Synthesis and endotoxin removal properties of a novel affinity sorbent with poly(1-vinylimidazole) as the ligand

Jianhua Li\textsuperscript{a,b}, Yuanyuan Zhang\textsuperscript{b}, Zhenghua Ping\textsuperscript{b}, Mizi Li\textsuperscript{a}, Qiqing Zhang\textsuperscript{a,*}

\textsuperscript{a} Institute of Biomedical and Pharmaceutical Technology, Fuzhou University, Fuzhou 350001, PR China
\textsuperscript{b} Key Laboratory of Molecular Engineering of Polymers, Ministry of Education, Department of Macromolecular Science, Fudan University, Shanghai 200433, PR China

\textbf{A R T I C L E   I N F O}

Article history:
Received 12 November 2010
Received in revised form 27 March 2011
Accepted 28 March 2011

Keywords:
Silica gel
LPS removal
Affinity adsorption
Poly(1-vinylimidazole)
Graft copolymerization
Ligand

\textbf{A B S T R A C T}

In this paper, a novel silica gel affinity sorbent with poly(1-vinylimidazole) (PVI) ligand was prepared for the selective removal of endotoxin (LPS) from a water or a BSA solution. The vinyl silane-modified porous silica gel particle SiO\textsubscript{2}–g–KH570 was used as macromonomer, and PVI was grafted on by heterogeneous graft copolymerization. First, the effect of reaction conditions on the grafted degree (GD) of PVI was studied and compared with those of His–g–SiO\textsubscript{2} affinity sorbent. The results showed that maximum LPS removal capacity was obtained with a GD about 2.5 wt% and the PVI–g–SiO\textsubscript{2} affinity sorbent had better selective removal of LPS compared to His–g–SiO\textsubscript{2} affinity sorbent in both water and BSA solution. The affinity mechanism between PVI and LPS is also discussed in this paper. All of our results indicate that PVI is a good ligand for LPS removal.

\textcopyright 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Endotoxin, also known as lipopolysaccharide (LPS), is derived from the outer cell wall of Gram-negative bacteria, e.g., Escherichia coli [1]. Due to the potential biological activity of pyrogenic and shock reactions in mammals even at 1 ng/kg in the body, LPS is a major concern during the course of downstream processing of target proteins and in healthcare and environmental protection. The LPS threshold limit set by pharmacopoeias for intravenous applications is 1 endotoxin unit (EU)/mL, which corresponds to 0.1 ng/mL [2]. Therefore, the selective elimination of LPS from biomedical products and fluids has always been a challenge, especially in situations in which LPS binds product proteins.

Among a variety of methods developed for the purpose of LPS removal, affinity adsorption has proven to be the most effective technique [3,4]. The high selectivity of this unique technique comes from the strong interaction between the ligand immobilized on the support and the target ligate through the synergy of multiple forces [5], especially the ionic/polar and hydrophobic interactions for LPS binding [6]. Although the ligand is generally the key factor for the success of affinity separation, properties of the carrier may also influence the ligate binding effect directly or indirectly through an impact on the accessible density or activity of the ligand. The support of an affinity sorbent for LPS removal is usually made from natural polymeric soft gel, synthetic polymer particles or inorganic oxide gel. Silica gel is considered an ideal matrix because of its good biocompatibility, high rigidity, thermal stability and good resistance against organic solvents and microbial attack [7,8]. However, few studies on silica-based affinity sorbent for LPS removal have been reported.

To date, many LPS affinity ligands have been reported, histamine, histidine (His) [9], polymyxin B [10], dimethylamine [11] and polyethyleneimine [12]. Among these ligands, His is most widely used due to its stability, innocuity and affordability and has been immobilized on Sepharose–4B or nylon membrane for LPS removal. Our group has also previously prepared a novel LPS adsorbent on His-immobilized silica gel with a saturated adsorption capacity of about 1.2 mg/g and an apparent dissociation constant ($K_d$) of 1350 μg/L [13], which were close to or even better than those of removal materials reported in the literature [14]. However, the LPS adsorbing efficiency of the His-immobilized adsorbent dropped remarkably with an increase of the ionic strength in testing solutions, which was found in both the literature and our work [13]. In addition, complicated steps are usually required to introduce His or other mentioned ligands on supports, and the amount of introduced ligand is always difficult to control.

