Synthesis and Modification of Supports with an Alkylamine and Their Use in Albumin Adsorption

CESAR G. GOMEZ, MIRIAM C. STRUMIA

Dpto. de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Haya de la Torre y Medina Allende, Edificio de Ciencias II, Ciudad Universitaria, 5000 Córdoba, Argentina

Received 8 June 2007; accepted 17 December 2007
DOI: 10.1002/pola.22587
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The morphological effect of polymeric networks (R) modified with terminal amino groups was studied on the adsorption of bovine serum albumin (BSA). Networks of ethylene glycol dimethacrylate and 2-hydroxyethyl methacrylate [poly (EGDMA-*co*-HEMA)] were synthesized by suspension polymerization, using different EGDMA contents and agitation speeds. These matrices were characterized by FTIR, mercury intrusion porosimetry, SEM, and swelling degree. The increase of the EGDMA concentration led to the formation of networks with the highest crosslinking degree and porosity. An earlier phase separation yielded a higher aggregation of rigid microspheres, also forming stable pore systems. The increase in coalescence frequency, together with the impeller speed, and the decrease of the stabilizer molecules led to an increment in drop size. Large fused aggregates of microspheres were formed with additional loss of small pores as the stirring was increased, attaining also a higher pore volume (V_p) and a slight decrease of the surface area. Once characterized, networks were activated with butanediolglycidyl ether (BDGE), and then reacted with hexamethylenediamine (HMDA) through coupling reaction. Only the R-BDGE-HMDA networks synthesized with the highest EGDMA content and agitation speed showed BSA adsorption. Their base matrices exhibited a V_p higher than 1.4 mL/g, which allows easier protein diffusion into the support. © 2008 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 46: 2557–2566, 2008

Keywords: albumin; adsorption; heterogeneous networks; macroporous polymers; swelling

INTRODUCTION

The aim of most of the research in the field of ion exchange is to enhance functional properties such as selectivity, capacity, and rate, which have direct impact on the applicability of the resins.[1] These supports can also be employed as anion exchangers[2–5] or in affinity chromatography, after attaching a ligand.[6–9] Thus, matrix derivatives with alkyl amines are used in affinity chromatography for the purification of amine oxidases[10] and other proteins with affinity for the amino group.[11,12] An example of the latter is the use of the ligand albumin for bilirubin.[13] At the present time, the search for new and more efficient supports for affinity chromatography is a topic of great, scientific and technological interest. Additionally, the synthesis of macroporous networks with interconnected pores that allow diffusion of large solutes and that provide a wide surface area for further chemical modifications (high network capacity)[14–22] is a subject of discussion within our research group.

Correspondence to: M. C. Strumia (E-mail: mcs@fcq.unc.edu.ar)

Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 46, 2557–2566 (2008)

These matrices are generally synthesized by suspension polymerization using a monovinyl and a polyvinyl monomer (crosslinker) and a porogen agent (diluent). In previous works,[17–22] we have studied reaction variables such as kind of porogen, monovinyl monomers and crosslinkers, and radical initiator content, which have an influence on the development of the porous structure. Therefore, in this opportunity the influence of both EGDMA content and stirring speed on the formation of macroporous networks was analyzed. Then, the poly(EGDMA-*co*-HEMA) supports (R-OH) with different pore structures were modified by amine coupling reaction using hexamethylenediamine (HMDA). These adsorbents were employed to study the influence of network morphology on the degree of bovine serum albumin (BSA) adsorption, employing this protein as a representative of a large biological substrate.

EXPERIMENTAL

Reagents and Equipments

The following reagents were purchased and used: 2-hydroxyethyl methacrylate (HEMA), ~95% (GC) Fluka, Switzerland; ethylene glycol dimethacrylate (EGDMA), >97% (GC) Fluka; polyvinylpyrrolidone (PVP), Kollidone 90, Fluka; cyclohexane (Cyc), p.a. Anedra, Argentina; hydroxy-methylaminomethane (Tris), p.a. Anedra; hydrochloric acid (HCl), Merck, Germany; bovine serum albumin (BSA), p.a. Merck; 1,4-butanediol diglycidyl ether (BDGE), 62% by GC, Sigma; potassium chloride (KCl), p.a. Mallinckrodt; hexamethylenediamine (HMDA), purified by distillation at reduced pressure at 100 °C/20 mmHg, Fluka; benzoyl peroxide (BPO), Riedel de Haen, Germany, purified by crystallization from chloroform/methanol mixture, and then dried at room temperature before use.

The polymeric networks were synthesized using a magnetic stirrer "04644-Series Digital Hot Plate/Stirrer (Cole Parmer)." In the Department TEMADI, CAB-CNEA (Bariloche, Argentina), an "Auto-Pore 9220 Micromeritics Inst. Corp" was used in the characterization of dry samples by mercury intrusion porosimetry. Using this technique, parameters such as pore size distribution, specific surface area, and pore volume in the pore size range of $4 - 4 \times 10^5$ nm could be analyzed. Scanning electron microscopy (SEM) assays were performed on an "EVO 40 XVP, LEO (2003) apparatus," at the laboratories in Centro Regional de Investigaciones Básicas y Aplicadas de Bahía Blanca (CRIBBAB), CONICET, Argentina. The BSA concentration was obtained by UV–vis spectroscopy, by measuring absorbance at 280 nm with a UV–vis Shimadzu UV-260 spectrophotometer.

