Adsorption of IgG on Spacer-Arm and L-Arginine Ligand Attached Poly(GMA/MMA/EGDMA) Beads

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ABSTRACT: This work presents data on human immunoglobulin G (HIgG) adsorption onto L-arginine ligand attached poly(GMA/MMA/EGDMA)-based affinity beads which were synthesized from methyl methacrylate (MMA) and glycidiyl methacrylate (GMA) in the presence of a crosslinker (i.e., ethylene glycol dimethacrylate; EGDMA) by suspension polymerization. The epoxy groups of the poly(GMA/MMA/EGDMA) beads were converted into amino groups after reaction with ammonia or 1,6-diaminohexane (i.e., spacer-arm). With L-arginine as a ligand, it was covalently immobilized on the aminated (poly(GMA/MMA/EGDMA)-AA) and/or the spacer-arm attached (poly(GMA/MMA/EGDMA)-SA) beads, using glutaric dialdehyde as a coupling agent. Both affinity poly(GMA/MMA/EGDMA)-based beads were used in HIgG adsorption/desorption studies under defined pH, ionic strength, or temperature conditions in a batch reactor, using acid-treated poly(GMA/MMA/EGDMA) beads as a control system. The poly(GMA/MMA/EGDMA)-SA affinity beads resulted in an increase in the adsorption capacity to HIgG compared with the aminated counterpart (i.e., poly(GMA/MMA/EGDMA)-AA). The maximum adsorption capacities of the poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads were found to be 112.36 and 142 mg g^{-1}, and the affinity constants (K_d), evaluated by the Langmuir model, were 2.48 $\times 10^{-7}$ and 6.98 $\times 10^{-7}$ *M*, respectively. Adsorption capacities of the poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA were decreased with HIgG by increasing the ionic strength adjusted with NaCl. Adsorption kinetic of HIgG onto both affinity adsorbents was analyzed with first- and second-order kinetic equations. The first-order equation fitted well with the experimental data. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 672–679, 2007

Key words: beads; polymerization; affinity chromatography; amino acid; IgG; thermodynamics

INTRODUCTION

Affinity separations for protein purification have been developed extensively because they offer rapid isolations of proteins.[1–4] Since affinity chromatography exploits selective interactions between a ligand and a target biomolecule, it can therefore be used to simplify purification schemes, and hence one-step purification procedures are commonly employed.[5–7] The magnitude of the interaction of a protein with a chemically or biologically defined affinity surface is governed by the system free energy before and after the interaction and can be attributed to the changes in the corresponding enthalpy and entropy of the system. Most biospecific ligands in affinity chromatography, such as proteins and enzymes, are expensive and unstable in chromatographic systems. In order to overcome these problems pseudospecific liquids can be used (such as amino acids, polymer with pendant amine groups, metal chelates and textile dyes) instead of biospecific ligands.[8,9] Amino acids have been reported to be fairly selective and efficient immobilized ligands for the purification of a variety of proteins. Among them, L-arjinine is a basic amino acid, and it has been known to have interesting bioactivity, such as antiplatelet activity, angiogenesis, wound healing promotion, nerve regeneration, etc.[10] L-Arjinine could be used as an affinity ligand for adsorption and separation of protein.

In the previous studies, different types of affinity ligand molecules, such as triazidine dyes,[11,12] immobilized metal ions,[5,13] and L-histidine amino acid,[14,15] have been used for adsorption and purification of proteins. In the present work, epoxy group containing poly(GMA/MMA/EGDMA) beads were prepared by suspension polymerization. The epoxy groups of the beads were modified into amino group using ammonia or 1,6-diaminohexane or both as a spacer-arm. L-Arginine was then covalently immobilized after activation of the aminated or spacer-arm attached beads, with glutaric dialdehyde as coupling agent. The effect of spacer-arm on the performance of L-arginine ligand was tested using a model protein HIgG from solution. The adsorption parameters, such as pH, temperature, initial HIgG concentration,

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and ionic strength, were investigated in a batch system for both affinity beads. Finally, the adsorption kinetic and isotherm of HIgG on the affinity beads were studied, and thermodynamic parameters were also evaluated.

