

Polyacrylate guanidine and polymethacrylate guanidine as novel cationic polymers for effective bilirubin binding

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Abstract The effect of polycationic polymers of polyacrylate guanidine (PAG) and polymethacrylate guanidine (PMAG) on bilirubin absorbance were studied in phosphate buffer (pH 7.4). It was shown that the change in absorbance spectra of bilirubin in the presence of PAG/PMAG can be associated with the formation of a bilirubin-polymer complex and dissociation of tetramers on bilirubin monomers. Also, the organic-inorganic composite materials based on silica gels and guanidine polymers were synthesized via the sol-gel technique. The incorporated guanidine polymers have a big influence on particle size distribution of silica gel due to their high cross-linking ability. The infrared spectroscopy revealed the presence of guanidine polymers inside solid networks of silica gel. The bilirubin adsorption process onto a guanidine functionalized silica surface was investigated. The Langmuir and Redlich-Peterson isotherm models were tested to explain the adsorption mechanism. The analysis of the adsorption isotherms confirms the possibility of electrostatic interactions of bilirubin molecules with guanidine polymers incorporated inside silica matrix. We conclude that cationic guanidine polymers might be effectively applied for bilirubin removal.

Keywords Guanidine polymers · Complex · Interactions · Sol-gel · Bilirubin · Adsorption

Introduction

Guanidine polymers are a new class of nanotechnological polymers which can be applied in different branches of science

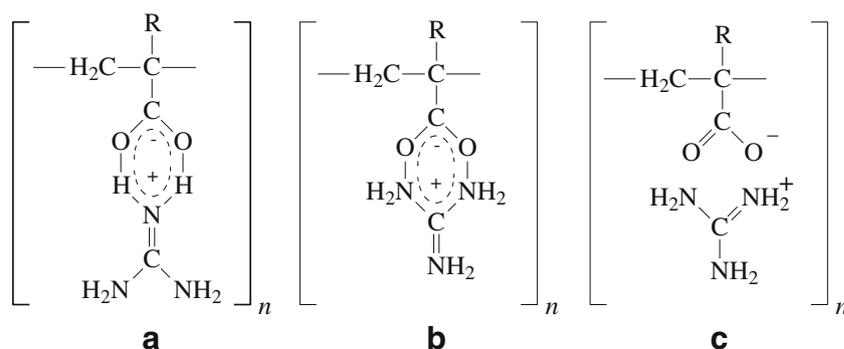
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[1–3]. The structure of the guanidine polymers based on guanidinium fragments and the branched units are built from methyl acrylate (Fig. 1). Careful study of the literature reveals that these polymers are used as biocides in the synthesis of materials with antimicrobial properties [4–6]. As is pointed out by Timofeeva and others, microbial cells generally carry a negative net charge on the surface while guanidine polymers are polycations; therefore, they are able to attach easily to the microbial cells [7]. Because of their antimicrobial properties, these polymers became commercially produced [8–10]. However, despite of their antimicrobial properties, these polymers might be used as polycations in electrostatic binding with different negatively charged bioorganic molecules [11].

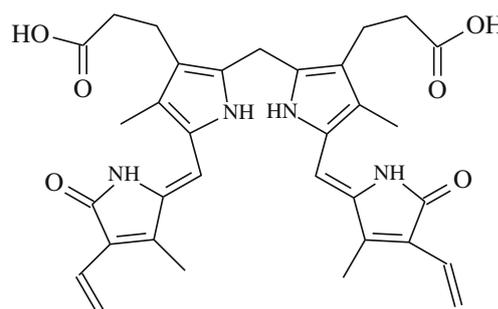
For instance, Funhoff et al. showed that polyplexes based on poly(3-guanidinepropyl methacrylate) were able to transfer organic cells in the absence of serum due to adsorption of negatively charged proteins on the surface modified by poly(3-guanidinepropyl methacrylate) [12]. These guanidine polymers were also used in ion complexation processes for selective removal of different ions from water [13]. Carlos et al. have shown that guanidine-containing polymers are more effective for cellular delivery of cargo than their primary amine analogs [14]. Qian et al. obtained the polyelectrolyte (PE) complex based on guanidine polymers adsorbed onto cellulose fibers. This PE complex is formed by electrostatic interactions between guanidine polymers with high cationic charge density and anionic carboxymethyl cellulose [15]. Thus, the guanidine polymers have already been successfully applied in inter- and intramolecular binding with different negatively charged molecules via electrostatic or hydrogen interactions and, furthermore, these polymers have good solubility and reactivity [16]; therefore, it makes them suitable for targeting, catalysis or as drug delivery systems due to their unique physicochemical properties [17].

