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Evaluation of two different Concanavalin A affinity adsorbents for the adsorption of glucose oxidase

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Abstract

Concanavalin A (Con A) was selected as ligand and thus immobilized onto two different supports, namely the polymeric Toyopearl and the inorganic silica, with the protection of its binding sites provided during the coupling procedure. The prepared Con A affinity adsorbents were then employed to evaluate their adsorption behaviour for the enzyme glucose oxidase (GOD). The immobilization kinetics showed that the immobilization of Con A on silica supports was much faster than that on Toyopearl supports, which could highly reduce the possibility of the denaturation of Con A. The optimal adsorption conditions for binding of GOD onto the ligand were determined in terms of the pH value and the ionic strength of the adsorption medium. The adsorption isotherms for binding GOD onto two Con A affinity adsorbents fitted well with the Langmuir equation. The maximum adsorption capacity q_m of Toyopearl Con A and silica Con A were 7.9 mg/ml and 4.9 mg/ml, with a dissociation constant K_d of 4.8×10^{-7} M and 2.6×10^{-6} M, respectively. Due to the less diffusive resistance, silica Con A showed both higher adsorption and desorption rates for GOD when compared with Toyopearl Con A. The nonspecific adsorption of GOD was less than 8% for both end-capped Toyopearl and silica supports. The dynamic adsorption of GOD for five times repeated processes showed a high stability for both prepared adsorbents. All the results indicate a good suitability of both Con A adsorbents for affinity adsorption of GOD.

Keywords: Affinity chromatography; Affinity adsorbents; Concanavalin A; Glucose oxidase; Adsorbent optimization

1. Introduction

With the growing expansion of the biotechnology industry, it is becoming increasingly evident that there is still considerable scope for the technological development in the manufacturing of biological products. Due to the high complexity of the processing suspensions, particularity, instability of and the rigorous demand for biological products, downstream processing, a complicated series of isolation and purification steps of the target products, could account for up to 80% of the total manufacturing cost [1]. Hence, efficient and well-designed product recovery methods are very essential in developing a downstream process. In recent years, (bio)affinity chromatography has developed into a powerful tool for the purification, separation and analysis of enzymes, hormones, antibodies, proteins, and other macromolecules [2–4]. Affinity chromatography is based on the unique bio-specificity inherent in a ligand-target molecule interaction. The absolute dependence of affinity interaction on biological recognition rather than physico-chemical properties implies that this technique could preserve the biological and immunological activities of the isolated products [4].

The chromatographic performances of immobilized affinity ligands depend on many factors such as the properties of the matrix, the characteristics of the ligand, and the ligand coupling chemistry [5,6]. Numerous approaches have been taken to immobilize bioactive molecules onto solid matrices including adsorption, covalent coupling, and tethering via an intermediate linker. Studies show that the properties of the surface and the

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Table 1
Physical and chemical properties of employed supports

Supports	Particle size (μm)	Pore size (Å)	Specific surface area (m^2/g)	Spacer	Functional group
Toyopearl	40–90	1000	42	No	Tresyl
Silica	90–130	2500	15–16	No	Aldehyde

methods of immobilization have profound effects on the resulting bioactivity of the immobilized ligands. The hydrophobicity, charge, and chemical properties of the surface material could influence the stability and orientation of the immobilized ligands [7]. The immobilization method could also affect ligand activity through the chemical modifications of its amino acids especially when the coupling sites are close to the active binding sites [8].

Among the versatile bioaffinity ligands, lectins play an important role in affinity separations. Lectins are proteins that interact specifically and reversibly with certain sugar residues. Lectin affinity chromatography is carried out under very mild conditions which highly preserves biological activities of the target molecules. Thus it has been extensively employed for the isolation, fractionation, structure characterization and immobilization of glycoproteins and other biologically important glycoconjugates [3,9,10].

Concanavalin A, Con A, a lectin from *Canavalia ensiformis* (Jack bean), is the most extensively studied lectin for the affinity chromatography due to its ability to form complexes with molecules containing mannosyl or glucosyl residues. In an aqueous medium, Con A can be a monomer, dimmer, or tetramer, depending on the pH value and temperature [11]. Con A is a metallprotein and each Con A subunit contains two metal sites for a successful saccharide binding. One of them is suitable for the binding of a transition metal such as manganese, whereas the other one binds calcium. The crystal structures of Con A with a series of carbohydrates have been extensively studied; the results show that the affinity binding is mainly based on hydrogen bonds and Van der Waals interactions [12,13]. Immobilized Con A is widely employed for the affinity purification and separation of glycoenzymes [14–16]. Glucose oxidase, GOD, is a glycoprotein with a high mannose-type carbohydrate content [17]. GOD is of considerable importance in industrial processes. The most important application of GOD is its use as a molecular diagnostic tool such as the quantitative determination of glucose in body fluids, foodstuffs, beverages, fermentation products or other samples [18].

In this study, two kinds of Concanavalin A affinity adsorbents with different chemical and physical properties were evaluated for the adsorption of glucose oxidase. Con A was first immobilized onto two activated matrices, the polymeric Toyopearl and inorganic silica supports, to produce Con A affinity adsorbents. The immobilization kinetics were compared between these two supports. The optimal GOD adsorption conditions were investigated in terms of the pH value and the ionic strength of the adsorption buffer. The evaluation of Con A adsorbents for the adsorption of GOD includes the investigations of the adsorption isotherms, adsorption and desorption kinetics, nonspecific adsorption properties (which is an indirect measure of the power for employment of the adsorbents in sorptive separation processes), and static and dynamic adsorption of GOD onto the different Con A adsorbents.

