J Sol-Gel Sci Technol (2013) 67:297–303
DOI 10.1007/s10971-013-3079-5

ORIGINAL PAPER

Sol–gel synthesis of mesoporous silicas containing albumin and guanidine polymers and its application to the bilirubin adsorption

A. S. Timin · E. V. Rumyantsev

Received: 3 April 2013 / Accepted: 22 May 2013 / Published online: 4 June 2013

Abstract The organic–inorganic composite materials based on mesoporous silica were synthesized using sol–gel method. The surface area of silicas was modified by bovine serum albumin (BSA) and guanidine polymers: polyacrylate guanidine (PAG) and polymethacrylate guanidine. The mesoporous silicas were characterized by nitrogen adsorption–desorption analysis, Fourier transform infrared spectroscopy, transmission electron microscopy. The obtained materials were used as adsorbents for selective bilirubin removal. It was shown that the structural properties and surface area of modified materials depend on the nature of polymers. Incorporation of polymers in silica gel matrix during sol–gel process leads to the formation of mesoporous structure with high pore diameter and a BET surface area equals to 346 m^2/g for SiO_2/BSA and 160 m^2/g for SiO_2/PAG. Analysis of adsorption isotherms showed that modification of silica by BSA and guanidine polymers increases its adsorption ability to bilirubin molecules. According to Langmuir model, the maximum bilirubin adsorption capacity was 1.18 mg/g.

Keywords Sol–gel · Mesoporous silicas · Bovine Serum Albumin · Guanidine polymers · Bilirubin · Adsorption

1 Introduction

Sol–gel technology is well known process for obtaining nano- and mesoporous materials using different precursors based on metal alcoholates [1–3]. The sol–gel process is a fairly example of self-organization of molecular systems. One of the most important advantages of sol–gel method is to inherit new properties of the obtained materials by the immobilization of different organic and inorganic compounds inside a silica matrix. Moreover, sol–gel technique is one of the simplest methods for protein encapsulation [4, 5] with the following application of new obtained hybrid materials in different branches of nanobiotechnology.

Nowadays, several methods have been developed for an effective removal of different toxins from human plasma, such as plasma exchange, hemodialysis and phototheraphy [6, 7]. However, hemoadsorption has become the most promising technique [8]. There are generally used neutral sorbents (activated carbon, silica, alumina gels, neutral-polymers) in hemoadsorption [9]. As a result these sorbents are not so effective for removal of different toxins because most of them are microporous and are not capable to remove biomolecules with high molecular mass such as bilirubin [9].

The aim of this study is to synthesize mesoporous hybrid materials based on silica modified by bovine serum albumin (BSA) and guanidine polymers which can be used for the selective removal of bilirubin (BR) from water solutions (pH 7.4) simulated human plasma. BR is a tetrapyrroledicarboxylic acid that is formed in the normal metabolism of heme proteins in red blood cells, and it is normally conjugated with albumin to form water-soluble complex [10]. The free BR is toxic and high concentrations of bilirubin can evoke hepatic and permanent brain damage [11] (Fig. 1).

2 Experimental

2.1 Reagents and materials

Bovine serum albumin (“Agat-Med”, M = 66430.3), bilirubin (“Agat-Med”, M = 584.7) were obtained from

A. S. Timin (✉) · E. V. Rumyantsev
Department of Inorganic Chemistry, Ivanovo State University of Chemistry and Technology (ISUCT), 7, Sheremetevsky prosp., Ivanovo, Russian Federation
e-mail: a_timin@mail.ru

