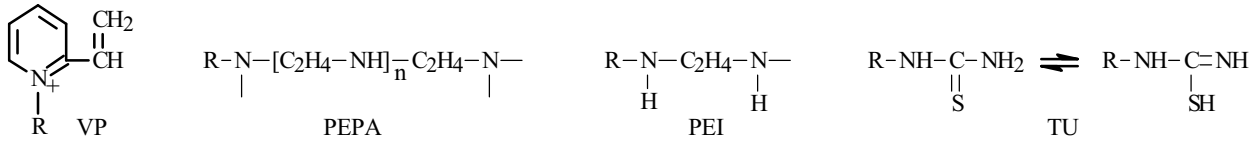
Adsorption is the fundamental physical chemical operating principle of the immune system of a living organism. A wide range of sorption materials, carbohydrate, ion-exchange, affinity, immune, etc., is applied with varying efficiency [1, 2]. Accumulated experience in their clinical use for hemosorption has shown that mineral sorbents are not selective enough compared with others and absorb from blood useful substances and toxins. However, their versatility, availability, and satisfactory compatibility with blood enabled them to be solidly entrenched in medical practice [3]. If it is considered that hemosorption, in contrast with other methods, including dialysis, can isolate from blood water- and lipid-soluble compounds, bacteria, and viruses, its efficiency can be increased only by creating strictly specific sorbents for particular toxins, the specificity of which is determined not only by the nature of the active functional groups responsible for the extraction but also by the permeability of the matrix for the absorbed substrates.

The goal of our work was to investigate the detoxification properties of new ion-exchange phytosorbetns based on grafted copolymers of wood and lignin for blood serum of patients with various ophthalmic pathologies, including diabetic retinopathy. Tables 1 and 2 give the biochemical composition of blood serum before and after contact with the ion exchangers and the synthesis conditions and principal physical chemical characteristics of the phytosorbents.

Because bilirubin acts as a weak organic acid at physiological pH values, the use of strongly basic anion-exchange sorbents to extract it is most effective. This enables the macromolecule to be bound specifically by ion exchange. This hypothesis was completely validated using the sorption by an ion exchanger based on lignin that was aminated by vinylpyridine and contained a quaternary pyridine N atom as an example. The degree of absorption reached 86.06%. The bilirubin level in serum was reduced from 14.2 to 1.98 μmol/L.

Reducing the basicity of the sorbents decreased their extraction capability. Thus, the completeness of isolation of the desired component decreased to 50.0-24.65% on going from anion exchanger with a quaternized N atom (VP) to ion exchangers enriched with tertiary, secondary, and primary amino groups (PEPA, PEI, TU):

L—DGE—VP > W—DGE—PEPA > L—DGE—PEPA > L—DGE—PEI > W—DGE—TU > L—DGE—TU



where R is the epoxidized biopolymer produced by modification of wood or lignin with DGE [4-6]; W, wood; L, lignin; DGE, diglycidyl ether of dihydroxydiphenylpropane; PEI, polyethyleneimine; PEPA, polyethylenepolyamine; VP-2, vinylpyridine; and TU, thiourea. This shows the efficiency of the electrostatic mechanism of absorption upon sorptive extraction of bilirubin.

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TABLE 1. Static Detoxification Activity of Phytosorbents Based on Wood and Lignin

Serum component	Initial level	Biochemical composition of blood serum after contact with sorbents/degree of extraction, %						
		lignin-DGE-PEI	lignin-DGE-PEPA	lignin-DGE-VP	lignin-DGE-TU	wood - DGE-PEPA	wood-DGE-TU	polyphepan
Cholesterol, mmol/L	7.30	5.60	5.50	5.20	5.37	3.81	4.93	6.39
		23.29	24.66	28.77	26.44	47.81	32.47	12.47
Bilirubin, μmol/L	14.20	10.70	7.10	1.98	10.60	6.89	10.55	14.3
		24.65	50.0	86.06	25.35	51.48	25.71	0
Sugar, mmol/L	7.40	5.00	6.00	6.50	5.10	5.90	6.90	6.90
		32.43	18.92	12.16	31.1	20.27	6.76	6.76
Total protein, g/L	66	66	66	66	66	66	66	66
		0	0	0	0	0	0	0

TABLE 2. Synthesis Conditions and Physical Chemical Properties of Ion Exchangers from Natural Polymers