MATERIALS AND METHODS

Materials

Human immunoglobulin G (HIgG) was supplied from the Sigma Chemical Co. (St Louis, MO) and used as received. Methyl methacrylate (MMA), glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA), and α-α′-azobisisobutyronitrile (AIBN) were obtained from Fluka AG (Switzerland). The monomers were distilled under reduced pressure in the presence of hydroquinone and stored at 4°C until use. 1,6-Diaminohexane, polyvinyl alcohol, and toluene were obtained from Sigma. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany).

Preparation of poly(GMA/MMA/EGDMA) beads

The poly(GMA/MMA/EGDMA) beads were prepared by suspension polymerization. The polymerization was carried out in an aqueous dispersion medium containing NaCl (0.2*M*, 400 cm^3), which was used to decrease the solubility of the monomers in the medium. The organic phase contained MMA (7.5 cm^3), GMA (5.0 cm^3), EGDMA (7.5 cm^3; crosslinker), and 5.0% polyvinyl (20 cm^3), as stabilizer, were mixed together with 0.2 g of AIBN as initiator in 20 cm^3 of toluene. The polymerization reactor was placed in a water bath and heated to 65°C. The reactor was then equipped with a mechanical stirrer, nitrogen inlet, and reflux condenser. The polymerization reaction was maintained at 70°C for 2.0 h and then at 80°C for 1.0 h. After the reaction, the resultant beads were filtered under suction and washed with distilled water and ethanol. The beads were sieved, and 75–150 μm size of fraction was used for further reactions.

Surface modification of beads

The epoxy groups carrying poly(GMA/MMA/EGDMA) beads were aminated with 0.5*M* ammonia or 1,6-diaminohexane solution (i.e., spacer-arm) at pH 10.0 and at 65°C in a reactor containing 10 g beads, and were stirred magnetically for 5 h. After the reaction, the aminated and/or spacer-arm attached poly(GMA/MMA/EGDMA) beads were washed with distilled water. The aminated (poly(GMA/MMA/EGDMA)-A) and spacer-arm attached (p(GMA-MMA)-S) beads (10 g) were equilibrated in phosphate buffer (20 cm^3, 50 m*M*, pH 7.0) for 6 h, and transferred to the same fresh medium containing glutaric dialdehyde (20 mL, 0.5% v/v). The activation reaction was carried out at 25°C for 12 h while continuously stirring the medium. After the reaction period, the excess glutaric dialdehyde was removed by washing sequentially with distilled water, acetic acid solution (100 m*M*, 100 cm^3), and phosphate buffer (100 m*M*, pH 7.0). Immobilization of L-arginine on the glutaric dialdhyde activated beads was carried out at 22°C in a shaking water bath for 8.0 h. Physically bound L-arginine was removed first by washing the supports with saline solution (40 cm^3, 1.0*M*) and then phosphate buffer (0.1*M*, pH 7.0).

Analysis and measurement

Scanning electron micrographs (SEMs) of the dried poly(GMA/MMA/EGDMA) beads were obtained using a JEOL, JMS 5600 SEM, after coating with gold under reduced pressure. The FTIR spectra of the affinity beads were obtained using an FTIR spectrophotometer (Shimadzu, FTIR 8000 Series, Japan). The average size and size distribution of the poly(GMA/MMA/EGDMA) beads were determined by screen analysis performed by using molecular sieves. The amount of available surface functional epoxy group's content of the poly(GMA/MMA/EGDMA) beads was determined by pyridine–HCl method as described previously.[16]

The amount of amine groups content of the aminated and/or spacer-arm attached beads was determined by titration. The aminated and/or spacer-arm attached p(GMA-MMA) beads (0.2 g) were allowed to soak in water (10 cm^3) for 24 h. Then 2*M* HCl (10 cm^3) was added to the mixture and shaken for 1 h. At the end of this period, the beads were filtered and assayed by titration with 2*M* NaOH solution.

The amount of immobilized L-arginine on both beads was determined by measuring the initial and final concentrations of amino acid within the immobilization medium at 230 nm by using a double-beam UV/vis spectrophotometer (Shimadzu, model 1601). A calibration curve constructed with L-arginine solution of known concentration was used in the calculation of amino acid in the solutions.