Synthesis of the Polymeric Network

Polymer beads were prepared by suspension polymerization in a 250-mL round bottom flask equipped with a reflux condenser and a magnetic stirrer. The reaction mixture was heated at 85 °C in a bath of water and stirred for 2 h with a magnetic stir bar 3-cm long and 1.5-cm wide. The influence of the stirring speed (450 or 750 rpm) on network formation was studied using a ratio of 25 mol % of crosslinking agent (EGDMA) to total vinyl monomers in the reaction mixture. Here, to obtain 10 g of dry polymer, a mol ratio of $3.0{:}1.0{:}9.3{:}2.5 \times 10^2$ of HEMA (6.2 mL), EGDMA (3.2 mL), Cyc (17.2 mL), and water (77.0 mL), respectively, was used in the reaction. Likewise, in all cases, the free radical initiator BPO (0.411 g, 2.44 mol %) was also added, using PVP (0.777 g, 10 mg/mL of total mixture) as suspension stabilizer. Afterwards, the resulting polymer beads were exhaustively washed first with distilled water and then with ethanol. This procedure removed the porogenic diluent, PVP, and some unreacted monomers. Next, samples were dried in an oven at 70 °C to reach a constant mass. The yield of each reaction was calculated as the percentage of dry matrix obtained versus the total grams of vinyl monomers used. Finally, the assays of network swelling in water, FTIR, SEM, and mercury intrusion porosimetry characterized the networks.

On the other hand, using the same procedure described earlier and a stirring of 450 rpm, the effect of the crosslinker content (6, 25, or 33 mol % of EGDMA) on network formation was studied.

Modification of the Polymeric Network

Activation Reaction of the Matrix

The hydroxyl groups of poly(EGDMA-*co*-HEMA) networks were reacted in a basic aqueous solution with oxirane groups of BDGE, to obtain terminal oxirane groups attached to the polymeric

chains. First, a sample (3.00 g) of dry network (R-OH) was swollen in dimethylformamide (5.80 mL/g) for 24 h at room temperature and then mixed with BDGE and a 0.74 N NaOH solution, yielding an epoxy-activated support (R-BDGE). The amount of hydroxyl groups per gram of dry network was considered in reference to the EGDMA content used in the synthesis of the network. Then, an equivalent ratio was used—(0.9–1.4):12:0.5 of hydroxyl groups, oxirane, and base, respectively, under stirring for 7.5 h at room temperature (Table 3). Finally, the poly (EGDMA-*co*-HEMA)-BDGE supports were purified by exhaustive washing with water and ethanol, and dried to reach a constant weight. Next, the amount of oxirane groups per gram of dry product by colorimetric acid–base titration was determined.

Amine Coupling on R-BDGE Supports

In the next modification step, a terminal amino group was attained by reaction of the oxirane on polymeric backbone with an amino group of the alkyl diamine. Here, the epoxy-activated matrix (2.00 g) was swollen in water (5.40 mL/g) for 24 h at room temperature, and then mixed and reacted during 24 h at 60 °C with a diamine (HMDA) dissolved in a 2 M Na_2CO_3 solution (Table 4). Here, a mol ratio of 1:40:72 of epoxy: diamine:Na_2CO_3, respectively, was used in the reaction mixture. The poly(EGDMA-*co*-HEMA)-BDGE-HMDA product was filtered, washed with 300 mL of distilled water, 0.1 N acetic acid solutions (200 mL), and then washed again with distilled water to reach the supernatant pH 7. Once the product was purified and dried, the amino group content was determined by colorimetric acid–base titration.

Methods

Mercury Intrusion Porosimetry

Using this technique, the pore size distribution ($dV/d\ \log\ d$), specific pore volume (V_p), specific surface area (S_s), apparent density (d_0), and porosity percentage (% P) of dry networks were obtained (pore diameter range: $4 - 4 \times 10^5$ nm).

Analysis of Network Swelling in Water

The assays of network swelling were carried out in distilled water (pH 6.5). In this process, a sample of the polymer bead (0.10 g) was left in contact with water (excess) in a special funnel (with a very small hole) inside a closed chamber for 24 h followed by a further 24-h drainage in the same closed chamber. Then, the wet sample was weighed on an analytical scale at different times (every 15 s for 7–8 min after the sample was removed from the chamber). The data were processed using a plot of swollen sample weight (g) versus evaporation time (s), where the swollen mass of the sample (W_{sw}) from curve extrapolation was taken at time zero. Subsequently, the samples were dried in an oven at 70 °C for 48 h, and then weighed to obtain the dry sample weight (W_{dry}). The equilibrium weight swelling ratio (Q_w) was also calculated according to eq 1.