2. Materials and methods

2.1. Materials

The polymeric support Toyopearl AF-Tresyl-650M was bought from Tosoh Bioscience (Stuttgart, Germany). The inorganic silica support activated with glutaraldehyde on the basis of Davisil® XWP2500Å 90–130 was a kind gift provided by Grace (Worms, Germany). The physical and chemical properties of these two supports are listed in Table 1 according to the data provided by the suppliers. Con A (type V), methyl-α-D-mannopyranoside, and Bradford reagent were purchased from Sigma (Munich, Germany). Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4) was obtained from Serva (Heidelberg, Germany). All other chemicals were of analytical reagent grade unless otherwise stated.

2.2. Preparation of Concanavalin A affinity adsorbents

Both Toyopearl and silica supports were first equilibrated in the coupling buffer (0.5 M phosphate buffer, containing 0.1 M NaCl, pH 8.0) for at least 10 min. Approximate 6 ml (settled volume) of each vacuum evacuated supports were then mixed with 4 mg/ml Con A with a volumetric ratio of 1:3 in the coupling buffer, which contained a 50-fold molar excess of methyl-α-D-mannopyranoside for the protection of the binding sites of Con A during the immobilization. The reaction was carried out for 4 h at room temperature with gentle shaking. To determine the immobilization kinetics the sample aliquots were taken from the reaction mixture at certain time intervals. After the immobilization the reacted supports were filtrated and extensively washed with the coupling buffer and then water. The filtrate together with the washing fraction was collected for the determination of the protein concentration by the Bradford method [19].

The residual functional groups on the supports were blocked with 18 ml 0.5 M Tris-HCl buffer, containing 0.1 M NaCl, pH 8.0, for 2 h at room temperature. After the blocking reaction, Toyopearl Con A adsorbents were thoroughly washed with 0.1 M acetate buffer, which contains 0.1 M NaCl and 1 mM Ca^{2+}, Mn^{2+}, and Mg^{2+}, pH 5.0, and then stored at 4 °C in the same buffer for later use. For silica Con A adsorbents, additional 20 mg $NaCNBH_3$ was introduced to the reaction mixture and allowed to react for another 1 h to eliminate the unstable C=N Schiff bases formed during the immobilization and blocking reactions. The following washing and storage steps to silica Con A adsorbents were carried out in the same way as for Toyopearl Con A.

2.3. Calculation of the coupling yield and ligand density

The coupling yield and ligand density were employed to characterize the efficiency of the Con A immobilization. The coupling yield y is defined as:

$$y = \frac{m_i}{m_0} \times 100\% \quad (1)$$

where m_i is the amount of Con A immobilized onto the supports (mg); m_0 is the total amount of Con A added for the immobilization (mg); both values were derived from the Bradford analyses.

The volumetric ligand density d_l is calculated according to Eq. (2):

$$d_l = \frac{m_i}{V_{ads}} \quad (2)$$

where V_{ads} is the volume of the Con A affinity adsorbents (ml).

2.4. Calculation of the surface coverage

The surface coverage is an approximate value and is just taken as a reference parameter to compare the immobilization performance combined with the support characteristics. It was calculated by assuming the average molecular dimension for a Con A tetramer as 124 Å × 129 Å × 67 Å, which was obtained according to the 3D crystal structure of Con A by X-ray diffraction [20]. Because the orientations of the immobilized Con A are unknown, the surface area covered by one protein molecule is calculated using the smallest and largest values of the protein dimensions generating a rectangle at the surface of the affinity adsorbents. Consequently, a covered surface area of 0.51 m^2 can be calculated for 1 mg Con A tetramer.

2.5. Batch experiments of glucose oxidase adsorption onto Concanavalin A affinity adsorbents

The adsorption of GOD onto Con A adsorbents was carried out by batch experiments. Some factors such as the pH values and the ionic strength of the adsorption buffer that affect the adsorption process were first studied. A 0.1 M acetate buffer was prepared for pH 5.0 and pH 6.0, while a 0.1 M Tris-HCl buffer was employed for pH 7.0. The effect of the ionic strength was analyzed by changing NaCl concentrations in the range of 0–0.5 M. All the adsorption buffers contained 1 mM Ca^{2+}, Mn^{2+}, and Mg^{2+} in order to maintain the activity and stability of Con A. All the experiments were performed at room temperature unless otherwise stated.

In the standard adsorption experiment, about 0.2 ml vacuum evacuated Con A adsorbents were equilibrated with 1 ml adsorption buffer for 2 h and then mixed with 4 ml GOD solution to obtain the final concentration of GOD in the range of 0.1 mg/ml to 2 mg/ml in the relevant adsorption buffer. All the measurements were carried out in a shaking bath at 120 rpm for 15 h. The amount of the adsorbed GOD onto Con A affinity adsorbents was determined from the difference of the initial and final concentration of GOD in the liquid phase of the adsorption buffer, which is shown in Eq. (3).

$$q^* = \frac{(c_0 - c^*)V_{GOD}}{V_{Ads}} \quad (3)$$

where q^* is the equilibrium adsorption capacity of the affinity adsorbent for GOD (mg/ml); c_0 and c^* are the initial and equilibrium concentration of GOD in the aqueous phase of the mixture, respectively (mg/ml); V_{GOD} is the total volume of the GOD aqueous solution (ml); and V_{Ads} is the volume of the Con A adsorbents (ml).