Sorbent	Synthesis conditions			N, S content, %		SEC for 0.1 N solution, mg-eq/g			TSEC, mg-eq/g
	[epoxypolymer]:[amine], mass ratio	T°, C	τ, h	N_{titr}/S_{titr}	N_{tot}/S_{tot}	HCl	NaCl	NaOH	
Lignin-DGE-PEI	1.0:1.50	40	2	10.85	12.84	6.75	1.00	0.00	7.75
				0.0	0.0				
Lignin-DGE-PEPA	1.0:0.75	40	2	7.77	8.95	4.95	0.60	0.00	5.55
				0.0	0.0				
Lignin-DGE-VP	1.0:1.50	60	1	4.90	6.64	0.00	3.50	0.00	3.50
				0.0	0.0				
Lignin-DGE-TU	1.0:1.50	40	2	5.18	6.72	3.70	0.00	1.37	5.07
				4.38	9.02				
Wood-DGE-TU	1.0:1.50	40	2	4.76	6.46	3.40	0.00	1.15	4.55
				3.68	8.53				
Wood-DGE-PEPA	1.0:0.50	40	3	5.60	6.97	4.00	0.50	0.00	4.50
				0.0	0.0				

SEC and TSEC are static and total static exchange capacities, respectively.

Amphoteric ion exchangers based on W—DGE—TU and L—DGE—TU, which contain complexing thioamide groups, typically have definite detoxification activity. This is obviously due to the presence in the phytosorbent structure of the chelating sulfamide moiety, the mutual steric placement of thiol and amide groups in which have a positive influence on the coordination of macroscopic bilirubin anions to sorption centers. These capacity characteristics may be due not only to salt exchange but also molecular sorption stabilized by hydrogen bonds.

Spectra of sorbents before and after their contact with biological fluid were studied in order to determine the role of the ion-exchanger functional groups in the sorption of bilirubin and the absorption mechanism (Table 3). Spectra of samples of epoxidized lignin and wood modified with PEI, PEPA, and VP exhibited overlap of δ_{NH} or ν_{Py+R} (for the pyridine sample) bands with ν of carbonyls conjugated to a benzene ring (α-CO) at 1653, 1650, and 1640 cm^{-1}, respectively. Such overlap did not occur in spectra of TU sorbents. Frequencies $\nu_{\alpha\text{-CO}}$ were situated at 1635 cm^{-1}. However, δ_{NH} bands of thioamide groups were masked by absorption of skeletal vibrations of aromatic rings of wood and lignin and were located at 1605 cm^{-1}. Absorption bands belonging to free ester C=O and C=O not conjugated with an aromatic ring (β-CO), which usually appear at higher frequencies (1700-1750 cm^{-1}) than α-CO, were missing in spectra of the modified derivatives because these groups were cleaved during their interaction with amines. Thus, judging from the spectral analysis of the starting samples, they contain NH, $Py^{+}R$, CO, and OH groups that may be involved in the extraction.

TABLE 3. IR Characteristics of CO, NH, Py^+R, OH, and CH Vibration Frequencies of Ion Exchangers from Wood and Lignin Derivatives Before and After Sorption of Bilirubin from Blood Serum

Ion exchanger	Group vibrations before/after bilirubin sorption, cm^{-1}		
	ν_{CO}, δ_{NH}*	ν_{OH}	ν_{CH}
L-DGE-PEI	1653 (vs)	3412 (vs), 3230 (vs)	2926 (m), 2851 (w)
	1658 (vs)	3400 (s), 3246 (s)	2926 (s), 2833 (m)
L-DGE-PEPA	1650 (vs)	3410 (vs), 3230 (vs)	2926 (m), 2851 (vw)
	1650 (sh)	3402 (s), 3240 (s)	2928 (m), 2833 (m)
L-DGE-VP	1640 (s)	3545 (s), 3463 (vs), 3412 (vs), 3234 (m)	2926 (w), 2852 (vw)
	1597 (vs)	3399 (s), 3240 (s)	2927 (m), 2833 (w)
L-DGE-TU	1606 (vs), 1635 (m)	3408 (vs)	2925 (m), 2853 (vw)
	1604 (vs)	3388 (s)	2926 (m), 2830 (w)
W-DGE-PEPA	1638 (vs)	3411 (vs), 3230 (vs)	2924 (m), 2850 (vs)
	1653 (sh)	3408 (vs)	2926 (m), 2854 (w)
W-DGE-TU	1605 (vs), 1635 (s)	3411(vs)	2924 (m), 2850 (w)
	1602 (vs)	3387(s)	2926 (m), 2850 (m)
Bilirubin	1690 (vs), 1611 (vs)	3411 (s), 3246 (m)	2921 (m), 2853 (w)

*ν_{Py+R} for pyridine ion exchanger; vs, very strong, s, strong; m, medium; w, weak; vw, very weak; sh, shoulder.