The equilibrium volume swelling ratio (Q_v) obtained from the sample volume in swollen equilibrium state (V_{sw}) and the volume of the sample in dry state (V_{dry}) was calculated as in eq 2. The measurement of values V_{sw} and V_{dry} was performed using graduated tubes, after the dry sample (particle size range 40–70 mesh) had been soaked for 24 h in an excess of distilled water. The assays were carried out four times, and the results generally showed an error of less than 5%.

The data of the Q_w obtained, the swelling liquid density ($\delta_1 = 1.00$ g/mL), and the true polymer density ($\delta_2 = 1.283$ g/mL) were used to calculate the network porosity percentage in swollen state, which is expressed in eq 3.[23]

$$Q_w = \frac{W_{sw}}{W_{dry}} \tag{1}$$

$$Q_v = \frac{V_{sw}}{V_{dry}} \tag{2}$$

$$P(\%) = \frac{(Q_w - 1)/\delta_1}{[(Q_w - 1)/\delta_1] + (1/\delta_2)} \times 100 \tag{3}$$

Quantification of Functional Groups on Polymeric Chains

The quantification of oxirane groups was performed by the pyridinium chloride method, which is a retrocession titration. The R-BDGE support (0.1 g) is put in a beaker with 2.0 mL of 0.05 N pyridinium chloride solution and left under agitation near 90 °C for 45 min. Then,

the reaction mixture is titrated at room temperature with 0.05 N NaOH aqueous solutions, employing phenolphthalein as colorimetric indicator.[24]

On the other hand, the content of amino groups present in R-BDGE-HMDA was also determined by colorimetric titration. Here, the modified polymer (0.1 g) with terminal amino groups was left in an Erlenmeyer flask with 4.0 mL of HCl aqueous solution (0.01 N) under stirring at room temperature for 1 h. The acid reacts with the amino groups and then the proton amount in the mixture decreases. Next, the supernatant (1.0 mL) was titrated with a 0.01 N NaOH aqueous solution up to the color change of phenolphthalein.[25]

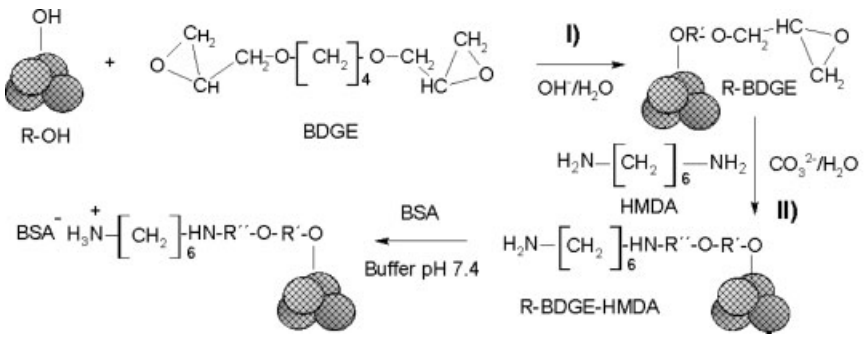
BSA Adsorption by Electrostatic Interaction with the Modified Supports

Four different active networks (I–IV) modified with terminal amino groups (R-BDGE-HMDA) were used to examine the influence of network morphology on the degree of BSA adsorption. Thus, 4.5 mL of buffered BSA solution (1.12 g/L) and 0.1 g of dry amino supports were left on agitation (200 rpm) for 80 min at room temperature in a system batch. Later, the mixture was centrifuged and the concentration of BSA in 2.0 mL of the supernatant was measured twice, and then the BSA retention was expressed as milligrams of protein per gram of dry support. This procedure was also performed twice on each support. The concentration of the BSA solutions was tested by measuring the absorbance at 280 nm, using an extinction coefficient [0.615 (cm^{-1} L)/g] obtained from an appropriate calibration curve, plotting absorbance against protein concentration. UV–visible spectra were recorded with a UV-260 Shimadzu recording spectrophotometer. The buffer used to carry out the studies of BSA adsorption was chosen from a previous work.[16] Then, a 0.01 M of Tris-HCl solution at pH 7.4 was prepared using an aqueous solution of 0.1 M KCl as solvent.

In other analysis, the kinetic of BSA adsorption on the modified support (III) was performed twice, using 0.20 g of dry sample and 15.0 mL of buffered BSA solution (0.45 g/L, pH 7.4) on stirring for 200 rpm at room temperature. In this assay, the BSA absorbance at 280 nm of the supernatant solutions was measured as a function of the adsorption time. During the first 2 h, every 20 min of process, the reaction mixture was stopped; it was then centrifuged and the absorbance to an aliquot of 2.0 mL of BSA supernatant solution measured. The same procedure was followed at intervals of 30 min for the next 3 h.

RESULTS AND DISCUSSION

Poly(EGDMA-*co*-HEMA) beads were prepared by suspension polymerization using different EGDMA contents and stirring speeds. These base supports consisted of white, smaller, densely crosslinked microspheres forming a porous mesh, which in turn formed larger macrospherical beads. In all cases, the polymerization yield was 95–98 w/w %. Next, the hydroxyl group present on the polymeric backbone was first substituted by an oxirane group (first reaction), and then modified with a terminal amino group (second pathway), as shown in Scheme 1. Later, the modified networks were employed in the BSA adsorption to analyze the influence of support morphology on the degree of protein retention. The latter was adsorbed by electro-



Scheme 1. Modification reactions on initial polymeric network. (I) Activation reaction. (II) Amine coupling reaction.

