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Surface modification on poly (EGDMA-*co*-HEMA) synthetic matrices to be used as specific adsorbents

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Abstract

The preparation and modification of ethylene glycol dimethacrylate/2-hydroxyethyl methacrylate copolymers [poly (EGDMA-*co*-HEMA)] were carried out to study their properties on Immobilized Metal Ion Affinity Chromatography (IMAC). For that purpose, the adsorption–desorption assays of a phosphoprotein, ovalbumin (OVA) on different ion-containing adsorbents were investigated. The metal ion-complexing agent used was iminodiacetic acid (IDA) and the metal ions chelated were Al^{3+} or Fe^{3+}. The efficiency on both modification reactions and OVA retention was analysed taking into account the pore volumes (V_p) and specific surface area (S_s) values in macropores zones and equilibrium volume-swelling ratio (q_v) from base matrices and final ion-containing supports. In general, Fe^{3+}-containing products resulted more efficient adsorbents in the OVA retention and although the adsorption assays on Fe^{3+} or Al^{3+}-containing supports were fast, the complete and faster OVA desorption was reached when Al^{3+}-containing matrix was used.

Keywords: Matrix chemical modification; Ion-containing supports; Ovalbumin retention

1. Introduction

Affinity chromatography is one of the most useful procedures to purify biological molecules such as proteins [1–4]. This biospecific isolation method uses the reversible specific interaction properties between biomolecules and 'ligands' bonded on polymeric matrices. IMAC profits by the properties of metal ions and proteins to act as electronic acceptor–donor components, respectively, where the metal ion is the chelated-ligand on an insoluble matrix and the protein is the specific replacement of the coordinated water molecules around the metal ion in aqueous medium. Therefore, the adsorption of a protein on an immobilized ion chelated is the result of coordinated binding and the formation of a complex that can be then be removed by using an alternative ligand, which competes with the immobilized ligand [5]. The benefits of IMAC (ligand stability, high protein loading, mild elution conditions, simple regeneration and low cost) are decisive features for developing large-scale purification procedures for industrial applications.

Related to the IMAC field, Pearson postulated [5,6] that metal ions could be classified into three categories: hard, intermediate and soft, based on their preferential reactivity towards nucleophiles. Fe^{3+} and Al^{3+} belong to the hard metal ion group and display preferential binding towards oxygen-containing side chains of amino acid residues [7]. Besides, Fe^{3+} and Al^{3+}-containing supports exhibit strong affinity by phosphate group of substrates, which allows for their selective retention from a complex mixture. Immobilised Fe^{3+} or Al^{3+} supports have been used for retention of phosphoamino acids and phosphoproteins [8–11]. Some of they play an important role in biological phenomena, such as in neurotransmitter control, biosynthesis related to signal transduction, cell division, and cancer [12].

OVA is composed of one component called A_1, which contains two phosphoserines residues, another component A_2 with one phosphoserine, and small amounts of A_3 which are absent from of phosphate groups [11]. It is the major protein in hen egg white, which has become a key reference protein in biochemistry. It is used as carrier protein in

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conjugo-immuno-determinations, as stabiliser, as blocking agent or standard, in drug and pharmaceutical processing and in the food industry [13].

The purification pathway of a biological molecule involves a series of steps, since an adequate choice of the base matrix and buffer solutions as necessary to reach the biological molecule in pure state. The porous structure type, mainly affects the adsorption phenomenon of different substances on polymeric matrices since the specific surface area, pore volume (macro, meso and micropores) and their ratio, rule the retention efficiency of organic compounds. Therefore, the polymeric matrix retention capacity is a function of the accessible matrix surface area and the effective ligand density attached to matrix surface. Thus, the optimisation of synthesis conditions and the modification reactions on polymeric matrices are essential procedures to obtain efficient sorbents [14,15]. Besides, for a particular system, the appropriate conditions for selective adsorption and elution on metal ion-containing sorbents must be ascertained [10].

The synthesis of macroporous matrices (that allow internal diffusion of great size solutes and that provide great surface area for further chemical modifications) to reach efficient adsorbents for affinity chromatography constitutes one of the topics of discussion in our research group [16–20]. In this paper, the synthesis and modification of poly (EGDMA-*co*-HEMA) base supports to obtain different metal ion-containing sorbents, as well as the study of their properties in retention and elution of OVA through IMAC are presented. For that purpose, the metal ion-complexing agent used was IDA and the metal ions used were Al^{3+} or Fe^{3+}. Besides, by using these IMAC sorbents [poly (EGDMA-*co*-HEMA)-IDA-Al^{3+} or Fe^{3+}], the more efficient conditions for OVA adsorption and desorption were investigated.