HIgG adsorption studies

Adsorption of HIgG on both affinity beads, p(GMA-MMA)-AA and p(GMA-MMA)-SA, was studied at various pHs, in either acetate (50 m*M* pH 4.0–5.5), in phosphate buffer (50 m*M*, pH 6.0–6.5), or in Tris-HCl buffer (50 m*M*, pH 7.0–8.0). The effect of temperature and ionic strength on HIgG adsorption was carried out in Tris-HCl buffer (50 m*M*, pH 7.0) at

four different temperatures (i.e., 4, 15, 25, and 37°C) and at three different NaCl concentrations (i.e., 0.1, 0.25, and 0.5*M*), respectively. To determine the adsorption capacities of both adsorbents, the initial concentration of HIgG was changed between 0.125 and 3.0 mg cm^{-3}. Desorption of HIgG was performed in a buffer solution (glycine–HCl buffer) containing 1.0*M* NaCl at pH 2.6. Desorption medium was stirred magnetically at 100 rpm at 25°C for 4 h.

Adsorption kinetic and isotherms models

The kinetic models (the first-order and second-order equations) can be used in this case, assuming that measured concentrations are equal to adsorbent surface concentrations.[17] It may be represented as follows:

$$dq_t/dt = k_1(q_{eq} - q_t) \quad (1)$$

where k_1 is the rate constant of the first order adsorption (min^{-1}), and q_{eq} and q_t denote the amounts of adsorbed HIgG at equilibrium and at time t (mg g^{-1}), respectively. Equation (1) can be integrated and rearranged to obtain a linear form:

$$\log(q_{eq} - q_t) = \log q_{eq} - (k_1 t)/2.303 \quad (2)$$

Ritchie proposed a second-order rate equation for the kinetic adsorption of gases on solids.[18] The second-order equation was applied for adsorption of solutes on the adsorbents.[19–21] The second-order equation based on adsorption equilibrium capacity may be expressed in the form:

$$1/q_t = 1/k_2 q_{eq} t + 1/q_{eq} \quad (3)$$

where k_2 is the rate constant for second-order adsorption

The equilibrium adsorption of a protein on an adsorbent is often described by the Langmuir equation [eq. (3)], with the assumption that a single-site, homogeneous interaction occurs between the protein and the ligand.[19,21–24]

$$q_{eq} = q_m C_{eq}/K_d + C_{eq} \quad (4)$$

The Freundlich isotherm is frequently used to describe the adsorption. It relates the adsorbed concentration as the power function of solute concentration. This empirical equation takes the form:

$$q_{eq} = K_F(C_{eq})^{1/n} \quad (5)$$

where C_{eq} is the equilibrium concentration of protein in solution, q_{eq} is the amount of protein adsorbed on the beads, q_m is the maximum binding (adsorption) capacity of the sorbent, and K_d is the dissociation constant. K_F and n are the Freundlich constant characteristic of the system. K_F and n are the indicators of adsorption capacity and adsorption intensity, respectively.

Evaluation of thermodynamic parameters

The equilibrium association constant ($K_a = 1/K_d$), extracted from the semireciprocal plot of Langmuir isotherm, was then employed for van't Hoff plot analysis. The value of the change in enthalpy during the binding process was determined from the gradient of the plots between ln K_a versus $1/T$ ($\ln K_a = (\Delta S/R) - (\Delta H/RT)$). From this plot, the thermodynamic parameters (ΔH, ΔS, and ΔG) were calculated. The $-\Delta H/R$ and $\Delta S/R$ should be equal to the slope and intercept, respectively. The value of ΔG can be estimated from the relationships $\Delta G = -RT \ln K_a$, where R is the gas constant (1.987 cal mol^{-1} K^{-1}).[25]

$$\Delta G_{assoc} = -RT \ln K_a \quad (6)$$

where R is the gas constant (1.987 cal mol K^{-1}).