Under the optimal adsorption conditions the adsorption isotherms of GOD onto different Con A adsorbents were studied and the results were fitted with different models. As an example the Langmuir model equation is presented in Eq. (4).

$$q^* = \frac{q_m c^*}{K_d + c^*} \quad (4)$$

where q_m is the maximum adsorption capacity (mg/ml) and K_d the dissociation constant (M), which are the characterizing model parameters.

The adsorption kinetic studies were carried out by mixing 1 ml Con A adsorbents with 30 ml 0.5 mg/ml GOD solution. After the adsorption, the saturated Con A adsorbents were vacuum evacuated and carefully washed with the adsorption buffer and then mixed with 20 ml 0.1 M methyl-α-D-mannopyranoside to investigate the desorption kinetics. The sample aliquots in the adsorption and desorption solutions were taken out at certain time intervals to determine GOD concentration at a wavelength $\lambda = 280$ nm in a UV spectrophotometer (Carl Zeiss, Jena, Germany).

2.6. Determination of the nonspecific adsorption of glucose oxidase onto end-capped supports

To elucidate the specific and nonspecific interactions in affinity separation, as the basis of affinity chromatography the specific interaction between the immobilized ligand and the target molecule has to be isolated. So, in principle any other non-covalent interactions between any molecule except of the target particle in solution and any part of the derivatized affinity support should be minimized. For example nonspecific hydrophobic adsorption results from interactions between nonpolar side chains of proteins and the support material, the spacer arms between support and ligand or from the ligand itself. Ionic nonspecific interactions can arise from the matrix, the spacer arm, the ligand or the coupling agent with the protein to be adsorbed off the active binding area [21]. Usually the nonspecific adsorption is regarded as the nonspecific adsorption of the supports, which is well accepted by the researchers [6,22]. Therefore, it is reasonable to measure the nonspecific adsorption by blocking the support's surface with small molecules.

The silica and Toyopearl supports each about 1 ml were reacted with 30 ml 0.5 M Tris-HCl buffer, pH 8.0, for 8 h to eliminate all the reactive functional groups. The carefully washed end-capped supports were then mixed with 10 ml 0.5 mg/ml GOD solution in a 15 ml tube for 15 h. The supernatant was withdrawn from the tube to determine the protein concentration

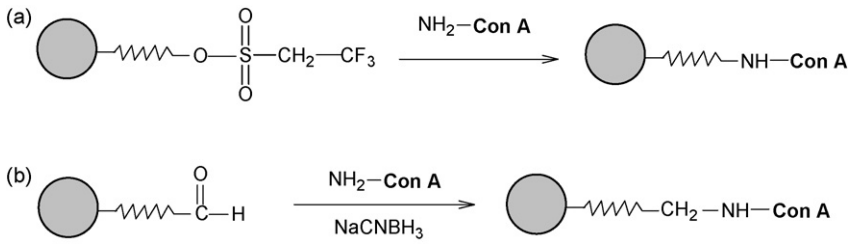

Fig. 1. Schemes of immobilization reactions on (a) Toyopearl supports and (b) silica supports.

by UV absorption at $\lambda = 280$ nm. The adsorbed GOD was calculated by the mass balance derived from a calibration curve coupling the spectrometric absorption with protein masses. The same procedure was carried out with silica Con A and Toyopearl Con A affinity adsorbents to evaluate the degree of the nonspecific adsorption of GOD in relation to the total adsorption capacity of Con A adsorbents. Here, in order to distinguish from the nonspecific adsorption, the adsorption capacity for binding GOD onto affinity adsorbents was defined as total adsorption capacity. Since the adsorption capacity was obtained according to the GOD concentration before and after the adsorption experiment, the total adsorption capacity of the affinity adsorbents actually were mostly composed of specific adsorption and also nonspecific adsorption.

2.7. Dynamic adsorption of glucose oxidase

The dynamic adsorption of GOD was performed by a self-assembled system containing a peristaltic pump (Bio-Rad, Munich, Germany) and a UV detector connected with a laptop computer. The UV signal was recorded by the software VirtualBench (National Instruments, Munich, Germany). After equilibration of the column with 0.1 M acetate buffer, containing 0.1 M NaCl, 1 mM Ca^{2+}, Mg^{2+}, and Mn^{2+}, pH 5.0 (adsorption buffer), 200 μl 5 mg/ml GOD was applied to 1.5 ml Con A adsorbents packed in a column with 8 mm inner diameter. The same buffer was employed to wash the column until the UV signal reached the baseline. The bound GOD was then recovered from the column with 0.1 M methyl-α-D-mannopyranoside in the adsorption buffer. Finally the column was regenerated with the adsorption buffer for the further use. The flow rate in all affinity separations was kept at 0.8 ml/min. The desorbed GOD was collected to determine the protein concentration by the Bradford method [19].

3. Results and discussion

3.1. Immobilization of Concanavalin A

The performance of the affinity adsorbents depends on many factors, such as the preparation method, the properties of the matrix and the ligand, and the orientation of the immobilized ligand. The selection of biological molecules as affinity ligands has to consider the immobilization method. It is particularly important because the activities of the ligands can be affected by the immobilization procedure as well as the support itself. In this work, silica supports with aldehyde functional groups and Toyopearl with Tresyl functional groups were selected to immobilize Con A. The schemes of immobilization reactions onto both supports are shown in Fig. 1.

