Journal of Non-Crystalline Solids 385 (2014) 81–88

Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

journal homepage: www.elsevier.com/ locate/ jnoncrysol

Synthesis and application of amino-modified silicas containing albumin as hemoadsorbents for bilirubin adsorption

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ARTICLE INFO

Article history:
Received 6 July 2013
Received in revised form 23 September 2013
Available online 23 November 2013

Keywords:
Sol–gel;
Mesoporous silicas;
Adsorption;
Bilirubin;
Protein immobilization

ABSTRACT

The organic–inorganic composite materials based on mesoporous silica containing bovine serum albumin (BSA) were synthesized using sol–gel method. According to the sol–gel process the surface of silica was modified by amino-groups using 3-aminopropyltrimethoxysilane (APTMS). This modification increased the selectivity to the albumin molecules due to special intermolecular interactions between amino groups of silica and carboxyl groups of BSA. The obtained silicas were characterized by nitrogen adsorption–desorption analysis, fourier transformed infrared spectroscopy, scanning electron microscopy. It was shown that encapsulation of the BSA inside silica matrix leads to the formation of mesoporous materials with high pore diameter (10–14 nm for albumin modified silicas) and a BET surface area equals to 346 ± 17 m^2/g. The obtained materials were used as adsorbents for selective bilirubin removal. Analysis of adsorption isotherms showed that the adsorption ability to bilirubin depends on the amount of BSA grafted into silica particles. As a result, the amino-modified silica containing 70 mg/g of BSA is characterized by the highest bilirubin adsorption capacity. Thus, the obtained results showed the potential application of these adsorbents in medicine for toxins removal and treatment of hyperbilirubinemia.

1. Introduction

One of the most essential goals of modern chemistry is to synthesize inorganic–organic composite materials with the unique physical and chemical properties. Nowadays, the synthesis of bio-composite materials through sol–gel approach is one of the rapidly growing trends in a modern material science [1–6]. It was synthesized the bio-composite materials such as bio-ceramic implants using the sol–gel approach [7,8]. The sol–gel method is the most effective technique for immobilization of different biomolecules that exhibit drug and prolonged properties. The sol–gel technology has been applied for the synthesis of biocatalysts, biosensors, transport systems for drug delivery, etc. [9,10].

Application of silica gel in different adsorption processes is determined by the specific surface area of adsorbents [11]. New methods of surface modification via sol–gel process usually use organosilanes with different functional groups such as $-NH_2$, $-SH$, $-COOH$, etc. [12]. For instance, 3-aminopropyltriethoxysilane (APTES) and 3-aminopropyltrimethoxysilane (APTMS) are widely used for synthesis of amino-functionalized materials in order to provide strong binding interactions with functional groups of biomolecules including different peptide sequences [12]. Also the silica surface can be treated by different natural or synthetic polymers [13,14]. It was found that biomolecules such as proteins may interact with the silica surface which may lead to new physicochemical properties [15]. Although, the proteins may easily interact with silica surface, these types of interactions can alter their ability to fulfill their biological role [16]. Besides, the conditions of sol–gel synthesis may lead to protein denaturation [17]. Also some conformational changes in protein structure can significantly influence biological properties of the protein [16]. It already developed some methods of protein encapsulation inside silica matrix using sol–gel approach [18,19]. However, some disadvantages are found in these methods associated with manipulation of protein–surface interaction: silica–protein binding based on nonspecific van der Waals interactions (physical adsorption) [19]. Therefore, application of functional organosilanes in sol–gel synthesis for proteins immobilization provides more effective ways to encapsulate proteins via covalent, non-covalent or electrostatic interactions in mild conditions in order to keep their bio-activity [20,21]. The organo-functionalization of silica gel can be accomplished by two general routes: the post-modification through the reaction of organosilanes with surface silanol groups [22], and co-condensation of organosilanes with silica precursor [22,23]. These simple processes produce chemically modified silica surface with high loading functional groups which easily react with functional groups of proteins [16]. Thus, these novel functional bioorganic–inorganic materials can be applied in various fields of medicine and nanobiotechnology [24]. We can treat different toxicological diseases using such modified materials [25,26].

When the metabolism of human is disordered it may lead to the increase of the concentration of different toxics in human blood [27]. One of such toxics is bilirubin (Fig. 1). Bilirubin is a tetrapyrroledicarboxylic acid that is formed in the normal metabolism of heme proteins in red

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http://dx.doi.org/10.1016/j.jnoncrysol.2013.11.002

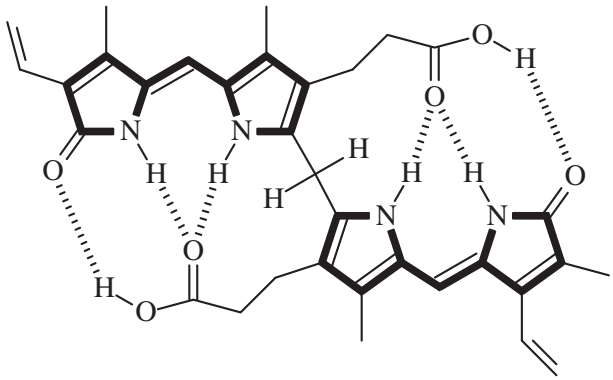

Fig. 1. Structural formula of bilirubin in ridge-tile conformation stabilized by six intermolecular hydrogen bonds.

blood cells, and it is normally conjugated with albumin to form water-soluble complex [28]. The free bilirubin is toxic and high concentrations of bilirubin can provoke hepatic and permanent brain damage. Disorders in the metabolism of bilirubin may cause a yellow discoloration of the skin and other tissues [29].

