Synthesis and research of modified carbon sorbents with hydroxy acids

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Abstract

The results on the synthesis and study of modified materials for medical application based on nanodispersed carbon are given. Modification of the carbon sorbent was performed using the biologically active substances with biospecific properties – hydroxy acids (glycolic and lactic). The main results obtained by investigation of the modified carbon sorbents by physicochemical and biomedical methods are presented.

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Keywords: carbon sorbents; carbon black; nanodispersed carbon; synthesis; modification; hydroxy acids

1. Introduction

Nanodispersed carbon black is a unique subject of inquiry. Various carbon materials based on it exhibit specific properties and are widely used in science, engineering and medicine. The use of two structural modifications of graphite materials – nanosized carbon and low-temperature pyrolytic carbon – formed a basis for the development of the matrix synthesis technology at the Institute of Hydrocarbons Processing SB RAS (IHP SB RAS) \cite{1, 2}.

Chemical modification is a promising approach to impart biospecific (antibacterial, antifungal and other) properties to carbon materials. Chemical modification of carbon materials is carried out by functionalizing their

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surface with active (reactive) groups. This is accompanied by changes in the functional cover (chemical nature) of the surface [4, 5]. The development of this area allows creating a wide range of efficient sorbents for use in medicine and veterinary. Biologically active substances with biospecific properties are used as modifiers [3].

Hydroxy acids, such as glycolic and lactic acids, and their derivatives meet these requirements: they are biocompatible and non-toxic and have antibacterial properties [4]. In recent years, interest in hydroxy acids increased significantly due to the growing demand in traditional areas of their application and expansion of their use for the production of pharmaceutical and cosmetic preparations. Hydroxy acids play an important role in many biochemical processes. The biologically significant form of lactic acid for animal and human is L-isomer. It should be noted that lactic acid is the key biological substrate that maintains the optimum of biological homeostasis in the female body microecosystem, stabilizes pH, promotes the accumulation of glycogen and has microbicidal and immunocorrective properties [6]. Lactic acid at a concentration of not less than 0.5% exhibits antibacterial activity and provides a low pH of the medium, 3.7-4.5. The acid environment is necessary for the normal microflora (female genitalia, stomach) and pernicious for pathogenic microorganisms – causative agents of infectious diseases [7].

The indicated hydroxy acids can be involved in polycondensation reaction to form biocompatible and biodegradable aliphatic polyesters of the acids or their copolymers. Among the advantages of these compounds are adjustable physicochemical and mechanical properties, biocompatibility, non-toxicity, biodegradability, antimicrobial activity, and ability to reduce the pH of the environment [4, 8, 9]. They are employed as drug carriers and components of implants, surgical sutures and wound coverings [10,11]. Various long-acting preparations with sustained release formulations containing a copolymer of glycolic and lactic acids as an auxiliary substance have been developed and registered in Russia. These are the anti-inflammatory agents such as Buserelin depot, Lucrin depot, Zoladex and others.

The objective of the study was to develop biospecific carbon sorbents modified with oligomers of hydroxy acids (a copolymer of glycolic and lactic acids and a lactic acid oligomer) and to investigate physicochemical and biomedical properties of the modified sorbent samples.

2. Materials

Carbon sorbents developed and synthesized at the IHP SB RAS are the promising supports for the synthesis of modified carbon materials with biospecific activity. Carbon hemosorbent VNIITU-1 (Technical Conditions 9398-002-71069834-2004) has a smooth surface of 0.5-1.0 mm spherical granules. The surface and pores of the sorbent are free of dust, it is chemically pure: the content of carbon in hemosorbent is not less than 99.5 %; mass fraction of ash is not more than 0.15 %; mass fraction of sulfur is not more than 0.30 %. The material has high strength of the granules and the developed mesoporous structure with the dominant pore size of 50-60 nm (the surface area is 300-400 m²/g according to nitrogen adsorption).