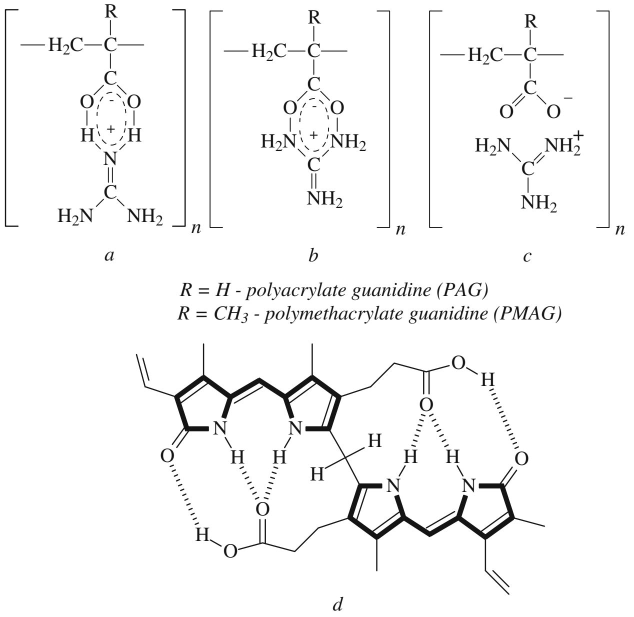

Fig. 1 Structural formulas of guanidine polymers (**a**, **b**, **c**) and bilirubin (**d**) in ridge-title conformation stabilized by six intermolecular hydrogen bonds

commercial sources. TEOS (tetraethyl orthosilicate, 99 %) was purchased from closed-company "Ecos-1". All the above materials were used without further purification. Deionized water was used throughout this work. The guanidine polymers: PGA (M = 400,000) and PMAG (M = 500,000) were synthesized at the department of macromolecular compounds of the Kabardino-Balkar State University by N.M. Berbekova. They were recrystallized from a water–acetone mixture with the following drying under vacuum at 60 °C.

2.2 Synthesis of non-modified silica

A typical synthetic procedure of non-modified silica used TEOS and water in relative molar ratios of 1:4 [12]. In a typical synthesis, 4 g TEOS was mixed with 1.668 g water and stirred for 2 h. The ammonia buffer (pH 9) was added as a base catalyst. The final product was transferred into a Petri dish for solvent evaporation at room temperature. The obtained powder sample was dried under vacuum at 50 °C during 2 days.

2.3 Synthesis of silica modified by BSA and guanidine polymers

It has already described some similar synthetic routes of albumin immobilization inside silica gel matrix [4, 5]. They used the acid conditions for obtaining albumin-containing glasses. In our case the synthesis of silicas modified by BSA, PAG and PMAG was similar to the one described above but we used basic conditions to obtain ultra dispersive powders. For this aim it was used the same molar ratio of TEOS/H_2O such as in previous synthesis. The different content of BSA (from 0.02 to 0.08 g) was added in water during sol–gel process to prepare solutions with necessary concentration of BSA. Then prepared solutions were mixed with TEOS and stirred for 2 h. The same process of synthesis was applied for silica modified by guanidine polymers with a content of the PAG ranged from 0.1 to 0.3 g and the PMAG from 0.1 to 0.2 g. The ammonia buffer was used as a base catalyst. The pH value equals to 9 and was measured by pH-meter U-500 ("Aquilon", Russia). The final product was transferred into a Petri dish for solvent evaporation at room temperature. The obtained powder samples were dried under vacuum at 50 °C during 2 days.

2.4 Characterization of mesoporous adsorbents

The obtained materials were characterized by common analytical techniques. Nitrogen adsorption measurements were carried out at 78 K using Micromeritics ASAP 2020 analyzer. Before the experiment all samples were heated at 500 °C for 8 h. This procedure was used for removing of all organic molecules from silicas (all samples were degassed) in order to calculate pore size, surface area and pore volume. The specific surface area was calculated by employing the Brunauer-Emmett-Teller (BET) method [13] in the range of relative pressure from 0.05 to 0.25. Average pore sizes (d_{BJH}) were estimated using the BJH method [14], while pore size distribution from desorption branches of isotherms using the same approach. FTIR spectra were obtained on Avatar 360 FTIR spectrometer by the KBr pellet method. Transmission electron microscope (TEM) images of obtained samples were performed using electron microscope (EMW-100 L) operated at 50 kV.