To obtain an optimal estimate of the time for the completion of the ligand immobilization, the immobilization kinetics of Con A was measured for these two supports. As shown in Fig. 2, the immobilization of Con A onto silica supports already reached the equilibrium in 1 h with the final coupling yield of 72%. However, the immobilization onto Toyopearl supports was not completed even after 4 h with the coupling yield of 75%. It only finished in 15 h with a final coupling yield of 80% (data not shown in the graph). The difference of the immobilization kinetics for these two supports are due to the differences in the chemical properties of the activated supports and most probably due to the increased diffusive resistance of Con A into the pores of the Toyopearl support which provides a mean pore size of 1000 Å compared to 2500 Å for silica support [23]. The shorter the reaction time, the less likely the denaturation of the ligand becomes. From this point of view, the immobilization of Con A on silica supports is preferential for maintaining the activity of Con A, and generally for sensitive ligands.

3.2. Investigation of the adsorption conditions for glucose oxidase

The interaction involved in affinity separation is a combination of electrostatic, hydrophobic, hydrogen binding and Van

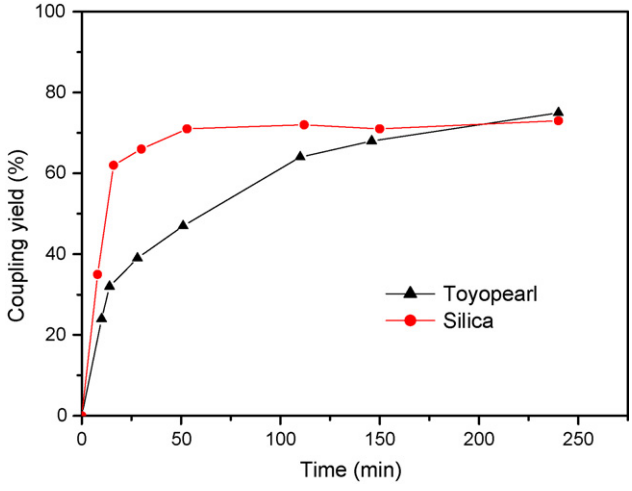

Fig. 2. Immobilization kinetics of Con A onto two different supports. Coupling buffer: 0.5 M phosphate buffer, 0.1 M NaCl, pH 8.0; Con A concentration: 4 mg/ml. Immobilization was carried out at room temperature.

der Waals interactions [24–26]. The pH value and ionic strength of the adsorption medium may have significant influence on the interaction between the affinity ligand and the adsorbate [14]. Therefore, it is necessary to study the effects of these factors on the adsorption capacity in order to obtain optimal adsorption conditions.

3.2.1. Effect of pH on the adsorption of glucose oxidase

The adsorption experiments for binding GOD onto Toyopearl Con A and silica Con A were carried out in buffers of various pH values, where both Con A and GOD show good stability and activity. These buffers were prepared without additional NaCl to adjust the ionic strength. The effect of the pH value on the adsorption capacity for GOD is presented in Fig. 3. Both Con A adsorbents show the similar trend with respect to the adsorption capacity in adsorption buffers with different pH values. The adsorption capacity decreased remarkably with increasing the pH value from 5.0 to 7.0. For both Con A adsorbents, the maximum adsorption capacity was obtained at pH 5.0, where GOD shows its notably high activity [27]. Therefore, pH 5.0 was selected as the appropriate value for the further GOD adsorption experiments unless otherwise stated. Here the enzyme provides a satisfactory stability.

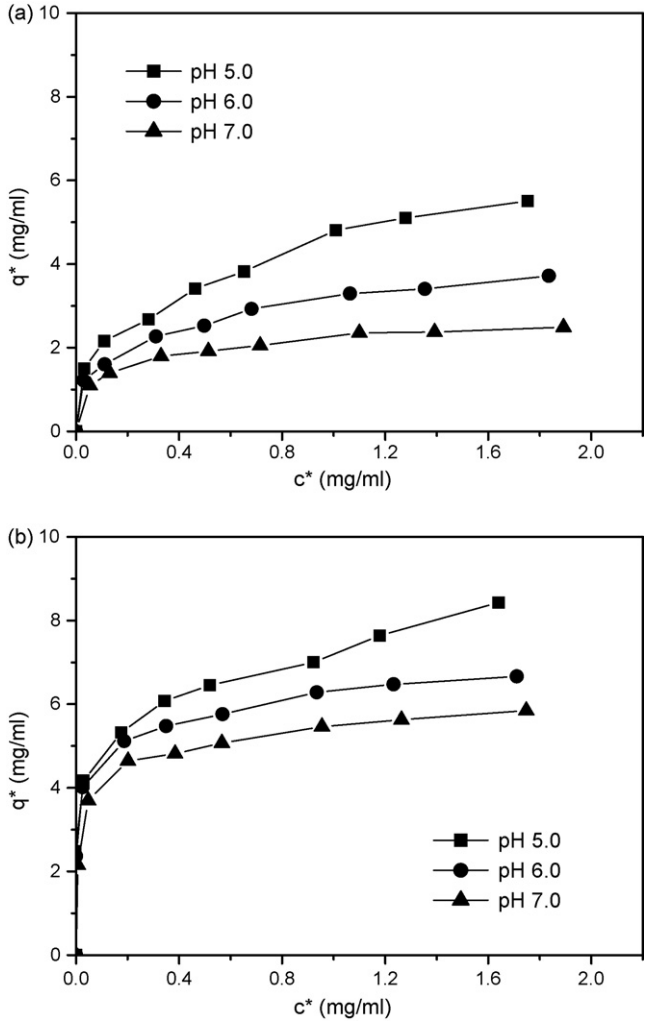

Fig. 3. Effect of pH on the adsorption capacity for binding GOD onto different Con A adsorbents. (a) Silica Con A with a ligand density of 9.2 mg/ml; (b) Toyopearl Con A with a ligand density of 8.8 mg/ml.