Treat et al. suggested significant potential for guanidine-containing methacrylamide (co)polymers as drug delivery

Fig. 1 Constitutional structure of guanidine polymers in different conformational forms (*a*, *b*, *c*) and bilirubin



R = H - polyacrylate guanidine (PAG)
R = CH₃ - polymethacrylate guanidine (PMAG)



vehicles in targeted therapies [18]. To sum up, the guest molecule can be strongly attached in the host matrix of guanidine polymers via electrostatic interactions. The nature of the guest molecule can be different, which provides a great opportunity for treatment of different toxicological diseases.

One of the best-known endogenous toxins is bilirubin [19]. Bilirubin, a dicarboxylic acid, circulates in human blood plasma where it is bound to serum albumin to form a water-soluble complex [20]. It is transported to the liver as a complex with albumin, where it is normally conjugated with glucuronic acid and excreted into bile [21]. But a dysfunction in bilirubin metabolism leads to high concentrations (hyperbilirubinemia) of free bilirubin in the blood plasma. The increase of free bilirubin concentration may be due to several reasons including genetically caused Krigler-Najjar, Gilbert, and Rotor syndromes; neonatal and hepatitis jaundice; or insufficient albumin activity. Nowadays, it is established that hyperbilirubinemia can cause irreversible brain damage, so finding a way to remove bilirubin excess from organisms is still relevant.

In water solution when the pH ranges from 7 to 9, bilirubin is present as a dianion [22], and negatively charged bilirubin might be attached to the polycations. Since guanidine polymers are non-toxic for humans, they might be used for effective bilirubin removal [23]. Also, due to good solubility, they can be introduced into a sol-gel matrix for designing hybrid materials with unique physicochemical properties [24].

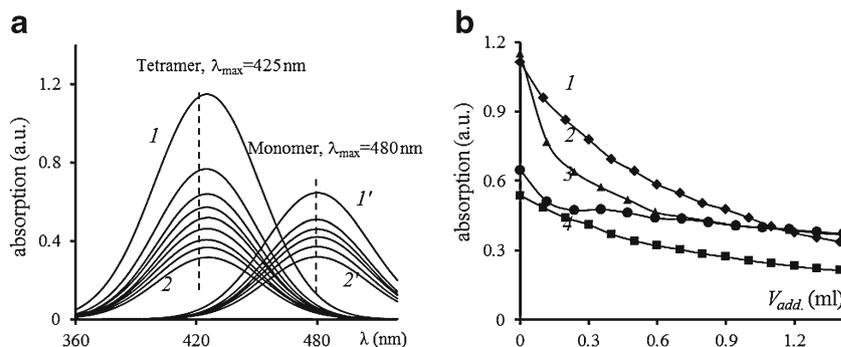
Nowadays, the wide potentialities of the sol-gel method are demonstrated by the synthesis of silica functionalized with different polymers [25–27]. The sol-gel method provides a

possibility to design the materials while keeping control over their chemical and physical properties [28] and, based on this, the silica surface treated with biopolymers may serve as a host matrix for bilirubin binding. And, importantly, guanidine polymers do not undergo any conformational changes such as proteins molecules [29]. During the sol-gel process, the formation of protein-silica interactions may lead to protein denaturation [30] because the structure of protein molecules is very sensitive to any changes and it may reduce their bioactivity. Therefore, the guanidine polymers can serve as an analogue of protein for preparation of sol-gel materials with reactive binding ability relative to different toxins like bilirubin.