2.5 The adsorption of bilirubin from aqueous solution

The magnitude of BR adsorption on the surface of non-modified and modified silicas was determined as the difference between its initial and equilibrium concentrations in a water solution (pH 7.4) after contact with a sorbent from the formula

$$Q = \frac{(C_o - C_{ads}) \cdot V}{m} \tag{1}$$

where Q is the magnitude of BR adsorptions, mg/g; C_o and C_{ads} are the initial and equilibrium of BR in a water solution, mg; V is the volume of the solution, ml; and m is the mass of the sorbent sample, g. The UV-visible spectra of solutions and suspensions were recorded on SF-104 spectrophotometer ("Aquilon", Russia). All adsorption experiments were performed in darkness. Initial solutions were prepared by dissolving bilirubin in a small amount of alkaline solution with the following addition of phosphate buffer (pH 7.4). In a typical adsorption system, 5 ml of prepared solutions containing different concentrations of BR was incubated with 45 mg of particles, at 25 °C for 2 h. The pH values were measured by a pH-meter U-500 (“Aquilon”, Russia).

3 Results and discussion

3.1 Synthesis and characterization of the obtained materials

It is well known that albumin is amphoteric molecular whose surface charge depends on the pH value of the medium. The isoelectric point of BSA equals to 4.7 [15] so at a pH bellow its isoelectric point, protein surface has positive charge; above its isoelectric point the protein surface has negative charge. The conformation of albumin can be changed due to changes in pH value (Fig. 2). During the sol–gel process, negative charges of silica surface should cause repulsion of albumin molecular. However, the interaction between albumin molecules and silica surface is a very complex process, which is driven by not only electrostatic forces but also including van der Waals, hydrophobic and hydrophilic forces [16]. Soft proteins such as albumin undergo conformational changes under the influence of hydrophobic (dispersive) forces [17]. Previously it was already discussed in [18] that significant amount of immobilized protein is observed for slightly hydrophilic particles even under repulsive electrostatic condition. Thus, hydrophobic/hydrophilic effects are playing more important role than electrostatic interactions. Therefore, since the surface charges of silica are negative, the interaction between silica surface and BSA is based on hydrogen bonds (between Si–OH and –COOH of BSA) and hydrophobic/hydrophilic effects [19].

The guanidinium cation ($C(NH_2)_3^+$) in the guanidine polymers can react with nucleophilic agent in a wide range of pH value (from 6 to 11) [20]. As a result, during the sol–gel process negative charges of silica surface can easily interact with positive charge of guanidinium cation via electrostatic interactions.

The TEM images of the obtained materials are shown in Fig. 3. All investigated samples are spherical particles. The size of particles ranges from 20 to 1,000 nm. The non-modified silica is characterized by the smallest particle size distribution. It is clearly seen that the addition of a large amount of BSA (from 0.06 to 0.08 g) inside the inorganic matrix of silica leads to the particles aggregation of particles. The same result is obtained for silicas modified by guanidine polymers.

The obtained materials were examined by FTIR to characterize the modification by adding BSA and PAG/PMAG in the silica matrix during sol–gel process. The FTIR patters of all obtained adsorbents showed similar locations and appearance of the major bands (Fig. 4). The FTIR spectrum of non-modified silica has following important bands: 3,470 ÷ 3,420, 1,630, 1,390, 1,060 ÷ 1,220, 960 and 798 cm^{-1}. The features around 960 and 1,060 cm^{-1} are assigned to the Si–O–Si stretching vibrations [21, 22]. The vibrations of Si–OH appeared around 1,630 and 3,470 cm^{-1} [23]. As it was described in [24] the FTIR spectrum of pure BSA is characterized by a set of several peaks at 3,430, 3,062, 1,650 and 1,531 cm^{-1} which are assigned to the stretching vibration of –OH, amide A (–NH stretching vibrations), amide I (C=O stretching vibrations) and amide II (N–H bending vibrations). The FTIR spectrum of silica modified by BSA the most important peaks are the amide-I band (C=O stretching vibration) localized at 1,650 cm^{-1}, and the amide-II band (N–H bending vibration) at 1,540 cm^{-1} (Fig. 3). The FTIR spectra of silicas containing PAG and PMAG show the same peaks which correspond Si–O–Si and Si–OH vibrations. The peak at 860 cm^{-1} corresponds to the CH_2=C non-planar bending vibrations. The bands around 1,680 and 1,656 cm^{-1} correspond to the N=C stretching vibrations and $–NH_2$ bending vibrations [25].Thus, we conclude that BSA and guanidine polymers have been successfully grafted onto the silica skeleton.

