Synthesis and Characterization of Novel Nano-,Micro- and Macroporous Lignin Sorbents for Purification of Biological Fluids

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Abstract: Novel nano-, micro- and macroporous ion-exchangers based on hydrolytic lignin of cotton husk have been synthesized by two-step process including *o*-alkylation of biopolymer with diglycidyl ether of dioxydiphenylpropane and subsequent amination of formed glycidyl derivative. The optimal conditions of synthesis, composition, structure and physical, chemical properties of ion-exchangers were investigated by FTIR, SEM, porosimetry and potentiometric titration method. Textural characteristics of lignin and sorbents were also studied. It has been established that alkaline activated lignin shows an increase of S_{BET} from 17.7 to 20.9 m^2/g while modification leads to decrease of S_{BET} more than double (from 14.5 to 9.2-5.2 m^2/g) that of an untreared sample. Synthesized ion-exchangers are characterized by approximately identical porous structure and mainly contained a pore size of 10-14 nm. The results show the efficiency of lignin based sorbents for the removal of water and lipid soluble toxic metabolites from blood serum of diabetic retinopathy patients. Considerable detoxication effects of sorbents have potential for prevention and treatment of diabetes.

Keywords: Hydrolytic lignin, nano-micro-macroporous sorbent, static exchange capacity, sorption, biological fluid, detoxication.

1. INTRODUCTION

Lignin, one of the main constituents of lignocellulosic biomass, is the second abundant biopolymer on the earth [1, 2]. It is aromatic compound constituting from 15 to 30% of plant dry weight. More than 30 million tons per year of lignins are produced world wide by pulping and hydrolysis of lignocelluloses. However, only approximately 2% of the total lignins are used in industry, agriculture, pharmaceutics and medicine for production of fine chemicals (dispersants, binding agents, fuels and etc.), fertilizers, food additives and dietary supplements [3,4]. Huge amounts of isolated lignins qualified as a massive byproduct of plant processing. The development and exploitation of such bioresources has become increasingly important for practical, ecological and socio-economic reasons.

The outstanding aspects of lignins are merits of natural abundant, renewability, good biodegradability and biocompatibility, low-cost, environmentally friendly and most importantly containing various functional groups including phenolic and aliphatic hydroxyl, carbonyl, carboxyl and methoxyl groups [1,2]. These groups impart to lignin a capability to complex a wide range of compounds, from metals ions to pesticides and humic substances. Due to their large number of active sites for interaction with different contaminants and also unique porous structure, lignins have proven to be excellent adsorbents [3-30]. Some resent studies demonstrated numerous applications of lignin in the treatment of industrial wastewaters [3-5], decontamination of oil spills and soil [6-8], removal of heavy metals ions [9-13] and various organic pollutants including pesticides [14, 15], dyes [16, 17], surfactants [18], phenols and other aromatic compounds [19-21], as well as in medicine and veterinary for adsorption and elimination of bile acids, glucose, cholesterol, proteins, creatinine, urea, ammonia and other metabolites from biological fluids and living organisms [3, 4, 19, 22-30]. Besides sorption ability to heavy metals ions, radioactive isotopes and allergens [23, 24, 28], lignins exibit high antibacterial [31-34], antiviral [35] and antioxidant [33, 34, 36] activities.

Due to these advantages one of the most promising lignin utilization ways is manufacture of medical enterosorbents. Recently on the basis of hydrolytic lignin various commercially available medical preparations such as Polyphepanum, Polyphepanum plus, Polyphan, Lignosorb, Entegnin, Filtrum-STI, Laktofiltrum, Latkofiltrum ECO are produced. These preparations are prepared from wood wastes in tablet, powder, granule and pasta form by two Russian plants (Saintek ltd., Kirov Biochemical Plant) [3, 4, 23, 24]. Enterosorbents are recommended for prevention and treatment of different diseases, caused by exo- and endogenic intoxication of an organism, including alcohol, medicinal and food poisonings, infectious diseases (salmonellosis, dysentery, diarrhea, virus

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hepatitis, peritonitis), dysbacteriosis, dyspepsias, allergies, bronchial asthma and etc. Further clinical experiments have proven to use them for treatment acute and chronic lungs, liver, kidney and gastro-intestinal diseases such as protracted pneumonia, giperbilirubinemiya, hyperazotemia, cholecystitis, pancreatitis, diabetes, steatohepatitis and different disorders of fat metabolism (hypercholes-terolemia, dyslipidemiya) [23-25, 27, 32, 37-39]. Also they can be used for correction of radiating, chemical and thermal defeats [23, 24].

In order to improve detoxication properties and specificity to toxins, lignin-based sorbents were modified by carbonization [40, 41], oxidation [42], immobilization of special compounds [27, 43, 44] and incorporating oxygen- [42], nitrogen- [19] or sulfur-containing [35] functional groups. M.G. Ismailova *et al*. developed high-performance carbon sorbent AU-L on the basis of cotton lignin [40] and studied its pharmacological properties [41]. It was suggested that preparation of pharmaceutical compositions of enterosorbent with prebiotics [27, 43] and iodine [44] provides occurrence of additional prebiotic properties and increasing bactericidal activity of lignins. Amination of lignins showed enhanced sorption ability towards heavy metals, iodine, phenol, bile acids and cholesterol [19]. Sulfoalkylated lignins in comparison with initial kraft lignin possess anti-AIDS virus activity. They are cost-effective, less toxical than publicly approved drugs and can be used as effective antiviral agents for prevention and therapy of AIDS [35].

