Macroporous Bead Modification with Polyethylenimines of Different Molecular Weights as Polycationic Ligands

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ABSTRACT: Polyethylenimines (PEIs) with different molecular weights [number-average molecular weights (M_n's) = 60,000, 1200, and 423] were coupled onto macroporous beads. These rigid and spherical beads were prepared by the crosslinking of 2-hydroxyethyl methacrylate and ethylene glycol dimethacrylate. The PEI attachment was carried out through epoxy groups yielded in a previous activation step with epichlorohydrin on matrix hydroxyl groups. Different initial concentrations of PEI were assayed. The supports so obtained were characterized by several techniques (Fourier transform infrared spectroscopy, scanning electron microscopy, thermogravimetric analysis, and mercury intrusion porosimetry). All of the PEI-containing beads were used to analyze the influence that the molecular weight, the shape of the polycationic ligand (PEI), and the degree of coupling onto the matrices may have had on the efficiency of the retention of the bovine serum albumin protein used as a model biomolecule. In these assays, the PEI-modified beads with M_n = 60,000 showed better results than those modified with PEIs with M_n's of 1200 and 423. The presence of sparse and long chains of PEI 60,000 onto the matrix, by reason of their highest accessibility toward the large protein, may have resulted in a better disposition of functional groups, whereas more short chains in the other PEIs (M_n's = 1200 and 423) used as ligands would not have. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2857–2865, 2010

Key words: functionalization of polymers; macroporous polymers; structure–property relations; supports; surfaces

INTRODUCTION

The purification of biomolecules has been a topic of research by several investigators because biological macromolecules are of great interest as commercial bioproducts. Chromatography is the technique of separation most commonly used for the isolation and purification of biomolecules. A wide range of chromatographic techniques has been used for large-scale biopurifications: size-exclusion, ion-exchange, hydrophobic interaction, reversed-phase, and affinity chromatography.[1]

The success of the purification of biomolecules is due to several factors. These factors should be taken into account in the design of a material capable of separating biomolecules from a mixture or biological fluid.[2] For the choice of a matrix, two parameters must be considered: a knowledge of the matrix itself (surface area per unit volume, pore size, pore structure, and ligand density potential) and a knowledge of the target molecule (size, shape, and nature of binding with the ligand in solution).[3] Macroporous crosslinked polymers are effective and efficient base materials for many separation processes because of their physical properties. Therefore, they can be used as starting materials for adsorptive and chromatographic separation and for many other applications such as immobilization techniques and cell culturing.

Various parameters, such as particle size, porosity, surface area, swelling, pore volume, and specific residual functional groups, have been taken into account to describe the overall performance of polymer beads in these applications.[2–5]

The selectivity of the specific ligand is perhaps the most important parameter. It was demonstrated that the attachment of polymer chains onto the surface of porous polymer beads to form tentacle-type supports could sufficiently increase the adsorption capacity of proteins.[6–9] The enhanced adsorption capacity of these modified polymer adsorbents apparently arose because the tentacles could extend away a sufficient distance from the support surface

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to allow proteins to penetrate the polymer layer and bind in multiple layers. With tentacle-type chains, the contact between the protein and the base support surface was also markedly reduced; this minimized undesirable hydrophobic interactions between the protein and the base support surface.[6–9] Amino-carrying polymers can be used as tentacles ligands in affinity chromatography for biomolecules with an affinity for amine groups and also in ion-exchange chromatography by the presence of positively charged groups at certain pH values.[10]

Polyethylenimine (PEI) exists as both a branched polymer and in linear form. The branched form of PEI shows a theoretical ratio of primary to secondary to tertiary amine groups of 1 : 2 : 1. These amines have pK_a values spanning the physiological pH range; this results in a buffering capacity. Branched PEI is one of the most extensively investigated polycations as a nonviral gene carrier and for gene delivery.[11,12] A PEI with long spacers can be immobilized on a surface; this allows for flexible and multifunctional sites on the surface in a biological environment. Various antecedents, in which PEI was covalently bound on polymeric matrices and used as tentacles for different purposes, have been reported. PEI-immobilized adsorbers (Sepharose 4B; flat-sheet and hollow-fiber membranes or cellulose fibers) have been used for the removal of *Escherichia coli* derived endotoxin from solutions.[8,13–15] PEI or polymyxin B ligands were immobilized on cellulose beads for the extracorporeal removal of endotoxin from plasma. A similar degree of efficacy was attained with the two different ligands (PEI or polymyxin B), but a higher biocompatibility was obtained when PEI was used.[7] Supermacroporous monolith columns with immobilized PEI, polymyxin B, and lysozyme were shown to be efficient in the capture of bacterial endotoxins from protein solutions and, hence, for the decontamination of these solutions.[16] Also, PEI-attached poly(*p*-chloromethylstyrene) beads were used as sorbents in DNA adsorption,[17] and Cu^{2+} chelated on PEI magnetic poly(2-hydroxyethylmethacrylate) beads was used for cytochrome c adsorption.[6] Thus, bilirubin adsorption from human serum was assayed with PEI-containing poly(glycidyl methacrylate–methyl methacrylate) microspheres.[18]