It has been shown that the LPS-adsorbing function of His is mainly attributed to its imidazole ring [13]. Therefore, we can expect that other imidazole-containing compounds may function as ligands for LPS removal. 1-Vinylimidazole (VI) is a good candidate...
for this purpose. It is easily introduced onto a support by controllable graft polymerization, and the graft degree is easily controlled by adjusting the reaction conditions. Compared to His, PVI does not contain any negatively charged group in its structure, which will strengthen the electrostatic interaction between the imidazole ring and LPS. Moreover, the longer and flexible aliphatic chains of PVI will facilitate binding to large LPS molecules (1–50 nm).

In this study, a novel affinity sorbent was developed based on silica gel support and immobilized with PVI ligand. The grafting reaction conditions and the LPS adsorption properties in aqueous and BSA solutions were studied. Finally, the LPS adsorption properties of PVI-g-SiO2 were compared to those of His-g-SiO2 under several conditions.

2. Materials and methods

2.1. Materials

Macroporous silica gel was obtained from Qingdao Meigao Chemical Co., Ltd. The specific surface area, mean pore diameter and particle size were 180 m²/g, 40 nm and 50 μm, respectively. γ-Methacryloxypropyltrimethoxysilane (KH570) was purchased from Nanjing–Crompton Shuguang Organosilicon Specialties Co. Ltd. and used as received. α,α′-Azobis(isobutyronitrile) (AIBN) was purchased from Shanghai Chemical Reagent Corp. and recrystallized in ethanol three times before use. 1-Vinylimidazole (VI; 99%) was purchased from Alfa Aesar without further purification. LPS standard samples (from Escherichia coli O111: B4) and the limulus amebocyte lysate assay (LAL) chromogenic kit for LPS detection were purchased from Shanghai Yihua Clinical Medicine Technologies Company. Pyrogen-free pure water was prepared using the Purelab System (PALL Co.; America). Glass columns of Ø 0.6 cm × 5 cm were supplied by Zhongshan Hospital. Bovine serum albumin (BSA) was supplied by Bio Life Science and Technology Co. (Shanghai, PR China). Other organic solvents were used directly without further purification.

2.2. Preparation of the affinity sorbent

2.2.1. Pretreatment of silica gel

Pretreatment of silica gel is necessary to eliminate impurities and activate the silanol groups on the surface before silanization [15]. The silica gel particles were first immersed in 0.5 M HCl for 30 min under an ultrasonic condition. After rinsed with pure water to neutrality, it was dried at 80 °C for 12 h before use. Elemental analysis (VARIO EL III, Elementar Co.) showed that the treated particles contained 1.17% wt% hydrogen, which corresponded to about 11 mmol/g hydroxyl group.

2.2.2. Preparation of SiO2-g-KH570

SiO2-g-KH570 was synthesized by the following process. 10 g of treated silica gel and 80 mL of anhydrous toluene containing 10 wt% KH570 were charged into a 250-mL three-neck flask equipped with a reflux condenser. The flask was then moved into a 110 °C oil bath for 6 h under magnetic stirring. After completion of the reaction, the activated particles (containing vinyl end groups) were thoroughly washed with toluene and methanol and dried at 40 °C under a vacuum. The components of SiO2-g-KH570 were characterized by FT-IR.

2.2.3. Preparation of PVI-g-SiO2 affinity sorbent

A series of PVI-g-SiO2 affinity sorbents were prepared through free–radical graft polymerization with AIBN as an initiator. The influence of the synthesis conditions to PVI grafted degree was studied. Typical synthesis conditions were as follows: 5 g SiO2-g-KH570 in 25 mL DMF was charged into a three-neck flask equipped with a reflux condenser. Then, 10 mL DMF, which contained 4–27 wt% VI was added into the reaction mixture. After 30 min of bubbling with nitrogen, AIBN (0.005–0.025 mol/L) was imported, and the flask was sealed. The reaction was performed at 60–85 °C for 2–12 h under a nitrogen atmosphere. After cooling to room temperature, the product was filtrated and washed thoroughly with DMF and methanol to completely remove non-anchored polymer on the silica gel. Finally, the product was dried at 40 °C under a vacuum.

The synthetic scheme is shown in Fig. 1.