Table 1. Morphologic Properties of the Networks in Swollen and Dry State

R	EGDMA (mol %)	Q_w[a]	Q_v[a]	P[a] (%)	d_0 (mL/g)	Total V_p (mL/g)	Total S_s (m^2/g)	P[b] (%)
I[c]	6	3.82	1.59	78	0.38	1.95	30.2	71
II[c]	25	6.22	1.20	87	0.20	4.08	30.2	83
III[c]	33	6.41	1.07	87	0.22	3.7	33.2	83
IV[d]	25	6.27	1.14	87	0.20	4.26	23.6	84

[a] Swelling assays of the network in water (eqs 1–3).
[b] The porosity percentage of the dry networks was calculated as % P = (Total V_p.d_0) × 100, using the parameters obtained from mercury intrusion porosimetry. Networks (R) obtained at [c]450 or [d]750 rpm of agitation.

static interaction with the ammonium groups on the polymeric backbone, since this protein is a representative of a large substrate.

Synthesis and Characterization of the Networks

Three heterogeneous networks (I–III) were prepared by suspension polymerization using an agitation of 450 rpm and different contents of EGDMA (6, 25, and 33 mol %). Moreover, the agitation speed was also varied during network formation, and a fourth matrix IV was synthesized using a 25 mol % of EGDMA at 750 rpm. Next, the products were purified, and then the dry networks were characterized by mercury intrusion porosimetry, SEM, and FTIR. Thus, the assays of network swelling in water provided information on the morphology of the matrix in swollen state.

Variation of the Crosslinker Content During Network Formation

Table 1 shows that an increase of the EGDMA concentration in the reaction mixture leads to the formation of networks (II, III) with the highest porosity in dry state (nearly 83%). Figure 1 shows that bimodal pore size distributions (dV/d log d) against pore diameter (d) are attained, which also demonstrates that heterogeneous networks with microspheres and their aggregates are formed. It is well known that cyclization reactions increase together with the crosslinker content during polymerization, which is due to the fact that the number of pendant vinyl groups increases on growing chains.[26] Thus, an earlier phase separation with respect to the conversion degree was generated, which had an influence on the particle system produced. During polymerization, the nuclei that are nonporous and constitute the highly crosslinked regions of the network are generated first. Beads contain large agglomerates of microspheres (100–200 nm). Each microsphere consists of smaller nuclei (10–20 nm) that are more or less fused together.

Moreover, SEM micrographs of the networks (Fig. 2) confirmed the formation of heterogeneous surfaces corresponding to irregular agglomerates of spherical particles (microspheres), and their aggregates resemble in some cases a cauliflower. Figure 3 shows that the highest surface areas belong to pores system formed between the interconnected microspheres, while no area is verified for the pore size distribution (10–400 μm) of the particles agglomerates mentioned earlier. Therefore, we will only focus on the particle system that presents substantial changes

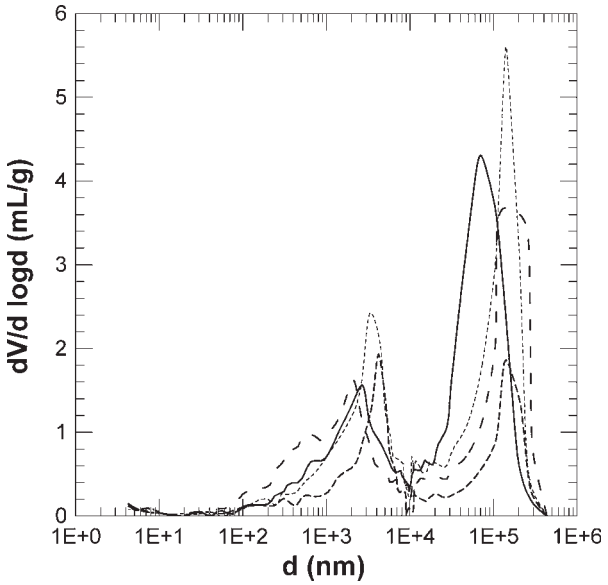


Figure 1. Pore size distribution of the porous poly (EGDMA-*co*-HEMA) beads synthesized at 450 rpm of agitation with 6 (– – –), 25 (—), and 33 mol % of EGDMA (– –), or at 750 rpm and 25% of EGDMA (—).