RESULTS AND DISCUSSION

Characterization of the adsorbents

The total polymerization yield was 78%. The beads were sieved, and 75–150 μm size of fraction was used in the further reactions. The specific surface area of the poly(GMA/MMA/EGDMA) beads was measured by BET method and was found to be 11.4 m^2 g^{-1} beads. The amount of available epoxy groups on the poly(GMA/MMA/EGDMA) beads was determined to be 0.98 mmol g^{-1} beads. The content of the amino group of the poly(GMA/MMA/EGDMA)-A and poly(GMA/MMA/EGDMA)-S beads was found to be 1.14 and 1.06 mmol g^{-1}, respectively. The L-arginine contents of the poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA beads were found to be 35.3 and 57.8 μmol g^{-1} beads, respectively.

SEM micrographs shows that the beads have a porous surface structure (Fig. 1). The porous surface properties of the poly(GMA/MMA/EGDMA) beads would favor higher adsorption capacity for the protein because of increase in the surface area.

The FTIR spectra of poly(GMA/MMA/EGDMA) beads had the characteristic stretching vibration band of hydrogen-bounded alcohol of MMA at ~ 3500 cm^{-1}. Among the characteristic vibrations of both MMA and GMA was the methylene vibration at ~ 2930 cm^{-1}. The vibration at 1740 cm^{-1} represented the ester configuration of both MMA and GMA. The epoxide group gave the band at 910 cm^{-1} (epoxy ring vibrations). The most important adsorption bands at

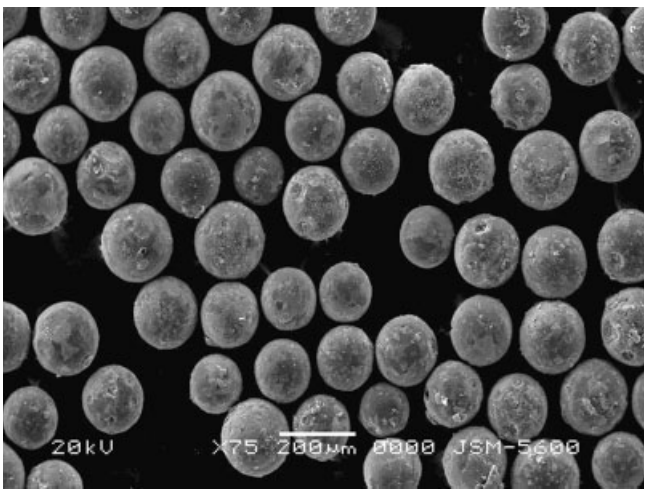

Figure 1 The SEM micrograph of poly(GMA/MMA/EGDMA) beads.

1550 cm^{-1} was representing N—H bending, which is due to aminated and/or spacer-arm bonded to the poly(GMA/MMA/EGDMA) beads.

Effect of spacer-arm on adsorption capacity of affinity beads for HIgG

To minimize the involvement of the support surface force in the affinity interactions, the chemical groups of the ligand molecules critical in the interaction with the target macromolecule must be sufficiently distant from the support surface. Thus, the application of spacer-arm on affinity systems could affect the performance of immobilized ligand. If an affinity ligand is coupled directly to the support surface, steric hindrance with the ligand interaction will occur. This problem could be solved by incorporation of a spacer-arm between support surface and ligand molecule.[26,27] 1,6-Diaminohexane as a spacer-arm was placed between support and L-arginine amino acid. It was expected that incorporation of spacer-arm on the beads surface could prevent steric hindrance between HIgG and surface of support. As expected, HIgG adsorption capacity of spacer-arm attached poly(GMA/MMA/EGDMA)-SA beads was higher (140.9 mg g^{-1}) compared with the poly (GMA/MMA/EGDMA)-AA beads (106.6 mg g^{-1}). Thus, the HIgG adsorption capacity of the spacer-arm attached adsorbent significantly increased (about 1.32-fold) with respect to poly(GMA/MMA/EGDMA)-AA beads. From these observations, it can be concluded that incorporation of spacer-arm on the adsorbent steric hindrance will become small and the capacity of HIgG adsorption can increase. By this way, the incorporation of spacer-arm might prevent different interactions between ligand and protein, and thus increased the likelihood of homogeneous adsorption of HIgG on the spacer-arm and L-arginine ligand attached poly(GMA/MMA/EGDMA) beads.