2.3. Characterization of the affinity sorbent

The chemical compositions of the silica gel before and after grafted degree were analyzed using a FT-IR spectrophotometer (Magna 550, Nicolet). The grafted degree (GD) of PVI was defined as the mass ratio of the grafted PVI to the silica gel support, which was calculated according to the weight loss percentage of the samples measured by thermogravimetry (TG, Pyris 1, Perkin Elmer) in a dry nitrogen atmosphere. The temperature ranged from 100 °C to 650 °C, and the heating rate was 25 °C/min. Typical TGA curves are shown in Fig. 2. LPS adsorption experiments were conducted in dynamic and static mode. The LPS content in solution was quantitatively analyzed using the LAL assay (endpoint method) following the supplier’s instructions. The detection limit of the assay was 0.015 LPS units (EU)/mL, which corresponded to 1.5 × 10⁻¹² g/mL. Before measurements, all glassware was baked at 260 °C for at least 3 h. The plastic apparatus was
treated with 33% H₂O₂ and rinsed with pyrogen-free water before being dried at 80 °C for 8 h.

2.3.1. Effect of the PVI grafted degree on LPS adsorption capacity

The effect of the PVI grafted degree on LPS adsorption capacity was determined by using a static adsorption model. Adsorbents with grafted degrees from 1.1 wt% to 15 wt% (50 mg of each) were filled in 25 mL of 30 EU/mL LPS solution. After being shaken at 37 °C for 2 h to reach equilibrium, the remaining LPS concentrations in the supernatants were analyzed.

2.3.2. Determination of equilibrium adsorption isotherm

The LPS adsorption isotherms for two representative adsorbents with PVI grafted degrees of 2.5 wt% and 8.4 wt% were determined by a batchwise method. A series of 25 mL LPS solutions with concentrations from 10 EU/mL to 6600 EU/mL were prepared in 50 mM Na₂HPO₄–NaH₂PO₄ buffer solution (pH 7.0). After the 50 mg affinity sorbent was filled, each LPS solution was continuously shaken for 3 h at 37 °C to reach equilibrium. The remaining LPS concentration was determined using the LAL assay (endpoint method) following the supplier’s instructions.

The adsorption capacity of the sorbent was evaluated by LPS removal efficiency (E%) and LPS-binding capacity (q), which were calculated by formulas (1) and (2), respectively.

\[
E\% = \frac{C_0 - C_f}{C_0} \times 100 \quad (1)
\]

\[
q(\text{EU/mg}) = \frac{C_0 - C_f}{V_f} \quad (2)
\]

In these equations, \(C_0\) is the original LPS concentration (EU/mL) in the sample solution, and \(C_f\) is the LPS concentrations in the sample solution after adsorption by affinity absorbent. \(M\) is the weight of sorbent (mg) used in each sample solution, and \(V\) is the volume of each sample solution (mL). \(C_0\) and \(C_f\) were determined using the LAL method and a UV spectrophotometer (752S; Shanghai Lengguang Tech. Co., Ltd.) to measure the absorbance at 280 nm, which was recorded on a UV–vis spectrometer.

2.3.3. Comparison of LPS adsorption ability between PVI-g-SiO₂ (PVI-s) and His-g-SiO₂ (His-s)

After all basic characteristics were clarified above, the LPS removal capacity of PVI-g-SiO₂ (PVI-s, CD = 1.45, 0.148 mmol/g) was compared with the His-g-SiO₂ (His-s, 0.15 mmol/g) under various conditions to further evaluate the LPS adsorption performance of the novel PVI-s. His-s was prepared by our group [13]. Every experiment was repeated three times, and the results were averaged.

2.3.3.1. Effect of absorbent dose. To compare the effect of absorbent dose to LPS adsorption ability for both PVI-s and His-s, a certain amount of absorbents (the dose varying from 1 to 30 mg/mL) were filled into 15 mL of LPS solution (15 EU/mL). The mixtures were shaken at 37 °C for 3 h, and the LPS concentrations in the supernatants were analyzed. The relationship between absorbent dose and endotoxin removal efficiency was studied.

2.3.3.2. Effect of LPS initial concentration. A series of 25 mL LPS solutions were prepared with concentrations from 10 EU/mL to 500 EU/mL. Then, 50 mg sorbent of PVI-s or His-s was filled in each solution. After shaking at 37 °C for 3 h, the LPS concentrations in sample solutions (\(C_c\)) were determined by the LAL method, and the q was calculated using the formula (2).