To investigate a removal of the template molecules (BSA, PAG and PMAG) from the silica surface, the modified silicas were extensively washed in aqueous solution (pH 7.4) for 4 h at room temperature. It was determined that surface modification by a high amount of albumin (more than 0.06 g of BSA) leads to the removal of polymer molecules from silica surface which was confirmed by UV-spectroscopy. The UV-spectrum of water suspension of silica modified by 0.08 g of BSA showed the characteristic peak at $\lambda = 280$ nm which is typical for pure albumin. Also it was determined that the limited content of

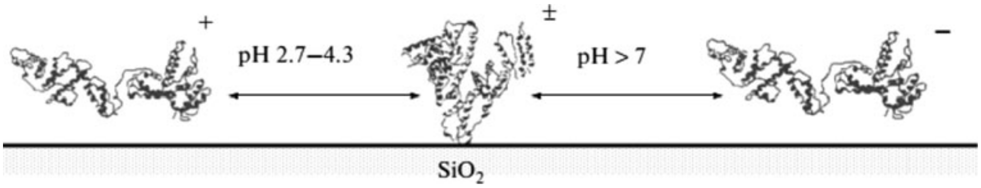

Fig. 2 Stability of bovine serum albumin at different pH values

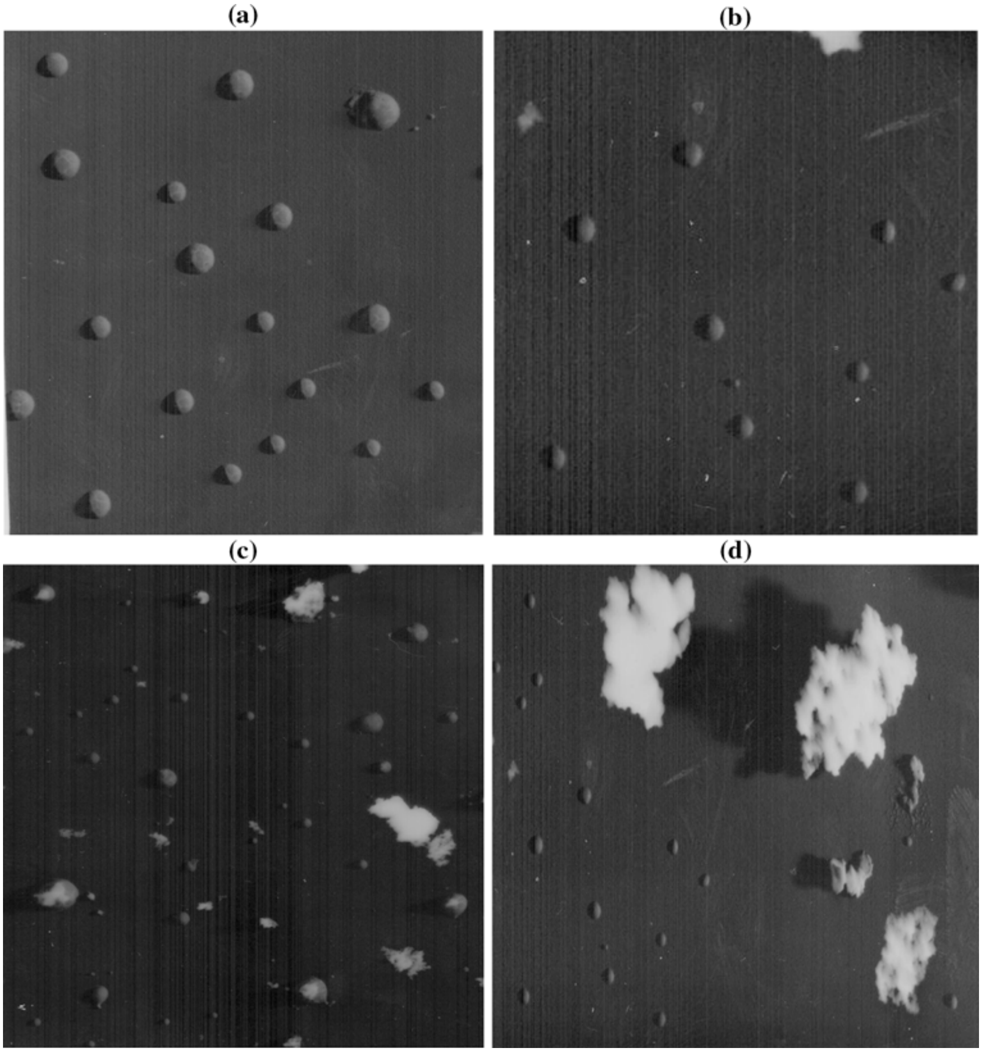

Fig. 3 TEM images of the obtained materials: non-modified silica (X38000, **a**); BSA-modified silica with a content of BSA of 0.02 g (X35000, **b**); BSA-modified silica with a content of BSA of 0.04 g (X19500, **c**); BSA-modified silica with a content of BSA of 0.06 g (X19500, **d**)

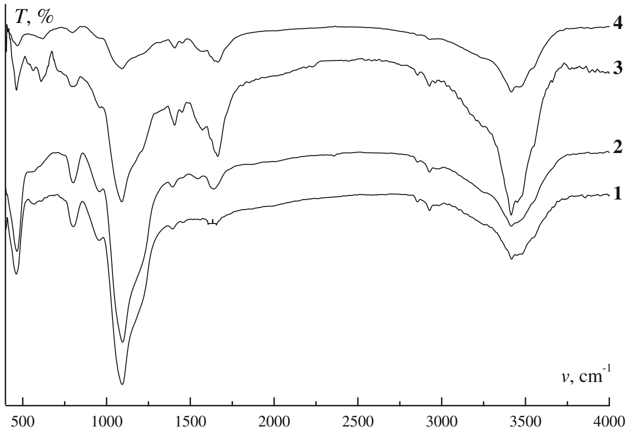

Fig. 4 A FTIR spectra pattern of the obtained adsorbents: non-modified silica (*1*), BSA-modified silica with a content of the BSA of 0.06 g (*2*), PAG-modified silica with a content of the PAG of 0.3 g (*3*), PMAG-modified silica with a content of the PMAG of 0.2 g (*4*)

guanidine polymers inside silica matrix should not be more than 0.2 g for PMAG and 0.3 g for PAG.

The nitrogen adsorption–desorption isotherms for non-modified and modified silicas are shown in Fig. 4. According to BDDT (Brunauer, Deming, Deming and Teller) classification, the isotherms **2** and **3** are referred to fourth type of isotherms which are typical for mesoporous materials. The form of hysteresis is related to H3 type which corresponds to adsorbents with slit-shaped pores [14].