At present, several methods have already been developed such as plasma exchange, hemodialysis, phototherapy and hemoperfusion. Hemoperfusion is the most promising technique [27]. This method based on application of hemoadsorbents for removing of different toxics (such as bilirubin) from human blood [30–32]. However, there are generally used neutral adsorbents (activated carbon, silica, alumina gels, and neutral polymers) which are not so effective for bilirubin removal because most of them are microporous and bilirubin removal from the human blood occurs only through physical adsorption via non-specific van der Waals interactions [33]. Thereby, it is very essential to develop new methods of synthesis and modification of these hemoadsorbents for the effective bilirubin removal from human plasma. Due to the sol–gel technique which focuses on the protein encapsulation and surface functionalization, it can be used for synthesis of highly effective hemoadsorbents.

In present study, we have produced amino-modified silicas containing different amounts of albumin for the effective bilirubin removal from water solutions (pH = 7.4) simulating human plasma and their physical and chemical properties were investigated.

2. Experimental techniques

2.1. Materials and sample preparation

2.1.1. Reagents

Bovine serum albumin (M = 66430.3), bilirubin (M = 584.7) were supplied by "Agat-Med" (Russia). APTMS (3-aminopropyltrimethoxysilane, 97%) and TEOS (tetraethyl orthosilicate, 99%) were purchased from "Ecos-1" (Russia). All of the mentioned materials were used without further purification. Deionized water was used throughout this work.

2.1.2. Synthesis of non-modified silica

A typical synthetic procedure of non-modified silica used TEOS and water in relative molar ratios of 1:4 [34]. In a typical synthesis, 4 g of TEOS was mixed with 1.668 g of water and vigorously stirred for 2 h. Ammonia buffer solution was added every 25 min as a base catalyst (pH value equals to 8). The final product was transferred into a Petri dish for solvent evaporation at room temperature. The obtained powder sample was dried under vacuum at 40 °C during 2 days.

2.1.3. Synthesis of silica modified by BSA

The synthesis of silica modified by BSA was similar to the one described above. It used the same molar ratio of TEOS/H_2O as in a previous synthesis. The different quantities of BSA (from 0.02 g to 0.08 g) were added in water before sol–gel process to prepare four solutions with necessary concentration of BSA. Then prepared solutions were mixed with TEOS and stirred for 2 h. Ammonia buffer solution was added every 25 min as a base catalyst to increase the pH to 8. The pH values were measured by a pH-meter U-500 ("Aquilion", Russia). The final products were transferred into a Petri dish for solvent evaporation at room temperature. The obtained powder samples were dried under vacuum at 40 °C during 2 days.

2.1.4. Synthesis of amino-modified silica containing BSA

The synthetic procedure used TEOS and APTMS in relative molar rations of 4:1. In our case we divided the sol–gel synthesis into two steps. In the first step, acid hydrolysis was used. For this aim, 4 g of TEOS and 1.668 g of H_2O containing required amount of BSA (from 0.08 g to 0.12 g) were mixed and stirred for 30 min. Then HCl solution was added as an acidic catalyst to adjust the pH to 5. In the second step, base catalysis was used. For this reason, after hydrolysis of TEOS 0.86 g of APTMS was added in 30 minutes and simultaneously ammonia buffer solution was added to increase the pH to 8. This prepared solution was stirred for 1 hour 25 min. Then the final products were transferred into a Petri dish for solvent evaporation at room temperature. The obtained powder samples were dried under vacuum at 40 °C for 2 days. The synthesis route is shown in Fig. 2.

The weights of TEOS, APTMS and the quantity of BSA loaded into silica particles are shown in Table 1.

2.2. Characterization of mesoporous adsorbents

2.2.1. Scanning electron microscopy

Scanning electron microscope (SEM) images of obtained samples were performed using electron microscope (EMW-100L, Russia) operated at 50 kV.

2.2.2. Fourier transformed infrared spectroscopy (FTIR)

The FTIR spectra were obtained on Avatar 360 FTIR spectrometer ("Thermo Nicolet," USA) using KBr-disc pellet method (about 2–3 mg sample was mixed with 100 mg KBr, then this mixture was pressed under a pressure of 5 tons for 6 min). Spectroscopic grade KBr salt was used in pellet preparation as a non-absorbing matrix and background. Background and the sample spectra were acquired from 400 to 4000 cm^{-1}. The processing of spectra was achieved using SPECVIEW program and Origin Pro 8.5.

2.2.3. Nitrogen adsorption and desorption isotherms

The obtained materials were characterized by common analytical techniques. Nitrogen adsorption measurements were carried out at 78 K using Micromeritics ASAP 2020 analyzer ("Norcross," GA, USA). Before the experiment all samples were outgassed at 60 °C under vacuum. The analysis was carried out with approximately 0.3 g of sample using nitrogen as the absorbing gas. The specific surface area was calculated by employing the Brunauer–Emmett–Teller (BET) method [35] in the range of relative pressure from 0.05 to 0.25. The pore volume and pore size distributions were calculated using the Barrett–Joyner–Halenda (BJH) model [36] on the desorption branch. Error in determining of BET surface area and pore volume do not exceed 5%.

2.3. Bilirubin adsorption from aqueous solution

The magnitude of bilirubin adsorption on the surface of non-modified and modified silicas was determined as the difference