Table 1 Amount of reagents and precursors used in the sol-gel synthesis of guanidine functionalized silicas

Sample	Sol-gel formulation (weight ratio, g)	The quantity of immobilized polymer, mg/g
PMAG-functionalized silicas		
SiO ₂ -PMAG-a	TEOS:PMAG=4:0.1	87
SiO ₂ -PMAG-b	TEOS:PMAG=4:0.15	130
SiO ₂ -PMAG-c	TEOS:PMAG=4:0.2	174
PAG-functionalized silicas		
SiO ₂ -PAG-a	TEOS:PAG=4:0.1	87
SiO ₂ -PAG-b	TEOS:PAG=4:0.15	130
SiO ₂ -PAG-c	TEOS:PAG=4:0.2	174

Fig. 4 Gaussian components for absorption spectra of bilirubin titration by PAG (1a) and PMAG (2a) and their corresponding titration curves of tetramers with PMAG (1b) and PAG (2b); monomers with PMAG (3b) and PAG (4b), respectively



Laser diffraction analysis

The particle size distribution analysis of the obtained materials was carried out on a laser diffraction particle size analyzer Analysette 22 Micro TecPlus (Fritsch, Germany). For laser diffraction analysis, the prepared sample (2 g) was introduced into the dispersion unit device of the laser particle analyzer for measurement; it contained 400 ml of deionized water. The measurable range of size distribution is from 0.01 to 1,000 μm .

Bilirubin adsorption from aqueous solutions

Bilirubin adsorption was studied in an aqueous solution at pH 7.4, simulating blood plasma. All adsorption experiments were performed in darkness. In a typical adsorption system, 5 ml of prepared solution containing bilirubin was incubated with 45 mg of synthesized sol-gel materials, at 25 $^{\circ}\text{C}$ for 2 h. The pH values were measured with a pH-meter U-500 (Aquilon Impex Ltd., Russian Federation).

The amount of adsorbed bilirubin was determined using a mass balance equation expressed as

$$Q_e = \frac{V \cdot (C_o - C_e)}{m}, \quad (1)$$

where Q_e is the amount of adsorbed bilirubin onto the silica surface; C_o is initial bilirubin concentration in solution (mg/l); C_e is equilibrium concentration of bilirubin in solution after adsorption (mg/l); V is volume of initial bilirubin solution used (l); and m is a mass of adsorbent used (mg). Then the experimental isotherm curves are built.

In order to compare the validity of two isotherm equations, a normalized standard deviation $\Delta q_e(\%)$ is calculated as follows:

$$\Delta q_e = 100 \sqrt{\frac{\sum [(q_{e,\text{exp}} - q_{e,\text{cal}}) / q_{e,\text{exp}}]^2}{(N-1)}}, \quad (2)$$

where N is the number of data; $q_{e,\text{exp}}$ is the experimental amount of adsorption at equilibrium; $q_{e,\text{cal}}$ is the amount of adsorption calculated from Langmuir and Redlich-Peterson equations.

Sample preparation

The pure silica gel was prepared using the sol-gel method which is described in [31]. For this synthesis, TEOS and H_2O were used in relative molar ratios of 1:4. In this typical synthesis, 4 g of TEOS and 1.40 g of H_2O were mixed simultaneously and stirred at 20 $^{\circ}\text{C}$ for 2 h by adding 0.05 ml of ammonia solution (pH=8) every 25 min as a base catalyst. Then the obtained solid precipitates were collected by filtration, washed with deionized water and dried at 80 $^{\circ}\text{C}$ under vacuum. The synthesis of guanidine functionalized silicas was similar to the above described; the same molar ratios of TEOS and H_2O were used varying the quantity of guanidine polymers (PAG and PMAG) in sol-gel synthesis. The weight of TEOS, H_2O and guanidine polymers are shown in Table 1. Also, the final solid products were recovered by filtration and dried at 80 $^{\circ}\text{C}$ under vacuum for 3 days. The synthesis route is shown in Fig. 2. During the hydrolysis reaction of TEOS which consists of replacing the alkoxide groups ($-\text{OC}_2\text{H}_5$) with hydroxyl groups ($-\text{OH}$), the smallest sol-gel particles are formed that are able to react with each other via silanol groups ($\text{Si}-\text{OH}$) to form the silica matrix. During the sol-gel process, the guanidine polymers can easily