99 wt.% glycolic acid (GA) and 80 wt.% lactic acid (LA) (Merck Schuchardt OHG, Germany) were used as the modifiers.

3. Methods

Physicochemical properties of the synthesized samples were studied by both the standard methods and the techniques specially designed for these materials [12]. Polycondensation was controlled spectroscopically using 13C and 1H nuclear magnetic resonance (NMR) on an Avance-400 (Bruker, USA) spectrometer. The surface geometry and morphology of the carbon sorbent samples were examined by SEM on a JSM-6460LV (JEOL, Japan) electron microscope. Textural characteristics of the carbon sorbent samples (specific surface area, total pore volume, volume of micro- and mesopores) were determined by the low-temperature nitrogen adsorption on a Gemini 2380 (Micromeritics, USA) analyzer. The process of carbon sorbent modification with lactic acid and a mixture of lactic and glycolic acids was monitored using differential thermal analysis (TG-DTA-DTG) on a DTG-60H (Shimadzu, Japan) instrument [13-15]. CHNOS elemental analysis was performed on a Vario EL Cube (Elementar, Germany) analyzer. The qualitative composition of functional groups on the surface of the samples was studied by infrared spectroscopy. IR transmission spectra were recorded on a NICOLET-5700 (Thermo Fisher Scientific, USA) spectrometer and processed using the Origin software for baseline correction and background smoothing.
The desorption (migration) of the lactic acid oligomer supported on the carbon sorbent after contact with physiological saline (a 0.9% NaCl aqueous solution) was studied by the technique based on pH measuring and NMR spectroscopy [16].

In vitro biomedical testing the sorbents was made at the Omsk State Medical University (OmSMU). The microbiological studies were performed with the following microorganisms:

- Gram-positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus faecalis;
- Gram-negative organisms: Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Acinetobacter calcoaceticus;
- yeast-like fungal culture of the genus Candida: Candida albicans and Candida krusei;
- a mixture of bacterial cultures: St. aureus + Ps. aeruginosa; St. aureus + Kl. pneumoniae; St. aureus + Es. coli; St. aureus + Kl. pneumoniae + Ps. aeruginosa + Es. coli; St. aureus + Kl. pneumoniae + Es. coli; St. aureus + Ps. aeruginosa + Kl. pneumoniae; Es. coli + Ps. aeruginosa; Es. coli + Kl. pneumonia; Ps. aeruginosa + Kl. pneumonia;
- an association of bacterial and fungal cultures: S. aureus + C. albicans.

Identification of microorganisms was carried out on the PLIVA-Lachema Diagnostika (Czech Republic) test kits using the computer program Microbes Automatic.

All mixtures of the test cultures were prepared by mixing equal volumes of the working concentrations of microbial cells under stirring. A Vortex shaker (ELMI, Latvia) was used to stir mixtures of the cultures.

The antibacterial and antifungal properties of carbon sorbents were assessed by direct inoculation of culture media and by the disk diffusion method (agar diffusion method) [17]. The efficiency of carbon sorbents was determined by measuring the diameter of growth inhibition zone. When the diameter of such zone ranges from 10 to 15 mm, the sorbent exhibits a weak antibacterial (antimycotic) action. The diameter of growth inhibition zone from 15 to 20 mm corresponds to a moderate effect. And the diameter greater than 20 mm indicates that the sorbent possesses the pronounced biospecific properties. The absence of microbial growth inhibition zone around the disks testifies to the absence of antibacterial or antifungal properties.

The following working dilutions were used in the experiment with direct inoculation: bacterial cells ~ 103 CFU (colony forming units) in 1 ml, and Candida genus culture – 280-360 CFU in 1 ml. Inoculation with working dilutions of the tested cultures served as the control. In the experiments performed by the agar diffusion method the working dilutions were as follows: bacterial cells – a 0.5 McFarland dilution of $1.5 \times 10^8$ CFU in 1 ml; and cultures of the genus Candida – $500 \times 10^6$ CFU/ml.