The specific surface area of the adsorbents was determined using the multipoint BET measurement. The pore diameter and the average pore diameter of the adsorbents were determined using BJH method [14]. The structural properties of the adsorbent samples are shown in Table 1.

The surface area, pore volume and pore size of non-modified silica are all lower than the modified silicas

Table 1 Physical properties of the obtained adsorbents

No.	Sample	Specific area (m^2/g)	Total pore volume (cm^3/g)	Pore size (nm)
1	SiO_2	40	0.015	2.56
2	SiO_2/PAG (0.3 g)	160	0.27	9.2–15
3	SiO_2/BSA (0.06 g)	346	0.42	10–16

containing BSA and PAG. According to the IUPAC classification, the form of isotherms and physical properties (total pore volume) show that non-modified silica is nonporous material whereas silicas containing BSA and PAG are mesoporous. As a result, the modification by BSA and guanidine polymers increases the surface area, pore volume and pore size of the obtained adsorbents. The silica modified by BSA is characterized by the highest surface area and pore size. It might be explained by the fact that molecules of polymers can be used as templates to form necessary pore size. The pore size distributions of the obtained materials are shown in Fig. 5. Incorporation of the polymers inside silica matrix increases pore size of the obtained adsorbents. The pore diameter of silica containing BSA varies over a range from 10 to 16 nm and the average pore diameter equals to 14 nm. The pore diameter of silica modified by PAG varies over a range of 9–15 nm and the average pore diameter equals to 12 nm.

3.2 The adsorption isotherms of the modified and non-modified silicas

Langmuir equation was used to analyze the experimental adsorption isotherms in Fig. 6.