2.3.3.3. Effect of ionic strength. To study the effect of ionic strength on LPS adsorption, LPS solutions of 30 EU/mL were prepared with NaCl solution at different ionic strengths, i.e., 0, 0.5, 1, 1.5, 2 mol/L. After 40 mg PVI-s (or His-s) was filled, the mixtures were incubated for 30 min at 37 °C. The residual LPS concentration was then determined by the LAL method, and the endotoxin removal efficiency was calculated using the formula (1).

2.3.3.4. pH effect. To study the effect of pH on the LPS removal efficiency of PVI-s and His-s, 25 mL of 30 EU/mL LPS solutions were prepared separately with buffers at various pHs from 3.0 to 8.0. The buffers were acetate buffer (pH 3.0, 4.0 and 5.0) and phosphate buffer (pH 6.0, 7.0 and 8.0). The concentration of all buffer solutions was 0.05 M. After 40 mg of PVI-s (or His-s) was filled into each LPS solution, the mixtures were incubated for 30 min at 37 °C. Then, residual LPS concentrations were determined using the LAL method, and the endotoxin removal efficiency was calculated using the formula (1).

2.3.3.5. Column-mode LPS removal from BSA–LPS mixture. The selective removal of LPS in a BSA protein solution was conducted using the chromatographic column method. 20 mg of PVI-s and His-s absorbents were separately added into a glass column to form a packed bed. The formed column was then washed three times with LPS-free water until the LPS adsorbed on the absorbent could not be physically detected. The feed solution (containing 0.05 M NaCl, 30 EU/mL of LPS and 1 mg/mL of BSA) was perfused through the column at a flow rate of 1 mL/min by a peristaltic pump. The effluent (0.1 mL) was collected at a certain time points to determine the LPS and BSA concentrations. The BSA concentrations in the tested solutions were determined using the absorbance intensity at 280 nm, which was recorded on a UV–vis spectrometer.

3. Results and discussion

3.1. Characterization of PVI-g-SiO₂

The chemical compositions of the SiO₂, SiO₂-g-KH570 and PVI-g-SiO₂ were determined by FT-IR and the results are shown in Fig. 3. A characteristic peak at 968 cm⁻¹ was observed in the spectrum of the SiO₂ support, which was attributed to the –OH stretching vibration on SiO₂ (Fig. 3a). After KH570 immobilization, the strength of the peak at 968 cm⁻¹ was significantly decreased, and a new band was detected at 1727 cm⁻¹, which was attributed to the C=O stretching mode of the –COO group in the KH570 structure (Fig. 3b). In the PVI-g-SiO₂ spectrum (Fig. 3c), a new peak at 1500 cm⁻¹ was attributed to the C=C stretching vibration in the imidazole ring groups, and the peak at 665 cm⁻¹ was attributed to the C=N torsion stretching vibration in the imidazole ring groups, which indicated that PVI was successfully grafted on SiO₂-g-KH570 by free radical polymerization.

3.2. Effect of grafting conditions on the PVI graft degree

The adsorption capacity of the affinity sorbent in a certain solution generally depends on the amount of accessible ligand immobilized on the support. So the ligand grafted degree has a significant influence on the properties of an affinity sorbent. In this paper, the influence of reaction conditions on PVI grafted degree was systematically studied, and the results are shown in Fig. 4. Under a 70 °C reaction temperature and 3 h reaction time, the VI concentration in the reaction solution was 16 wt%, and the grafted degree of PVI onto the silica gel improved with the increasing AIBN concentration. However, when the AIBN concentration exceeded 0.02 mol/L, the PVI grafted degree decreased with the increasing AIBN concentration. When the AIBN concentration in the reaction solution was 0.02 mol/L, the PVI grafted degree increased with the increasing VI concentration. However, after the VI concentration exceeded 16.7 wt%, the grafted degree of PVI decreased with the increasing VI concentration. Because the abundance of the radical led to its participation in the termination of the growing polymer when the AIBN concentration exceeded 0.02 mol/L, the grafted...
degree decreased. When the PVI grafted degree exceeded 16.7 wt%, the VI concentration in the reaction solution accelerated the homopolymerization, rather than graft polymerization, of the VI monomer, which led to the decreased PVI grafted degree with the increase of the VI concentration on the silicon gel. A similar phenomenon has been reported by Fu [17] and Teke and Baysal [18].