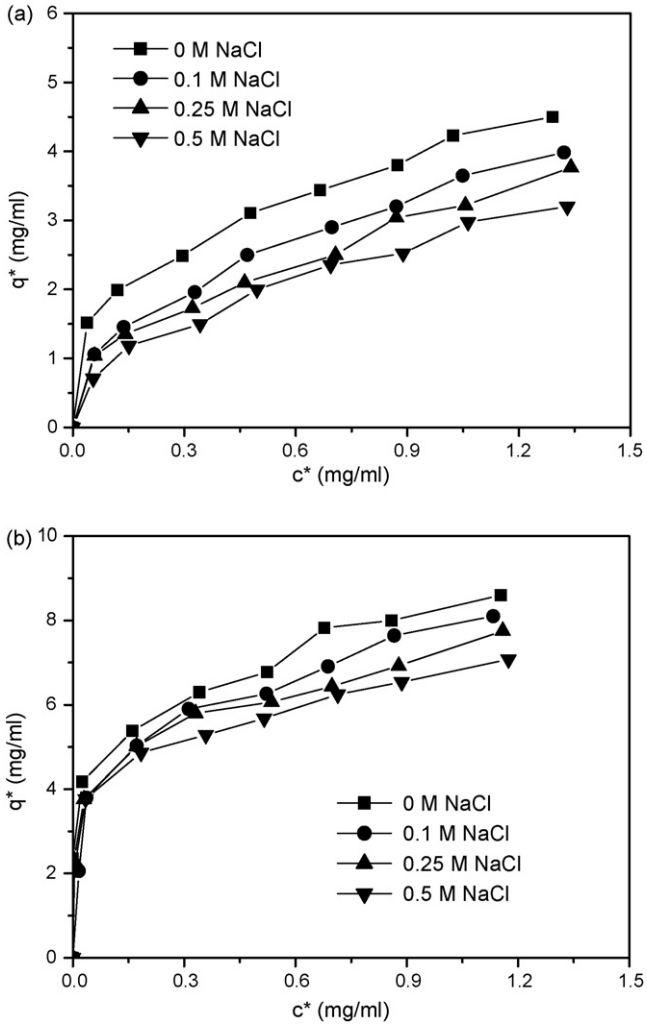

Fig. 4. Effect of ionic strength on the adsorption capacity for binding GOD onto different Con A adsorbents. (a) Silica Con A with a ligand density of 9.2 mg/ml; (b) Toyopearl Con A with a ligand density of 8.8 mg/ml.

3.2.2. Effect of ionic strength on the adsorption of glucose oxidase

The affinity interaction between lectin-carbohydrate is mainly based on hydrogen bonds and Van der Waals interactions [13], which may be influenced by the ionic strength. The investigation of the effect of the ionic strength on the adsorption of GOD was performed in 0.1 M acetate buffer, pH 5.0, by introducing NaCl with its concentration varying between 0 and 0.5 M. The results are given in Fig. 4. The adsorption capacity for binding GOD onto both Con A adsorbents reached the maximum in the absence of NaCl. The slight increase of the ionic strength of the adsorption buffer induces the decrease of the adsorption capacity for binding GOD. For example the adsorption capacities in 0.5 M NaCl solution were 40% and 15% of that in the NaCl-free solution in the lower loading area (c^* ~0.4 mg/ml) for silica Con A, and Toyopearl Con A,

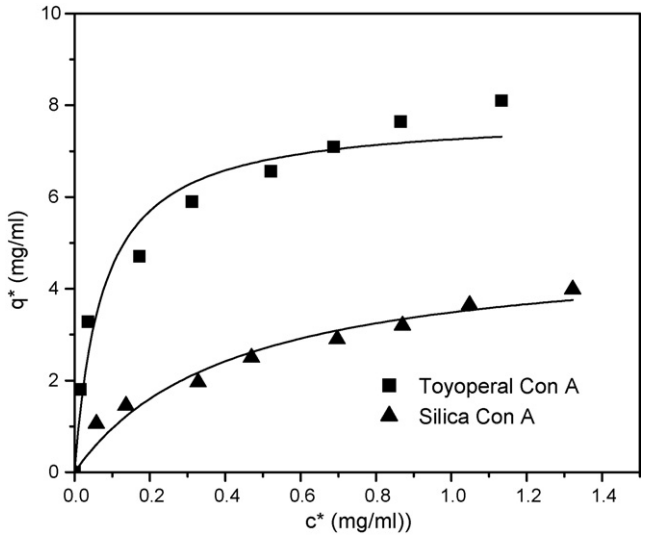

Fig. 5. Adsorption isotherms for binding GOD onto Toyopearl Con A and silica Con A. The adsorbents employed are the same as described in Fig. 4. Solid lines were fitted by the Langmuir equation.

respectively. This indicates stronger ionic forces involved in the formation of the ligand–glycoprotein (GOD) complex. Increasing of the ionic strength of the medium could reduce the static electronic interactions, which finally also may contribute to the nonspecific adsorption in multi component mixtures [28]. To minimize the nonspecific affinity adsorptions, moderate salt concentration was required [21,29]. Since relatively high adsorption capacity could still be obtained in 0.1 M acetate buffer, which contains 0.1 M NaCl, pH 5.0, this buffer was chosen for the further GOD adsorption experiments.