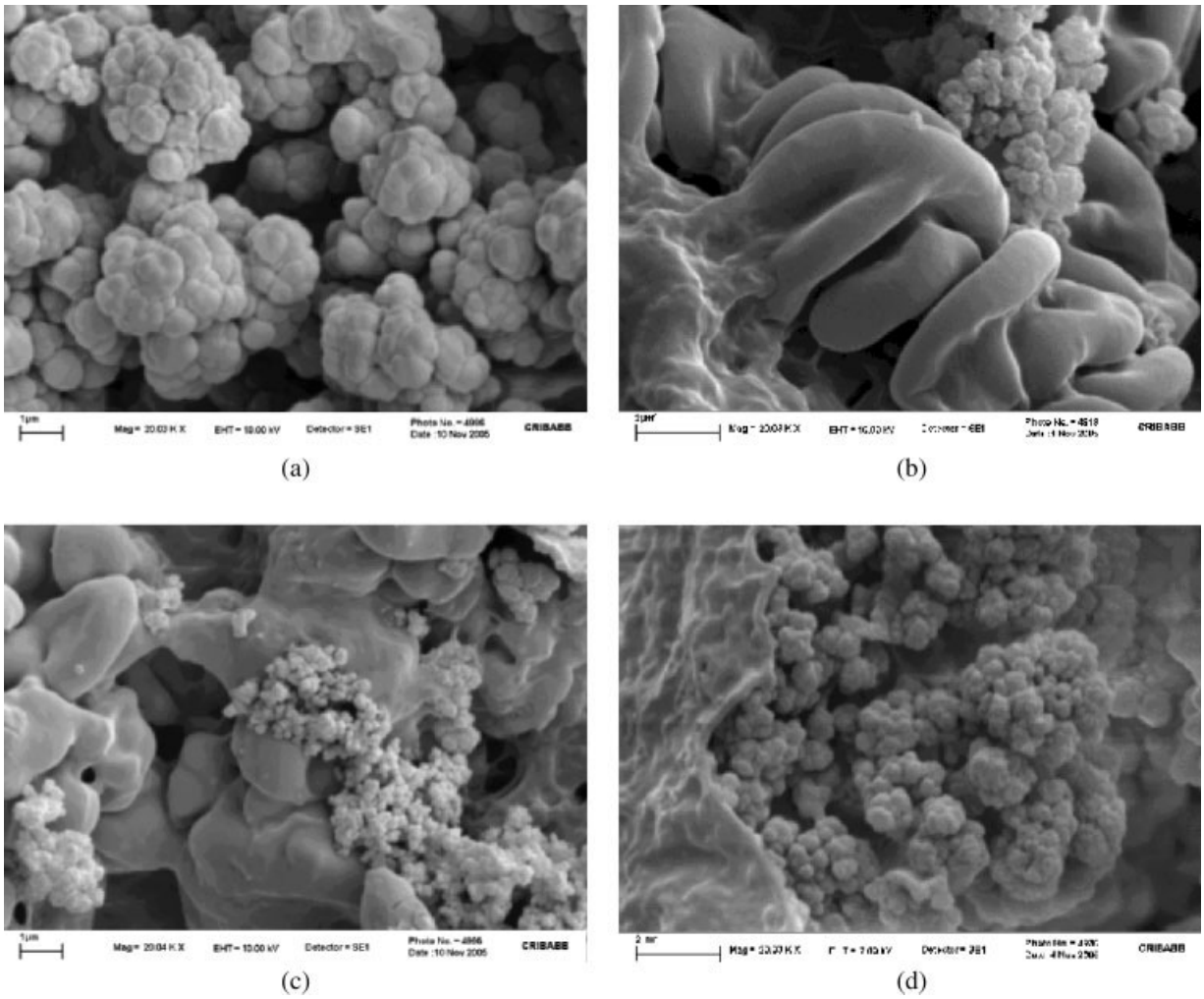


Figure 2. Micrographs of the surface and interior of the unmodified beads I–IV (a)-(d). Magnification scale is shown inside each photograph.

in pore size distribution (50 nm–10 μm) and specific surface area when polymerization conditions vary (Figs. 1 and 3). Increase of the crosslinker content leads to a shift in the curve maximum (50 nm–10 μm) toward lower pore size (Fig. 1), which indicates that smaller microspheres are formed during the polymerization. This behavior is supported by the values of average pore diameter (D) exhibited in Table 2. The values of V_p and S_s (pore size range 50 nm–10 μm) of the networks I–III increase together with the EGDMA content (Table 2), which demonstrates that the number of smaller microspheres also increases. Thus, an increase of the crosslinker content in the synthesis of copolymers creates nuclei so rigid that the number of micropores increases and their size decreases.[26] As a consequence, microspheres of smaller diameter and higher aggregates of constituent particles of the network are obtained, generating also more stable pores, and then higher S_s as well as V_p are reached. Network morphology in swollen state was analyzed by a swelling assay in water. It is well known that two separate processes rule the swelling of polymeric networks. One of them consists in the filling of pores with the swelling liquid determined by the diluent volume separated out of the phase network during polymerization. The other process consists in the solvation of network chains determined by the volume of the remaining diluent in the structure of the network. The solvation process depends on both crosslinking density and interaction between the swelling liquid and the network chains.[26] Accord-

