

# Optimization of ligands coupling on poly(EGDMA-co-HEMA)-BDGE matrices through esterification reactions

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## Summary

The optimization of coupling reactions from hexamethylenediamine tetrapropanoic acid (HMDTP) or disodium ethylenediamine tetraacetate (EDTA) on poly(ethylene glycol dimethacrylate-2-hydroxyethyl methacrylate)-butanediol diglycidyl ether [poly(EGDMA-co-HEMA)-BDGE] matrix was carried out through esterification. Then, the chelation of metal ions on products [poly(EGDMA-co-HEMA)-BDGE-HMDTP and poly(EGDMA-co-HEMA)-BDGE-EDTA] was assayed. The metal ions complexing agent in chlorhydrate form (HMDTP.HCl) was synthesized and characterized. Namely, hexamethylenediamine and acrylonitrile reacted to give a cyanide-derivative through Michael addition reaction. Next, the compound was hydrolyzed with HCl aqueous solution to obtain the final product.

## Introduction

The preparation of supports involving immobilized ligands covalently attached to activated polymeric adsorbents, involves a series of convenient coupling reactions that depend on the type, density and accessibility of interacting functional groups and the nature of both, matrix and ligand [1]. Various ligand-containing supports have been used in removal of heavy metals [2-4] while others have been widely used in affinity chromatography [1, 5-8] or as chelators of metal ions that possess the property of binding proteins, as a basic tool in immobilized-metal affinity chromatography (IMAC) [9-12]. The yield of coupling reactions plays an important role in the choice of the immobilization technique since the major density of bound ligand usually raises the performance for the proposed use. Thus, the development of adsorbents synthesis [13-15], modification techniques to activate themselves or to accomplish coupling reaction of a chelating ligand on an adsorbent [1, 16-19] as well as the choice of different metallic ions to act as adsorption center [11-12, 16] was studied.

As advanced by Porath [11], the metal ion-complexing agent bound to a matrix, contains electron donor atoms (N, O), and fixes by coordination the metal ion (electron-pair acceptor). Besides, the chelator ligand modulates the metal affinity specificity of the adsorption center and its capacity. As it is known, iminodiacetic acid (IDA), tris-carboxymethyl-ethylene-diamine (TED), nitrilotriacetic acid (NTA) and carboxymethylated aspartic acid (CM-Asp) are the most common chelating ligands used [10-12]. Ordinarily, chelators are bound to an oxirane polymer through their amino or thiol groups, while esterification reaction can be used to bind a carboxyl

group-containing chelator in the presence of a basic amine type catalyst [19-22]. In this case, the optimization of the reaction must be taken into account to avoid any side reactions. The reaction from epoxy-activated matrices and a ligand-containing carboxyl groups to reach hydroxyethyl ester groups in aqueous medium constitutes an alternative and efficient method of coupling that supplies good conversion rate [23]. In general, porous synthetic polymers with a high crosslinking degree are used for derivatization reactions (activation and coupling of a ligand), since they possess a sufficiently large specific surface area from which functional groups are accessible to react [15, 24-26].

In this work, a metal ions complexing agent (HMDTP.HCl) was synthesized and characterized. Then, the optimization of coupling from HMDTP or EDTA on poly(EGDMA-co-HEMA)-BDGE matrix through esterification reactions was carried out. Finally, the chelation of metal ions such as  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  on the final products [poly(EGDMA-co-HEMA)-BDGE-HMDTP and poly(EGDMA-co-HEMA)-BDGE-EDTA] was assayed.

## Experimental

### *Reagents and methods*

The following chemicals were purchased and used: 2-hydroxyethyl methacrylate (HEMA) (95 %), Fluka (St.Louis, USA); ethylene glycol dimethacrylate (EGDMA), (90 %) Fluka (St.Louis, USA); poly(vinylpyrrolidone), (PVP K 90) Fluka Chemie AG (Buchs, Switzerland); benzoyl peroxide (BPO), Fluka (Switzerland) (purified by crystallization from methanol); 1,4-butanediol diglycidyl ether (BDGE) (70%), Sigma Chemical Co. (St.Louis, USA); disodium ethylenediamine tetraacetate (EDTA) (p.a.), Mallinckrodt (USA); acetic acid glacial (99.7 %), Eclair (Argentina); triethylamine (TEA), Cicarelli (Argentina); hexamethylenediamine (HMDA), Fluka (Switzerland), purified through vacuum distillation (100 °C / 20 mm) [27]; chlorhydric acid (37 %), J.T. Baker (Xalostoc, Mexico); sulfuric acid (98 %), Eclair (Argentina); silica gel 60 (0.063-0.200 mm), Merck (Darmstadt, Germany); acrylonitrile (AN), Carlo Erba (Milane, Italy); dimethylformamide, (p.a.) Cicarelli (Argentina); methanol (MeOH), Merck (Darmstadt, Germany); chloroform, Cicarelli (Argentina) and ethyl acetate, Cicarelli (Argentina). Solvents were purified by fractional distillation.

