IMMOBILIZATION OF AMINES, AMINOACIDS AND PROTEINS ON THE CARBON

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INTRODUCTION

Sorption detoxification during various grave diseases and poisonings has become recently well known as a new rapidly developing field of modern medicine [1]. It is noteworthy that sorption therapy has become widespread in our country to a great extend thanks to the elaboration and industrial invention of the so-called «SCN» (nitrogen-containing spherical carbonites) hemosorbents, prepared on the basis of synthetic carbons with regular spherical grains, which are free of mineral admixtures and have well-developed porosity and high catalytic activity in catalase- or oxidase-like enzymatic reactions [1,2]. Further possibilities in development of sorptive processes are connected with elaboration and application of new types of sorbents, particularly carbon hemosorbents, modified by immobilization of some substances.

In the present work we have tried to synthesize and investigate biospecific carbons with chemically immobilized amines (ammonium, hydrazine ethylenediamine, pentaethylenediamine, hexaethylenediamin, tris(hydroxymethyl)aminomethane), aminoacids (lysine, tyrosine, tryptophan, phenylalanine), house domestic and pollen allergens, proteins (insulin, albumines, immuno-globulines, protein A from S.Aureus). Physico-chemical, structural-adsorptive and biospecific properties of initial and modified sorbents were tested by modern methods.

EXPERIMENTAL

Active carbons prepared from porous copolymers and resins (SCN and SCS types), fruit and coconut stones (KAU type) by pyrolysis and activation were used as a matrix in this work. Carbon is inert material. For its further chemical modification it is necessary to create initial stable chemical groups on the carbon surface. Oxidization by nitric acid in fluid phase is simple and convenient method to prepare oxygen-containing stable groups with movable proton atom on carbon surface. This fact determines the method of immobilization of some ligands on carbon surface. The methods of immobilization of ligands (carbodiimide, chloranhydride and p-nitrophenol) were optimized conformably to carbons of SCN, SCS and KAU types.

Surface functional groups are analyzed pH-metrically and by XPS. Structure-sorptive investigations: a) macro- and mesopores distribution by their radii was determined by mercury porosimetry method; b) determination of the sorptive pore volume (SPV) by benzene vapor adsorption at 20 °C, by the thermostatted vacuum adsorption unit with a quartz spring balance; c) determination of specific surface area (SSA) chromatographycally by nitrogen thermal desorption; d) determination of adsorption capacity of sorbents by spectrophotometric data. Biospecific activity of modified sorbents was tested in specific biological reactions.

RESULTS AND DISCUSSION

Modification of carbon's surface by amines change the chemistry. Almost all carbons, modified by amines and aminoacids sorbents are ampholytes, and their isoelectric point shifted to pH values of neutral solutions. As compared with the initial oxidized carbon, the modified ones have higher anion-exchange capacity (>0.6-0.8 mmole/g). Cation-exchange capacity is consequently decreased to the same extent. This reflects essential alterations in the surface chemistry of carbon adsorbent.

Establishing the chemical structure of the immobilized surface groups is a fairly complex problem since carbon sorbents are difficult for spectra investigations. XPS can

provide information on the energetic state of particular atoms. As the energetic state of nitrogen atoms in the initial oxidized carbons has been studied well enough [3], we have analyzed the state of nitrogen atoms in carbons modified by amines and aminoacids. The 2p-electron absorption band of nitrogen in XPS of modified carbons turns out to be 0.5 - 1 eV shifted to the low energies as compared with that of pyridine-like hybridized electron of nitrogen in the initial carbon. This proves the appearance (as a result of modification) of groups with nitrogen in a low energetic state, which is typical for nitrogen (III)-containing fragment of amines, aminoacids. After introduction of sulfophenyl groups into a carbon's surface, XPS of 3p-electrons of sulfur add to the evidence of a successful surface modification, as there are now sulfur atoms in the initial carbon.

Since porous materials have been used for modification, studying the sorptive ability of oxidized and modified by proteins carbons was of particular interest. Oxidation and particular protein immobilization on inner pore surfaces make for considerable deterioration in the sorption characteristics of the initial materials. In the specimens with an oxidized surface, the SSA proper decreases down to 25-30 % though reduction in the sorption pores volume is considerably lower, i.e. 5-10 %. Under albumin and IgG immobilization the SSA reduces to 60-80% and SPV down to 40-60 % of the their initial values. Porosigrams of the initial and protein-containing carbons (PCC) demonstrated considerable decrease in volume and SSA of meso- and macropores (down to 10%). Distribution on the equivalent radii of the initial and PCC is actually equal. Sorption from the solutions of substances-markers, i.e. methylene blue, vitamin B-12, ovalbumine, bovine serum albumin on the initial and PCC was studied. Substances with low molecular mass (methylene blue, vitamin B-12), that basically sorbed in the super-micropores, decreased in sorption values and correlates with reduction in the SPV after oxidization or immobilization of ligands. It should be noted, however, that in the case with methylene blue sorption on the carbons with an immobilized IgG, the sorption values preferably exceed these of the initial sorbents which is, probably, explained by the fact an immobilized protein polymer acts as a acceptor of the low-molecular substances. The effect of increase in sorption of metabolites with low molecular mass was earlier reported when carbons have been micro encapsulated with polymer film [4]. Immobilization of proteins on carbon surface reduce ability of PCC to adsorb proteins from liquids. It may be explained by the fact that the protein inoculated molecules are proportionate with the pore diameter of transport ones and are a sterical obstacle for the protein molecules penetration from solution volume into the sorption pores [5].

Biospecific activity of modified carbon sorbents was investigated too. Carbon, modified by amines (ethylenediamine, TRIS) can be used for correction of pH of blood in treating of acidosis or in rehabilitation of properties of stored blood. Biospecific activity of PCC has been tested in several ligand - substrate systems: domestic dust allergen - IgE, protein A - IgG, anti IgG - IgG, insulin - antibodies to insulin. In all the cases PCC demonstrated increased selectivity (3-5 times) compared to initial carbon (in vitro and in vivo) [6].

CONCLUSIONS

It was shown, that combination of physico-chemical, adsorptive and biospecific properties of carbons SCN, SCS or KAU types modified by amines, or aminoacids, or proteins allow the sorbents to be successfully used in medicine as sorbents for extracorporeal detoxification and in biotechnology.

REFERENCES

1. V.G. Nikolaev, V.V. Strelko. Hemosorption with Activated Carbons. Kiev: Naukova Dumka.-288 p. (1979) (Russ).
2. V.Strelko. Int.Carbon Conf. «Carbon - 90» Paris. (1990).
3. V.Strelko, V.Nemoshkalenko, N.Kartel et al. Sorption and adsorbents.-11 (1983) (Russ).
4. Patent N 3983053 USA. Covered adsorptive materials.
5. O.Bakalinskaya, N.Koval. Proceed. Carbon'92, Essen, Germany, 1992.
6. V.Strelko, S.Mikhalovsky, O.Bakalinskaya. Proceed. Europ. Soc. Artif. Organs, Amsterdam, 1993.