Although there have been several studies that have used PEI as a ligand, the influence of the length or molecular weight of PEI and coupling degree on its efficiency as a ligand in affinity chromatography has not yet been studied extensively. In this way, when tentacle ligands are used, it is important to establish how the surface architecture affects the binding capacity, to optimize the efficiency of the support for use in some chromatographic techniques. Thus, the main aim of this study was to examine the influence of the molecular weight, the shape of the polycationic ligand (PEI), and the degree of coupling onto the matrices on the efficiency of the retention of bovine serum albumin (BSA) (Merck, Darmstadt, Germany) used as a model biomolecule. Therefore, crosslinked hydroxyethyl methacrylate-*co*-ethylene glycol dimethacrylate copolymer beads were synthesized, activated with epichlorohydrin (Ech), and modified by coupling with different PEIs [number-average molecular weights (M_n's) = 60,000, 1200, and 423].

EXPERIMENTAL

Materials

The following chemicals were purchased and used: 2-hydroxyethyl methacrylate (HEMA; Fluka, USA), ethylene glycol dimethacrylate (EGDMA; Fluka), cyclohexanol (Cicarelli, Buenos Aires, Argentina), azobisisobutyronitrile (AIBN; Aldrich, Steinheim, Germany), polyvinylpyrrolidone (PVP K 90; Fluka Chemie AG, Buchs, Switzerland), Ech (Mallinckrodt, Baker, Inc., Phillipsburg, NJ), PEI with an M_n of 60,000 (50% w/w aqueous solution; Sigma, USA), PEI with an M_n of 1200 (50% w/w aqueous solution; Aldrich), PEI with an M_n of 423 (Aldrich), BSA (Merck, Darmstadt, Germany), and sodium carbonate (Cicarelli).

Synthesis and activation of macroporous beads

A suspension polymerization was performed to obtain spherical crosslinked hydroxyethyl methacrylate-*co*-ethylene glycol dimethacrylate copolymer [poly(HEMA-*co*-EGDMA)] beads. The continuous phase was comprised of an aqueous solution (250 mL) of 1% w/v PVP. The discontinuous organic phase consisted of HEMA (21.6 mL) and EGDMA (8.4 mL). Cyclohexanol (42.3 mL) was used as a porogen. The polymerization was initiated by AIBN and kept under stirring (450 rpm) for 3 h at 70°C. After cooling, the beads were purified by Soxhlet with ethanol and then with acetone to remove any unreacted monomer or the porogen and dried *in vacuo* until constant weight was reached. The total level of monomer conversion in the products (yield) was found by the determination of the dry weight of the beads. The yield was calculated as the ratio of the dry weight of product to the initial monomer weight, including crosslinker. Poly(HEMA-*co*-EGDMA) beads were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and mercury intrusion porosimetry. The equilibrium volume swelling ratio (q_v) was calculated as the ratio of the volume of the samples in the

swelling equilibrium state (V_{sw}) in water to the volume of the samples in the dry state (V_{dry}). The q_v value was obtained with graduated tubes after the sample had been soaked for 24 h in an excess of distilled water.

The hydroxyl groups of poly(HEMA-*co*-EGDMA) were activated with Ech in a basic medium. The activation step was carried out with a molar ratio of 1 : 2 : 3 of hydroxyl groups to epoxide groups to 10*M* NaOH, respectively. The beads were left in water for 24 h before this reaction, which was carried out under stirring for 7.5 h at room temperature. After this, the activated beads were washed with distilled water and ethanol, dried *in vacuo* until a constant weight was reached, and studied by TGA and SEM. Oxirane group quantification assays were performed with the pyridinium chloride method.[19]

PEI ligand attachment onto the activated beads

The activated dry beads (0.8 g) were added to an aqueous solutions (12 mL) containing 2*M* sodium carbonate and various concentrations of PEIs of different M_n's (60,000, 1200, and 423) and kept under agitation for 7 days at room temperature. The experimental conditions are summarized in Table I. Then, the supports were extensively washed with distilled water and ethanol to remove any physically adsorbed PEI on beads. The modified beads were dried at room temperature under reduced pressure. The final products were named as shown in Table I. TGA, elemental analysis, and SEM techniques were used to characterize the PEI-containing beads. Their free amino content was determined by acid–base titration. For this purpose, the different PEI-modified beads (0.1 g of each) were treated with a 0.1*M* standard HCl solution (5 mL) for 24 h at room temperature under stirring. After the consumption of HCl by the free amine groups of the modified beads, the mixture was filtered, and 1 mL of the final HCl concentration in the aqueous medium was determined (in duplicate) by titration with 0.037*N* NaOH. The amino content of the PEI-modified beads was calculated from the differences in the acid contents of the initial and final solutions. The results are summarized in Table I.