2. Experimental

2.1. Reagents and equipment

The following chemicals were purchased and used: 2-hydroxyethyl methacrylate, HEMA (Fluka, St Louis, USA, p.a.); ethylene glycol dimethacrylate, EGDMA (Fluka, St Louis, USA, p.a.); benzoyl peroxide, BPO (Fluka, Switzerland, p.a., purified by recrystallization from chloroform solution with methanol); 1,4-butanediol diglycidyl ether, BDGE (Sigma Chemical Co., St Louis, USA, purity: 62% by GC); iminodiacetic acid, IDA (Merck, Darmstadt, Germany, p.a.); ammonium thiocyanate (Merck, Darmstadt, Germany, p.a.); ammonium iron (II) sulphate hexahydrate (Analítica, Argentine p.a.); potassium cyanide (Sigma, St Louis, USA, p.a.); methyl cellosolve (Merck, Darmstadt, Germany, p.a.); ninhydrin (Merck, Darmstadt, Germany, p.a.); ferric chloride hexahydrate (Anedra, Argentine, p.a.); HCl (37%, J T Baker, Xalostoc, Mexico) and ovalbumin, OVA, (Sigma, St Louis, USA, p.a.). tris(hydroxymethyl)aminomethane, TRIS, (Anedra, Argentine); cyclohexane, Cyc (Cicarelli, Argentine); glacial acetic acid, (Anedra, Argentine); poly(vinylpirrolidone), PVP (PVP K 90, Fluka Chemie AG, Buchs, Switzerland); dimethylformamide, (Cicarelli, Argentine).

The copolymers were synthesised using a 04644 Series Digital Hot Plate/Stirrer (Cole Parmer, Illinois, USA). IR spectra were recorded on a Nicolet 5-SXC spectrometer (Madison, USA) on KBr disks. Scanning electron microscopy (SEM) was performed on a Phillips SEM 501 B instrument (Holland), at the laboratories in Centro de Investigaciones de Materiales y Metrología (CIMM), Argentine. The specific pore volumes (V_p) and specific surface areas (S_s) of the starting matrices were studied by mercury intrusion porosimetry using a Micromeritics Autopore III 9410 (Norcross, USA). The porosity percentage (*%P*) was estimated as Eq. (1), where d_0 is the apparent density:

$$\%P = \text{Total } V_p d_0 \times 100 \tag{1}$$

Equilibrium volume-swelling ratio (q_v), was calculated [16] by the volume ratio (Eq. (2)) of the sample in swelling equilibrium state (V_{sw}) and in dry state (V_{dry}). It was performed using graduated tubes after the samples had been soaked 24 h in excess of distilled water.

$$q_v = V_{sw}/V_{dry} \tag{2}$$

Oxirane groups quantification assays were performed using the pyridinium chloride method [21] whereas the number of carboxyl groups was analysed by retrocession titration with 0.05N NaOH and 0.05N HCl aqueous solutions, using phenolphtalein as indicator.

UV–visible spectra were recorded with a Shimadzu recording spectrophotometer UV-260 (Kyoto, Japan). Ammonium thiocyanate was used as analytical reagent for colorimetric trials of Fe^{3+}:1.0 mL fractions separated from supernatant, before and after metal ions in contact with each sorbent, were diluted (50–500 μg/100 mL) in acid medium (0.25 mL of concentrated H_2SO_4 and 2.0 mL of HCl: water [1:1]) and a drop of 0.2N $KMnO_4$ was added. Samples of 5.0 mL were mixed with 1.0 mL of NH_4SCN (400 mg/mL) and their absorbance values were monitored at $\lambda = 480$nm. The ferric ion concentration was then calculated using the extinction coefficient obtained from an appropriate calibration plot of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ solutions (50–500 μg/100 mL). Atomic absorption analyses for Al^{3+} were performed on a Polarizing Zeeman Atomic Absorption spectrophotometer Hitachi (Tokyo, Japan). The OVA amount present in solutions was tested by absorbance measurements at $\lambda = 280$nm using an appropriate calibration plot. The OVA retention assays were carried out using the following buffers: 0.2 M acetic acid/sodium acetate, pH 3.00; 0.2 M acetic acid/sodium acetate, pH 5.30; 0.2 M

acetic acid/sodium acetate, pH 5.85; 0.05 M Tris/HCl, pH 7.20 or 0.05 M Tris/HCl, pH 8.50.