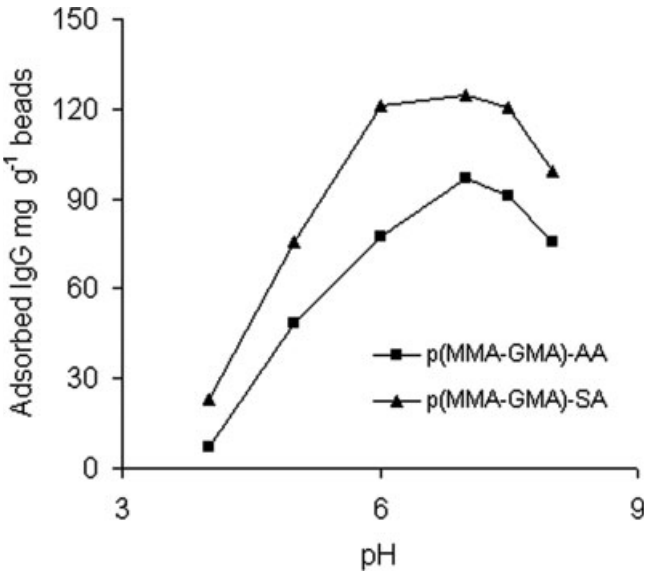

Figure 2 Effect of pH on HIgG adsorption onto poly (GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads (initial concentration of HIgG: 1.0 mg cm^{-3}; temperature: 25°C, pH: 7.0).

Effect of pH

The pH and the ionic strength play an important role in the affinity chromatography system by favoring hydrophilic or hydrophobic interactions involved at interface.[28] The distribution of charged amino acid

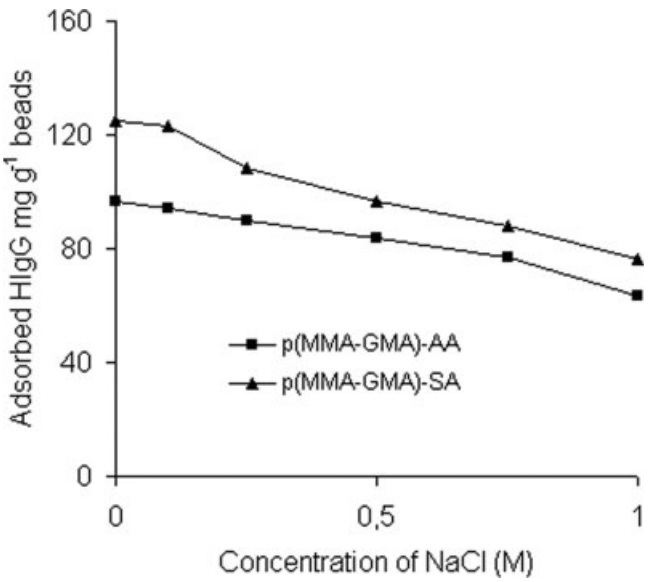

Figure 3 Effects of ionic strength on HIgG adsorption onto poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads (initial concentration of IgG: 1.0 mg cm^{-3}; temperature: 25°C, pH: 7.0).

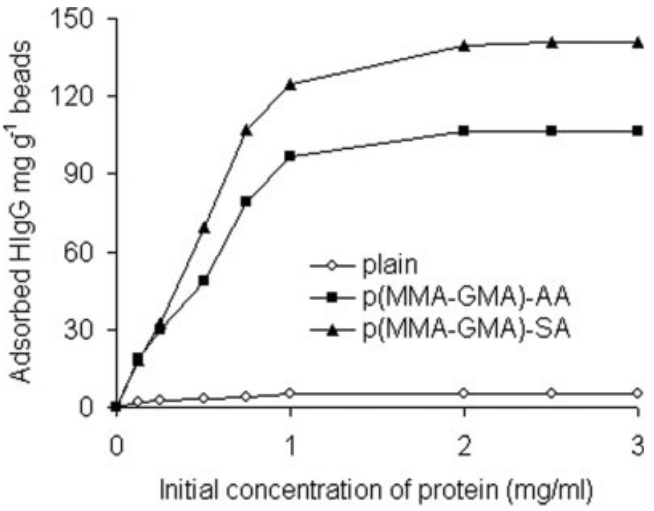

Figure 4 The experimental adsorption isotherm curves of HIgG onto poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads.