FTIR spectra were recorded on a Nicolet 5-SXC FTIR spectrometer (Madison, USA) on KBr disks. Mass spectra were performed using a Finnigan 3300 F-100 spectrometer (Sunnyvale, California, USA) while  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  were obtained using a Bruker AC-200 spectrometer (Silberstreifen, Germany). Atomic absorption analyses were performed on a Polarizing Zeeman Atomic Absorption spectrophotometer Hitachi (Tokyo, Japan). The copolymer was synthesized using a 04644 Series Digital Hot Plate/Stirrer (Cole Parmer, Illinois, USA). The mercury intrusion porosimetry was made with an AutoPore III Micromeritics 9410 (Norcross, USA). Oxirane group assay was performed using the pyridinium chloride method [21] and the amount of carboxyl groups was back-titrated with aqueous 0.05 N NaOH and 0.05 N HCl using phenolphthalein as indicator.

### *Synthesis of the metal ions complexing agent*

Hexamethylenediamine tetrapropanoic acid in chlorhydrate form (HMDTP.HCl) was synthesized from HMDA and AN, as shown in Scheme 1.

### *Cyanoethylation (Michael reaction)*

To synthesize hexamethylenediamine tetraethylcyanide (HMDTEC), AN in MeOH (20 % v/v) was slowly dropped on a solution containing HMDA dissolved in MeOH (8.8 % w/v). The different reaction conditions assayed are shown in Table 1. The product was purified by column chromatography (using silica gel), in a proportion of 1g of reaction raw / 40 g of silica. The solvents of elution were chloroform and mixtures of chloroform: ethyl acetate up to a final ratio of 2:8, respectively. HMDTEC resulted in a transparent viscous liquid, soluble in chloroform, ethyl acetate, ethanol, methanol and insoluble in water.

*FTIR*: (KBr) stretching vibrations corresponding to 2930 and 2860  $\text{cm}^{-1}$  ( $\nu$  C-H, -CH<sub>2</sub>); 2247  $\text{cm}^{-1}$  ( $\nu$  R-C $\equiv$ N), were found.

<sup>1</sup>H-NMR: (CDCl<sub>3</sub>, 200MHz),  $\delta$ : 2,85 ppm (t; 8 H, N-CH<sub>2</sub><sup>b</sup>-);  $\delta$ : 2.50 ppm (m; 12 H, CH<sub>2</sub><sup>a,c</sup>-);  $\delta$ : 1.41 ppm (m; 8 H, -CH<sub>2</sub><sup>d,e</sup>-).

<sup>13</sup>C-NMR: (CDCl<sub>3</sub>, 50 MHz),  $\delta$ : 118.6 ppm (-CN);  $\delta$ : 53.25 ppm (N-CH<sub>2</sub><sup>c</sup>-);  $\delta$ : 49.62 ppm (N-CH<sub>2</sub><sup>b</sup>-);  $\delta$ : 27.33 (-CH<sub>2</sub><sup>e</sup>-);  $\delta$ : 26.79 ppm (-CH<sub>2</sub><sup>d</sup>-);  $\delta$ : 16.95 ppm (CH<sub>2</sub><sup>a</sup>-CN).

*Mass spectrometry*: (70-77 eV) (m/z: % R.I.), (289: 19.8); (248: 4.4); (205: 3.79); (166: 4.84); (137: 52.33); (136: 71.81); (97: 12.18); (83: 11.21); (69: 3.7); (54: 100); (42: 83.21); (28: 84.3).

### *Hydrolysis from cyanide to carboxyl groups*

HMDTEC product was submitted to acid hydrolysis reactions monitored by FTIR. The optimization of this step was carried out as follows: HMDTEC (6.2633 g, 76.4 meq -CN) was put to react with 6.5 N HCl aqueous solution (70 mL) during 15 h at 100 °C under stirring. Then, an excess of distilled water was added and the residue of HCl was eliminated as azeotropic mixture (HCl: water) by simple distillation at 108 °C. The white product (HMDTP.HCl), which crystallized at room temperature, was filtered and dried.