3.3. Adsorption isotherms

The adsorption isotherms obtained from the adsorption of GOD onto different Con A adsorbents under optimized adsorption conditions are shown in Fig. 5. The Langmuir model is most commonly used to describe the adsorption behaviour of affinity adsorbents. It mainly assumes that the adsorption is a monolayer adsorption and the binding sites are homogeneously distributed on the adsorbent surface. In the case of GOD adsorption onto Con A adsorbents, the experimental data fitted well with the Langmuir equation in the studied concentration range. The determined thermodynamic parameters of this model from the experiments are listed in Table 2. It can be observed that the adsorption behaviour varies significantly between these two affinity adsorbents. The theoretical maximum adsorption capacity q_m of Toyopearl Con A and silica Con A for binding GOD were 7.9 mg/ml and 4.9 mg/ml, with dissociation constants of 4.8×10^{-7} M and 2.6×10^{-6} M,

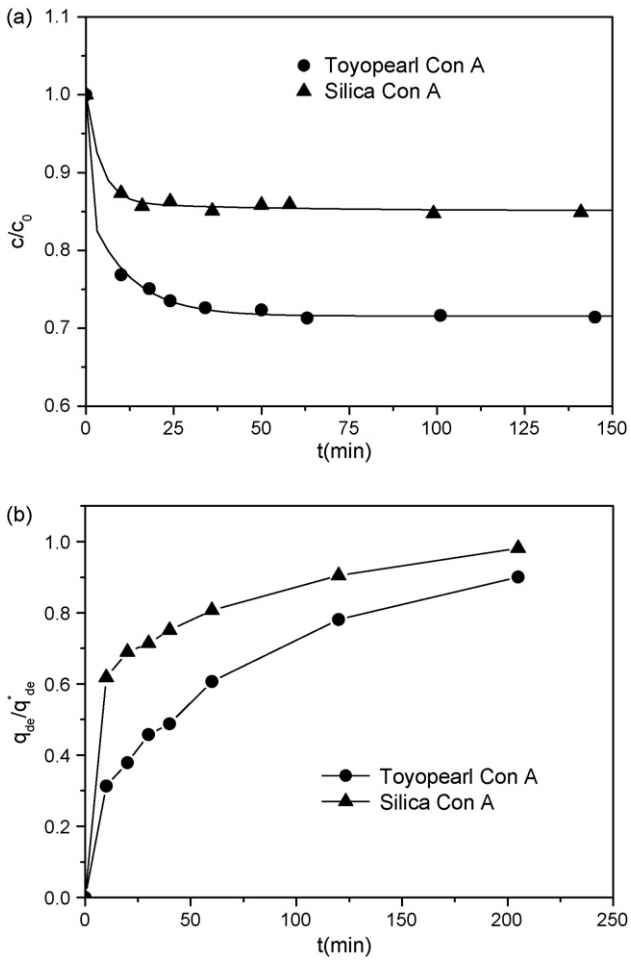

Fig. 6. Adsorption (a) and desorption (b) kinetics of GOD onto and from different Con A adsorbents. The ligand density was 9.8 mg/ml for silica Con A and 9.4 mg/ml for Toyopearl Con A.

respectively. The dissociation constants between immobilized Con A and GOD investigated in this study are almost in the same magnitude as previously reported [5,6]. The difference in the adsorption behaviour between these two adsorbents may be highly correlated with the immobilization chemistry and the physical properties of the matrix. Affinity adsorbents should have a dissociation constant below 10^{-5} M in order to avoid the ligate elution during the washing step [10,22,30]. Our results show that both Con A adsorbents were highly specific to GOD and therefore suitable for affinity separations. It is also worthwhile to point out that the deviation of the experimental data from the Langmuir isotherm become larger for the higher GOD concentration range, especially for Toyopearl Con A adsorbents. This may be caused by the multilayer adsorption resulting from GOD–GOD interactions; similar enzyme–enzyme interaction

Table 2
Parameters calculated from the Langmuir equation for different affinity adsorbents

Affinity adsorbents	q_m (mg/ml)	K_d (mg/ml)	K_d (mol/l)	R^2
Toyopearl Con A	7.9 ± 0.4	0.076 ± 0.021	$(4.6 \pm 1.3) \times 10^{-7}$	0.964
Silica Con A	4.9 ± 0.5	0.416 ± 0.111	$(2.6 \pm 0.7) \times 10^{-6}$	0.967