3.3. Effect of PVI grafted degree on LPS adsorption capacity

It is essential to eliminate LPS to a level lower than 100 pg/mL in fluids and therapeutic agents. Variations of pore size and LPS adsorption capacity for sorbents with different PVI grafted degrees are listed in Table 1. From these results, we can observe that the pore size of adsorbents reduces with the increase of PVI grafted degree on a silicon gel support. Though the residual LPS in solution after adsorption were all reduced below 1 EU/mL regardless of the PVI grafted degree, the highest LPS removal capacity was around the 2.5 wt% grafted degree under an LPS concentration of 30 EU/mL. This result was probably because the grafting chains on the sorbent became denser at higher grafted degrees, which badly shrank or even blocked the internal pores of the silica gel (Table 1). Consequently, diffusion of the large LPS molecules to approach the internal active sorption sites was hindered. Obviously, in such an instance a high GD is unfavorable for LPS removal. However, it should be noticed that a high PVI grafted degree would be effective once the pore size of the matrix was optimized.

3.4. Determination of equilibrium adsorption isotherm

The equilibrium adsorption isotherm is very important in the design of an adsorption system and can be calculated using Eq. (3). In this paper, the equilibrium adsorption isotherm of absorbents with PVI grafted degrees of 2.5 wt% and 8.4 wt% were determined, and the results are shown in Fig. 5.

The isotherms display a good fit for the Freundlich isotherm model, which is indicative of surface heterogeneity of the sorbent and can be linearized by logarithmic transfer [19]:

$$\log Q_e = \log K_f + \frac{1}{n} \log C_e$$

where $K_f$ and $n$ are Freundlich constants related to adsorption capacity and adsorption intensity, respectively. For a PVI absorbent with a grafted degree of 2.5 wt%, the $K_f$ value was calculated as 119.6 EU/mg and $n$ was 1.35 (for GD = 2.5 wt%). For a PVI absorbent with a grafted degree of 8.4 wt%, the $K_f$ was 114.4 EU/mg and $n$ was 1.09. These results partly verified the phenomenon presented in Table 1. The correlation coefficients for the linear fit of the Freundlich isotherm equation were above 0.991 for both curves.

It was also found that the adsorbents were far from adsorption saturation, and the maximum adsorption capacity was absolutely higher than 3500 EU/mg, or 0.35 mg/g (Fig. 5). Because LPS contamination in samples is usually below 1 µg/mL, this adsorption capacity fully satisfies the basic requirements for LPS removal.

3.5. Comparison of LPS adsorption ability between PVI-g-SiO$_2$ (PVI-s) and His-g-SiO$_2$ (His-s)

The ligands in His-g-SiO$_2$ and PVI-g-SiO$_2$ both contain an imidazole ring that can selectively adsorb LPS via an electrostatic interaction. Compared to the His ligand, the PVI ligand has a more advantageous chain structure feature. At the same grafted degree, the PVI in PVI-g-SiO$_2$ has a more regular structure compared to the His in His-g-SiO$_2$ and will support the formation of an optimal arrangement of opposite charges decisive for effective LPS adsorption. Additionally, the longer and flexible aliphatic chains in the PVI chains will facilitate binding to large LPS molecules. So we can infer that PVI-g-SiO$_2$ will be more effective in LPS removal than His-g-SiO$_2$.

To compare LPS adsorption abilities, PVI-g-SiO$_2$ (PVI-s, GD = 1.4 wt%, 0.148 mmol/g) and His-g-SiO$_2$ (His-s, 0.15 mmol/g) were used to remove LPS under various conditions.

3.5.1. Effect of sorbent dose

In general, at a certain initial concentration of adsorbent, the LPS removal percentage was improved, and the adsorption capacity was decreased with an increasing sorbent dosage. Therefore, the proportion of LPS–containing solution to the addition amount of adsorbent is very important and was systematically studied here. The change of LPS removal efficiency to absorbent dosage in 100 mL of solution is shown in Fig. 6. The LPS removal efficiency was increased with the enhancement of absorbent doses for both absorbents. However, PVI-s had a better LPS removal efficiency compared to His-s under the same dosage. When 0.25 g of PVI-s was filled into 100 mL of LPS solution, the LPS removal efficiency reached 97%, which was higher than His-s (83%). When the dose of PVI-s
Table 1
Effect of GD on the pore size of the PVI-bearing adsorbents and the LPS removal.