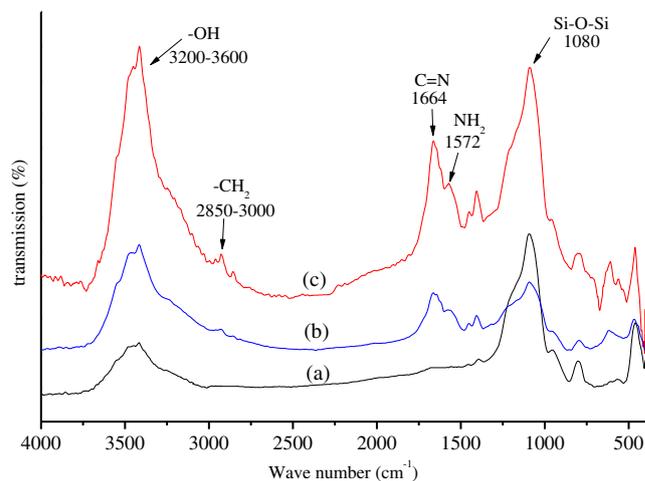


Fig. 5 FTIR spectra of synthesized silica gel: (a) pure silica gel; (b) silica gel modified with 174 mg/g of PMAG; (c) silica gel modified with 174 mg/g of PAG

interact with silanol groups via electrostatic and hydrogen bonding, and as a result, these guanidine polymers are immobilized into the silica surface as well as inside the silica matrix [13, 31].

Results and discussion

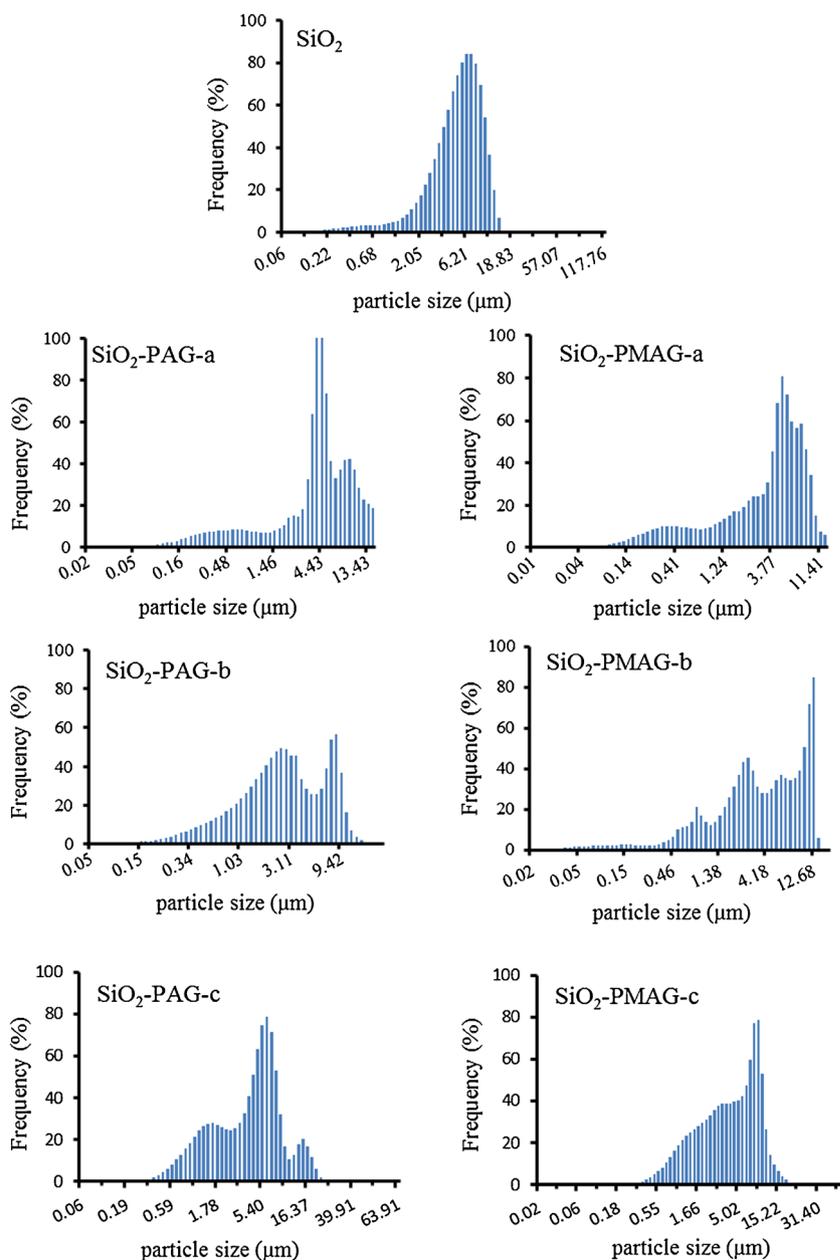
The effect of guanidine polymers on bilirubin absorption