According to the presented studies additional functionalization of lignins allows improving their detoxication properties and expands areas of medical application. The aim of the present work is development and characterization of a novel nano-, micro- and macroporous sorbents based on multy-tonnage waste of hydrolysis industry – hydrolytic lignin of cotton husk and evaluation of their efficiency for detoxication of blood serum of diabetic retinopathy patients.

2. MATERIALS AND METHODS

2.1. Solvents and Chemicals

Hydrolytic lignin of cotton husk was extracted with an alcohol-benzene solution (1/2, v/v), alcohol and distilled water as described in [45] for removal of ashes, resinous and other compounds, which are not chemically bind with cellular wall of vegetative tissue. Initial lignin was treated by 0.5-1.5% solution of NaOH for 1 hour at 60º and washed with distilled water until neutral. The composition of purified lignin samples were established [45] and shown in Table **1**. Alkaline treatment was carried out at 60ºC for 1 hour.

Polyethyleneimine (PEI) with molecular weight $(30\text{-}40)\cdot10^3$ was obtained from Fluka AG. Polyethylene-polyamine (PEPA) with molecular weight 265, epoxy-diane resin ED-20, containing 20.97% of epoxide groups, were purchased from Nizhnii-Tagil plant (Russia). Aliphatic polyamines were stored over granulated KOH and used without further purification. All other chemicals were of analytical grade and used as received.

2.2. Synthesis of Glycidyl Derivative of Lignin

The glycidyl derivative of lignin (Lignin-ED-20) was synthesized by *o*-alkylation of biopolymer with epoxy-diane resin ED-20 in the presence of triethylamine (TEA) as catalyst. Synthesis was carried out according to the following procedure: 1.0g of lignin treated with NaOH was added into three-necked round-bottom flask equipped with a paddle stirrer, thermometer and reflux condenser. TEA was mixed with 20% dimethyl-

Table 1: The Composition of Extracted and Activated Hydrolytic Lignin

Lignin	Content, %					Element Composition, %			Yield of Treated Lignin, %
	Polysaccharide	Lignin	Humic Compounds	Extractive Compounds	Ash	C	H	N	
Extracted	14.50	72.00	6.40	4.20	3.50	53.87	5.73	1.50	88.70
Treated with 0.5% NaOH	12.65	80.49	3.01	1.75	3.00	54.62	5.88	1.32	85.62
Treated with 1.0% NaOH	12.23	82.42	2.20	1.15	2.92	54.99	6.40	1.28	82.12
Treated with 1.5% NaOH	11.60	84.05	1.54	0.90	2.91	56.05	6.87	1.15	80.48

formamide (DMF) solution of ED-20 and transferred to the flask with lignin and vigorously stirred for 1-10 hours at 20-100°C. Synthesized glycidyl derivative Lignin-ED-20 was separated from the solution and purified with acetone in a Soxhlet apparatus after 6-8 hours and dried in a vacuum oven at 25°C. The content of epoxide groups in Lignin-ED-20 was determined according to the procedure [46]. The mass gain of the lignin was calculated by the formula:

$$(m - m_o) \cdot 100/m_o,$$

where m and m_0 are the masses of modified and initial polymers respectively. Equilibrium swelling degree (γ) was estimated by keeping alkylated samples in DMF up to an establishment of equilibrium swelling and calculated by the formula:

$$\gamma = (m\text{-}m_o)/m_o,$$

where m and m_o are the masses of swelled and dry samples respectively.

2.3. Preparation of Lignin Based Sorbents

To synthesize the sorbents the glycidyl derivative of lignin was aminated by 50% DMF solution of PEI and PEPA in a three-necked flask at 40-100ºC for 1-3 hours. After amination reaction mixture was put into porcelain crucibles and solidified in a muffle oven at 80°C for 10 hours. The final sorbents were treated with 5% HCl and then 5% NaOH aqueous solutions for 24 hours. After each treatment samples were washed with distilled water and dried in a vacuum oven at 50°C. The static exchange capacity (SEC) of sorbents was determined by keeping a 1.0 g of the samples in the OH^--form in 100 mL of the 0.1 M HCl solution for 24 hours and titration of aliquot of the filtrates (25 ml) with 0.1 M NaOH solution in the presence of methylene blue indicator [50]. The SEC was calculated by the formula:

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

where 100 is the volume of 0.1 M HCl solution, mL, V is the volume of 0.1 M NaOH solution spent for titration of the 25 mL of the acid solution and m is mass of sorbent, g.

2.4. Characterization of Sorbents

Sorbents were characterized by FTIR, SEM, porosimetry and potentiometric titration methods. Potentiometric titration was carried out using the method of separate weights [47]. The apparent dissociation constant pK of sorbents ionic groups was determined from the potentiometric titration data and was calculated by the Henderson–Hasselbalch equation. The pH of the aqueous solutions was measured with an S20-K ionmeter (Mettler-Toledo Inst., Switzerland). The composition and amount of functional groups were found from the inflection points of titration curves.

The FTIR analysis was performed on Nicolet 5700 FTIR spectrophotometer (France) using the KBr disk method in the spectral range between 4000 and 400 cm^{-1}.