IR spectra of the ion exchangers after bilirubin sorption showed much stronger (1.5-2 times) absorption for ν_{CH} than spectra of the starting ion exchangers. The band shapes in the region of OH and CH absorption (2800-3500 cm^{-1}) with maxima at 3400-3387, 3246, 2926, and 2833 cm^{-1} coincided with the spectrum of bilirubin itself, for which ν_{OH} and ν_{CH} appeared at practically these same frequencies. This fact and the increased strength of the CH bands indicated that the frequencies of CH absorption of bilirubin overlapped with analogous ones in the ion exchangers themselves and was consistent with its presence in the sorbents.

The band at 1640 cm^{-1}, which corresponds to α-CO and quaternized pyridinium, disappeared completely in the spectrum of the L—DGE—VP ion exchanger after bilirubin sorption. A new band at 1597 cm^{-1} appeared. The lack of maxima at 1611 and 1690 cm^{-1}, which are characteristic of NH and COOH groups of bilirubin, and the shift of the frequencies for ν_{CO} and ν_{Py+R} by 43 cm^{-1} to low frequency are obviously explained by involvement of both groups of the ion exchanger and the extracted substrate in sorption to form an ionic bond between quaternary N atoms of the pyridinium-based and bilirubin carboxylic groups and hydrogen bonds between CO and OH groups of the ion exchanger and bilirubin OH and NH groups. Significant broadening in the region of active H absorption at 3000-3600 cm^{-1} and the shift of the band maximum to low frequency indicated that H-bonds had formed.

The position of frequencies for δ_{NH} and $\nu_{\alpha\text{-CO}}$ at 1653 cm^{-1} did not change in the spectrum of L—DGE—PEI ion exchanger after bilirubin sorption. However, the substantial increase in their strength and the lack in the spectrum of ν_{COOH} bands of bilirubin was apparently the result of a shift of the COOH frequency of the sorbed molecule by 37-83 cm^{-1} into the region of α-CO and NH (1653 cm^{-1}) and aryl group (1606 cm^{-1}) absorption. The band for α-CO and NH groups involved in interaction with OH and COOH groups of bilirubin may have shifted into this same frequency range (1606 cm^{-1}). Broadening of ν_{OH} bands at 3100-3600 cm^{-1} with maxima at 3400 and 3246 cm^{-1} compared with analogous bands in the spectrum of the starting ion exchanger suggested highly that OH and CO groups of the sorbent were involved in the formation of H-bonds with NH and OH groups of bilirubin.

The band for $\nu_{\alpha\text{-CO}}$ and δ_{NH} in spectra of ion exchangers based on wood and lignin modified with PEPA changed shape upon sorption of bilirubin, in contrast with spectra of the ion exchanger based on PEI. It changed from a clearly defined band into a shoulder next to one at 1604 cm^{-1}. For wood, it shifted by 15 cm^{-1}. The increased strength of the band at 1608-1606 cm^{-1}, corresponding to absorption of aromatic groups of the lignocellulose complex, was probably a consequence of the shift of the absorbing groups to low frequency by 29-49 cm^{-1} and overlap with the ν_{Ar} band. In all instances the bands at 3000-3600 cm^{-1} became broad and smoothed after bilirubin absorption, in certain instances without clear maxima. This was evidently a result of forming a H-bond system.

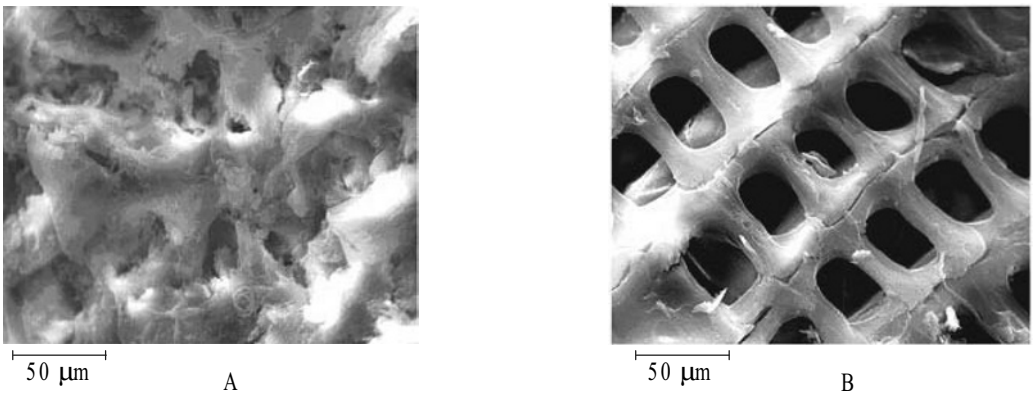

Fig. 1. Photomicrograph of lignin (A) and wood (B) surface (1000×).