UV-Vis absorption spectra of bilirubin titration in presence of guanidine polymers in buffer solution (pH 7.4) are shown in Fig. 3a. Bilirubin titration was performed by monitoring the absorbance at 438 nm ($\epsilon=52\,000\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$). As can be

observed from Fig. 3, the addition of PAG has changed the absorbance characteristics of the bilirubin solution. Figure 3 indicates a decrease of the bilirubin absorbance at 438 nm and appearance of a weak peak at 492 nm. The difference in experimental bilirubin titration curves with a water solution and with a solution of PAG (Fig. 3b) confirms the possibility of bilirubin-polymer interactions which indicates that these interactions between PAG and bilirubin have complex characteristics. The same results were obtained in the case of PMAG. This type of interaction might occur due to electrostatic forces of positively charged guanidinium cation [$\text{C}(\text{NH}_2)_3^+$] and bilirubin anions (BR^{2-}) [31, 32].

The UV-Vis spectra of bilirubin titration in the presence of PAG/PMAG were analyzed using a sum of two Gaussian

Fig. 6 The particle size distribution of the obtained materials: pure silica gel (SiO_2); silicas containing 80 mg/g of PAG ($\text{SiO}_2\text{-PAG-a}$); 130 mg/g of PAG ($\text{SiO}_2\text{-PAG-b}$) and 174 mg/g of PAG ($\text{SiO}_2\text{-PAG-c}$); silicas containing 80 mg/g of PMAG ($\text{SiO}_2\text{-PMAG-a}$); 130 mg/g of PMAG ($\text{SiO}_2\text{-PMAG-b}$) and 174 mg/g of PMAG ($\text{SiO}_2\text{-PMAG-c}$), respectively



contours (Fig. 4a). Decreasing concentration of bilirubin at 438 nm in the presence of PAG leads to a simultaneous increase in the shoulder at 492 nm. The bilirubin molecule is known to self-associate in aqueous solution, forming tetramers at high concentrations of bilirubin [33, 34]. Previously, it was proved that addition of serum albumin leads to formation of bilirubin monomers from tetrameric forms via formation of a water-soluble complex with albumin [35]. This fact is confirmed by the spectral changes in the absorption spectra: the bilirubin absorption maximum shifts from 438 nm (pure bilirubin in solution) to 480 nm (bilirubin-albumin complex) [33]. The same processes are observed in the presence of PAG/PMAG. From the deconvolution of the absorption spectra by Gaussian components (Fig. 4a), two maximum absorptions are observed at 480 nm and 425 nm corresponding to the bilirubin monomers and tetramers, respectively. So we can conclude that the interactions between guanidine polymers and bilirubin have similar characteristics as between serum albumin and bilirubin, in which bilirubin attaches to hydrophobic and positively charged fragments of the protein [34]. Apparently, the interaction of tetrameric form of bilirubin with hydrophobic cationic guanidine polymers leads to dissociation of the tetramer and the attachment of bilirubin monomers to the host molecule forming a guanidine-bilirubin complex. Moreover, electrostatic forces between guanidinium cation $C(NH_2)_3^+$ and COO^- groups of bilirubin provide additional stability to the formation of this complex.

However, the ratio peaks of tetramer and monomer depend on the type of polymers used during bilirubin titration: the ratio of monomers and tetrameric forms of bilirubin equals to 2:3 in the case of PAG and 1:3 for PMAG, respectively. This might be explained by the influence of methyl groups ($-CH_3$) in PMAG which may prevent electrostatic interactions between COO^- groups of bilirubin dianions and the guanidinium cation $C(NH_2)_3^+$ in polymethacrylate guanidine.