residues on the protein surface is a very important factor in ion-exchange interactions with the ligand on the affinity beads.[29,30] The optimal pH values for adsorption of HIgG on the affinity beads were investigated in the pH range of 4.0–8.0 (Fig. 2). In all cases investigated, the maximum adsorption of HIgG was observed at around pH 7.0. At this pH value, the maximum HSA binding capacities of the poly(GMA/MMA/EGDMA)-AA and the poly(GMA/ MMA/EGDMA)-AS beads were found to be 96.6 and 124.7 mg g^{-1} beads, respectively. It is well known that major interactions involved in protein adsorption can be classified as hydrophobic, electrostatic, and hydrogen bonding. The electrostatic contribution is most important, so that the adsorption is controlled by the surface charge.[31,32]

Effect of ionic strength and temperature

The adsorption of HIgG from aqueous solution was studied by varying the ionic strength between 0.0*M* and 1.0*M* NaCl at initial HIgG concentration 1.0 mg mL^{-1} (Fig. 3). As seen in the figure, HIgG adsorption capacities of the poly(GMA/MMA/EGDMA)-AA and the poly(GMA/MMA/EGDMA)-AS affinity beads were decreased by about 1.53- and 159-fold, respectively, with increasing ionic strength from 0.00 to 1.0. When the salt concentration increased in the adsorption medium, the surface charges are screened. These effects cause the electrostatic interactions between the L-arginine amino acid and HIgG molecules to decrease.

The adsorption capacity of the poly(GMA/MMA/EGDMA)-AA and the poly(GMA/MMA/EGDMA)-AS affinity beads was obtained at four different temperatures. The equilibrium adsorption of HIgG onto both L-arginine ligand immobilized beads significantly increased with increasing temperatures. From 4 to 37°C, the adsorption capacity of the poly (GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads for HIgG increased by about 1.48- and 1.39-fold, respectively. This indicated that the increase in the temperature was favorable to HIgG adsorption on both L-arginine immobilized affinity beads.

Initial protein concentration

The experimental adsorption isotherm curves are presented in Figure 4. An increase in the HIgG concentration in the adsorption medium led to an increase in the amount of adsorbed HIgG on both L-arginine ligand immobilized affinity beads. The relationship between the initial HIgG concentration and the HIgG adsorption capacity on both the affinity beads was almost linear when the initial HIgG concentration was no more than 2.0 mg cm^{-3}. The nonspecific adsorption of HIgG for acid-treated poly(GMA/MMA/EGDMA) as control support was negligible. The maximum HIgG adsorption capacity of the poly(GMA/MMA/EGDMA)-AA and poly (GMA/MMA/EGDMA)-SA affinity beads was 106.6 and 140.9 mg g^{-1}, respectively (Fig. 4).

Adsorption kinetic and isotherm models

To analyze the adsorption kinetic of HIgG onto poly (GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads, the first-order and the second-order kinetics models were applied to the experimental data. The fitted parameters for the first- and second-order kinetic equations are presented in Table I. The theoretical q_{eq} values estimated from the

TABLE I
The First-Order and Second-Order Kinetic Constants for the Adsorption of HIgG on the Poly(GMA/MMA/EGDMA)-AA and Poly(GMA/MMA/EGDMA)-SA Affinity Beads

		First-order kinetic			Second-order kinetic		
Affinity beads	Experimental q_{exp} (mg g^{-1})	q_{eq} (mg g^{-1})	k_1 (10^2 min^{-1})	R^2	q_{eq} (mg g^{-1})	k_2 (10^2 g mg^{-1} min^{-1})	R^2
Poly(GMA/MMA/EGDMA)-AA	106.6	102.7	4.04	0.990	146.7	2.84	0.972
Poly(GMA/MMA/EGDMA)-SA	140.9	145.5	5.27	0.993	160.8	6.58	0.991