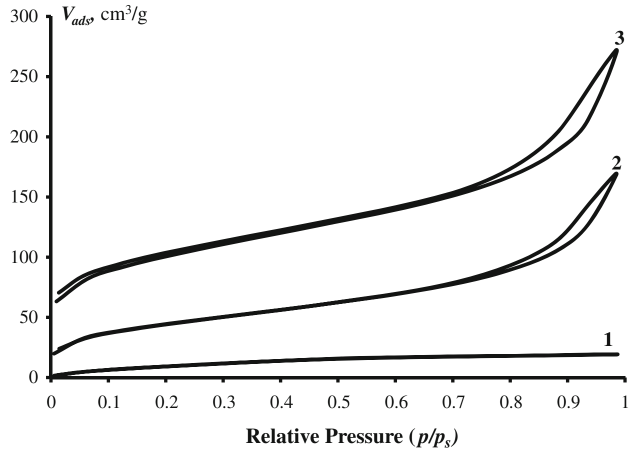

Fig. 5 Nitrogen adsorption–desorption isotherms of obtained adsorbents: non-modified silica (*1*), silica modified by PAG (*2*), silica modified by BSA (*3*)

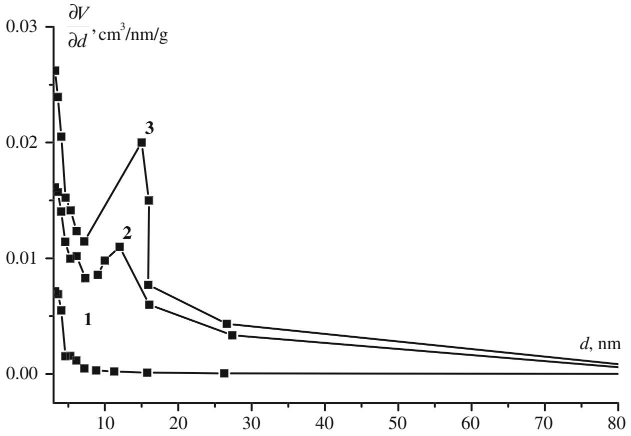

Fig. 6 A pore size distribution of the obtained adsorbents: non-modified silica (*1*), silica modified by PAG (*2*), silica modified by BSA (*3*)

$$Q = \frac{Q_{\max} bC}{1 + bC} \tag{2}$$

$$\frac{C}{Q} = \frac{1}{Q_{\max} b} + \frac{1}{Q_{\max}} C \tag{3}$$

where b is the Langmuir isotherm constant. The ratio of Q_{max} (mg/g) gives the theoretical monolayer saturation capacity of the adsorbent, C is the equilibrium concentration (mg/ml) of BR.

The isotherms of Langmuir model for BR adsorption on non-modified and modified silicas are displayed in Fig. 7. As it can be observed the adsorption of BR rapidly increased in the initial phase and a saturation value is achieved at a bilirubin concentration equals to 3.2 mg/ml. In accordance with Langmuir model, it is clear that

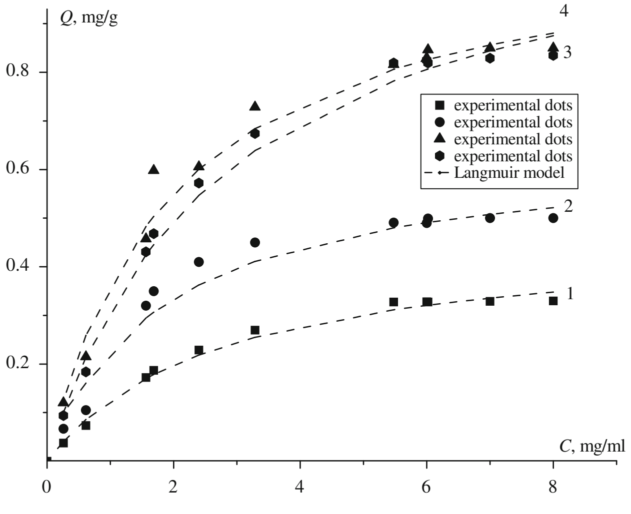

Fig. 7 Isotherms of BR adsorption on the surface: non-modified silica (*1*), PMAG-modified silica with a content of the PMAG of 0.2 g (*2*), PAG-modified silica with a content of the PAG of 0.3 g (*3*), BSA-modified silica with a content of the albumin of 0.06 g (*4*)