The concentration of microbial cells was measured on a Densi-La-Meter (PLIVA-Lachema, Italy) device for determining the turbidity of suspensions.

Prior to bench biomedical testing of the sorbents, they were sterilized with saturated steam at a gauge pressure of 0.11 ± 0.02 MPa and a temperature of (121 ± 1)°C in an autoclave.

1 ml of bacterial suspension was added to 0.5 ml of the modified sorbent, stirred on a shaker and incubated for one day. After 24 hours of the sorbent contact with pathogenic culture, 50 μl of supernatant fluid was withdrawn and inoculated onto sterile Petri dishes with GMF nutrient agar (meat-peptone agar) for bacteria, and Saburo agar for the yeast-like fungal culture. Petri dishes with inoculated crops were placed in a CO2 incubator 15AC and incubated upside down: the bacteria at $37 \pm 1°C$ for 24±2 hours and the yeast-like fungi for 48-72 hours.

Counting of the grown colonies is carried out visually with a stencil in five 1 cm² fields and recalculation for the plate area with the diameter of 90 mm. The number of colony forming units on the surface is estimated and compared with the control (the concentration is set on the device for measuring the turbidity). The result is expressed as the number of colony forming units in 1 ml of the test sample.

The average value of three parallel tests is considered as the result of testing.

4. Results and discussions

Carbon sorbents are intrinsically hydrophobic. A high content of reactive functional groups on the carbon surface is needed to enhance biospecific and hydrophilic properties of the sorbents. For this purpose, lactic and glycol acids
were used. The modifiers chosen to functionalize sorbents for medical applications meet the requirements of sorption therapy. They are non-toxic, the modifier monomers are water soluble and readily available. The structure of the monomers includes oxygen- and nitrogen-containing functional groups that can be involved in polycondensation/polymerization reaction with the formation of oligomers or polymeric chains causing either a low mobility of modifiers in the support pores (for hemosorbents) or a free migration of modifiers into water solution (for entero- and application sorbents).

The developed method for the production of the carbon sorbent modified with oligomers of hydroxy acids (a copolymer of glycolic and lactic acids and an oligomer of lactic acid) consists in the impregnation of the carbon sorbent with an aqueous solution of hydroxy acids (lactic acid or a mixture of lactic and glycolic acids) of different concentrations at a sorbent to hydroxyacids solution ratio of 1:1 (by volume) under static conditions at a temperature of 21-25°C for 20-24 hours. This is followed by a multi-stage heat treatment of the sorbent at normal pressure for 1.2 h at 105-135°C with subsequent heating in the presence of a desiccant at 145-175°C for 4-13 h and at 165-185°C under argon for 6-20 h in a tube furnace.

The properties of the modified sorbents were studied by various physicochemical methods (Table 1).

It was found that modification of the carbon sorbent with the indicated modifiers (lactic acid or a mixture of lactic and glycolic acids) followed by polycondensation leads to a significant reduction in the specific surface area and total pore volume, increases the oxygen content in the elemental composition, and changes the qualitative composition of functional groups of the sorbent. The physicochemical properties of the original sorbent are influenced mostly by such modification stages as impregnation and prolonged heat treatment.

It was established that the polycondensation conditions allow obtaining regular oligomers of hydroxy acids (with lactic and glycolic acids alternating in the polymer chain) that are soluble in acetone and chloroform. According to the analysis of NMR spectra, the oligomer samples do not contain lactic and glycolic acids and their low-molecular derivatives (dimers and trimers). The calculated NMR spectra gave the average number of monomer units of t (MW of the LA product ≥ 700 g/mol).