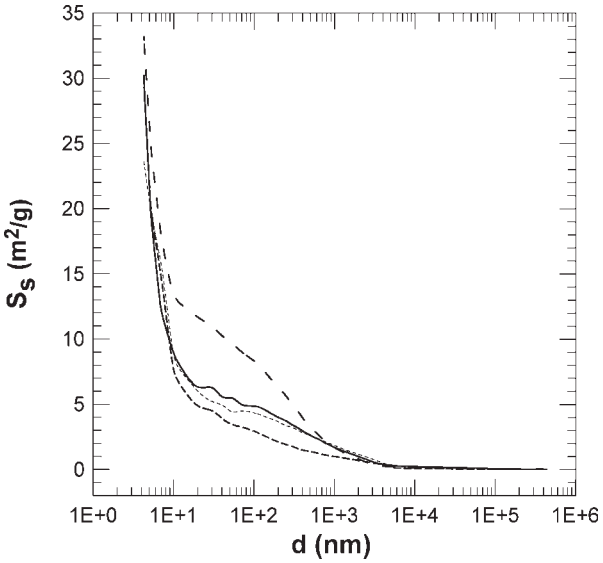


Figure 3. Specific surface area of the heterogeneous networks obtained at 450 rpm of stirring with 6 (- - -), 25 (—), and 33 mol % of EGDMA (– –), or at 750 rpm and 25% of EGDMA (—).

ingly, relative values of Q_w and Q_v (eqs 1 and 2) for heterogeneous networks provide information on the distribution of the diluent between the gel and diluent phases at the end of the network formation process.[26] The porosity of the swollen network (% P) was obtained from Q_w data and true polymer density according to eq 3. Measurements of swollen network porosity are shown in Table 1, as experimental points plotted as a function of the EGDMA content. This performance is similar to that found on the porosity of dry networks, porosity in the swollen state being ~4% higher than in dry state. Moreover, the Q_v value related to the network crosslinking degree decreases due to the fact that the increase of the EGDMA content produces a higher amount of pendants vinyl groups and also an increase of the crosslinking degree. These results demonstrate that networks with a highly stable system of pores were formed.

Analysis of the Effect of Agitation on Network Morphology

Faster agitation in the reaction mixture generates networks II and IV with higher microspheres, V_p and D as seen in Figures 1 and 2(b,d) and Table 2. Thus, a lower S_s (pore size range 50 nm–10 μm) value was reached (Table 2). Stronger agitation applies higher shear to the primary particles, causing them to aggregate at a lower conversion rate and to give irregular grains of higher porosity.[27] However, the increases coalescence frequency together with the impeller speed, in combination with the diminution of the stabilizer molecules, leads to an increase in drop size.[28] Large fused aggregates of microspheres are formed with additional loss of small pores as a consequence of their in-filling, resulting also in a decrease of S_s as the agitation increases.

On the other hand, it is important to note that the influence of stirring speed on all swelling parameters of the networks II and IV was weak, as shown in Table 1. This indicates that agitation did not have a significant effect first on phase separation and then on the crosslinking degree of these systems.

Derivatization of the Polymeric Networks

Poly(EGDMA-*co*-HEMA) networks (I–IV) were activated by substitution of the hydroxyl groups on backbone with oxirane groups of BDGE in a basic aqueous solution (Scheme 1). Here, the epoxy-activated matrices (R-BDGE) were obtained using a mol ratio of (0.9–1.4):12:0.5 of hydroxyl groups, oxirane, and base, respectively. In this study, the yield percentage was obtained from the theoretical hydroxyl content and the oxirane groups quantified on the network. Table 3 shows the effect of network morphology on the activation yield, where an increase in the EGDMA content is found to lead to a typical U-shaped activation curve, which is related to two opposed effects. One of them is the amount of hydroxyl group present on the matrix surface, and the other is the diffusion toward the interior of the network. The amount of hydroxyl decreases for networks with an EGDMA content from 6 to 25 mol % (Table 3), while the values of V_p and S_s (pore size range 50 nm–10 μm) increase

Table 2. Mercury Intrusion Porosimetry

Polymer	EGDMA (mol %)	V_p (mL/g)	S_s (m^2/g)	D[a] (nm)
I[b]	6	0.90	3.4	1066
II[b]	25	1.26	5.3	956
III[b]	33	1.57	9.6	656
IV[c]	25	1.45	4.3	1360

Network parameters correspond to the pore size distribution 50 – 1 × 10^4 nm.
[a] Average pore diameter (D: 4000 × V_p/S_s).
Networks synthesized at [b]450 or [c]750 rpm of agitation.

Table 3. Influence of Network Morphology on Activation Reaction

	Mol Ratio			R-BDGE[b]	
Matrix[a]	R-OH	BDGE	OH^-	μmol/g	Yield (mol %)
I	1.4	12	0.5	127 ± 11	1.8
II	1.0	12	0.5	44 ± 5	0.9
III	0.9	12	0.5	76 ± 1	1.7
IV	1.0	12	0.5	110 ± 6	2.2

[a] Porous poly(EGDMA-*co*-HEMA) beads.
[b] Total oxirane groups of the poly(EGDMA-*co*-HEMA)-BDGE supports.

(Table 2). Here, there is a prevalence of the former effect, which generates a lower amount of terminal oxirane groups in the polymer. On the other hand, the reaction activation increases together with the EGDMA content from 25 to 33 mol %, since the yield depends mainly of both V_p and S_s (pore size range 50 nm–10 μm), as a result of a higher diffusion and capacity of the network (Tables 2 and 3). Moreover, the base support (IV) synthesized using a faster agitation speed shows a higher amount of oxirane group than network (II) (Table 3). This performance is due to the fact that a higher BDGE diffusion into support is reached, since network IV presents a V_p value (Table 2) higher than that found in network II, a behavior supported by the average pore diameter (D).