Table 2 The adsorption capacities for BR of various adsorbents

Sample	Adsorption capacity (Q_{max}, mg/g)	R^2
SiO_2	0.47	0.984
SiO_2/BSA (0.02 g)	0.93	0.942
SiO_2/BSA (0.04 g)	1.02	0.948
SiO_2/BSA (0.06 g)	1.18	0.986
SiO_2/PAG (0.1 g)	0.94	0.991
SiO_2/PAG (0.2 g)	1.09	0.987
SiO_2/PAG (0.3 g)	1.10	0.987
SiO_2/PMAG (0.1 g)	0.62	0.904
SiO_2/PMAG (0.2 g)	0.64	0.951

equilibrium adsorption capacity for BR on non-modified silica, silica modified by PMAG (0.2 g), silica modified by PAG (0.3 g) and silica modified by BSA (0.06 g) was 0.47, 0.64, 1.10 and 1.18 mg/g, respectively. Table 2 shows the equilibrium adsorption capacity and the correlation coefficients for all analyzed samples. According to the correlation coefficients, Langmuir adsorption model is suitable. It can be seen that modification of silica surface by BSA and guanidine polymers increases adsorption capacities for BR molecules.

The adsorbents containing BSA and PAG are characterized by the highest adsorption ability relative to BR molecules. The high adsorption capacity of silicas modified by BSA is most probably due to the formation of strong intermolecular complex between bilirubin and albumin molecules grafted on the silica surface. This type of binding can occur through various non-covalent forces such as hydrogen bonds, van der Waals attractions and hydrophobic interactions [26, 27]. As for silicas modified by guanidine polymers, the increase of adsorption capacity is likely to be due to electrostatic interactions between $–COO^-$ groups of bilirubin and guanidinium cation $(C(NH_2)_3^+)$ in the guanidine polymers. Besides, the large specific surface area of modified silicas can also lead to the increase of adsorption capacity of bilirubin molecules. The adsorption capacity of PMAG-modified silica is not so high compared to the adsorbents containing BSA and PAG because of influence of $–CH_3$ group in PMAG which prevents the interaction between BR molecules and surface of silica matrix.

4 Conclusions

The experimental results show that mesoporous silicas containing natural and synthesized polymers were successfully prepared via sol–gel process. The results indicate that the addition of polymers in silica gel matrix increased physical properties of the obtained materials: specific surface area, pore volume and pore size. The bilirubin adsorption process on surface of the obtained adsorbents was studied. It was determined that incorporation of bovine serum albumin and guanidine polymers in silica gel matrix increases adsorption capacity for bilirubin molecules. Besides, it was discussed possible interactions between bilirubin and polymers grafted on the silica surface. The results of adsorption ability of these adsorbents show a potential application of the obtained materials as effective hemoadsorbents for bilirubin removal in hemoadsorption.

Acknowledgments We thank Dr. Khashirova S. Yu., department of macromolecular compounds, the Kabardino-Balkar State University by N.M. Berbekova, for synthesis of guanidine polymers. The work is supported by the Grant of the RFBR (Project No. 12-03-31309).