The possibility of desorption (migration) of the oligomers from the modified sorbent surface upon contacting with the 0.9% NaCl saline solution (the initial pH 6.3-6.5) for 30 days was studied by 1H NMR spectroscopy. The study showed that ca. 0.9% of GA and 0.1% of LA passed into solution from the modified carbon sorbent (on Fig. 3, low intensity signals at 2.1 and 4 ppm correspond to hydroxy acids; broad peaks in the region of 4-6 ppm are referred to the water signal). The signals of hydroxy acid dimers (less than 0.5%), which are the hydrolysis products of the GA and LA copolymer, were also recorded (Fig. 1). The pH of saline after contact with the modified sorbent samples reduced to 3.5-3.6, as determined by pH-metric method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Modifying parameters and conditions</th>
<th>Physicochemical characteristics</th>
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<tbody>
<tr>
<td></td>
<td>Content of hydroxy acid in impregnating solution, wt. %</td>
<td>Conditions of polycondensation: temperature (T,°C) and time (t, h)</td>
</tr>
<tr>
<td>GA LA</td>
<td>Impregnation time, hour</td>
<td>Stage 1</td>
</tr>
<tr>
<td>Sample 1. Sorbent modified with a copolymer of hydroxy acids</td>
<td>70</td>
<td>30</td>
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</table>
The desorption of lactic acid oligomers from the modified samples was studied at a temperature of 37±1°C (model conditions) [16]. After contacting with NaCl for 30 days, up to 10% of LA with dimer and trimer passed into solution from the carbon sorbent modified with a lactic acid oligomer (Fig. 2). The pH decreased to 1.7-1.9.

The thermal analysis makes it possible to directly control the process of carbon sorbent modification, to detect polycondensation products of hydroxy acids on its and quantify their mass (Figure 3). The differential thermal analysis (DTA) curve of the initial sorbent has no peaks in the region of 200-400°C. Degradation of the copolymer of glycolic and lactic acids occurs at 340-370°C (a weight loss of 45%), while degradation of the lactic acid oligomer, at 350-400°C. The observed exothermic peaks are quite homogeneous and uniform. This may indicate that the polycondensation products of hydroxy acids in all the samples have similar molecular weights. These results are consistent with the published data on the properties of GA and LA copolymer and LA oligomer [19-20]. The thermal analysis data confirm the completeness of the carbon sorbent modification with the oligomers of hydroxy acids.

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<table>
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<tr>
<th>Sample 2. Sorbent modified with an oligomer of lactic acid</th>
<th>50</th>
<th>24</th>
<th>130±5</th>
<th>2</th>
<th>150±5</th>
<th>4</th>
<th>170±5</th>
<th>20</th>
<th>29±5</th>
<th>0.116±0.043</th>
<th>30.6±0.4</th>
<th>4</th>
<th>1.9±0.3</th>
</tr>
</thead>
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Fig. 1. The $^1$H NMR spectrum of the aqueous solution after 28 days of contact with the sorbent modified with a copolymer of hydroxy acids.

Fig. 2. The $^1$H NMR spectrum of the aqueous solution after 28 days of contact with the sorbent modified with a lactic acid oligomer.
Thus, the application of a set of physicochemical methods demonstrated that modification of the carbon sorbent with hydroxy acids followed by polycondensation results in the formation of a copolymer of hydroxy acids and an oligomer of lactic acid on the carbon surface. The developed method for modification of carbon sorbents allows obtaining the materials with controlled characteristics (the amount of modifier, specific surface area, pore volume, composition of functional groups of the carbon sorbent and others). A distinctive feature of the proposed synthesis method is that polycondensation of hydroxy acids is carried out without toxic solvents or catalysts.

High antibacterial and antifungal properties of the modified carbon sorbent samples can be attributed to the acid-base properties of the applied oligomer of hydroxy acid: the contact of the oligomer with a NaCl solution decreases the pH value. According to pH-metry and NMR spectroscopy data, there is a local acidification of the medium due to hydrolysis of the oligomer of hydroxy acids that formed on the sorbent; such acidification is detrimental for pathogenic microorganisms.