2.2. Synthesis and modification of poly (EGDMA-co-HEMA) matrix

Poly (EGDMA-*co*-HEMA) matrices (I and II) were synthesised using a mole ratio of:3.0:1.0:9.3:2.5.10^2 of monovinylic monomer (HEMA), crosslinking reagent (EGDMA), cyclohexane and water, respectively. Poly (vinylpyrrolidone) was used as suspension stabiliser (10 mg/mL of total mixture). The reactions were kept under stirring (450 rpm) during 2 h at 70 or 85 °C for matrices I and II, respectively, using 1.22 mol% of BPO as initiator, referred to total mole of reagents (EGDMA, HEMA and BPO). Once obtained, the beads were exhaustively washed with distilled water and ethanol, dried in an oven at 70 °C up to constant weight and characterised by FTIR, swelling studies and mercury intrusion porosimetry.

Later, poly (EGDMA-*co*-HEMA) I–II were swollen in dimethylformamide for 24 h (172 mg/mL) and mixed with BDGE and solution of 0.6N NaOH to yield epoxy-activated matrices. Thus, an equivalent ratio of 1:12:0.5 of hydroxyl groups, oxirane and base, respectively, was used under stirring for 7.5 h at room temperature. Poly (EGDMA-*co*-HEMA)-BDGE I–II products were purified by exhaustive washes with water and ethanol, and dried to constant weight. Next, the epoxy equivalents per gram of dry product were determined. In the next modification step, the epoxy-activated matrices were swollen in water (83 mg/mL) for 24 h and then mixed during 24 h at 60 °C with IDA dissolved in 2 M Na_2CO_3, using a mol ratio of 1:40:72 of epoxy:IDA:Na_2CO_3, respectively. Poly (EGDMA-*co*-HEMA)-BDGE-IDA I–II were filtered and washed with water, 0.1N acetic acid solutions and exhaustive washes with water again until pH 7.00. Once the products were purified, dried and studied by IR, the acid group amount was determined by colorimetric titration.

To yield the optimum chelation time, four different samples of poly (EGDMA-*co*-HEMA)-BDGE-IDA I (0.100 g) were incubated in batch system with 3.0 mL of ferric chloride solution (137 ppm of Fe^{3+}, pH 2.26–2.27) at room temperature by stirring for different times: 3.5, 7, 10 and 14 h, obtaining poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I a, b, c and d, respectively.

On the other hand, chelating-containing matrices samples (2.000 g) were incubated batch wise with 60.0 mL of ferric (137 ppm of Fe^{3+}, pH 2.26–2.27) or aluminium chloride solutions (200 ppm of Al^{3+}, pH 4.12–4.13). These assays were achieved at room temperature by stirring for a period of 10 h to obtain poly(EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I c and poly(EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} II, poly(EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}I and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} II. Next, the unbound metal ion was removed by washes with 1.3 L of distilled water and dried to constant weight. Before and after chelation reaction, the metal ion amount in each solution was analysed.

Two samples of poly (EGDMA-*co*-HEMA)-BDGE-IDA I were washed with 0.1N NaOH and then with water until pH 8.5 and 10, respectively. Later, these samples (0.100 g) were dried and incubated with 3.0 mL of ferric chloride solution (137 ppm, pH 2.26–2.27) under stirring for 10 h at room temperature, yielding poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I e and f products, respectively.

2.3. OVA retention and desorption assays using poly (EGDMA-co-HEMA)-BDGE-IDA-Fe^{3+} II and poly (EGDMA-co-HEMA)-BDGE-IDA-Al^{3+} II

2.3.1. OVA retention assays at different pH or KCl concentration

Poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II (0.100 g) and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II (0.100 g) were incubated in a batch system with 4.0 mL of buffered-OVA solution (0.043 g/100 mL) at different pH (3.00, 5.30, 5.85, 7.20 or 8.50) under slow stirring for 3 h at room temperature followed by centrifugation. Before and after binding, the OVA amount in solutions was tested. Then, the same procedure was performed at pH 7.20 with poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II at different KCl concentrations (0.00; 0.20; 0.40 or 0.75 M).

