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Suitable for Immobilization Ferromagnetic Silica Modified by Carbonized Lignin and Carbodiimide: Preparation and Properties

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Abstract—The composition of native aluminosilicate used to prepare, by means of chemical modification, a functionalized ferromagnetic organosilicon sorbent suitable for immobilizing biologically active substances was studied.

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Various materials whose breadth of application is determined by their properties as regards cells and biomolecules with a certain structure are widely used in advanced bioengineering. A special place is held by highly dispersed silicas, which are distinguished by developed surfaces coated by hydroxide groups [1].

Synthesis of silica supports makes it possible to prepare sorbents with positive properties of inorganic matrices: chemical purity, nonswelling in solutions, considerable adsorption capacities, rigid frameworks, chemical and microbiological stability, and nontoxicity.

In this work, we prepared ferromagnetic immunosorbents based on a strong, easily modified matrix that has minimal nonspecific sorption.

METHODS

Chemical analysis of a silicate rock was carried out as described in [2]. IR spectra were studied on a Specord 75IR spectrophotometer in the wavenumber range 500–4000 cm^{-1}. The amino group concentration of the organosilicon sorbent was determined according to [3].

The specific surface area of the magnetic sorbent (MS) was determined by low-temperature nitrogen adsorption. The total pore volume and pore diameter were determined by mercury porometry on a Micromeritics Auto Pore-9200 instrument [4]. Sorbent microstructure was examined on an IMZ-T3000 electronic device as described in [5].

Fractionation of proteins (immunoglobulins G) with the use of poly(ethylene glycol) (PEG-6000, a high-molecular-weight water-soluble polymer) [6] was employed.

Proteins were quantified, as in [7], by comparing optical absorption of proteins at 280 and 260 nm.

EXPERIMENTAL

An MS with a high sorption activity was manufactured by generating the pore structure of the support in the presence of an organic polymer.

Native aluminosilicate (from the Astrakhan deposit) used in this work as the major structural component to form the framework of the sorbent composite had the following parameters: bulk density, 320 kg/m^3; water content at 110°C, 2.7%; and water capacity, 71%. Chemical analysis showed the following components in this aluminosilicate sample: SiO_2 (wt 78%), Al_2O_3 (wt 21%), Fe_2O_3 (0.8 wt %), magnesium (44 mg/kg), manganese (9.4 mg/kg), zinc (10 mg/kg), cobalt (0.4 mg/kg), and calcium (0.9 mg/kg).

Chemical modification of the sorbent was performed in the presence of carbonized lignin and carbodiimide. The magnetic component used in the synthesis was magnetite (Fe_3O_4). When the support is treated with an organic polymer (carbonized lignin containing carboxy groups), its surface is activated. Upon subsequent modification of the sorbent by carbodiimide (1-cyclohexyl-3(2-morpholinyl-4-ethyl)carbodiimidemethyl-*p*-toluenesulfonate), the product is activated by functionalities that are capable of interacting with protein ligands.

Magnetic sorbents were prepared as follows. To aluminosilicate (1.5 g), added were 1, 1.5, 2, 2.5, 3, or 4% aqueous solution of carbonized lignin (100 mL) and, then, magnetic Fe_3O_4 powder (0.5–2.5 g). The mixture was allowed to stand at (22 ± 2)°C for a period of 1 to 16 h. Gelling pH was 4.0–7.0. The product was dried at 100–115°C for 30 min, ground, and sieved to separate 80–120 μm fractions. Then, acetate buffer (pH 4.0; 9 mL) containing carbodiimide (40 mg) was added to the sorbent, and the mixture was incubated for 1 h. Then, the sorbent was carefully washed with acetate buffer and distilled water.

Table 1. Parameters of the MS as functions of magnetite amount used in the synthesis

Batch ratio (wt/wt)			Specific saturation magnetization, A m^2/kg	Specific surface area, m^2/g	Pore volume, cm^3/g	Pore radius, nm
SiO_2	Fe_3O_4	carbonized lignin				
1.5	0.5	1	8.8 ± 0.04	64.5 ± 0.74	1.21 ± 0.05	25.6 ± 0.49
1.5	1.0	1	10.2 ± 0.08	63.6 ± 0.49	1.23 ± 0.08	29.6 ± 0.49
1.5	1.5	1	12.4 ± 0.08	55.4 ± 0.83	1.25 ± 0.09	30.6 ± 0.49
1.5	2.5	1	17.7 ± 0.08	54.6 ± 0.47	1.29 ± 0.038	32.2 ± 0.6