TABLE I
Different Initial Concentrations of the Three PEIs Used (M_n's = 60,000, 1200, and 423) to Reach PEI-Containing Products and the Amount of Amine Groups Yielded by the Modification of Activated Beads with PEI

PEI-containing product[a]	C_i (% w/v)[d]	mequiv of amine/g of dry polymer
R1.PEI.60,000[a]	1.0	0.47
R2.PEI.60,000[a]	2.0	0.16
R3.PEI.60,000[a]	4.0	0.18
R4.PEI.60,000[a]	6.5	0.48
R5.PEI.60,000[a]	8.0	0.73
R1.PEI.1200[b]	1.0	0.28
R2.PEI.1200[b]	2.0	0.25
R3.PEI.1200[b]	4.0	0.35
R4.PEI.1200[b]	6.5	0.40
R5.PEI.1200[b]	8.0	0.58
R1.PEI.423[c]	1.0	0.58
R2.PEI.423[c]	2.0	0.43
R3.PEI.423[c]	4.0	0.47
R4.PEI.423[c]	8.0	0.63
R5.PEI.423[c]	10.0	0.96

[a] PEI $M_n = 60{,}000$.
[b] PEI $M_n = 1200$.
[c] PEI $M_n = 423$.
[d] C_i = initial concentration of PEI.

Assays of BSA adsorption

The PEI-containing beads were used as BSA adsorbents at pH 8.0 (0.02*M* Tris-HCl buffer) in a batch system for 3 h. So, the modified beads (0.15 g) were put in contact with BSA solutions (10 mL) of known concentration (0.45 or 0.72 g/L, in duplicate) under stirring. Unmodified beads (0.15 g for each assay) were put in contact with both BSA solutions (0.45 or 0.72 g/L) to serve as blank experiments.

Before and after adsorption, the amount of BSA in the solutions was calculated by absorbance measurements at 280 nm. Previously, a calibration curve was performed. After the retention assays, the supports were washed with 0.02*M* Tris-HCl buffer (pH 8.0) until the absorbance value (at 280 nm) of the withdrawn sample was zero. Later, the protein was desorbed with 0.02*M* Tris-HCl buffer prepared with 1*M* NaCl (pH 8.0).

Equipment

The characterization of the products by FTIR was performed with KBr pellets on a Nicolet (USA) 5-SXC spectrometer. The surface morphology and internal structure of the products were observed by SEM with a LEO model EVO 40 (Cambridge, UK) XVP in Centro Regional de Investigaciones Básicas y Aplicadas Bahía Blanca, Bahía Blanca, Argentina.

The porous properties of the beads were determined by mercury intrusion porosimetry with an Autopore 9200 Micromeritics (USA) in Centro Atómico Bariloche, Río Negro, Argentina. The TGAs were performed with a TA Instruments H1-Res TGA 2950 (TA Instruments, New Castle, DE). The samples (1.5–3.0 mg) were weighed in aluminum pans and heated at a scan rate of 10°C/min between 25/50 and 550/600°C in an atmosphere of air. Elemental analysis assays were performed in a Fisons ES-1108 instrument (Beverly, MA). For the protein absorbance measurements, a Shimadzu (Kyoto, Japan) UV-260 recording spectrophotometer was used.

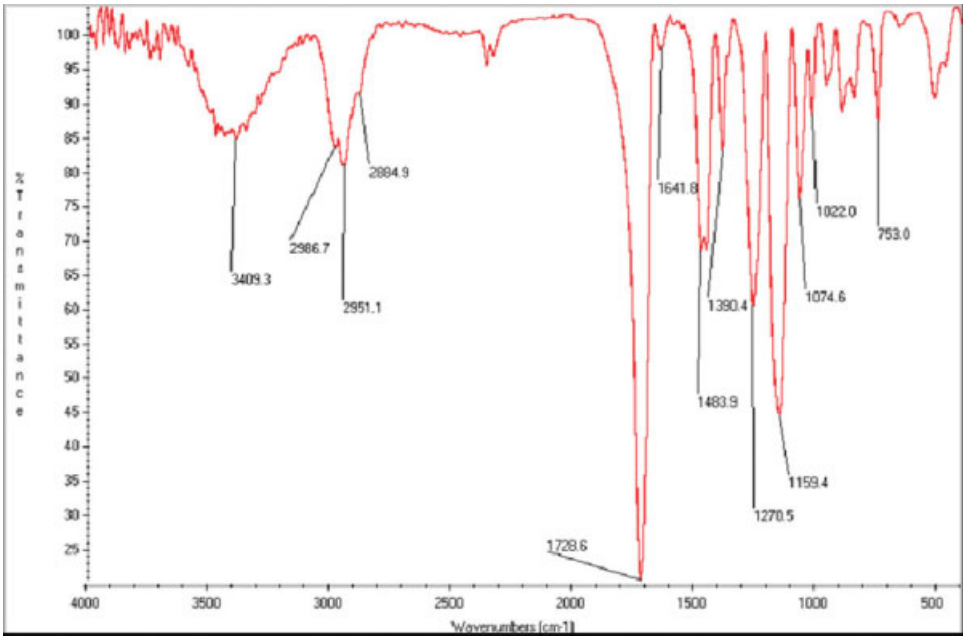

Figure 1 FTIR spectrum of the poly(HEMA-*co*-EGDMA) beads. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS AND DISCUSSION