Characterization of the guanidine functionalized silica particles

We obtained functionalized silicas with different amounts of guanidine polymers (PMAG and PAG) via hydrolysis and polycondensation of TEOS. FTIR spectroscopy was used for the characterization of the sol-gel matrices of the guanidine functionalized silica. In Fig. 5, FTIR spectra of pure silica gel and silicas modified with guanidine polymers (PMAG and PAG) are shown. The spectrum of pure silica (Fig. 5a) reveals several peaks which correspond to Si–O–Si bonds ($1,080$ and 450 cm^{-1}) [35] and Si–OH bonds (intensive peak at $3,500\text{ cm}^{-1}$ and low peak at 950 cm^{-1}) [36]. The spectra in Fig. 5b, c are associated with silica gels modified with 174 mg/g of PMAG and 174 mg/g of PAG, respectively. All of these spectra show peaks at $1,664$ and $1,572\text{ cm}^{-1}$ that are characteristic of the N=C stretching vibration bands and the

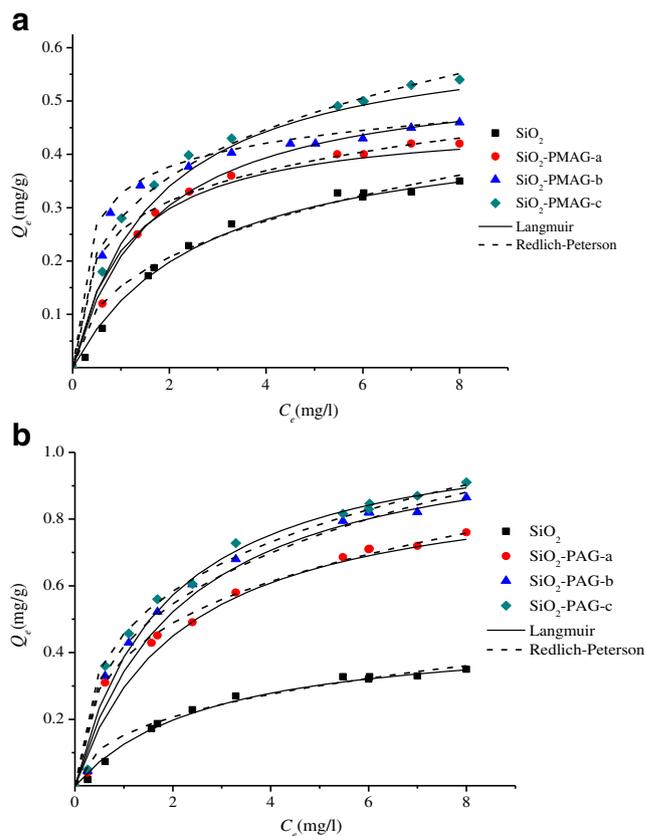


Fig. 7 Experimental data (points) and theoretical adsorption isotherm models (lines) for bilirubin onto surfaces of pure and functionalized silicas containing different quantities of PMAG (a) and PAG (b), respectively

presence of $-NH_2$ bending vibration bands [13, 31]. Also, the peaks at $2,850\text{--}3,000\text{ cm}^{-1}$ are associated with the presence of $-CH$ stretching vibration bands in guanidine fragments [13, 31, 37]. These results have clearly indicated the presence of guanidine polymers inside the silica gel matrix. Similar results were obtained for silica gels modifying with $87, 130\text{ mg/g}$ of PMAG and PAG, respectively.

To investigate the effect of guanidine polymers on the particle size of silica, all samples having different contents of PAG and PMAG were examined by laser diffraction analysis. Figure 6 shows the particle size distributions for pure and guanidine functionalized silicas. The obtained results indicate that guanidine modified silicas, besides presenting a larger size, exhibit a broader size distribution than pure silica. The particle size distribution of pure silica showed a sharp peak of

Table 2 Mathematics models of equilibrium adsorption

Adsorption model	The equation	Linear form of the equation
Langmuir	$Q_e = \frac{Q_{\max} \cdot K_L \cdot C_e}{1 + K_L \cdot C_e}$	$\frac{C_e}{Q_e} = \frac{1}{K_L \cdot Q_{\max}} + \frac{C_e}{Q_{\max}}$
Redlich-Peterson	$Q_e = \frac{K_R \cdot C_e}{1 + A_R \cdot C_e^\beta}$	$\ln\left(K_R \frac{C_e}{Q_e} - 1\right) = \ln A_R + \beta \ln C_e$