2.3.2. OVA adsorption and desorption kinetics

The retention assays for poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II were performed using 0.250 g of samples and 15 mL of buffered-OVA solution (0.018 g/100 mL, pH 7.20) under stirring at room temperature. For this assay, the values of absorbance ($\lambda = 280$ nm) of supernatant solutions were measured vs. time to follow the adsorption on supports. Samples were centrifuged and aliquots of OVA supernatant solutions were collected at different times (at intervals of 18 min for a period of 3 h) while their absorbance values were measurement. Later, it was followed by several washes with portions of 30 mL of buffered solution (pH 7.20) until supernatant absorbance value at $\lambda = 280$ nm was zero.

Once the OVA retention was assayed, desorption assays were carried out. For this purpose, the samples were incubated with 10 mL buffer solution pH 7.20 containing 2 M KCl, measuring the increase in absorbance values at $\lambda = 280$ nm at different times (at intervals of 18 min for a period of 3 h). Desorption of retained OVA on poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II was completed when 10 mL of buffered-solution of pH 3.00 was added and left for 30 min. All these assays were performed at room temperature under slow stirring followed by centrifugation.

2.3.3. OVA retention assays using poly (EGDMA-co-HEMA)-BDGE-IDA-Fe^{3+} I a, b, c, d, e, f and poly (EGDMA-co-HEMA)-BDGE-IDA-Al^{3+} I

Four mL of buffered-OVA solution (0.043 g/100 mL, pH 7.20) was incubated in a batch system for 3 h at room temperature under slow stirring with 0.100 g of each sample, followed by centrifugation. Before and after adsorption, the OVA amount in solutions was tested by absorbance measurements at $\lambda = 280$nm.

3. Results and discussion

3.1. Synthesis and modification of poly (EGDMA-co-HEMA) matrix

Poly (EGDMA-*co*-HEMA) highly porous matrices were yielded by suspension polymerization. Their structural properties are listed in Table 1. In general, both matrices possess low apparent density (d_0) and high porosity (%P) showing morphological properties of heterogeneous networks. As can be seen, at higher temperature of reaction (85 °C), a matrix (II) with less q_v value (1.1) and consequently lower expansion degree than matrix I (obtained at 70 °C) was reached. A major diffusion of the monomers at highest polymerization temperature would lead to higher conversion rate and crosslinking density. It must be marked that q_v parameter is closely related with the network stretching and network expansion degree as a function of the crosslinking density [16].

By poly (EGDMA-*co*-HEMA) I and II SEM analysis, it was found (Figs. 1 and 2) that both presented heterogeneous surfaces of conglomerates of spherical particles (nucleus and microspheres) resembling a cauliflower with high pores among conglomerates. IR spectra of poly (EGDMA-*co*-HEMA) matrices showed the following characteristic signals (cm^{-1}): 3650–3590 (–OH stretching vibration), 3100–2900 and 1635 (=C–H and C=C stretching vibrations, respectively), 1730 (ester group, C=O stretching vibration), 1320–1250 and 1200–1150 (C–O–C stretching vibrations), 1350–1260 (–OH group bending vibration) and 1050 (–C–O of C–OH stretching vibrations).

Matrices were efficiently modified following an activation process with BDGE, a posterior IDA ligand coupling and later chelation step using Fe^{3+} or Al^{3+}. The epoxy and carboxyl groups' amounts reached in product modification processes were measured and the results shown in Table 3.

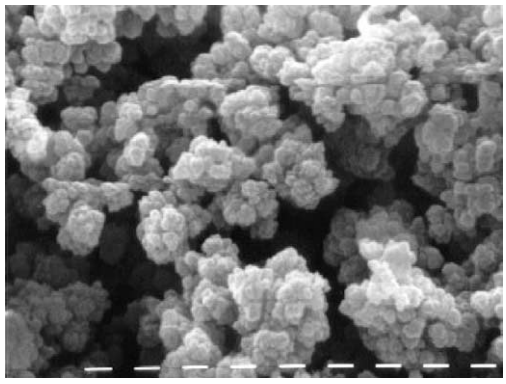

Fig. 1. Poly (EGDMA-*co*-HEMA) I SEM micrograph at a magnification of 5000.

It may be pointed out the pores morphology effect from matrices on reaction steps (activation, coupling and chelation) to reach the final products since poly (EGDMA-*co*-HEMA) I led higher reaction efficiency than poly (EGDMA-*co*-HEMA) II. It could be due to the fact that matrix I presents higher q_v value than matrix II and was able to expose the functional groups more easily since this parameter is related with the network expansion [16]. On the other hand, slightly higher V_p and S_s values in the macropores zone (diameter range: 1.10^3–6.10^3nm) (Table 2) on poly (EGDMA-*co*-HEMA) I, could indicate a higher possible reaction efficiency taking into account that this zone could act as limit of accessibility and diffusion in the network for the reagents used.