TABLE II
The Langmuir and Freundlich Isotherm Constants and Thermodynamic Parameters for Adsorption of HIgG onto Poly(GMA/MMA/EGDMA)-AA Affinity Beads from Aqueous Solutions at Different Temperatures[a]

Temperatures (°C)	Experimental q_{ex} (mg g^{-1})	Langmuir constants			Freundlich constants			ΔG (kcal mol^{-1})
		q_m (mg g^{-1})	K_d (10^7 M)	R^2	K_F	n	R^2	
4	88.2	80.6	12.30	0.998	64.4	2.46	0.933	−7.478
15	88.2	94.3	9.50	0.998	78.6	2.70	0.936	−7.961
25	106.6	112.4	6.98	0.997	94.2	3.68	0.946	−8.389
37	126.3	131.4	5.14	0.999	124.7	2.77	0.882	−8.913

[a] $\Delta H = 4.63$ kcal mol^{-1}; $\Delta S = 43.69$ cal mol^{-1}.

first-order kinetic model gave almost similar values compared to experimental values, and the correlation coefficients were also found to be greater than 0.990 for both affinity beads. This result suggests that the first-order mechanism is predominant, and the chemical adsorption may be the rate limiting step that controls the adsorption process. Thus, the first-order kinetic model well describes both affinity systems.

Two theoretical isotherm models (Langmuir and Freundlich) were used to analyze the experimental data. The fitted parameters for these two kinds of isotherms are summarized in Tables II and III. The obtained adsorption isotherm curves of HIgG onto poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads are of Langmuir type (Fig. 5). From the slopes, maximum HIgG adsorption capacity was found to be close to the experimental result for both affinity beads. Thus, the measured HIgG adsorption isotherm can be reasonably described by a Langmuir adsorption isotherm model (Fig. 4), with a maximum adsorption capacity of 106.6 and 140.9 mg g^{-1} for poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads at 298 K, respectively. The K_d values indicated that a moderate affinity is consistent with the easy and nondenaturing desorption of the bound HIgG and advantageous for the good recovery of nondenatured proteins.[30] The correlation coefficients of semireciprocal plots (R^2) was greater than 0.995 for poly(GMA/MMA/EGDMA)-AA and poly (GMA/MMA/EGDMA)-SA affinity beads (Tables II and III), respectively.

The Freundlich constants, K_F and n, were found to be between 64.4 and 161.3, and 2.44 and 3.95, respectively. Values of $n > 1$ for the L-arginine ligand indicate positive cooperativity in binding and a heterogeneous nature of adsorption. The magnitude of K_F showed easy adsorption of HIgG from the adsorption medium (Tables II and III).

Thermodynamic parameters

The dependency of the equilibrium association constant ($K_a = 1/K_d$) versus $1/T$ for the binding of affinity beads was analyzed in terms of van't Hoff plots. Changes in the apparent ΔH values for L-arginine ligand immobilized aminated and spacer-arm attached poly(GMA/MMA/EGDMA) beads, respectively, were 4.63 and 10.64 kcal mol^{-1}. The enthalpy change of adsorption process is smaller than typical chelating bonding and ionic bonding, but higher than electrostatic and van der Waal's forces. In addition, as seen in Tables II and III, the q_{exp} and q_{eq} increased with the rise of temperature, implying the existence of chemical adsorption. Therefore, it can be concluded that multi-interactions are included in the adsorption process of HIgG on both adsorbents. The values of ΔH were positive, which implied that the process involved was endothermic. The apparent ΔS values for the binding of HIgG onto poly(GMA/

TABLE III
The Langmuir and Freundlich Isotherm Constants and Thermodynamic Parameters for Adsorption of HIgG onto Poly(GMA/MMA/EGDMA)-SA Affinity Beads from Aqueous Solutions at Different Temperatures[a]

Temperatures (°C)	Experimental q_{ex} (mg g^{-1})	Langmuir constant			Freundlich constant			ΔG (kcal mol^{-1})
		q_m (mg g^{-1})	K_d (10^7 M)	R^2	K_F	n	R^2	
4	111.9	119.1	8.52	0.995	85.96	2.44	0.882	−7.603
15	126.9	132.8	6.37	0.997	122.74	2.79	0.870	−8.334
25	140.9	142.9	2.48	0.996	143.12	3.38	0.843	−8.973
37	152.8	154.4	1.21	0.999	161.03	3.95	0.856	−9.763

[a] $\Delta H = 10.64$ kcal mol^{-1}; $\Delta S = 65.87$ cal mol^{-1}.