The bench microbiological tests of the sorbents at the Omsk State Medical University showed that the unmodified sample has antibacterial activity against Gram-positive monocultures St. aureus (sensitive if contact time is at least 24 hours). The results obtained by the disk diffusion method indicate that the sorbent modified with a copolymer of hydroxy acids exhibits a moderate biospecific activity against pathogenic Gram-positive microorganisms (the diameter of the culture growth inhibition zone of 15-20 mm).

Sample 1 (the sorbent modified with a copolymer of hydroxy acids) exhibits antibacterial activity against pathogenic monocultures St. aureus, Ps. aeruginosa, E. coli, Kl. pneumoniae and mixtures thereof. Furthermore, it was shown that the modified sorbent is active against antibiotic-resistant Gram-negative bacteria Ac. calcoaceticus (the causative agent of serious infectious diseases, including meningitis, bacterial endocarditis, pneumonia, septicemia, etc.) and antibiotic-resistant yeast-like fungi C. krusei (the etiological agent of nosocomial infections, candidiasis, Candida peritonitis and others). Antibacterial and antimycotic effect of this sample is observed already after the first hour of contact with microorganisms. The data obtained by the disk diffusion method indicate that sample 1 exhibits a strong biospecific activity against pathogenic microorganisms (the diameter of the culture growth inhibition zone greater than 20 mm).
Sample 2 (the sorbent modified with lactic acid oligomers) showed a moderate antibacterial activity against microorganisms St. aureus, St. Epidermidis, St. pyogenes, Str. agalactiae, E. faecalis, and Ps. aeruginosa. Note that this sample exhibited a weak antibacterial action against microorganisms K. pneumoniae and E. coli. Sample 2 exerts a pronounced antifungal action on the cultures of yeasts C. albicans. It was found that the modified sorbent samples have strong antimicrobial properties against bacterial and fungal associations of cultures St. aureus + C. albicans. The results of the disk diffusion study indicate that sample 2 has strong biospecific activity against pathogenic microorganisms (the diameter of the culture growth inhibition zone greater than 20 mm).

The study demonstrated that the carbon sorbent samples modified with hydroxy acids and subjected to heat treatment exhibit high antibacterial and antifungal activity against pathogenic microorganisms: Gram-positive and Gram-negative bacteria St. aureus, St. epidermidis, St. pyogenes, Str. agalactiae, E. faecalis, Ps. aeruginosa, E. coli, Kl. pneumoniae, and mixtures thereof; Gram-negative bacteria Ac. calcoaceticus; C. kruzei yeasts and C. albicans. In comparison with the unmodified sample, samples 1 and 2 possess more pronounced antibacterial and antifungal properties against pathogenic yeast-like fungi of the genus Candida (the causative agent of nosocomial infections and infectious diseases in obstetrics and gynecology), Gram-negative bacteria Ac. calcoaceticus (the causative agent of infectious processes, including meningitis, bacterial endocarditis, sepsis, etc.) as well as associations of pathogenic microorganisms that are encountered most frequently in clinical practice.

5. Conclusion

Thus, the developed method of modifying a carbon sorbent with lactic acid or a mixture of lactic and glycolic acids and its subsequent polycondensation makes it possible to obtain materials with enhanced antibacterial and antifungal properties in comparison with the unmodified sample. The produced biospecific carbon sorbents are of great interest for medicine and veterinary as detoxifying and antimicrobial agents for the treatment of gastrointestinal and obstetric diseases and surgical pathology.

Acknowledgements

The authors express their gratitude to the Head of the Laboratory of physicochemical methods at IHP SB RAS and Center for collective use of the Omsk Scientific Center SB RAS Ph.D. V.A. Drozdov and his colleagues: V.P. Talzi for NMR studies, A.B. Arbuzov for infrared spectroscopy and scanning electron microscopy studies, A.V. Shilova for elemental analysis, and N.V. Antonicheva for thermal analysis.

The authors are grateful for microbiological studies performed under the guidance of Ph. MD, Professor T.I. Dolgikh and Ph. MD, Professor M.G. Chesnokova (Omsk State Medical University).

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