IR spectra of chelating-containing matrices presented a new carbonyl group band at 1610–1550 cm^{-1} assigned to carboxylate group.

The chelation of Fe^{3+} and Al^{3+} on chelating-containing matrices yielded poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} I–II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} I–II, orange and white-coloured products, respectively. The metal ion amount on chelating-containing matrices, expressed as μmol per gram of dry products, is shown in Table 3. Being M a metal ion, the ligand occupation (L) [22] was calculated and expressed as Eq. (3):

$$L = [\text{chelation capacity for } M^{3+} \text{ (μmol/g)/chelating} \times \text{ligand concentration (μmol/g)}] \times 100 \quad (3)$$

It can be noticed that in general, an increase in the chelation reaction time leads to a higher amount of

Table 1
Poly (EGDMA-*co*-HEMA) I–II structural properties

Matrix	Polymerization temperature (°C)	Total S_s[a] (m^2/g) (4–4.10^5nm)[b]	Total V_p[a] (mL/g) (4–4.10^5nm)[b]	d_0[a] (g/mL)	% *P*	q_v
I	70	54.14	3.39	0.236	80	1.3
II	85	48.70	3.73	0.220	82	1.1

[a] Calculated by mercury intrusion porosimetry.
[b] Pore diameter range.

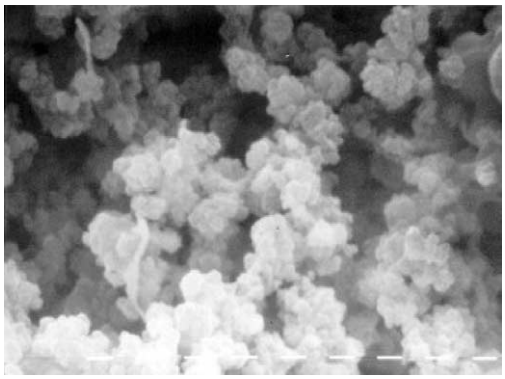

Fig. 2. Poly (EGDMA-*co*-HEMA) II SEM micrograph at a magnification of 5000.

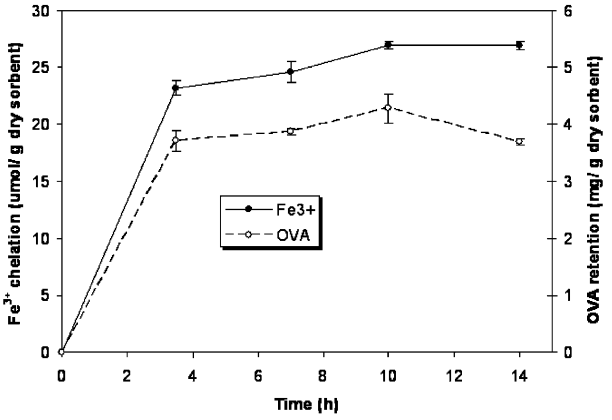

Fig. 3. Fe^{3+} chelation on poly (EGDMA-*co*-HEMA)-BDGE-IDA I at different times and results of OVA retention using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I a, b, c and d.

incorporated Fe^{3+}. As shown in Fig. 3, Fe^{3+} chelation on poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I at different times of incubation (products a, b, c and d), reached a plateau (26.9 μmol Fe^{3+}/g) at 10 h. This could be related with the ion diffusion into the network. On the other hand, those chelating-containing matrices submitted to washes with 0.1N NaOH and then with water up to pH 8.5 and 10 (e and f, respectively) were used for Fe^{3+} chelation by incubation during 10 h (Fig. 4) yielding 22.0 and 25.0 μmol Fe^{3+}/g, respectively. This last event indicated that the most efficient chelation is reached on supports when neutral pH is used for washes.

3.2. OVA retention on IMAC supports

Ferric and aluminium ions immobilised on IDA substituted agarose have been used as IMAC adsorbents for phosphoproteins [8,10,11,23,24] in a wide pH range. For adsorption, the presence of a primary phosphate ester group was required [4,25]. Phosphoserine residues have been attributed to the binding of metal ions to OVA presumably through the oxygen in the phosphate group [9,11].