The sorbents morphology was analyzed by SEM S-4800 (Hitachi, Japan). Samples were coated with a thin layer of gold or platinum for increased contrast and carbon for increased conductivity.

The porosity of lignin and sorbents was determined with analyzer «Accu Sorb» (Micromerifics, USA) at liquid nitrogen temperature and at 200 mm Hg pressure. The specific surface area (S_{BET}) was calculated by the Brunauer-Emmett-Teller (BET) method. The adsorption area of N_2 molecule in the filled monolayer was accepted as 0.162 nm^2. The total pore volume of samples was obtained from the nitrogen adsorption value.

2.5. Blood Serum Purification Procedure

Synthesized lignin-based sorbents were purified by boiling of samples in water for 2 hours. *In vitro* sorption studies were carried out from diabetic retinopathy patient's blood serum with a mass ratio of 1:12.5 in cells thermostated at 25°C for 1 hour. The total amount of bilirubin and cholesterol was determined quantitatively by measuring the optical density of the initial and equilibrated solutions on a UV-Visible Spectrophotometer (Specord 210plus, Germany) at 590 and 510 nm respectively.

The bilirubin concentration was established using diagnostic sets of reagents (Lachema, Czech Republic) [48]. 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. The glucose concentration in serum was determined using the glucooxidase method [48].

The total cholesterol and HDL-C content in serum was determined before and after contact with the sorbents by spectrophotometry for the enzymatic reaction with cholesterolesterase using a Vital-Europe

diagnostic set of reagents (Vital Diagnostics SPb, Russia) [48]. The level of HDL-C was determined in a supernatant after sedimentation of LDL-C and VLDL-C. The calculation used the formula:

$C_{Chol/HDL-C} = E_o/5.17 \cdot E$,

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

The concentration of VLDL-C and LDL-C was calculated by Friedvald formula [49]:

$C_{VLDL-C} = C_{TG}/2.2$

$C_{LDL-C} = C_{Chol} – C_{VLDL-C} – C_{HDL-C}$

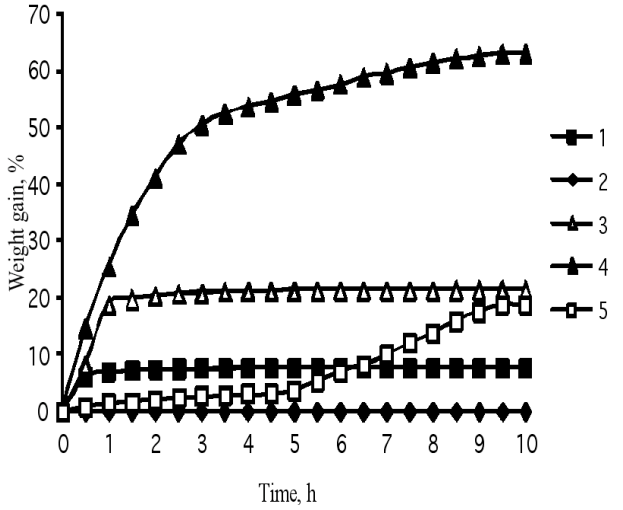
The cholesteric index of atherogenicity (CIA) of serum was determined as:

$CIA = (C_{Chol} – C_{HDL-C}) / C_{HDL-C}$

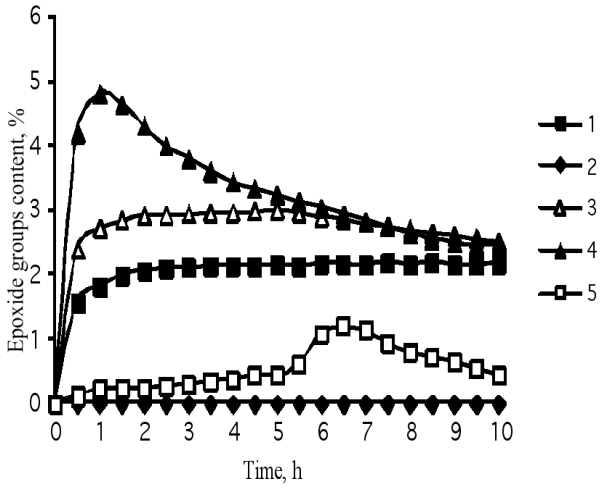
3. RESULTS AND DISCUSSION

3.1. Synthesis of Glycidyl Derivative of Lignin

Synthesis of glycidyl lignin derivative based on interaction of hydroxyl or carboxyl groups of lignin with epoxy rings of bifunctional epoxide resin ED-20. As a result ethers as well as grafted and crosslinked copolymers containing bulk epoxide rings were formed. The reaction rate of epoxide with biopolymer without catalysts is typically slow and inefficient (Figure **1**). It is established that lignin was not epoxidyzed at 20-50ºC for 0.5-10 h. Gravimetric and chemical analyses data testify to absence of weight gain and epoxy groups in samples (Figure **1a**, **b**). Therefore lignin etherification was performed in the presence of TEA catalyst (Table **2**).



(**a**)



(**b**)

Figure 1: Dependence of weight gain (**a**) and epoxy groups content in modified lignin (**b**) on duration of lignin alkylation with ED-20 in the presence (1, 3, 4) or without (2, 5) catalyst. 1 – 20ºC; 2 – 50ºC; 3 – 60ºC; 4,5 – 100ºC. [ED-20]: [TEA]=1,0:1,5 mol.