<table>
<thead>
<tr>
<th>GD of PVI (%)</th>
<th>Pore size of the sorbent (nm)</th>
<th>Residual LPS in solution (EU/mL) ±0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>34</td>
<td>0.36</td>
</tr>
<tr>
<td>2.5</td>
<td>30.1</td>
<td>0.07</td>
</tr>
<tr>
<td>4.2</td>
<td>28.2</td>
<td>0.105</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>0.47</td>
</tr>
<tr>
<td>8.4</td>
<td>7.56</td>
<td>0.7</td>
</tr>
<tr>
<td>10</td>
<td>7.23</td>
<td>0.68</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>0.84</td>
</tr>
</tbody>
</table>

- Measured using a Tristar 3000 gas adsorption analyzer.
- Characterized by a static adsorption mode: 50 mg adsorbents with different grafting degree were filled into 25 mL LPS solution, respectively; adsorption temperature: 37°C; adsorption time: 2 h; the LPS solution concentration: 30 EU/mL.
- Mean value ± standard deviation (n = 3).

Fig. 6. Effect of solid/liquid ratio on the adsorption efficiency of PVI-s (–■–) and His-s (–▲–). Initial concentration of LPS solution: 15 EU/mL; volume of LPS solution: 15 mL; pH: 7.0; temperature: 37°C; time: 3 h. Bar: mean ± SD (n = 3).

Increased to 1 g in 100 mL of LPS solution, the LPS removal efficiency reached nearly 99%. From these results, we can see that the appropriate dose for PVI-s was about 0.25 g of sorbent/100 mL of LPS solution, while for His-s the dose was about 2 g of sorbent/100 mL of LPS solution. Compared to His-s, PVI-s had a higher adsorption capacity because a smaller dosage produced a similar removal efficiency.

The dosages of the two affinity adsorbents were both fixed at 5 mg/mL in the following experiments.

3.5.2. Effect of LPS initial concentration

At relatively low initial LPS concentrations (10–500 EU/mL), the equilibrium adsorptions of His-s and PVI-s were determined and compared (Fig. 7). The results showed that PVI-s had a much higher adsorption capacity compared to His-s at all tested concentrations. Table 2 also indicates that at the same initial LPS concentration, PVI-s reduced LPS to a much lower level.

![Fig. 7. Equilibrium adsorption of PVI-s (–■–) and His-s (–▲–). Initial concentration of LPS solution: from 10 EU/mL to 500 EU/mL; volume of LPS solution: 25 mL; dosage of sorbent: 50 mg; temperature: 37°C; time: 3 h. Bar: mean ± SD (n = 3).](image)

PVI-s has a much higher adsorption capacity and can reduce LPS to a much lower level.

3.5.3. Effect of ionic strength

The effect of ionic strength on endotoxin removal efficiency (E%) of PVI-s and His-s is presented in Fig. 8. From these results we can infer that PVI-s had a higher removal efficiency compared to His-s at all ionic strengths. For PVI-s, when ionic strength increased from 0 to 2 M, the endotoxin removal efficiency decreased from 98% to 88%. However, for His-s, the endotoxin removal efficiency dropped significantly from 96% to 52% when ionic strength increased from 0 to 2 M. When the ionic strength increased from 1.5 to 2.0 M, the E% remained almost constant at 88% for PVI-s and 52% for His-s.

![Fig. 8. Effect of ionic strength on the endotoxin removal efficiency of PVI-s and His-s. Initial LPS concentration: 30 EU/mL; dosage of PVI (or His-s): 40 mg; absorption time: 30 min; temperature: 37°C. Bar: mean ± SD (n = 3).](image)

Table 2
Equilibrium LPS concentration after adsorption by PVI-s and His-s under different initial ET concentrations. Volume of LPS solution: 25 mL; dose of PVI (or His-s): 40 mg; adsorption time: 3 h; temperature: 37°C.