Matrices poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} II were assayed in OVA retention at different values of pH (Fig. 5), and several KCl concentrations at pH 7.20 (Fig. 6). As can be seen, the major OVA retention capacity in both metal-containing sorbents was found at pH 7.20, which is probably due to the formation of a more stable metal–phosphate group complex.

This behavior could be related to the pH in which the involved phosphate species favours maximum retention in absence of another competent anionic species with the metal. Besides, the retention at pH 7.20 leads to good yields in absence of KCl at least, under the conditions assayed, which reflects the influence of ionic strength.

Results of OVA adsorption at pH 7.2 using four different products: poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I a; b; c and d, are shown in Fig. 3. It can be noticed that in general an increase in the amount of chelated Fe^{3+} (produced by an increase in the chelation incubation time) leads to an increase in OVA retention. Nonetheless, the maximum value in OVA retention was reached when poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}I c (obtained at 10 h of chelation) was used. In Fig. 4, it can be noticed additionally, that an increase of pH in washes with water before Fe^{3+} was chelated, led to a lower amount of metal chelated and a consequently small quantity of OVA retained. Hence, several washes with water in neutral pH conditions are sufficient to yield higher Fe^{3+} chelation and posterior OVA retention.

As seen in Table 3, all obtained IMAC supports were capable to retain OVA although, in general, those obtained from poly (EGDMA-*co*-HEMA) II base-matrix were able to retain higher OVA amounts than those obtained from poly (EGDMA-*co*-HEMA) I. This tendency in OVA retention values was in agreement with their V_p values, as reported, between the major accessibility zone for the protein (6×10^3–4×10^5 nm), higher in the case of poly (EGDMA-*co*-HEMA) II (Table 2).

Actually, the chelation of Al^{3+} was more effective than Fe^{3+} for both poly (EGDMA-*co*-HEMA) I and poly (EGDMA-*co*-HEMA) II (Table 3). Yet, OVA adsorption values on poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} (I c and II) were higher in general than those found from poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} (I and II)

Table 2
S_s and V_p values from Poly (EGDMA-*co*-HEMA) I–II corresponding at different pore diameter range

Matrix	S_s (m^2/g) (1.10^3–6.10^3nm)	V_p (mL/g) (1.10^3–6.10^3nm)	S_s (m^2/g) (6.10^3–4.10^5nm)	V_p (mL/g) (6.10^3–4.10^5nm)
I	1.6	0.80	0.2	1.99
II	1.4	0.66	0.2	2.40

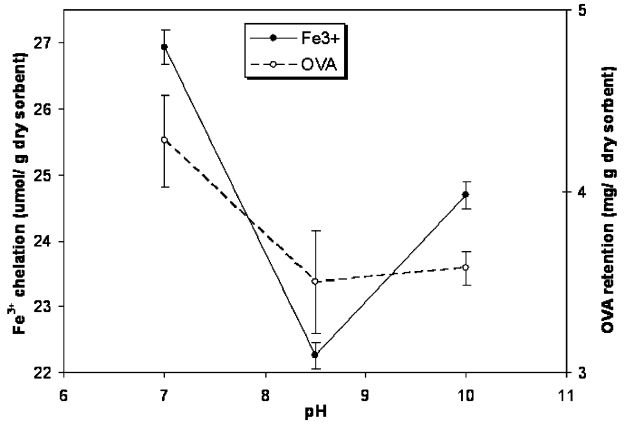

Fig. 4. Fe^{3+} chelation on poly (EGDMA-*co*-HEMA)-BDGE-IDA I (10 h of incubation) using washes at different pH before chelation and results of OVA retention using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} I c, e and f.

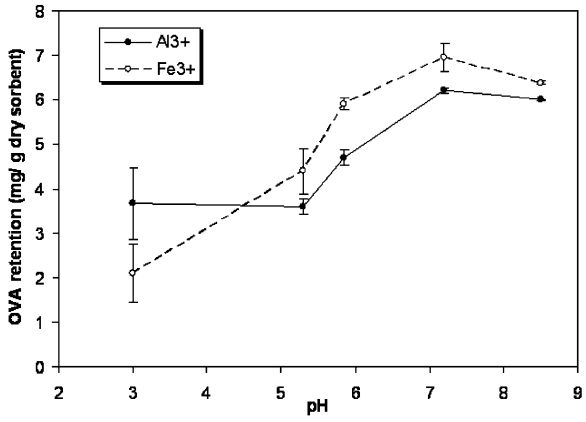

Fig. 5. OVA retention at different pH values using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} II.