Table 2: Influence of Catalyst Concentration on Composition and Swelling Degree (γ) of Glycidyl Derivative of Lignin (T=100°C, τ=1 ч)

[Epoxide]: [TEA], mol.	Elemental Composition, %			Epoxide Groups Content, %	Weight Gain, %	γ, %
	C	H	N			
1.0:0.00	56.10	6.47	1.25	0.00	0.00	53.67
1.0:0.05	58.40	6.82	2.21	2.76	12.35	86.00
1.0:0.1	58.82	6.88	2.25	2.93	13.40	87.75
1.0:0.5	60.26	7.10	2.70	4.05	20.53	102.0
1.0:1.0	63.50	7.20	2.85	4.65	26.10	114.3
1.0:1.5	63.12	7.22	2.83	4.79	25.85	121.8
1.0:2.0	62.92	7.19	2.62	4.20	25.20	99.50
1.0:5.0	60.66	7.09	2.17	2.20	21.40	58.75

The content of epoxide groups depends on concentration of tertiary amine in the reaction mixture. The amount of epoxide groups reached maximum at or near the equimolar ratio of resin to catalyst. Further increasing the TEA concentration leads to decrease of weight gain, epoxide and carbon contents in the modified polymers. Probably, this is due to extensive proceeding of homopolymerization of resin in the presence of a large amount of catalyst.

In FTIR spectrum of modified lignin new absorbance bands at 774, 832, 1035, 1183, 1249 cm^{-1} correspond to symmetrical and asymmetrical vibrations of C–O-, C–C- and C–H bonds of epoxy ring [50] (Figure **2**). Increase of the bands intensity of CH_3, CH_2 and OCH_3-groups ($\delta_{as,s}$, $\nu_{as,s}$ 1399, 1452, 2872, 2926, 2962 cm^{-1}) and skeletal vibration of aromatic groups (1509, 1603 cm^{-1}) is due to superposition of frequencies of similar groups in ED-20. Appearance of band of resin's ether bond C_{aryl}–O–C_{alkyl} (1297 cm^{-1}) confirms the existence of epoxy resin groups in modified lignin. In spectrum of epoxidized lignin decreasing of band intensity of COOH and phenolic OH-groups testify to participation of these groups in alkylation process.

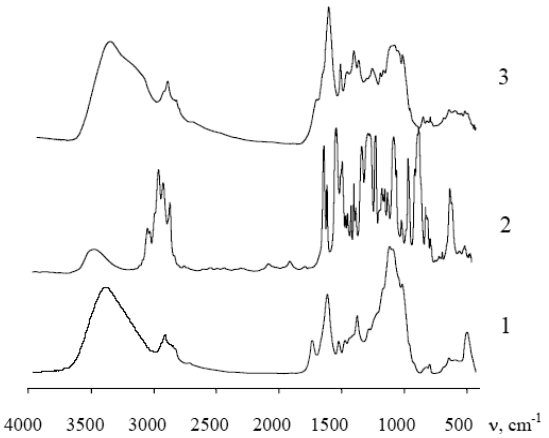


Figure 2: FTIR spectra of lignin (1), resin ED-20 (2) and glycidyl derivative of lignin (3).

3.2. Preparation and Investigation of Nano-, Micro- and Macroporous Lignin Based Sorbents

Glycidyl derivative of lignin was aminated with aliphatic polyamines to synthesize nitrogen-containing ion-exchangers. Composition and properties of sorbents depend on amination conditions. As seen from Table **3** the SEC of the sorbents increases regularly with increasing content of aminating agent in the reaction mixture. The highest SEC values are observed for mass ratios of Lignin-ED-20 to PEI 1.0:1.5 and PEPA 1.0:0.75, reaching 5.25 and 3.60 mequ/g with an active nitrogen content of 7.35 and 5.04% respectively. Further increasing of the amount of polyamines leads to decrease of the SEC and yield of final products due to formation of weakly cross-linked polyelectrolytes with high swelling degree ($V_{specific}$) and low mechanical strength. Increasing the amination temperature to 100°C slightly decreases the capacity, yield and nitrogen content in the modified lignin (Table **4**). Apparently this is due to the occurrence of parallel side reactions of the epoxide rings with hydroxyl groups in biopolymer than with amine groups of polyamines. Amination reaction is practically completed after 1 and 2 hours at 40°C for ion-exchangers based on PEPA and PEI respectively (Table **4**). A difference in process duration is caused by polymeric nature of the used amines, i.e. the higher molecular weight of PEI and its lower segmental mobility compared with PEPA.