<table>
<thead>
<tr>
<th>C_0 (EU/mL)</th>
<th>10</th>
<th>50</th>
<th>130</th>
<th>300</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_e (EU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVI-s</td>
<td>0.06</td>
<td>0.28</td>
<td>0.63</td>
<td>0.81</td>
<td>0.96</td>
</tr>
<tr>
<td>His-s</td>
<td>0.08</td>
<td>1.46</td>
<td>2.25</td>
<td>2.53</td>
<td>2.85</td>
</tr>
</tbody>
</table>

C_0: the original LPS concentration (EU/mL) in sample solution.
C_e: the LPS concentrations in sample solution after adsorption by affinity absorbent.
The decrease in endotoxin removal efficiency with the increase of ionic strength can be attributed to the decrease of electrostatic interaction between the positively charged functional groups in the sorbents and the phosphorylated lipopolysaccharide of the LPS molecules. The removal efficiencies of PVI-s and His-s at high ionic strengths may be due to the hydrophobic interaction from the long aliphatic chains of PVI and the spacer region of His-s with the LPS lipid region. The hydrophobic aliphatic chains of PVI are longer than the spacer region of His-s at the same grafted degree, so PVI-s has a stronger hydrophobic interaction with LPS and a higher endotoxin removal efficiency compared to His-s. In addition, as the imidazole content of the two adsorbents were almost equal, the density of the PVI ligand was clearly lower, and the cationic imidazole rings were distributed along the backbone chain, which would result in an easier combining of the PVI ligand with LPS.

3.5.4. pH effect

The effect of pH on LPS removal was studied, and the results are shown in Fig. 9. Within the pH range of this study, PVI-s had a higher LPS removal efficiency of above 98% and was less affected by pH values. In contrast, a pH of 5 was the optimum for His-s, and the efficiency decreased under more basic or acidic conditions. These results might be explained as follows. LPS is an amphipathic molecule composed of a hydrophilic polysaccharide chain covalently linked to a hydrophobic lipid moiety (lipid A). Lipid A is the toxic center and partially phosphorylated. The presence of both LPS molecules and the ligands. LPS ionization increases and the positive charge density at the ligands gradually decrease with the increase of pH. These factors simultaneously affect the ionic interaction between LPS and the ligand and lead to the occurrence of a maximum adsorption toward LPS at a particular pH for both adsorbents.

From pH 7–8, PVI-s can still eliminate LPS with a very high efficiency, even though more than 90% of the imidazole groups are in their neutral form and only 10% are in a positively charged imidazolium form according to Bayramoglu et al. [16,22]. This characteristic further illuminates that the adsorption of LPS by PVI-s is induced not only by ionic/electrostatic bonding but also by non-polar synergistic interactions, e.g., hydrophobic interaction, Van der Waals bonding or interactions from complementary effects of structures (e.g., heterocyclic character) [23]. More importantly, the polycationic characteristic of PVI may support the formation of an optimal arrangement of opposite charges decisive for effective LPS adsorption. Generally, PVI can be applied over a wider pH range.

3.5.5. Column-mode LPS removal from BSA–LPS mixture

A prerequisite for decontamination of protein solutions from ET is an efficient and selective binding of endotoxin to the adsorbent without affecting the recovery of the protein. BSA, a slightly acidic protein with a pI of about 4.7 [24], was used as a model protein. The LPS removal ability of PVI-s and His-s in presence of BSA was tested by a dynamic method under a physiological environment, and the results are shown in Fig. 10.

Residual LPS in effluent through PVI-s was always below the regulated safe level (1 EU/mL), while residual LPS in the case of His-s was much higher than this value when the effluent volume exceeded 1000 mL. This result means that PVI-s can process much more LPS–BSA solution to meet the LPS limit. The BSA recovery levels for both adsorbents were above 98% in all cases, which indicated that both PVI-s and His-s had good selectivity for LPS in protein solutions with a low BSA loss.

4. Conclusions

A novel affinity sorbent with PVI ligand on silica gel was prepared for LPS removal. The highest LPS adsorption capacity was attained when the grafted degree of PVI was about 2.5 wt%. Under the same testing conditions, the capacity, specificity and applicability in terms of pH and salt concentrations of PVI-g-SiO2 to adsorb LPS were superior compared to His-g-SiO2. This difference may be a result of the suited nature and good configuration of the PVI ligand to the silica gel. The convenient introduction and easy controllability of PVI makes PVI-g-SiO2 promising in this application.

Acknowledgements

Financial support from the Scientific Research Foundation at Fuzhou University (2010-XQ-17, 022284), the Scientific Major Research Project of Fujian Province in China (2010NZ0001-1), the Scientific Key Research Project of Fujian Province (2010N0015) and
the Natural Science Foundation of Fujian Province (2009J05075) are acknowledged.

References