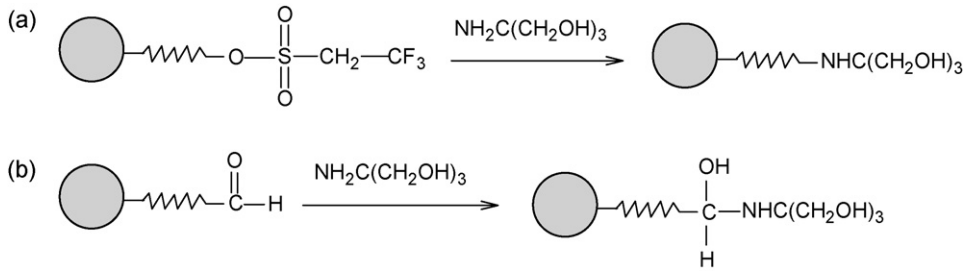

Fig. 7. Schemes of end-capping reactions on (a) Toyopearl supports and (b) silica supports.

was observed in previous investigations [31]. Castilho described the same phenomenon during the adsorption of IgG to affinity Protein A membranes [30].

3.4. Adsorption and desorption kinetics

The adsorption kinetics for the binding of GOD onto Con A adsorbents is presented in Fig. 6(a). The curves indicate that the adsorption proceeds very fast to silica Con A; the c/c_0 reached a constant value after about 15 min. The adsorption of GOD to Toyopearl Con A developed also very fast in the first 20 min, then the velocity decreased till a constant value of c/c_0 was reached after 60 min. The saturation time for the adsorption of GOD onto two different Con A adsorbents vary largely because of the different diffusion behaviours of GOD into the pores of the Con A adsorbents, strongly dependent on their pore sizes. In other words, the larger pore size of silica Con A significantly reduced the diffusive mass transfer resistance, as expected.

The Con A adsorbents with bound GOD were thoroughly washed to remove the GOD nonspecifically adsorbed onto the surface, diffused in the pore of the adsorbents, and staying unadsorbed, respectively. Then 20 ml 0.1 M methyl-α-D-mannopyranoside was mixed to the GOD–Con A adsorbents complex for the desorption of GOD. The sample aliquots were withdrawn from the supernatant at time intervals to investigate the desorption kinetics. The results are shown in Fig. 6(b). The desorption capacity of the saturated Con A adsorbents after 10 h's incubation in the elution solution was taken as the equilibrium desorption capacity, denoted as q^*_{de}. The desorption capacity at time intervals is denoted as q_{de}. In the first 10 min, the desorption capacity q_{de} reached 31% of its q^*_{de} for Toyopearl Con A, while 62% for silica Con A. As the desorption time increased, the difference in the desorption rates as well as the loading heights between the two Con A adsorbents gradually reduced. For example, after 200 min the q_{de}/q^*_{de} value was 90% for Toyopearl Con A, and 98% for silica Con A, respectively. In conclusion, both the adsorption and desorption rate of GOD onto and from silica Con A were much faster than that of Toyopearl Con A. Silica Con A adsorbents are expected to have higher potential to be used to purify unstable glycoproteins since short processing time could reduce the possibility of protein denaturation. The higher kinetic rates accelerate the dynamic process and thus increase economic efficiency, a prerequisite for industrial downstream applications.

3.5. Nonspecific adsorption of glucose oxidase

Nonspecific adsorption normally reduces the purity of the target compound in bioseparation processes, and increases the number of processes to be applied for high product purity, respectively, which finally results in higher capital and consequently product costs. Thus it is very important to investigate the nonspecific adsorption properties of employed supports. As described in the experimental section, the nonspecific adsorption of GOD onto end-capped adsorbents (without Con A ligand) was determined and compared with the total adsorption of GOD onto Con A affinity adsorbents (as applied in the adsorption process). The schemes of the end-capping reactions are shown in Fig. 7 and the adsorption results in Table 3. By static adsorption of 5 mg GOD (10 ml of a 0.5 mg/ml GOD solution) 0.17 mg GOD adsorbed nonspecifically onto 1 ml silica supports without the ligand Con A, while at 1 ml unmodified Toyopearl supports 0.35 mg GOD were bound nonspecifically. The adsorption capacity for the binding of GOD increased significantly after the immobilization of Con A onto the supports: 2.18 mg/ml for silica Con A, and 4.88 mg/ml for Toyopearl Con A, respectively. The degree of the nonspecific adsorption capacity of the end-capped adsorbents to the total adsorption capacity of Con A adsorbents was less than 8% for both adsorbents indicating that both synthesized Con A adsorbents are well suitable for affinity separations.

3.6. Static and dynamic processes of glucose oxidase

Following the reaction conditions (column 2-4) as shown in Table 4, the immobilization of Con A resulted in a slightly

Table 3
Unspecific adsorption of GOD onto end-capped adsorbents and total adsorption of GOD onto Con A affinity adsorbents

Supports	Immobilized Con A density (mg/ml)	Unspecific adsorption capacity of GOD (mg/ml)	Total adsorption capacity of GOD (mg/ml)	Percentage of unspecific to total adsorption capacity (%)
Silica	11.7	0.17 ± 0.01	2.18 ± 0.14	7.7 ± 0.4
Toyopearl	11.8	0.35 ± 0.02	4.88 ± 0.04	7.1 ± 0.2

Table 4
Comparison of immobilized Con A affinity adsorbents

Affinity adsorbents	Supports employed (ml)	Con A employed (mg)	Coupling time (h)	Con A density (mg/ml)	Con A density (mg/g)	Con A surface coverage (%)	GOD adsorption capacity[a] (mg/ml)
Silica Con A	5	60	1	9.8 ± 0.1	25.9 ± 0.3	86 ± 1	4.8 ± 0.3
Toyopearl Con A	6	72	4	9.4 ± 0.2	37.1 ± 0.6	44 ± 1	7.0 ± 0.5