In a second derivatization pathway (Scheme 1), the oxirane group of the R-BDGE supports was reacted in a basic aqueous solution with the amino group of an alkyldiamine such as HMDA, generating also terminal amino groups on polymeric chains. Table 4 shows that the amine content of the derivatives displays a performance similar to that found during the activation. However, derivative II showed the highest amine coupling yield as the modified supports I–IV were compared. This behavior depends on the oxirane group content and the accessibility of the substrate to the reaction center. Oxirane groups able to react on the network surface are mostly available up to a pore size equal to or larger than the substrate. Table 2 shows that support I has a higher D value than II; however, the former presents also a high network expansion degree (Q_v), which is strongly dependent on the ionic force. It is well known that this type of network shows a low efficiency under unfavorable swelling conditions. On the other hand, although matrix III presents a high crosslinking degree, its D is lower than in II, which makes the oxirane groups less available for the reaction. It is clear that in each derivatization pathway the accessibility of the substrate toward the reaction center is controlled by its size, and its polarity and affinity toward the polymeric backbone should not be discarded either.

Adsorption of BSA on Derivatives with Terminal Amino Groups

The adsorption of BSA by electrostatic interaction (Scheme 1) between ammonium groups on polymeric chains as a function of support morphology was analyzed in batches. The derivatives of R-BDGE-HMDA were mixed with a buffered-solution 1.12 g/L of BSA at pH 7.4 (0.01 M Tris/HCl; 0.1 M KCl) and stirred at room temperature for 80 min. Then, the concentration of BSA in the supernatant was obtained from absorbance measurement at 280 nm by UV–vis spectroscopy. Table 4 shows that the protein is adsorbed in those conditions only by derivatives III and IV, where III presents the highest adsorption value. This behavior is related to the size of the protein and its accessibility toward the ammonium group, as explained earlier. Table 2 displays also that the base supports of derivatives III and IV have a V_p (pore size distribution 50 nm–10 μm) higher than 1.4 mL/g, which gives the BSA molecule an easier diffusion into the network. Considering the high degree of substrate retention shown by the modified support III, the BSA adsorption kinetic study was performed. Figure 4 displays the protein adsorption at pH 7.4 on derivative III as a function of time, using a BSA concentration of 0.45 g/L in buffered solution. It can be seen that

Table 4. Amine Coupling Reaction and the Effect of Network Morphology on BSA Adsorption

R-BDGE-HMDA Derivatives	Terminal Amine Groups Yield		BSA Adsorption (mg/g)
	μmol/g	mol %	
I	113 ± 10	89	0
II	42 ± 2	96	0
III	53 ± 5	70	4.6 ± 0.3
IV	64 ± 8	58	1.8 ± 0.2

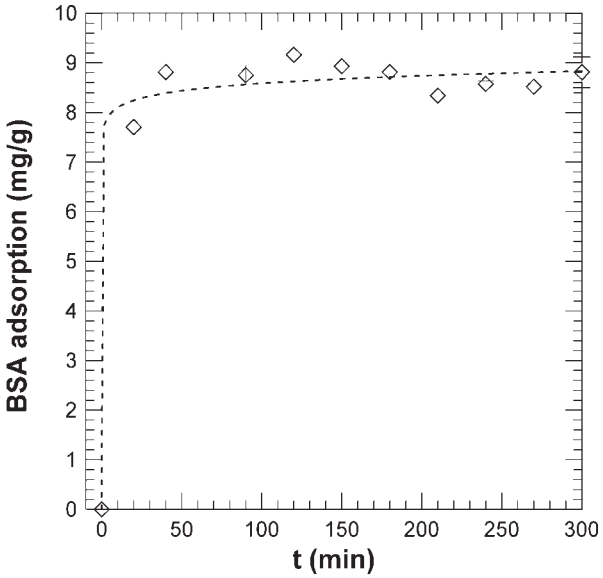


Figure 4. Protein adsorption kinetic on derivative III modified with HMDA.

the adsorption curve reaches a plateau (8.5 mg/g) at 35 min, which is related to the accessibility of the protein to the interior of the network (Fig. 4). BSA enters the network up to a pore size equal to or higher than the conformation of the protein, and the electrostatic interaction takes place with the ammonium group corresponding to HMDA. Here, the available ammonium groups are present on that support surface existent between pore sizes larger than the substrate, and the protein reaches also a faster access (0.385 mg/min) to the interaction center. This performance would confirm the effect that the disposition of the coupled-diamine on the matrix surface has on the accessibility of the protein, as a consequence of network morphology.

CONCLUSIONS

Four porous poly(EGDMA-*co*-HEMA) beads were synthesized by suspension polymerization using different EGDMA contents and agitation speeds. The network III synthesized with the highest EGDMA content (33 mol %) showed the highest values of porosity as well as of V_p and S_s (pore size range 50 nm–10 μm), giving a high diffusion and capacity to the matrix. Cyclization reactions led to an earlier phase separation, yielding a higher aggregation of smaller rigid microspheres, by which stable pore systems were also formed.