The $\nu_{\alpha\text{-CO}}$ band disappeared completely on going to the TU sorbents. As noted earlier, the α-CO band in the thioamidated samples was not masked by absorption of other groups in the ion exhangers, for example, NH or Py^+R, like for PEI, PEPA, and VP sorbents. Therefore, its disappearance indicated with high probability that CO groups were involved in the sorption. They were apparently involved in the absorption in other ion exchangers whereas the degree of involvement of N-containing groups was determined by their basicity, which was consistent with the gradual disappearance of the NH or Py^+R bands that appeared in spectra of the ion exchangers together with α-CO or aromatic groups on going from TU to VP sorbents. It can be assumed that the ion-exchange mechanism of absorption dominates over the H-bond one with increasing basicity of the ion exhcangers from TU, PEI, and PEPA to VP. This also determines the efficiency of the sorption process.

Comparison of Tables 1 and 2 showed that not only the nature of the functional groups but also the nature of the polymeric matrix, namely the surface structure of the biomaterials (Fig. 1), has a strong influence on the sorption properties of the anion exchangers for bilirubin. Thus, the slight increase of bilirubin extraction on wood with grafted PEPA groups compared with the anion exchanger based on lignin, which has an ion-exchange group of the same type but differs by their high quantitative content, may be explained by the different porosity of the starting macromolecular framework. Statistical treatment of photomicrographs showed a substantial difference in the average pore diameters of wood and lignin of 20.52 and 11.17 μm, respectively. These differences are reflected in the sorption properties of the samples. The volume of the pore space required to bind bilirubin is greater in wood than in lignin. This improves the absorptivity of the extracted substrate (Fig. 1).

The influence of the nature of the polymeric matrix became even more evident for cholesterol extraction. Thus, the degree of lipid sorption in wood ion exchangers was at the 32-47% level whereas the lignin sorbents differed little from each other in this property and extracted it at 23-28%. The cholesterol content in serum was reduced from a pathological level to acceptable (5.2-6.5 mmol/L) by using the latter and the industrial enterosorbent polyphepan. Its concentration decreased on wood ion exchangers to the ideal value (<5.2 mmol/L). Such a trend is apparently due to the large pore diameter of the lignocellulose preparations compared with lignin (Fig. 1). This increased the availablity of active sorption centers and reduced diffusion resistance to a minimum upon absorption of the rather bulky cholesterol molecules (7.5-70 nm [1]). This caused the effect of the chemical nature of the functional groups to dominate the process.

Protein sorption was suppressed in the studied samples apparently due to competing absorption of other water- and lipid-soluble components. However, carbohydrates were somewhat absorbed. The ion exchangers based on wood and lignin with aliphatic amino groups were able to lower the sugar level in serum to the physiological mean (4.2-6.0 mmol/L).

Thus, comparison of the detoxification capabilities of the synthesized phytosorbents for hemosorption showed that moderately basic ion exchangers based on α-oxide derivatives of wood and lignin with grafted PEI, PEPA, and TU groups are significantly effective for extracting particular pathogenic components of blood serum. They can eliminate excessive amounts of endotoxins, in particular, cholesterol, the content of which is increased by various diseases, including diabetes, and causes inflammation of vessel walls and destroys the normal functioning of cell membranes. These sorbents, in contrast with lignin anion exchanger with pyridinium groups, can adequately normalize the biochemical composition of blood serum, lowering the level of metabolites to physiological values. The practically complete absorption of bilirubin by the strongly basic ion exchanger with onium sorption centers limits its use for hemosorption becuase a low level of this lipid-soluble compound, which is a precursor of other vitally necessary compounds (bile acids, provitamins, vitamins, hormones, etc.) can have a negative effect on the vitality of an organism in general [7].

EXPERIMENTAL

Natural polymers were extracted beforehand with an alcohol:benzene mixture and distilled water as before [8]. Purified lignin and wood fractions sieved to 0.1 and 0.2 cm, respectively, were used for the modification.