Table 3 Equilibrium characteristics calculated from the Langmuir model

Sample	The value of Langmuir parameters				Equation
	K_L	Q_e (mg/g)	r^2	$\Delta q_e(\%)$	
SiO ₂	0.369	0.466	0.989	4.16	$Q_e=0.172C_e/(1+0.369C_e)$
SiO ₂ -PAG-a	0.458	0.943	0.983	11.9	$Q_e=0.431C_e/(1+0.458C_e)$
SiO ₂ -PAG-b	0.459	1.092	0.981	11.7	$Q_e=0.501C_e/(1+0.459C_e)$
SiO ₂ -PAG-c	0.544	1.101	0.985	11.6	$Q_e=0.598C_e/(1+0.544C_e)$
SiO ₂ -PMAG-a	0.881	0.477	0.981	12.3	$Q_e=0.412C_e/(1+0.881C_e)$
SiO ₂ -PMAG-b	0.635	0.524	0.918	15.5	$Q_e=0.332C_e/(1+0.635C_e)$
SiO ₂ -PMAG-c	0.579	0.634	0.950	6.87	$Q_e=0.367C_e/(1+0.579C_e)$

pH=7.4, weight of the adsorbent=45 mg, sample volume=5 ml, equilibrium time=2 h

6.21 μm with narrow range indicating monodisperse silica particles. Moreover, one small broad peak around 0.39 μm is observed between 0.20 μm and 0.76 μm. The mean particle size is about 6.21 μm, corresponding to a cumulative volume frequency of 84 %. The size distribution curves of guanidine functionalized silicas always show a bimodal or trimodal distribution. The particle size distribution of the silica containing 80 mg/g of PAG (SiO₂-PAG-a) was trimodal and extended from 0.08 μm to 13.51 μm. Two large peaks of 4.43 μm and 8.62 μm and one small peak of 0.45 μm were observed. The silica containing 130 mg/g of PAG (SiO₂-PAG-b) is characterized by particle sizes between 0.15 and 13.13 μm; the mean particle size is about 2.49 and 8.43 μm, respectively. Three broad peaks of 1.43 μm, 6.03 μm and 14.65 μm were observed between 0.30 μm and 20.43 μm in case of silica containing 174 mg/g (SiO₂-PAG-c). The shift of the particle size distribution toward larger size with the addition of guanidine polymers reflects the formation of non-uniform clusters of silica particles. This might be caused by the high cross-linking ability of guanidine polymers: the guanidine fragments, in particular, the guanidinium cation in polymers, can easily react with silica particles and the silica surface via hydrogen bonds or electrostatic forces and form big clusters during the gel formation, as evidenced from the corresponding

particle size distribution curves (Fig. 6). Similar results were observed in the case of silica modified by PMAG: the particle size distributions of the silicas with different amounts of PMAG also have several broad peaks which confirmed the formation of silica particles with different mean size.

Bilirubin adsorption isotherms of the pure and functionalized silicas

The obtained experimental isotherms of equilibrium adsorption of bilirubin onto pure silica gel and silicas modified with different quantities of guanidine polymers are presented in Fig. 7a, b. Langmuir and Redlich-Peterson models were used to analyze the experimental adsorption isotherms in Fig. 7a, b. The parameters of adsorption isotherms were calculated from linear forms of Langmuir and Redlich-Peterson equations (Table 2) and are listed in Tables 3 and 4.

As it can be seen from Fig. 7, the adsorption values of bilirubin increased as the concentration of bilirubin increased. All of the obtained materials, pure silica and silicas with different contents of guanidine polymers, have shown different potentials for bilirubin adsorption. It is clear that incorporation of guanidine polymers inside the silica matrix leads to an increase of the adsorption ability of bilirubin and it was

Table 4 Equilibrium characteristics calculated from Redlich-Peterson model

Sample	The value of Redlich-Peterson parameters					Equation
	K_R	A_R	β	r^2	$\Delta q_e(\%)$	
SiO ₂	0.978	5.397	0.646	0.978	8.41	$Q_e=0.978C_e/(1+5.397C_e^{0.981})$
SiO ₂ -PAG-a	2.897	6.573	0.723	0.989	4.32	$Q_e=2.897C_e/(1+6.573C_e^{0.723})$
SiO ₂ -PAG-b	2.996	6.14	0.698	0.991	4.01	$Q_e=2.996C_e/(1+6.14C_e^{0.698})$
SiO ₂ -PAG-c	3.234	6.086	0.728	0.989	4.32	$Q_e=3.234C_e/(1+6.084C_e^{0.728})$
SiO ₂ -PMAG-a	1.878	6.290	0.810	0.994	3.38	$Q_e=1.878C_e/(1+6.290C_e^{0.810})$
SiO ₂ -PMAG-b	2.254	5.935	0.897	0.996	4.29	$Q_e=2.254C_e/(1+5.935C_e^{0.897})$
SiO ₂ -PMAG-c	2.345	7.344	0.723	0.987	5.01	$Q_e=2.345C_e/(1+7.344C_e^{0.723})$