Table 2. Structural parameters of the MS as functions of gelling time

Batch ratio (wt/wt)			Gelling time, h	Specific surface area, m^2/g	Pore volume, cm^3/g	Pore radius, nm
SiO_2	Fe_3O_4	carbonized lignin				
1.5	1	1	2	63.0 ± 0.84	1.23 ± 0.09	29.7 ± 0.29
1.5	1	1	4	65.0 ± 0.51	1.23 ± 0.08	25.6 ± 1.12
1.5	1	1	8	69.6 ± 0.6	1.21 ± 0.02	23.7 ± 0.45
1.5	1	1	16	71.4 ± 0.52	1.21 ± 0.08	21.6 ± 0.49

The hydrogel experiences dehydration during ripening and syneresis, with thickening and a decrease in volume. Heat treatment converts the hydrogel to a xerogel, with the hydrogel volume decreasing by a factor of 8–15. The final stages of the synthesis of the composite MS ensure the separation of a highly disperse fraction and its activation by functionalities for subsequent immobilization of protein ligands. The resulting composite MS is built of silica particulates linked to one another in a three-dimensional framework and coated with a polymer (carbonized lignin). The particulate size determines the MS specific surface area, and the particulate packing density determines the pore volume and pore radius.

RESULTS AND DISCUSSION

Our results show a good precision of technology developed for manufacturing lignin-containing composite MSs with standard composition and structural parameters. For optimizing the structural parameters of the composite MSs, we varied the batch ratio between the components (aluminosilicate, carbonized lignin, and Fe_3O_4), as well as the gelling time and pH.

The results of our experiment on optimization of the structural parameters of the MS showed that with increasing magnetite amount in the batch, the specific surface area of the sorbent slightly decreased whereas its pore volume and pore diameter increased (Table 1). A likely reason for this is the stabilizing influence of magnetite, which opposes the process associated with particulate coarsening in the composite sorbent. Table 2 illustrates the effect of the gelling duration on the structural parameters of lignin-containing organosilicon composite MSs. In our opinion, the optimal gelling time during sorbent manufacture is 2 h; with increasing gelling time, the synthesis lengthens and the MS pore size decreases, which leads to a decrease in the degree of ligand immobilization with covalent binding in sorbent pores.

Proceeding from the results of our study, we can recommend the following optimal parameters for the synthesis of lignin-containing MSs: batch ratio, SiO_2 : Fe_3O_4 : carbonized lignin = 1.5 : 1 : 1; gelling time, 2 h; gelling pH, 7.0.

A well-defined spongy, porous structure was observed in the samples thus synthesized. The additive (magnetic powder) enhanced globule agglomeration.

Magnetic sorbents were manufactured as highly disperse microgranules of irregular shapes with well-defined magnetic properties, with good wettability and efficient sedimentation in solution, and without a tendency to agglomerate.

Determination of the amino group concentration in the aluminosilicate sorbent with occluded iron oxide showed that this concentration was within 0.45–0.54 mg-equiv/g sorbent.

Bands at 3750 and 3680 cm^{-1} in the IR spectra of the aluminosilicate sorbent were due to free and bound hydroxide groups, respectively. After the aluminosilicate support was chemically modified with carbonized lignin, the absorption intensity in the region of the 3750 cm^{-1} band was reduced. At the same time, an absorption band appeared at 1650 cm^{-1} in the region of the stretching vibrations of CH groups, carboxy groups (COOH), and amino groups (NH_2); an absorption band at 900 cm^{-1} appeared due to the vibrations in aromatic

rings. In addition, the sorbent spectrum contained strong bands at 2900 cm^{-1} due to the CH vibrations assigned to methylene groups [8].

X-ray powder diffraction showed unoxidized metal (copper) and oxide (Al_2O_3, CuO, and ZnO) phases in the aluminosilicate sorbent; aluminum oxide is the major phase as follows from the intensity of its reflections. This agrees with chemical analysis of aluminosilicate sorbent samples.

To prepare magnetic immunosorbents, specific ligands were immobilized on magnetic sorbents. These specific ligands were immunoglobulins G fractionated by poly(ethylene glycol)-6000 and isolated from mouse anti-West Nile virus immune ascitic liquid and immunoglobulins IgG isolated from the blood of patients suffering from borreliosis [6].

The kinetic study of immobilization and quantification of covalent binding of immunoglobulins with the sorbents showed that 2 mg/mL is the optimal ligand protein concentration for complete saturation of the sorbent with antibodies in a 0.2-mL aliquot of 10% suspension. With protein concentrations in immunoglobulins higher than 2 mg/mL, the adsorption capacity of the magnetic immunosorbent was lower. In all probability, the dependence of the adsorption capacity on the amount of the immobilized protein is due to steric factors [8–12]. The kinetic study of immobilization and the assessment of protein ligand binding to the sorbent showed that 2 h is sufficient for complete saturation of the MS with the protein with a solution pH of 6–7 and a temperature of 22–37°C.

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