Synthesis and activation of the macroporous beads

One of the topics of discussion within our research group[5,20–26] is the synthesis of macroporous matrices that provide surface area for chemical modification to reach efficient adsorbents for affinity chromatography and that allow internal diffusion of great size solutes. On this occasion, the poly(HEMA-*co*-EGDMA) beads, with an initial molar ratio of 4 : 1 of HEMA : EGDMA in the medium of the reaction, were synthesized by suspension polymerization to generate porous crosslinked spherical beads. PVP, AIBN, and cyclohexanol were used as the stabilizer, initiator, and porogen, respectively. These constituted a white solid whose yield was 100%. The IR spectrum (Fig. 1) showed the following characteristic signals (KBr, cm^{-1}): 3409 (—OH stretching vibration), 1728 (ester group, C=O stretching vibration), 1270 (—OH group bending vibration), and 1074 (—C—O of C—OH stretching vibrations). q_v (q_v = 1.3) was calculated as the ratio of V_{sw} to V_{dry}. The matrix showed a very low q_v in water and retained the shapes of the beads. This might have indicated that the resulting matrix was rigid and suitable for use as a starting material in chromatography. It possessed a high porosity (74%), and its specific surface area and the total pore volume value were 32.3 m^2/g and 2.14 mL/g, respectively. As was mentioned, the reaction yield was 100%, for which all hydroxyl groups present in the sample were aroused by the amount of HEMA used for the reaction. Thus, the density of hydroxyl groups was 5.56 mmol of hydroxyl groups/g or 172.22 μmol/m^2. Mercury porosimetry was used to measure the distribution of pores with a radius larger than 50 Å (corresponding to macropores). As shown by the mercury intrusion porosimetry analysis (Fig. 2), the beads had a monodispersed population of macropores. These characteristics help in the diffusion of reagents used for chemical modification.

Figure 3 shows by SEM the morphology of the unmodified poly(HEMA-*co*-EGDMA) beads, which corresponded to a structure formed by spheres, which agglomerated in large entities enclosing holes.

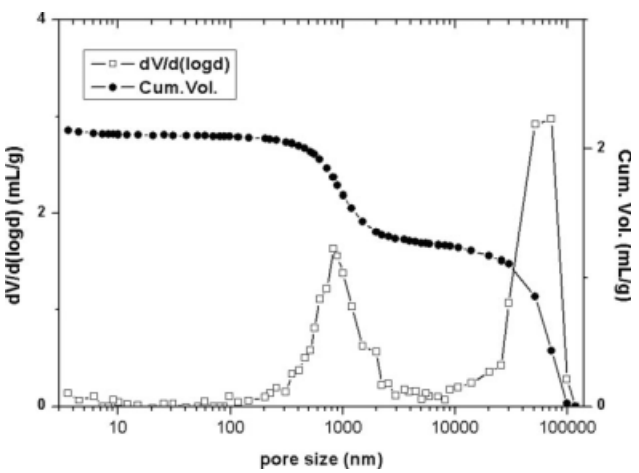

Figure 2 Differential and cumulative curves of the pore size distribution of the poly(HEMA-*co*-EGDMA) beads. dV/d(log d), pore volume distribution function; where V is the cumulative pore volume and d is pore diameter; Cum.Vol., cumulative pore volume.

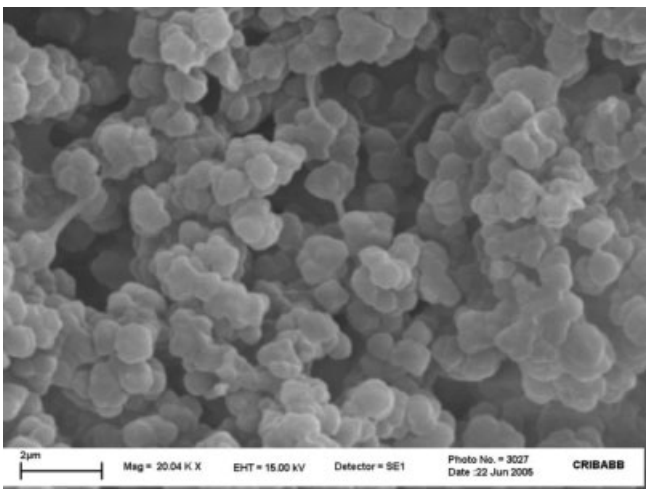

Figure 3 Electron micrographs of the unmodified poly (HEMA-*co*-EGDMA) beads (20 mx; size of the bar = 2 μm).