pH=7.4, weight of the adsorbent=45 mg, sample volume=5 ml, equilibrium time=2 h

found that adsorption capacity of adsorbents depends on the type and quantity of guanidine polymer incorporated into the silica matrix. As it was previously shown, the effect of guanidine polymers on bilirubin absorption is that the increasing adsorption capacity of guanidine modified silicas compared to pure silica can be associated with stronger electrostatic interactions between guanidine polymers grafted onto the silica surface and bilirubin molecules. Also, it can be observed that the adsorption efficiency of silica gels modified by PAG is higher than silica gels containing PMAG. The silica with 174 mg/g immobilized PAG is characterized by the highest adsorption ability to bilirubin molecules. Considering that initial masses of PAG and PMAG used in sol-gel synthesis were the same, the difference in adsorption capacity of silica-PAG and silica-PMAG might be related to various interactive ability of PMAG and PAG with respect to bilirubin molecules. These experimental data correlate with previously obtained results where it was found that the influence of methyl groups ($-\text{CH}_3$) in PMAG can deteriorate the electrostatic interactions between bilirubin molecules and guanidinium cations $[\text{C}(\text{NH}_2)_3^+]$.

The relationship between the amount of adsorbed bilirubin and bilirubin concentration remaining in solution is described by the mathematic isotherms which describe the distribution of the adsorbate species among liquid and solid phases based on a set of assumptions that are related to the heterogeneity/homogeneity of solid surface, the type of coverage and possibility of interaction between adsorbate and silica surface [38]. Tables 3 and 4 show the equilibrium characteristics calculated from linear equations of Langmuir and Redlich-Peterson models. According to the value of the correlation coefficient, the Langmuir model is the most suitable for describing bilirubin adsorption on a pure silica surface ($r^2=0.989$) which confirms the monolayer sorption onto a surface with a finite number of identical sites [39, 40]. The Langmuir equation also showed a good fit for guanidine functionalized silicas. However, it is clearly shown that all the r^2 values for Redlich-Peterson were higher and their corresponding $\Delta q_e(\%)$ values were lower than this in Langmuir isotherm equations in the case of guanidine functionalized silicas which indicate that the Redlich-Peterson model provides a better fit to the experimental data than Langmuir. The Redlich-Peterson isotherm model combines elements from Langmuir and Freundlich equations and the mechanism of adsorption is unique and does not follow ideal monolayer adsorption [41]. This type of isotherm reflects the sorption on heterogeneous surface [41]. It is suggested that irreversible coulombic (electrostatic) attraction is the mechanism for bilirubin adsorption. It is possible to imply that electrostatic interactions can occur via creation of a negatively charged bilirubin molecule and positively charged guanidinium cation in guanidine polymers incorporated onto a silica surface.

Conclusions

The bilirubin-guanidine interactions in aqueous solution at pH 7.4 were studied and it was shown that polyacrylate guanidine and polymethacrylate guanidine have good bioactivity for bilirubin binding. The analysis of UV-Vis spectra of bilirubin in the presence of guanidine polymers indicates a similar nature of interactions as between serum albumin and bilirubin, in which the bilirubin molecule attaches to the protein surface via hydrophobic and electrostatic forces. Based on this result, the guanidine functionalized silicas were prepared via a very simple homogeneous sol-gel method. The FTIR spectroscopy results confirm the presence of guanidine polymers inside the silica matrix. Because of strong electrostatic interactions between guanidinium cations of PAG/PMAG on the surface of silica gel and carboxyl groups of bilirubin, the guanidine functionalized silicas exhibit higher adsorption ability against bilirubin than that of pure silica. The obtained adsorption isotherms of bilirubin adsorbed onto the guanidine functionalized silica surface fit the Redlich-Peterson equation which confirms the non-ideal monolayer adsorption. The main adsorption for bilirubin is affected by the influence of electrostatic, hydrogen-bond and hydrophobic interactions between chemically modified silica surfaces and bilirubin molecules. Thus, these guanidine polymers can be successfully used in hemo-adsorption and hemodialysis systems for bilirubin removal.

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