Phytosorbents were prepared by *o*-alkylation of pine wood and cotton-husk lignin from the Shymkent hydrolysis plant with DGE and subsequent amination of grafted α-oxide copolymers PEI, PEPA, VP, and TU [4-6, 9-12].

Sorbent capacities for anion- and cation-exchange groups were determined by storing accurately weighed samples in the OH^- and H^+ forms (1 g) in HCl or NaOH solution (100 mL, 0.1 N) for 24 h and titrating an aliquot of the solution (25 mL) with NaOH or HCl solution (0.1 N), respectively, in the presence of methylene blue mixed indicator [13]:

$$SEC = (100 - 4 \cdot V)/10 \cdot m,$$

where V is the volume of NaOH or HCl solution (0.1 N) consumed in the titration (mL) and m is the mass of ion exchanger (g).

The SEC for strongly basic groups of ion exchanger in the OH form (1 g) was established in contact with NaCl solution (100 mL, 0.1 N). After reaching equilibrium (12 h), an aliquot of the solution was titrated with HCl solution (0.1 N) [14]:

$$SEC = 4V/10 \cdot m,$$

where V is the accurate volume of HCl solution (0.1 N) consumed in the titration (mL) and m is the sample mass (g).

The sorption was carried out under static conditions without stirring with a mass ratio of 1:10 in cells thermostatted at 25°C.

Total bilirubin, cholesterol, and protein were determined quantitatively by measuring the optical density of the starting and equilibrium solutions on a KFK-3 spectrophotometer at 590, 510, and 540 nm, respectively.

The bilirubin concentration was established using diagnostic sets of reagents (Lachema). The determination was based on reaction of the azo-bond of bilirubin with diazotized sulfanilic acid to form a colored solution of an azo dye that was measured photometrically.

Analytical Procedure. Serum (0.2 mL) was treated with reagents 1, 4, and 3 (0.2, 0.05, 1.0 mL) consisting of sulfanilic acid (30 mM) in HCl (150 mM), $NaNO_3$ (0.025 M), caffeine accelerator (0.26 M), and C_6H_5COONa (0.52 M), respectively. After thorough stirring for 10-50 min, sodium-potassium tartrate buffer (1 mL, 0.93 M) in NaOH (1.9 M) was added. The optical density of the sample was measured.

The total cholesterol content in serum was determined before and after contact with the sorbents by spectrophotometry for the enzymatic reaction with cholesterolesterase using a Vital-Evropa diagnostic set of reagents (Vital Diagnostics SPb). The calculation used the formula

$$C_{chol} = E_0/5.17 \cdot E,$$

where E_0 and E are the extinctions of the sample and standard measured relative to a control sample.

Analytical Procedure. Serum (0.02 mL) was mixed with reagents 1 and 2 (2 mL) consisting of phosphate buffer solution, phenol, detergents, activators, stabilizers, and lyophilizate (cholesterolesterase, cholesteroloxidase, peroxidase, chromogens, activators, stabilizers), respectively. The reaction mixture was thoroughly stirred, incubated at least 5 min at 20-25°C or 37°C, and measured photometrically.

The amount of total protein was established using a biuret method based on the ability of proteins to form a complex with copper ions in basic medium, the color intensity of which is directly proportional to the total concentration of protein in the sample [14]. Measurement of the optical density of the analyzed solution determined the content of the studied component in it:

$$C_{total\ protein} = (A_1/A_2) \cdot C_{st},$$

where A_1 and A_2 are absorption of the sample and standard, respectively, and C_{st} is the concentration of the standard, g/L.

Analytical Procedure. Serum (0.05 mL) was diluted with distilled water (0.5 mL) and acetone (5.0 mL). The mixture was shaken vigorously for 1 min and centrifuged. The supernatant liquid was decanted. The protein precipitate was dissolved in reagent R1 (2.5 mL, Lachema) consisting of $CuSO_4$ (12.0 mM), potassium—sodium tartrate (31.9 mM), NaOH (0.6 M), and KI (31.0 mM). The absorption of the sample was measured for 30 min.

The glucose level in serum was determined using glucooxidase.

Surfaces of natural polymers were observed using reverse-scattered electrons on a JNCA Energy (England) energy-dispersive spectrometer equipped with a Superprole 733 electron-probe microanalyzer (Japan). Samples were coated with a thin layer of gold before exposure in order to increase the contrast.

IR spectra of sorbent samples in KBr disks were recorded before and after contact with blood serum on a Nicolet 5700 FT-IR spectrophotometer.

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