Figure 4 shows the thermogram of the unmodified poly(HEMA-*co*-EGDMA) beads. The decomposition peak temperatures of the unmodified poly(HEMA-*co*-EGDMA) beads were found to be 246 and 363°C. The overall degradation involved two steps, due probably to the presence of two different monomers in the network.[27] The sample began to decompose at 207°C and lost 48.4% of its weight up to about 267°C. Beyond 267°C, there was a second loss of weight (51.6%) up to 550°C.

The poly(HEMA-*co*-EGDMA) beads were modified by an activation reaction to couple PEI. Poly (HEMA-*co*-EGDMA) was chosen because of its mechanical strength, surface area, and biological and chemical stability[27] and because of the presence of hydroxyl groups from HEMA, which supply hydrophilicity. Other advantages for the possibility of chemical modification were originated by the presence of HEMA. Examples of the derivatization of HEMA-containing matrices to bind different ligands have been described by several authors. In the study in ref. 6, ester groups in magnetic poly(2-hydroxyethyl methacrylate) were converted to amine groups by reaction with PEI in the presence of NaH. On the other hand, poly(HEMA-*co*-EGDMA) beads were grafted with poly(glycidylmethacrylate), and their epoxy groups were modified into sulfone groups for the immobilization of *Candida rugosa* lipase.[28] In addition, glutaraldehyde activation on poly(HEMA-*co*-EGDMA) was used to couple β-cyclodextrin (for the removal and preconcentration of phenolic species)[27] and Concanavalin A (for cell affinity applications).[29]

In this study, Ech was used as an activator agent for the attachment of PEI to the beads and spacer arm between the surface of the beads and the functional groups of PEI. The activation of the hydroxyl groups of beads with Ech was carried out according to the experimental conditions previously studied, which yielded satisfactory results.[21,22,25] This reaction yielded 0.3 mmol of epoxy groups/g of dry polymer, for which the density of epoxy groups on the porous surface was 9.29 μmol/m^2 and the estimated reaction efficiency was 5.4%. After the activation, the beads presented a porous morphology similar at that previously described for unmodified beads. The thermal studies demonstrated that the network became more stable because it began to decompose at 254°C. Its decomposition peak temperature was found to be 277°C. Its thermogram showed only one broad band.

PEI ligand attachment onto the activated beads

The activated beads were successfully modified with an amino-carrying polymer as PEI. The long and flexible ligand was covalently attached to the surface of the activated poly(HEMA-*co*-EGDMA) beads. This was carried out via the nucleophilic addition of primary and secondary amino groups of PEI to epoxy groups present in the activated matrices under basic conditions.[3,30] This chemical reaction and the two possible final structures are represented in Figure 5. After the PEI derivatization process, the white beads changed to a yellow color; this was the first indication that PEI had attached to the beads.

The amount of amino groups yielded in the coupling step from different initial PEI concentrations is given in Table I. PEIs with three different molecular weights were used as follows: 60,000, 1200, and 423. In general, with the use of any one of the three different PEIs at a greater initial PEI concentration of 2% w/v (for products R2–R5.PEI.60,000, R2–R5.PEI.1200, and R2–R5.PEI.423; Table I), a higher amino content in the modified beads was reached. However, when the reaction was carried out with a

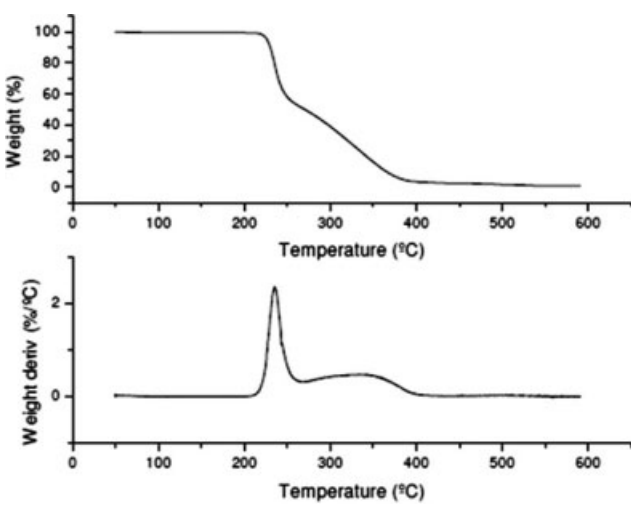

Figure 4 TGA thermogram of the unmodified poly (HEMA-*co*-EGDMA) beads.