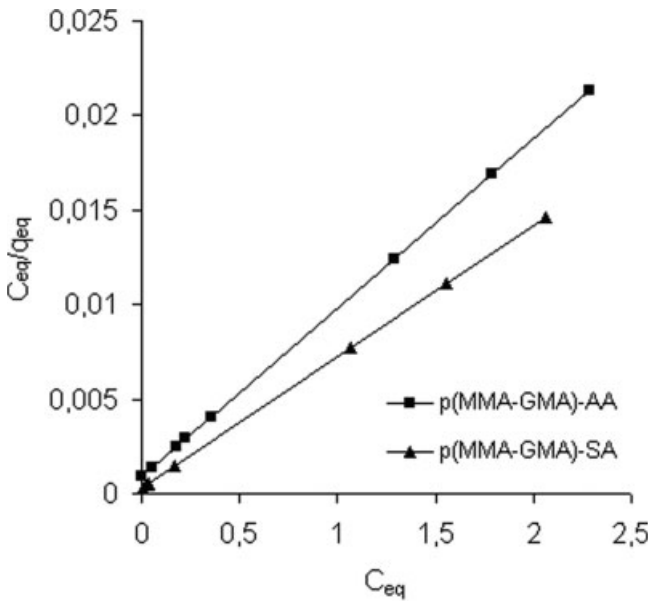

Figure 5 The Langmuir isotherm plots for adsorption of HIgG onto poly(GMA/MMA/EGDMA)-AA and poly (GMA/MMA/EGDMA)-SA affinity beads.

MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads were 43.69 and 65.87 cal mol^{-1}, respectively. Positive values of the apparent ΔS were obtained in all cases, indicating an increase in the total disorder of the system during adsorption. The positive values of the apparent entropy implied that some form of structural changes could have taken place in the interface between the protein and adsorbent during the adsorption process. The ΔG values for HIgG adsorption onto poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads were calculated for each temperature, and in accordance with adsorption being a favorable process, the derived ΔG values tabulated in Tables II and III were all negative. The negative ΔG values for the two different adsorbent system implied that the adsorption process was spontaneous in nature.[32]

Desorption and reusability of affinity beads

The desorption of the adsorbed HIgG from poly (GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads was studied in a batch system. The HIgG-loaded affinity adsorbents were placed within the desorption medium containing 1.0*M* NaCl in glycine–HCl buffer at pH 2.6, and the amount of HIgG released in 4 h was determined. For all the tested adsorbents more than 93% of the adsorbed HIgG was removed. To show the reusability of the affinity beads, adsorption–desorption cycle of HIgG was repeated eight times by using the same adsorbents. The adsorption capacity of the affinity beads did not change during the repeated adsorption–desorption operations.

CONCLUSIONS

The adsorption parameters of HIgG onto poly(GMA/MMA/EGDMA)-AA and poly(GMA/MMA/EGDMA)-SA affinity beads were investigated using various reaction conditions and compared for the adsorption HIgG from aqueous solution. The effect of spacer-arm on affinity systems for HIgG affected the immobilized ligand. As a result, the maximum adsorption capacity on the spacer-arm attached beads was higher compared with the aminated forms of the affinity beads. The experimental data can be well fitted by the Langmuir isotherm. The theoretical q_{eq} values for all the tested adsorbent systems were very close to the experimental q_{eq} values in the case of first-order kinetics. The first-order kinetics best described the data. The poly(GMA/MMA/EGDMA)-SA affinity beads are therefore suitable for separation and purification of a target protein from biological fluid.

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