In the FTIR spectra of lignin based anion-exchangers all absorbance bands of epoxide rings are absent that testify to their availability and high reactivity (Figure **3**). The bands of δ_{NH} (1630-1670 cm^{-1}) and ν_{C-N} (1220 cm^{-1}) confirm the existence of amine groups in modified lignin. In spectra of ion-exchangers increase of width and intensity of ν_{CH} (2962, 2925, 2850 cm^{-1}) and $\delta_{as,s\ CH}$ (1452, 1400-1385,1354 cm^{-1}) of lignin alkyl and alkylene groups testifies the superposition of

Table 3: Dependence of Composition, Yield and Capacity of Sorbents on Reactant Ratio ($T_{amination}$=80°C, $\tau_{amination}$=3h, $T_{solidification}$=80°C, $\tau_{solidification}$=10 h)

Polyamine	Epoxidized lignin: polyamine, mass. part	SEC in 0,1N HCl, mequ/g		N content, %		$V_{specific}$, ml/g	Yield, %
		HCl	NaCl	$N_{titrable}$	$N_{elemental}$		
PEI	1.00:0.50	3.75	0.25	5.25	11.69	5.13	99.27
	1.00:1.00	4.75	1.00	6.65	11.82	7.02	97.83
	1.00:1.50	5.25	1.20	7.35	12.13	7.56	97.70
	1.00:2.00	4.00	0.75	5.60	10.87	4.92	92.45
PEPA	1.00:0.50	3.00	0.15	4.20	7.15	3.61	93.30
	1.00:0.75	3.60	0.50	5.04	7.34	3.87	92.15
	1.00:1.00	3,70	0.40	5.18	7.98	4.12	87.71
	1.00:1.50	3.70	0.00	5.18	7.17	4.92	75.00

Table 4: Effect of Temperature and Duration of Amination on the Composition and Capacity of Lignin Based Sorbents ($T_{solidification}$=80°C, $\tau_{solidification}$=10 h)

Polyamine	[Lignin-ED-20]: [Polyamine], mass. part	Amination conditions		SEC in 0.1 N HCl, mequ/g		N content, %		Yield, %
		T°, C	τ, h	HCl	NaCl	$N_{titrable}$	$N_{elemental}$	
PEI	1.0:1.50	40	3	6.75	1.10	9.45	12.90	99.90
		60	3	6.00	1.00	8.40	12.60	99.64
		100	3	5.00	1.00	7.00	11.92	95.73
		40	1	600	0.90	8.40	12.67	97.88
			2	6.75	1.00	9.45	12.84	99.90
PEPA	1.0:0.75	40	3	4.95	0.60	6.93	8.96	90.50
		60	3	3.96	0.50	5.54	7.42	91.30
		100	3	3.30	0.40	4.62	6.59	90.07
		40	1	4.95	0.60	6.93	8.91	90.13
			2	4.95	0.60	6.93	8.95	90.20

frequencies of similar groups in polyamines. Absence of the bands of carboxyl and β-carbonyl groups at 1700-1720 cm^{-1} is due to their transformation into compounds with C=N, C(O)N bonds, which bands (1640-1690 cm^{-1}) shift in the field of absorbance of amine groups.

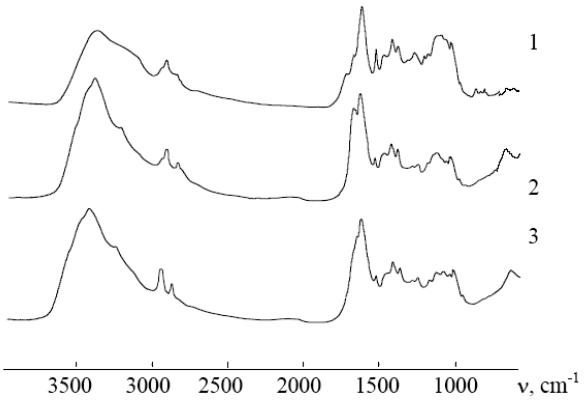


Figure 3: FTIR spectra of glycidyl derivative of lignin (1) and sorbents aminated with PEI (2) and PEPA (3).

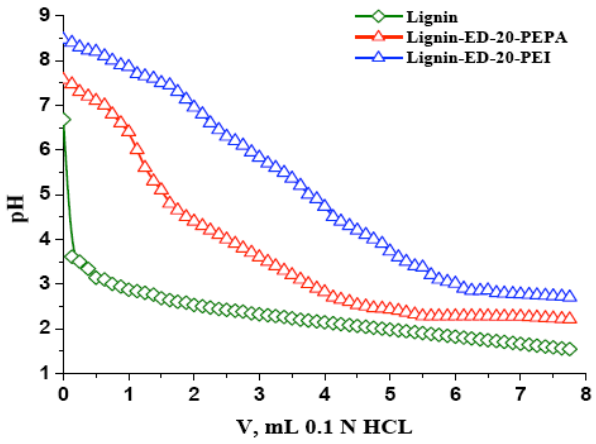
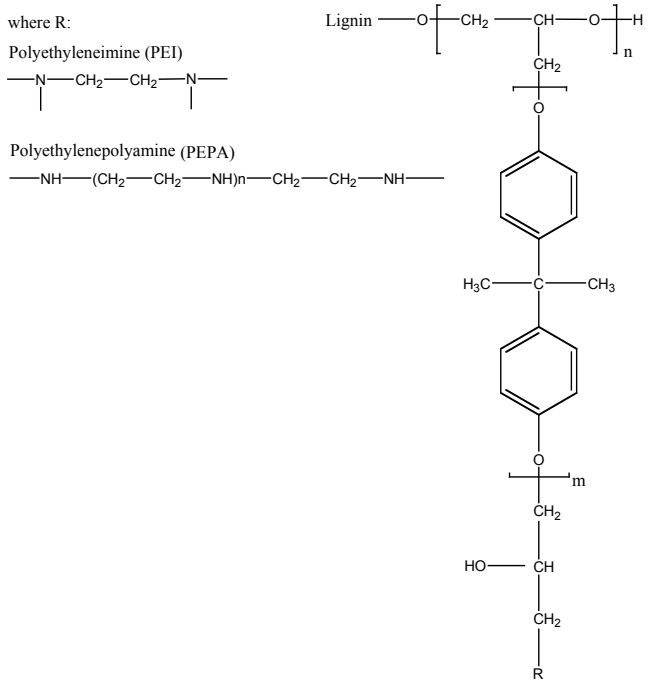


Figure 4: Potentiometric titration curves of sorbents based on lignin.