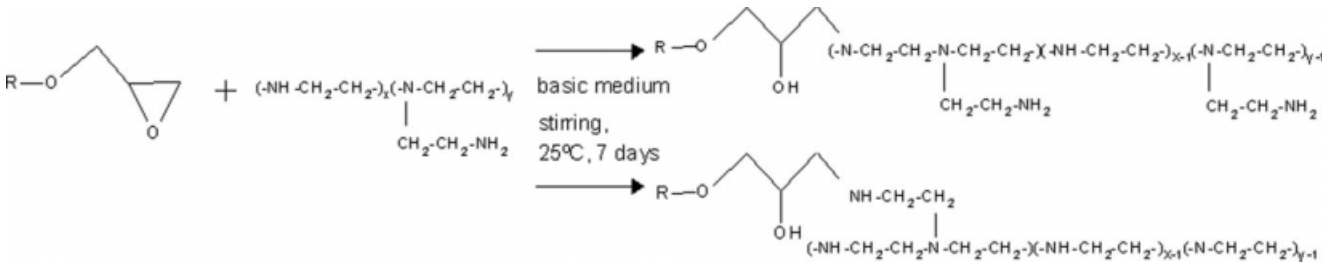

Figure 5 Scheme of the chemical reaction between activated beads and PEI. The nucleophilic addition of the secondary (upper) and primary (inferior) amino groups of PEI to the epoxy groups are represented by the two possible final structures.

1% w/v initial PEI concentration (for products R1.PEI.60,000, R1.PEI.1200, and R1.PEI.423; Table I), a higher amino content than expected was obtained. The explanation for this might be that a lower concentration of PEI involved fewer polymeric chains in solution, which could have been easily distributed with major diffusion and minor steric hindrance through the surface of the porous matrices.

For any given initial concentration of PEI, the highest milliequivalents of amine content per gram of dry polymer was reached when PEI with an M_n of 423 was coupled (0.43–0.96 mequiv/g). These values proved similar to those found by other authors.[17] Comparatively, lower values were obtained when PEI with an M_n of 60,000 was used for the attachment (0.16–0.73 mequiv/g). These behaviors could have been due to the molecular size of the different PEIs used because chains of PEI of low molecular weight could have been disposed in a dense hairy or brush form, whereas chains of PEI with a high molecular weight could have been either in a more extended and sparse brush or in a multipoint disposition because of its higher molecular size. In this last case, fewer PEI tails could have been bound and fewer amino groups could have been attached to the beads.

Also, because of the molecular shape of the different PEIs used, the chains of PEI with a high molecular weight could have had major steric hindrance for the attachment with respect to chains of PEI with a low molecular weight.

Figure 6 shows the morphology of R5.PEI.60,000. It was possible to observe again the porous structure of these beads that were modified with the PEI polymeric ligand. The micrographs of R5.PEI.1200 and R5.PEI.423 (not shown) were similar to that of R5.PEI.60,000.

TGA thermograms of PEI-containing beads were obtained to characterize and compare them with those of the unmodified and activated poly(HEMA-*co*-EGDMA) beads. Figure 7 shows the thermograms in which weight (%) versus temperature were plotted. Figure 8 shows the curves of derivative weight versus temperature of the unmodified and activated poly (HEMA-*co*-EGDMA) and PEI-containing beads. As shown in Figure 8, the coupling of PEI onto activated beads yielded products whose curves of derivative weight versus temperature showed a degradation process occurring with wide bands and probably involving at least two nondistinguished steps. In general, PEI-containing beads (R4.PEI.60,000, R4.PEI.1200, and R4.PEI.423) began their

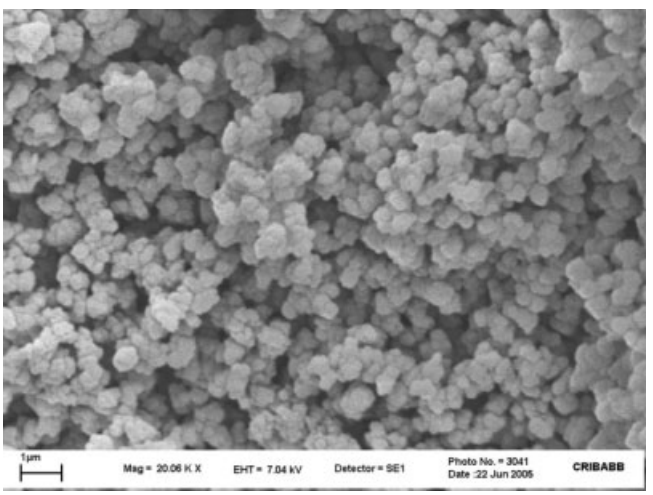

Figure 6 Electron micrographs of R5.PEI.60,000 (20 mx; size of the bars = 1 µm).

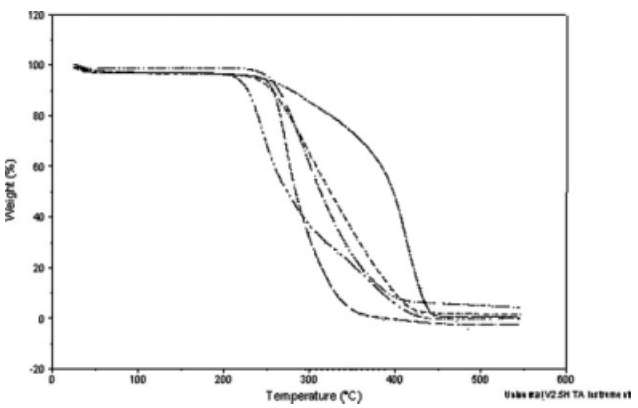

Figure 7 TGA thermograms of the (—— –) unmodified poly(HEMA-*co*-EGDMA), (——) activated poly(HEMA-*co*-EGDMA), and PEI-containing beads: (—·) R4.PEI.60,000, (– – –) R4.PEI.1200, and (—) R4.PEI.423.