References

1. Brinker J, Scherer W (1990) Sol–gel science: the physics and chemistry of sol–gel processing. Academic Press, Waltham, p 908
2. Rankin S, Macosko C (1998) Reaction engineering of co-condensing (Methyl) ethoxysilane mixtures: kinetic characterization and modeling. AIChE J 44(5):1141–1156
3. Rankin S, McCormick A (2000) The kinetics of alkoxysilane sol–gel process. J Chem Eng Sci 55:1955–1967
4. Menaa B, Menaa F, Aiolfi-Guimaraes C, Sharts O (2010) Silica-based nanoporous sol-gel glasses: from bioencapsulation to protein folding studies. Int J Nanotechnol 7:1–45
5. Menaa B, Miyagawa Y, Takahashi M, Herrero M, Rives V, Menaa F, Eggers DK (2009) Bioencapsulation of apomyoglobin in nanoporous organosilica sol–gel glasses: influence of the siloxane network on the conformation and stability of a model protein. Biopolymers 91(11):895–906
6. Zhu X, Brown G, St-Pierre L (1990) Adsorption of bilirubin with polypeptide-coated resins. Biomater Artif Cells Artif Organs 18:75–83
7. Ahmad N, Arif K, Faisal S, Neyaz M, Tayyab S, Owais M (2006) PLGA-microsphere mediated clearance of bilirubin in temporarily hyperbilirubinemic rats: an alternate strategy for the treatment of experimental jaundice. Biochim Biophys Acta 1760: 227–231
8. Yu Y, He B, Gu H (2000) Adsorption of bilirubin by amine-containing crosslinked chitosan resins. Artif Cells Blood Substit Immobil Biotechnol 28:307–316
9. Pyanova L (2011) Carbon sorbents in medicine and proteomics. Chem Sustain Dev 19:113–122
10. Lee K, Wendon J, Lee M, Lim S, Tan K (2002) Predicting the decrease of conjugated bilirubin with extracorporeal albumin dialysis MARS using the predialysis molar ratio of conjugated bilirubin to albumin. Liver Transpl 8:591–603
11. Tiribelli C, Ostrow J (2005) The molecular basis of bilirubin encephalopathy and toxicity: report of an EASL single topic conference, Trieste, Italy, 1–2 October. J Hepatol 43:156–158
12. Larry L, Hench Jon K (1990) The sol–gel process. West Chem Rev 90:33–72
13. Brunauer J, Emmet P, Teller E (1938) Adsorption of gases in multimolecular layers. J Am Chem Soc 60:309–319
14. Barrett EP, Joyner LG, Halenda P (1951) Determination of pore size distribution by gas adsorption. Ibid 73:373–380

15. Norde W (2003) In: Malmsten M (ed) Driving forces for protein adsorption at solid surfaces. Biopolymers at interfaces. Marcel Dekker, New York
16. Hofs B, Brzozowska A, de Keizer A, Norde W, Cohen Stuart MA (2008) Reduction of protein adsorption to a solid surface by a coating composed of polymeric micelles with a glass-like core. J Colloid Interface Sci 325:309–315
17. Eltekova NA, Eltekov AY (2008) Protein adsorption on silica molecular sieves. J Stud Surf Sci Catal 174:611–614
18. Lynch I, Dawson K (2008) Protein-nanoparticle interactions. Nano Today 3:40–47
19. Nakashi K, Sakiyamma T, Imamura K (2001) On the adsorption of proteins on the solid surfaces, a common but very complicated phenomenon. J Biosci Bioeng 91(3):233–244
20. Sivov NA, Khashirova SY, Malkanduev YuA, Baidaieva MH (2008) Biocide and toxicological properties of synthesized guanidine containing polymer and their structure. Mod Tendencies Org Bioorg Chem 27:310–335
21. Cheng Z, Yasukawa A, Kandori K, Ishikawa T (1998) FTIR study on incorporation of CO_2 into calcium hydroxyapatite. J Chem Soc FaradayTrans 94:1501–1505
22. Xu Y, Axe L (2005) Synthesis and characterization of iron oxide-coated silica and its effect on metal adsorption. J Colloid Interface Sci 282:11–19
23. Shengju Wu, Li Fengting, Ran Xu, Wei Shihui, Li Guangtao (2010) Synthesis of thiol-functionalized MCM-41 mesoporous silicas and its application in Cu(II), Pb(II), Ag(I) and Cr(III) removal. J Nanopart Res 12:2111–2124
24. Huang P, Zhiming L, Hengyao H, Daxiang C (2010) Synthesis and characterization of bovine serum albumin-conjugated copper sulfide nanocomposites. J Nanomater. doi: 10.1155/2010/641545
25. Sivov N, Khashirova S, Malkanduev Yu, Baidaeva M (2008) Biocide and toxicological properties of synthesized guanidine containing polymer and their structure. Mod Tendencies Org Bioorg Chem 27:310–335
26. Lee KH, Wendon J, Lee M, Da Costa M, Lim SG, Tan KC (2002) Predicting the decrease of conjugated bilirubin with extracorporeal albumin dialysis MARS using the predialysis molar ratio of conjugated bilirubin to albumin. Liver Transpl 8:591–601
27. Chen J, Song G, He Y, Yan Q (2007) Spectroscopic analysis of the interaction between bilirubin and bovine serum albumin. Microchim Acta 159:79–85