Figure **4** shows potentiometric curves of anion-exchangers. The absence of clear inflections and the sharp slope of the curves do not allow evaluating amount of primary, secondary, tertiary amine groups and indicate on the polyfunctional character of ionites. The pK of active groups of PEI and PEPA were equal to 6.76 and 6.20 respectively. These data correspond to literary data and testify to weakbasic nature of synthesized anion-exchangers [51]. In contrast to the modified analogues, the initial lignin does not contain functional groups capable of anion exchange. Therefore, it does not possess ion-exchange properties.

On the basis of FTIR and pH-titration data the structure of aminoepoxide units grafting into lignin surface is presented below:



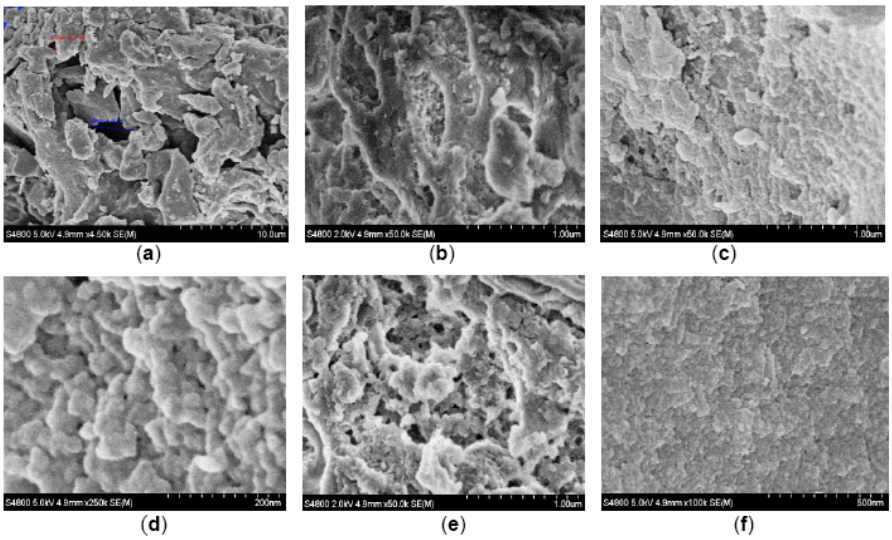


Figure 5: SEM images of lignin (**a,b**) and ion-exchangers with groups of PEI (**c,d**) and PEPA (**e,f**).

Table 5: Adsorption Characteristics of Lignin and Ion-Exchangers

Sample	Extracted Lignin	Lignin activated with 0,5%NaOH	Lignin activated with 1,0%NaOH	Lignin activated with 1,5%NaOH	Lignin-ED-20-PEI	Lignin-ED-20-PEPA
S_{BET}, m^2/g	14.7	17.53	19.8	20.9	5.2	9.2
ΣV_{pore}, mL/g	0.14	0.153	0.156	0.16	0.13	0.12

As a biopolymer, lignin saves the porous structure of initial vegetative raw material. SEM images testify the nano-, micro- and macroporous structure of initial lignin and ion-exchangers due to the presence of pores of varying diameter from 0.82-4.48μm (Figure **5a**), 8-714 nm (Figure **5b**), 12-300 nm (Figure **5c**), 7-16 nm (Figure **5d**), 32-333 nm (Figure **5e**), 3-27 nm (Figure **5f**).

Adsorption characteristics of lignin and sorbents were investigated by method of nitrogen vapor adsorption using BET model. The BET surface area (S_{BET}) of lignin and ion-exchangers is shown in Table **5**. Low size of a specific surface and high total pore volume (ΣV_{pore}) is specific for macroporous structures of samples. The results indicate that alkaline activation of lignin leads to increase of S_{BET} (from 14.7 to 20.9 m^2/g) and the total pore volume (from 0.14 to 0.16 mL/g) in comparison with an extracted sample. Increasing of adsorption characteristics of activated samples depends on concentration of used sodium hydroxide at the same treatment temperature. That structural improvement is caused by removal of extractive and humic compounds and polysaccharides from vegetative tissue after alkaline treatment, which blocked pores and channels, i.e. inner and outer surface of hydrolytic lignin.

Alkaline activation provided the development of mesoporosity (Figure **6**). Maxima of pore size distribution curves shifted from 5.14 nm (extracted sample) to 6.27, 7.75 and 8,06 nm for samples activated with 0.5, 1.0 and 1.5% NaOH respectively indicating the decreasing of pore size by a factor of 1.21-1.57. Superior quantity of transport macropores and development of sorption mesopores after alkaline treatment considerably improves the structural properties of lignin for its further modification.