On the other hand, when the influence of stirring in network morphology was analyzed, it was observed that the base matrix IV obtained at 750 rpm of agitation displayed a V_p (pore size range 50 nm–10 μm) higher than that of network II, since the size of the microspheres increased together with the stirring speed. Here, large fused aggregates of microspheres were formed with additional loss of small pores, attaining also a higher pore volume (V_p) and a slight decrease of the specific surface area.

Then, the modification of the network hydroxyl with terminal amino groups from an alkyldiamine as HMDA was carried out to study the influence of support morphology on the degree of BSA adsorption. This study clearly demonstrated that derivatives III and IV, whose initial matrices present a V_p (pore size range 50 nm–10 μm) higher than 1.4 mL/g, were able to adsorb the protein, which highlights the importance of network morphology in the adsorption degree. BSA enters the network up to a pore diameter equal to or higher than its conformation and then the ammonium group adsorbs the protein.

We thank students Natalia Gonzalez and Julieta I. Paez for their help in laboratory work. Moreover, we gratefully acknowledge the financial assistance of Consejo Nacional de Investigación Científica y Tecnológica (CONICET), Fondo para la Investigación Científica y Tecnológica (FONCYT), and Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECYT).

REFERENCES AND NOTES

1. Ismail, I. M.; Nogami, M.; Suzuki, K. Sep Purif Technol 2003, 31, 231–239.
2. Zhou, X.; Xue, V.; Bai, S.; Sun, Y. Biochem Eng J 2002, 11, 13–17.
3. Dragan, E. S.; Avram, E.; Axente, A.; Marcu, C. J Polym Sci Part A: Polym Chem 2004, 42, 2451–2461.
4. Houen, G. D.; Olsen, T.; Hansen, P. R.; Petersen, K. B.; Barkholt, V. Bioconj Chem 2003, 14, 75–79.
5. Fuentes, M.; Batalla, P.; Grazu, V.; Pessela, B. C. C.; Mateo, C.; Montes, T.; Hermoso, J. A.; Guisan, J. M.; Fernandez-Lafuente, R. Biomacromolecules 2007, 8, 703–707.
6. Uzun, L.; Say, R.; Denizli, A. React Funct Polym 2005, 64, 93–102.

7. Wang, R.; Zhang, Y.; Ma, G.; Su, Z. Colloids Surf B 2006, 51, 93–99.
8. Kracalikova, K.; Tishchenko, G.; Bleha, M. J Biochem Biophys Methods 2006, 67, 7–25.
9. Gong, B.; Zhu, J.; Li, L.; Qiang, K.; Ren, L. Talanta 2006, 68, 666–672.
10. Houen, G. J Biochem Biophys Methods 2001, 49, 189–197.
11. Machold, C.; Schlegl, R.; Buchinger, W.; Jungbauer, A. J Biotechnol 2005, 117, 83–97.
12. Han, B.; Specht, R.; Wickramasinghe, S. R.; Carlson, J. O. J Chromatogr A 2005, 1092, 114–124.
13. Baydemir, G.; Andacü, M.; Bereli, N.; Say, R.; Denizli, A. Ind Eng Chem Res 2007, 46, 2843–2852.
14. Nguyen, A. M.; Irgum, K. Chem Mater 2006, 18, 6308–6315.
15. Fan, J. W.; Deng, J. Y.; Xing, C. M.; Yang, W. T. J Polym Sci Part A: Polym Chem 2006, 44, 653–658.
16. Zhou, X.; Xue, B.; Sun, Y. Biotechnol Prog 2001, 17, 1093–1098.
17. Gomez, C. G.; Alvarez, C.; Strumia, M.; Reyes, P.; Rivas, B. J Appl Polym Sci 2001, 79, 920–927.
18. Gomez, C. G.; Alvarez, C.; Strumia, M. Polymer 2005, 46, 6300–6307.
19. Gomez, C. G.; Alvarez, C.; Strumia, M. J Biochem Biophys Methods 2001, 49, 141–151.
20. Gomez, C. G.; Alvarez, C.; Strumia, M. Polym Bull 2003, 49, 425–432.
21. Gomez, C. G.; Alvarez, C.; Strumia, M. Polymer 2004, 45, 6189–6194.
22. Gomez, C. G.; Alvarez, C.; Strumia, M. J Biochem Biophys Methods 2003, 55, 23–36.
23. Hradil, J.; Horák, D. React Funct Polym 2005, 62, 1–9.
24. Lee, H.; Neville, K. Handbook of Epoxy Resins; Mc-Graw-Hill: New York, 1967; Chapter 4, p 17.
25. Kasgoz, H.; Ozgumus, S.; Orbay, M. Polymer 2003, 44, 1785–1793.
26. Okay, O. Prog Polym Sci 2000, 25, 711.
27. Yuan, H. G.; Kalfas, G.; Ray, W. H. JMS-Rev Macromol Chem Phys 1991, C 31, 2–3, 231–248.
28. Georgiadou, S.; Brooks, B. W. Chem Eng Sci 2005, 60, 7137–7152.