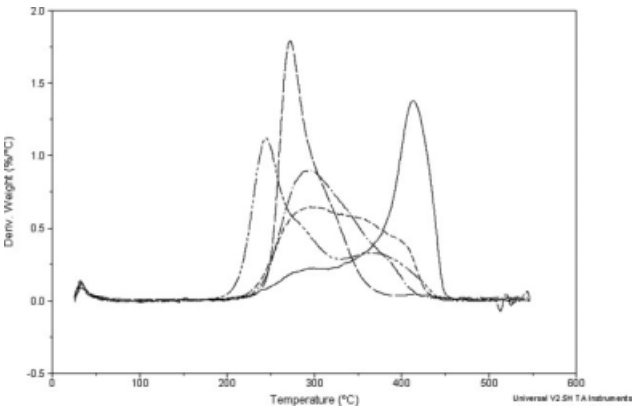

Figure 8 Curves of the derivative weight versus temperature of the (—— –) unmodified poly(HEMA-*co*-EGDMA), (——) activated poly(HEMA-*co*-EGDMA), and PEI-containing beads: (—·) R4.PEI.60,000, (– – –) R4.PEI.1200, and (—) R4.PEI.423.

decomposition at almost the same temperature as the activated beads. In the curves of the derivatives (Fig. 8), bands could be observed whose maximum decomposition peak shifted at higher temperatures when the PEI of lower molecular weight (423) was used. The maximum decomposition peaks registered for R4.PEI.60,000, R4.PEI.1200, and R4.PEI.423 were 289, 300, and 410°C, respectively. The most stable product was R4.PEI.423.

Elemental analysis assays were performed on the following PEI-containing beads, to determine their nitrogen content (in percentage): R5.PEI.60,000, R5.PEI.1200, and R5.PEI.423. The percentage nitrogen present in these samples (0.73, 0.64, and 1.12%, respectively) proved proportional to the amount of milliequivalents of amine per gram of dry polymer previously informed in Table I (0.73, 0.58, and 0.96 mequiv of amine/g of dry polymer for R5.PEI.60,000, R5.PEI.1200, and R5.PEI.423, respectively). The results are shown in Table II.

TABLE II
Results of the Elemental Analysis Assays Performed with Some PEI-Containing Products

PEI-containing product	C (%)	H (%)	O (%)	N (%)
R5.PEI.60,000	53.12	8.46	37.69	0.73
R5.PEI.1200	53.18	8.69	37.49	0.64
R5.PEI.423	52.47	8.50	37.91	1.12

Assays of BSA adsorption

The adsorption assays of the different PEI-containing beads were carried out with BSA as a model protein. The tentacle-type polymeric ligand PEI is composed of amine functional groups. Therefore, at a certain pH, it is able to bind proteins with a net negative charge, such as BSA (pI 4.8). In these assays, pH 8.0 and a low ionic strength were used. At this pH, the PEI polymeric ligand functioned as a weak anion-exchange polyligand, and BSA was negatively charged. Ion-exchange interaction forces could have been the most dominant interactions, achieved via polyion complexation and involved to a large extent in the adsorption mechanism.[31]

TABLE III
Values of BSA Adsorption

PEI-containing product	BSA (mg/g of dry polymer)[a]	
	C_i[b] = 0.45 g/L	C_i[b] = 0.72 g/L
Unmodified beads	0.0	0.0
R1.PEI.60,000	21.9	24.0
R2.PEI.60,000	19.0	6.0
R3.PEI.60,000	17.0	19.0
R4.PEI.60,000	21.0	20.0
R5.PEI.60,000	25.8	34.4
R1.PEI.1200	12.1	12.5
R2.PEI.1200	2.0	9.0
R3.PEI.1200	15.0	9.0
R4.PEI.1200	15.0	17.0
R5.PEI.1200	15.6	15.1
R1.PEI.423	7.8	7.7
R2.PEI.423	0.0	4.0
R3.PEI.423	0.0	4.0
R4.PEI.423	5.0	5.0
R5.PEI.423	8.9	10.7

[a] Amount of BSA retained.
[b] C_i = initial concentration of BSA in the solution.

Two different initial concentrations of BSA were used in the assays: 0.45 and 0.72 g/L. The variation in the BSA adsorption capacity with the two different initial BSA concentrations is given in Table III for the PEI-containing beads. The unmodified poly (HEMA-*co*-EGDMA) beads used as blank material did not adsorb BSA. In several cases, as shown in Table III, the BSA adsorption capacity increased slightly when a greater initial concentration of BSA solution (0.72 g/L) was used.