[a] Measured in 5 ml 2 mg/ml GOD solution (0.1 M acetate buffer, 0.1 M NaCl, pH 5.0) for 0.2 ml Con A adsorbents at room temperature for 20 h.

higher volumetric ligand density on silica (9.8 mg/ml) than that on Toyopearl (9.4 mg/ml). The static adsorption of GOD provides an adsorption capacity for the binding of GOD onto Toyopearl Con A of 7.0 mg/ml, and of silica Con A of 4.8 mg/ml. This could be strongly related with the ligand surface coverage on the adsorbents. A higher surface coverage of silica Con A (86%) induces a compact Con A ligand density on the silica surface. This may reduce the ligand accessibility and thus the adsorption capacity. On the other hand, the surface coverage of Toyopearl Con A (44%) seems also to be satisfactory in preventing the steric hindrance during the adsorption of GOD (Table 4). It should be pointed out that an appropriate ligand surface coverage must be taken into account during the adsorption of macromolecules in order to get a high adsorption capacity and thus a high utilization efficiency of affinity ligands [32].

Each Con A adsorbents (1.5 ml) with a similar ligand density was packed into columns to determine the dynamic adsorption capacity. For each run 200 μl of 5 mg/ml GOD was applied to the column and 0.1 M methyl-α-D-mannopyranoside was employed to desorb the bound GOD. Fig. 8 shows the adsorption capacity for binding GOD onto both Con A adsorbents for five repeated separations. The difference in the dynamic adsorption capacity between the two Con A adsorbents was much less than that of the static adsorption capacity as shown in Fig. 4 and Table 4. The mean dynamic adsorption capacity for silica Con A and Toyopearl Con A was 0.35 mg/ml and 0.42 mg/ml, respectively. The dynamic adsorption capacity was much smaller than the static one; for silica Con A it was calculated as 7.3% of the static adsorption capacity, and for Toyopearl Con A as 6.0%.

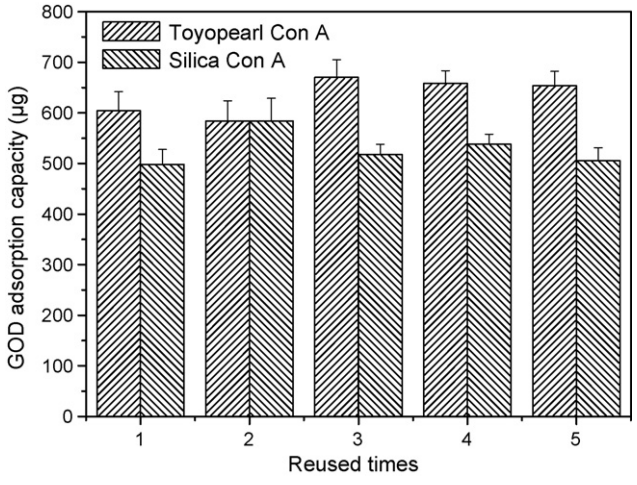

Fig. 8. Dynamic adsorption capacity for binding GOD onto Con A adsorbents. Ligand density of Con A adsorbents are the same as described in Table 3.

As suggested by Avramescu et al., the axial and radial diffusion, the sorption kinetics and the non-uniform porosity of the adsorbents can affect the dynamic adsorption performance of affinity adsorbents significantly. The full adsorption capacity can only be achieved when the mass transfer may be accelerated, and also the flow rate is slow enough to allow most adsorbate molecules to diffuse to and bind onto the active binding sites [33].

Fig. 8 also shows that both Con A adsorbents highly maintained their adsorption capacity for binding GOD after five times processes. This indicates that the separation procedure was successful and also proves the stability of the immobilized Con A adsorbents, confirming former results [34].

4. Conclusions

In this study, we evaluated two different supports for the immobilization of a bioligand and the affinity adsorption of the glycoprotein enzyme glucose oxidase. Con A, a plant lectin, was immobilized as the bioligand onto inorganic Davisil® silica and polymeric Toyopearl supports. The immobilization kinetics of Con A was investigated for these two supports. The results showed that silica supports activated with glutaraldehyde exhibited a much faster immobilization rate and a similar volumetric ligand density when compared with Toyopearl supports. The immobilized Con A adsorbents were then employed for the adsorption of GOD. The optimal adsorption conditions were determined in terms of the pH value and the ionic strength of the adsorption medium, wherein the adsorption mechanism was preliminarily discussed. The adsorption isotherms proved that Toyopearl Con A possessed much higher static adsorption capacity when compared with silica Con A. However, because of the less diffusive mass transfer resistance, silica Con A showed faster adsorption and desorption kinetics. Both Con A adsorbents exhibited:

(1) high affinity to GOD with a K_d value of 4.8×10^{-7} M for Toyopearl Con A and 2.6×10^{-6} M for silica Con A, respectively;
(2) low nonspecific adsorption capacity of GOD with 0.35 mg/ml and 0.17 mg/ml for Toyopearl and silica adsorbents, respectively.

The dynamic adsorption of GOD revealed similar values for both Con A adsorbents and also a high stability for them. Furthermore the results indicated their good suitability for the application of affinity separations.

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