(Table 3). Therefore, Fe^{3+}-containing products resulted in more efficient supports in the OVA retention. The maximum OVA adsorption values found on Fe^{3+} and Al^{3+}-containing IMAC supports (at pH 7.20) were 6.98 and 6.21 mg/g, respectively (Fig. 5, Table 3). The cause of this behavior could be explained by two different reasons. In the first place, this behavior was previously postulated [26] since *O*-phosphoserin (OVA component residue, responsible for the binding with Fe^{3+} and Al^{3+}-containing adsorbents) exhibits relatively higher affinity towards Fe^{3+} than Al^{3+}, as a result of the difference in the interaction forces between metal and protein-containing phosphate groups. In the second place, the q_v values in water for Fe^{3+}-containing products (Table 3) were increased compared to those found in base-supports I and II (Table 1). It is probably due to particle electrostatic repulsion on whose surface ferric ions were chelated. Concurrently, formation of hydration spheres occurred around them, leading to support expansion with the consequent increase in the ability to expose available sites to bind the protein [20]. Nevertheless, Al^{3+}-containing products maintain their structure without suffering expansion. This behavior could be due to several types of coordination complexes or arrangements, other than the simply 1:1 M*Lig*-type (being M and *Lig*, metal and immobilized chelating ligand), as MLig_2. In this last species, two ligands could bind a single Al^{3+} ion, provided the ligands are in close proximity [26] without the correct Al^{3+} exposure for binding to the protein to bind (influencing protein interaction during IMAC process).

3.3. OVA adsorption and desorption kinetic assays

OVA adsorption and desorption kinetic assays were carried out at pH 7.20 with poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} II using a buffered ovalbumin solution (0.018 g/100 mL). The adsorption kinetics was accomplished following the decrease in supernatant solution absorbance values ($\lambda = 280$nm) vs. time, as the adsorption on matrices was increased (Fig. 7). As can be seen, the adsorption for both ion-containing matrices was fast, reaching maximum adsorption rates approximately at 72 min after the start (Fig. 7).

Besides, from OVA adsorption kinetic assays, it can be noticed that lower retention values than those reported in Table 2 (previously assayed) were reached. This can be explained since a lower concentration in the buffered-OVA solution was used for this last experiment. This behavior confirms the influence of the concentration used in the solutions on the retention capacity. Besides, OVA adsorption was the highest again when Fe^{3+}-containing adsorbent was used.

On the other hand, desorption assays were carried out measuring the increase in absorbance values at $\lambda = 280$nm at different times (Fig. 8). It can be noticed that the higher

Table 3
Chemical modifications on matrices and OVA retention values at pH 7.20

Adsorbent	Oxirane groups (μeq/g of dry product)	Carboxyl groups (μeq/g of dry product)	Chelated M^{3+} (μmol/g of dry product)	*L* (%)	q_v	OVA (mg/g of dry product)
Poly(EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} (I *c*)	89	106	26.9	51	1.8	4.28
Poly(EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} (I)	89	106	31.4	59	1.3	3.23
Poly(EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} (II)	65	79	17.4	44	1.7	6.98
Poly(EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} (II)	65	79	20.3	51	1.0	6.21

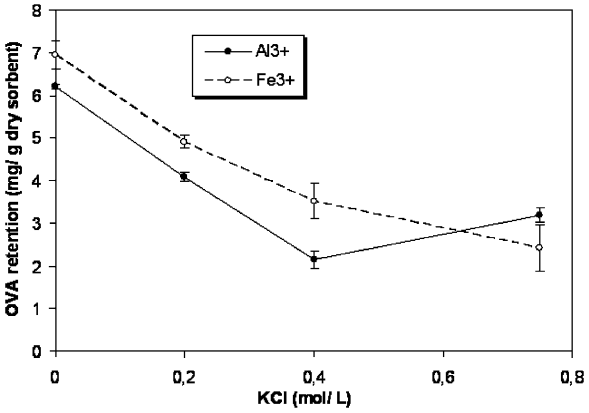

Fig. 6. OVA retention at different KCl concentrations (pH 7.20) using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+} II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+} II.