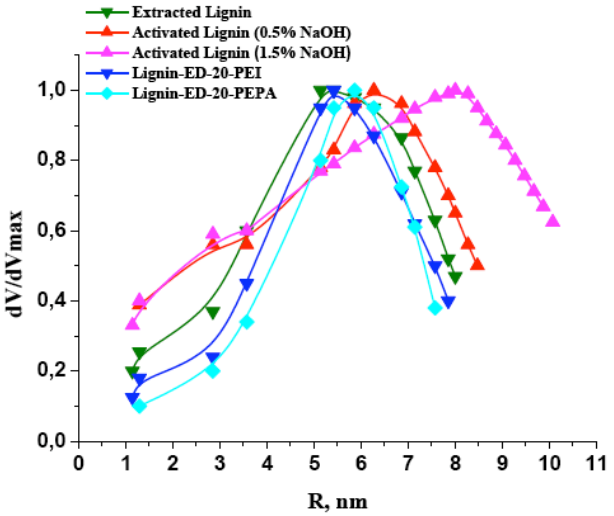


Figure 6: Pore size distribution curves of lignin and ion-exchangers.

As shown in Figure **6**, alkylation and amination processes lead to decrease of S_{BET} of modified lignin more than double (9.2 and 5.2 m^2/g) that of an extracted (14.5 m^2/g) and activated (20.9 m^2/g) samples. Probably, this is due to incorporation of epoxy and polyamine groups into lignin surface. As seen from experimental data, increasing of molecular weight of polyamines leads to decreasing of S_{BET} and increasing of microporosity of sorbents. Pore size of ion-exchangers based on PEI and PEPA decreased to 5.42 and 5.86 nm in comparison with activated sample (8.06 nm). Synthesized lignin based ion-exchangers are characterized by approximately identical porous structure and mainly contain pores with diameter ranging from 10 to 14 nm.

Existence of sorption-active nano- and micropores and transporting macropores plays important role in sorption ability, selectivity and kinetic properties of synthesized ion-exchangers. According to resent studies [52-56] using of amines as ligands improves sorption ability and selectivity of sorbents in relation to water-, lipid-soluble, protein-bound and other compounds. Due to fine porous structure, set of functionally active groups [57], nontoxicity [58] and availability synthesized nitrogen-containing biosorbents are used for removal of toxic metabolites from blood serum of diabetic retinopathy patients.

3.3. Purification of Blood Serum of Diabetic Retinopathy Patients Using Lignin Ion-Exchangers

Diabetes mellitus is a common metabolic disease in which the concentration of glucose, total cholesterol, including its most atherogenic fractions such as LDL-C, VLDL-C, TG in the blood is above the standard level [59]. This is due to insulin deficiency or functional disturbance of the receptors, which causes blood glucose to rise and induce disorders in the metabolization of fat and proteins. The number of patients with diabetes mellitus has grown each year. According to the vital statistics from International Diabetes Federation (IDF), 6.0% of the world population had diabetes in 2007 [60,61]. Due to the increasing of number of patients, the percentage of diabetes prevalence is expected to reach 7.3% by the end of 2025. For this reason, there is an urgent need to create new methods of prevention and therapy of diabetes mellitus and its micro- and macrovascular complications such as diabetic retinopathy, nephropathy, neuropathy, diabetic foot and etc.

Current therapeutic strategies of diabetes mellitus are aimed at reducing the risk of acute and late vascular complications by means of normalization of glycemia, hypercholesterolemia and dyslipidaemia. The adequate lipidemic and glycemic control should be supplemented by screening for and stable compensation of risk factors of vascular disorders. It may help to prevent or delay the development of vascular complications.

In these study sorption correction of carbohydride-lipid profile of diabetic retinopathy patients have been carried out by using lignin sorbents. Biochemical indicators of blood serum before and after sorption are shown in Figure **7**. As seen, blood serum of ophthalmological patients is typically characterized by high level of total cholesterol and LDL-C, VLDL-C, TG, glucose and low content of angioprotected HDL-C. Average concentration of these metabolites exceeded physiological normal values by factors of 1.67, 2.02,

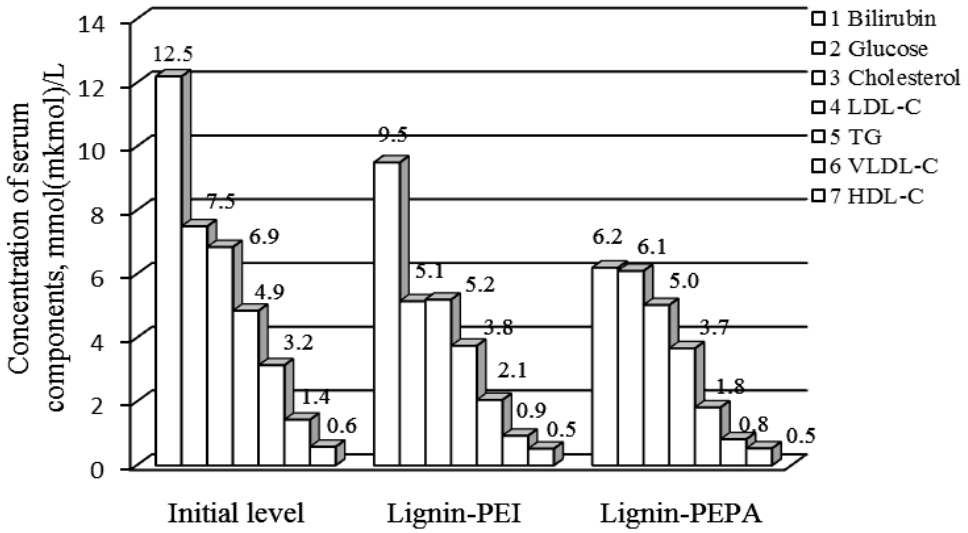


Figure 7: Detoxification of blood serum of diabetic retinopathy patients by lignin sorbents.