With respect to the distinct molecular weights of the PEI used (423, 1200, or 60,000), three different behaviors were observed. The matrices modified with PEI with an M_n of 423 were inefficient in the adsorption of BSA (for the two different initial concentrations of BSA used in the assays, given in Table III) because zero or low values of retention of the protein were obtained. The matrices modified with PEI with an M_n of 1200 reached values of retention of BSA larger than those beads containing PEI with an M_n of 423. In accordance with the values of retention observed in Table III, we concluded that a possible saturation of the PEI ligands could have been reached when 0.45 g/L was used as the initial concentration of BSA. Despite the fact that the matrices modified with PEI with an M_n of 60,000 showed, in general, the lowest amino content (than the 423-containing beads), they were able to retain the highest

BSA amount (for both initial concentration of BSA assayed, given in Table III) and reached values of 25.8 and 34.4 mg of BSA/g of dry R5.PEI.60,000 support when 0.45 or 0.72 g/L was used, respectively, as the initial concentration of BSA. The highest values of BSA retained (25.8 and 34.4 mg/g) proved similar to those reached by other authors[32] with macroporous polyacrylamide matrices with grafted polymer brushes of *N,N*-dimethylaminoethyl methacrylate.

Influence of the surface architecture on the binding capacity

It is known that the surface topography of these chromatographic supports is a factor more important than the surface charge in the determination of the binding capacity. It depends on the size, shape, and initial concentration of the polymer tentacle and on the precise methods applied during support derivatization. Some authors reported that the degree of penetration of PEI into porous beads is strongly dependent on its molecular mass.[33] The molecular masses of the branched PEIs used in this study for coupling onto the activated beads were 60,000, 1200, and 423. According to Theodossiou and Thomas[33] and Horn,[34] the hydrodynamic radius of a PEI molecule with a molecular mass (M_r) of 50,000 was between 80 and 90 nm at pH 11. Thus, exclusion of the high-M_r PEI species from much of the pore volume of the used beads was likely, whereas the PEI 1200 and 423 were expected to gain access to most of them. Thus, in general, the grafted degree (expressed as milliequivalents of NH_2 per gram of dry polymer) increased when the molecular weight decreased (Table I). Nevertheless, in accordance with the protein-binding capacity, the order of importance of the different PEIs (attached to the activated beads) for a better possible disposition toward the protein was 60,000 > 1200 > 423.

As reported by Theodossiou and Thomas,[33] the molecular weight and concentration of PEI used in the coupling reaction affect the conformation that a given PEI molecule adopts when it is covalently attached to a surface. The physical size and shape of branched PEIs are strongly dependent on the environment. In free solution, short PEI chains have a flat disclike shape, whereas larger molecules exist as coiled spheres. According to Horn,[34] short polymers with a high charge density tend to adsorb on surfaces in a flattened conformation, whereas larger molecules with fewer cationic charges adsorb via short polymer stretches or trains; the rest of the molecule extends away from the surfaces into the bulk phase as loops or tails. When the coupling reactions occurred at the high PEI concentration (R5, Table I), larger molecules in an extended form bonded to the bed through a minimum number of sites. The union increased when the molecular mass decreased. In contrast, when the PEI concentration was low (R1, Table I), the molecule was flattened against the surface via multisite covalent interaction. These sites increased when the molecular mass of PEI decreased. Therefore, the number of accessible sites for the coupling of BSA was higher at high PEI concentration (R5, PEI 60,000) and decreased with the molecular mass of PEI. The steric effect of the BSA also played an important role in the adsorption.

We concluded that the presence of sparse and long chains of PEI 60,000 onto the matrix, by reason of their highest accessibility toward the large protein, may have resulted in a better disposition of functional groups, whereas more short chains of the other PEIs (M_n's = 1200 and 423) used as ligands would not have. The long polymer chains were probably arranged as sparsely disposed tentacles, capable of multipoint interactions with negatively charged BSA molecules and apt to change their conformation to adapt for the BSA binding.[32] On the contrary, the possible dense and shielded arrangement of the shorter polymer chains could have provided less or poor accessibility toward the large protein.

CONCLUSIONS

Poly(HEMA-*co*-EGDMA) beads were obtained by suspension polymerization. The spherical beads showed high porosity and a monodispersed population of macropores. After the reaction with Ech, the activated beads were modified with PEIs of different molecular weights. For all of the concentrations of PEI assayed, when PEI with an M_n of 423 was immobilized, the products showed the highest values in milliequivalents of amine per gram of dry polymer.

In the assays of the retention of BSA as a model protein, the M_n = 60,000 PEI-modified beads showed better results than those modified with PEIs with M_n's of 1200 and 423. The presence of sparse and long chains of PEI 60,000 on the matrix, by reason of their highest accessibility toward the large protein, may have resulted in a better disposition of functional groups, whereas more short chains in the other PEIs (M_n's = 1200 and 423) used as ligands did not. So, long but few chains of PEI coupled onto the matrix contributed to major yield in the BSA retention.

These materials were studied as a way to obtain new tentacle-containing products that would be potentially useful as affinity chromatography supports.

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