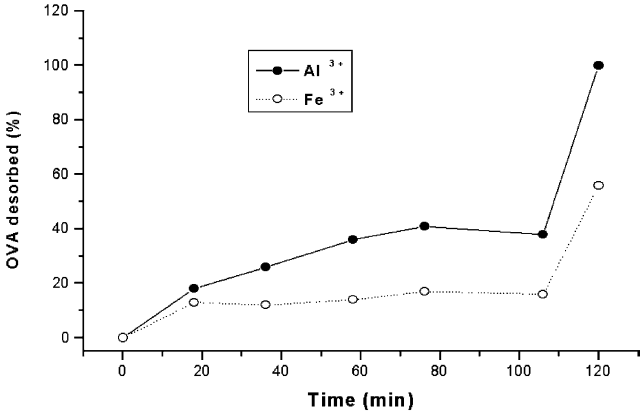

Fig. 8. OVA desorption kinetic using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II (pH 7.20)
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OVA proportion from desorption assays for both ion-containing matrices was reached approximately between 120 and 150 min of beginning the experiment.

As shown in Fig. 8, the entire desorption of retained OVA was not fully reached in the desorption-buffer used (pH 7.20) when poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II was assayed. It was completed when a significant drop in pH values (buffered-solution of pH 3.00) was detected (not shown in Fig. 8). So, it was concluded that the desorption assays were more efficient when Al^{3+}-containing adsorbent was used reaching the total release of retained OVA and that a significantly higher major OVA affinity for Fe^{3+} than Al^{3+} occurred.

4. Conclusions

The morphological influence of poly (EGDMA-*co*-HEMA) I–II base supports for later chemical modifications using BDGE and IDA to attain adequate Fe^{3+} or Al^{3+}-containing adsorbents and their IMAC properties in retention of a phosphoprotein, OVA, were studied. Poly (EGDMA-*co*-HEMA) products showed the presence of high macropore amounts and efficiency in the modification reactions, which allowed attaining adsorbents with high OVA retention capacity.

Higher values of q_v in poly (EGDMA-*co*-HEMA) I than poly (EGDMA-*co*-HEMA) II could lead to higher reaction efficiency. Besides, slightly higher V_p and S_s values in the macropores zone (diameter range: 1.10^3–6.10^3nm) on poly (EGDMA-*co*-HEMA) I, could indicate a major possible reaction efficiency taking into account that this zone may act as a limit of accessibility and diffusion in the network. Nevertheless, IMAC supports obtained from poly (EGDMA-*co*-HEMA) II base-matrix were able to retain higher OVA amounts than those obtained from poly (EGDMA-*co*-HEMA) I, which were in agreement with V_p values found between the higher accessibility zone for the protein (6.10^3–4.10^6nm), major in the case of poly (EGDMA-*co*-HEMA) II. This showed the influence of V_p in the OVA retention.

Metal chelated amounts were higher in Al^{3+} than Fe^{3+}-containing supports, although these last retained higher OVA quantity. The higher OVA retention capacity in both types of adsorbents was found at pH 7.20. In general, the adsorption assays using both supports poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II were fast, although the whole and faster OVA desorption was reached when Al^{3+}-containing adsorbent was used. In the case of poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II, the entire desorption was reached only when pH was lowered at 3.00. This indicated that OVA binding was stronger with Fe^{3+}-containing adsorbents.

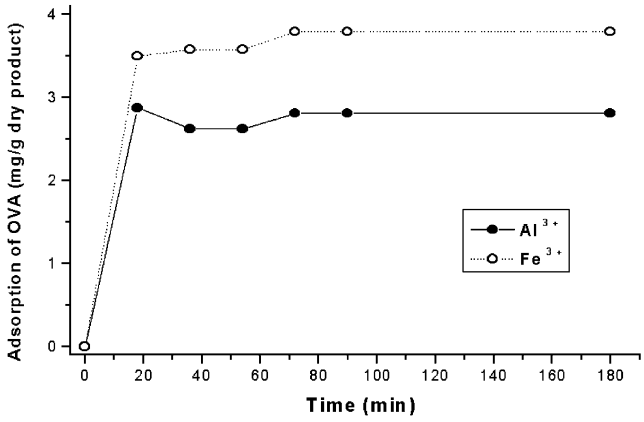

Fig. 7. OVA adsorption kinetic using poly (EGDMA-*co*-HEMA)-BDGE-IDA-Fe^{3+}II and poly (EGDMA-*co*-HEMA)-BDGE-IDA-Al^{3+}II (buffered-OVA solution: 0.018 g/100 mL, pH 7.20).

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