4.60, 2.09 and 1.23 respectively. Content of HDL-C was below more than twice. After contact with sorbents the total cholesterol level and average concentration of VLDL-C and LDL-C decreased to the level of optimum compensated diabetes (<4.8–6.0 mmol/L, <3.0–4.0 mmol/L respectively). Concentration of HDL-C insignificantly reduced from 0.6 to 0.5 mmol/L. Purification of serum blood proceeded not only by removal of atherogenic fractions of cholesterol, but also by adsorption of large amounts of other lipids and carbohydrates such as TG, bilirubin and glucose. After extraction concentration of TG decreased 1.5 (PEI) and 1.8 (PEPA) times than initial levels. Established TG concentration (2.1 and 1.8 mmol/L) corresponded to the level of optimum compensated diabetes (<1.7–2.2 mmol/L). Efficiency of bilirubin adsorption depended on the basic properties of used ion-exchangers and increased in the following order: Lignin-ED-20-PEI<Lignin-ED-20-PEPA.

To evaluate serum delipidization degree and sorbents efficiency to removal of lipid substances the cholesteric index of atherogenicity (CIA) and lipid parameters are used. Their values are appreciably reduced after contact of serum with anion-exchangers, but not after contact with Polyphepanum (Table **6**). Decreasing of above mentioned parameters when using amine-containing lignin is caused by the high adsorption of atherogenic fractions of cholesterol (LDL-C, VLDL-C) and insignificant extraction of angioprotective cholesterol (HDL-C). In contrast to ion-exchangers Polyphepanum is not selective to LDL-C, VLDL-C and extract low amount of atherogenic and angioprotected fractions of cholesterol and TG.

Synthesized ion-exchangers are able to remove of superfluous amount of glucose. Effective glucose extraction leads to reduce its content to physiological norm (< 4.2 – 6.1 mmol/L). The initial level of glucose was equal to7.50 mmol/L. Adsorption of metabolites is probably due to formation of macromolecular complexes, stabilized by H-bonds between carbonyl, hydroxyl, amine, ester or ether groups of sorbents and metabolites. High sorption activity of amine groups of ion-exchangers is caused by their existence in deprotonated form at pH of serum equal to 7.4.

Thus, nano-, micro- and macroporous lignin sorbents based on by-product of domestic hydrolytic industry possess considerable hypolipidemic and hypoglycemic effects in comparison with Russian enterosorbent Polyphepanum. This is due to the high concentration and availability of sorption active groups selective to toxic metabolites. They have the remarkable potential to use in medicine, veterinary medicine, biology and biotechnology as effective enterosorbents for correction of metabolic disorders at diabetes and other disease and purification of various biological fluids. Application of these sorbents for prevention and therapy of metabolic disease may improve of life quality and expectancy of patients and minimize the socio-economic losses.

4. CONCLUSION

Novel nano-, micro- and macroporous sorbents have been synthesized by catalytic *o*-alkylation of hydrolytic lignin of cotton husk with epoxide resin ED-20 followed semiproduct by amination of synthesized glycidyl derivative. It has been established that alkaline activation of lignin in contrast to surface function-alization leads to increase of surface area and total pore volume in comparison with extracted sample. The pores of synthesized ion-exchangers were found to be around 10-14 nm in diameter. The results show the efficiency of lignin sorbents for the removal of water and lipid soluble toxic metabolites from blood serum of diabetic retinopathy patients. Samples decreased the pathological level of lipids such as total cholesterol and

Table 6: Lipid Parameters and CIA of Blood Serum of Diabetic Retinopathy Patients before and after Sorption with Lignin Sorbents

Lipid parameters and CIA	Physiological norm	Prolypherative diabetic retinopathy	Initial level	Lipid parameters and CIA of serum after contact with sorbents		
				Lignin-PEI	Lignin-PEPA	Polyphepanum
CIA	1.56	3.9	10.81	9.00	8.49	13.63
$\frac{C_{Chol}}{C_{HDL-C}}$	2.6±0.17	4.9±0.11	11.81	10.00	9.49	14.63
$\frac{C_{LDL-C}}{C_{HDL-C}}$	–	3.45	8.36	7.21	6.92	11.34

its atherogenic fractions (LDL-C, VLDL-C) and TG to the level of optimum compensated diabetes without significant removal of angioprotected HDL-C. The level of glucose was reduced to physiological norm. Considerable hypolipidemic and hypoglycemic effects of sorbents allow to their use for the metabolic treatment of diabetes by means of compensation of carbohydrate-lipid status of an organism. Competitive advantages of these sorbents are the renewability, availability and nontoxicity in comparison with some synthetic or carbon and mineral adsorbents.

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