*FTIR*: (KBr) stretching vibrations corresponding to 3300-2500  $\text{cm}^{-1}$  ( $\nu$  O-H, -COOH); 2930 and 2860  $\text{cm}^{-1}$  ( $\nu$  C-H, -CH<sub>2</sub>-); 1710  $\text{cm}^{-1}$  ( $\nu$  C=O, -COOH) were found.

<sup>1</sup>H-NMR: (CDCl<sub>3</sub>, 200MHz),  $\delta$ : 3.58 ppm (t; J: 6.58 Hz; 8 H, N-CH<sub>2</sub><sup>b</sup>-);  $\delta$ : 3.31 ppm (m; 4 H, N-CH<sub>2</sub><sup>c</sup>-);  $\delta$ : 3.00 ppm (t; J: 6.58 Hz; 8 H, HOOC-CH<sub>2</sub><sup>a</sup>-);  $\delta$ : 1.88 ppm (m; 4 H, -CH<sub>2</sub><sup>d</sup>-);  $\delta$ : 1.52 ppm (m; 4 H, -CH<sub>2</sub><sup>e</sup>-).

<sup>13</sup>C-NMR: (CDCl<sub>3</sub>, 50 MHz),  $\delta$ : 176.46 ppm (-COOH);  $\delta$ : 53.18 ppm (N-CH<sub>2</sub><sup>c</sup>-);  $\delta$ : 50.13 ppm (N-CH<sub>2</sub><sup>b</sup>-);  $\delta$ : 29.81 ppm (HOOC-CH<sub>2</sub><sup>a</sup>-);  $\delta$ : 25.50 ppm (-CH<sub>2</sub><sup>e</sup>-);  $\delta$ : 23.29 ppm (-CH<sub>2</sub><sup>d</sup>-).

*Mass spectrometry*: (70-77 eV) (m/z: % R.I.), (188: 0.74); (172: 2.91); (158: 2.69); (144: 0.56); (128: 3.13); (112: 4.41); (102: 54.34); (99: 12.05); (98: 18.95); (83: 2.64); (82: 1.37); (72: 64.52); (70: 7.19); (69: 1.67); (57: 4.19); (55: 74.44); (44: 39.89); (41: 17.89); (30: 86.01); (27: 100).

### *Synthesis of poly(EGDMA-co-HEMA) and activation reaction*

Copolymerization reaction to obtain poly(EGDMA-co-HEMA) matrix, was performed using a mole ratio of (3.0: 1.0: 9.3: 2.5  $10^2$ ) of monovinyllic monomer (HEMA), crosslinking reagent (EGDMA), cyclohexane and water, respectively [25]. Poly(vinylpyrrolidone) (PVP) was used as stabilizer of the suspension using a ratio of 10 mg / mL of total mixture. The reaction was kept under stirring (450 rpm) during 2 h at 85 °C using 5.00 % of BPO [which 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 characterized.

*FTIR:* (KBr) 3650-3590  $\text{cm}^{-1}$  ( $\nu$  -OH), 3100-2900 and 1635  $\text{cm}^{-1}$  ( $\nu$  =C-H and C=C, respectively), 1730  $\text{cm}^{-1}$  (ester group,  $\nu$  C=O), 1320-1250 and 1200-1150  $\text{cm}^{-1}$  ( $\nu$  C-O-C), 1350-1260  $\text{cm}^{-1}$  ( $\sigma$  -OH) and 1050  $\text{cm}^{-1}$  ( $\nu$  -C-O, R-OH).

For the activation reaction, poly(EGDMA-co-HEMA) (3.00 g) was swollen in dimethylformamide, for 24 h (172 mg / mL). Then, the sample was mixed under stirring for 7.5 h at room temperature with BDGE and 0.6 N NaOH aqueous solution at an equivalent ratio of 1: 12: 0.5 of hydroxyl, epoxy and base, respectively. Poly(EGDMA-co-HEMA)-BDGE product was purified by washes with water and ethanol, and dried up to constant weight. Its amount of epoxy groups was determined.

### *Coupling reactions*

Poly(EGDMA-co-HEMA)-BDGE was swollen in dimethylformamide (67 mg/mL) and submitted to reaction with the chelating agent at different equivalents ratio of EDTA (or HDMTP): epoxy-activated matrix: 0.2 M NaOH (Table 2), using 10 % of TEA (referred to total mole of reagents) at 60 °C for 24 h (Scheme 2). Next, products poly(EGDMA-co-HEMA)-BDGE-EDTA and poly(EGDMA-co-HEMA)-BDGE-HMDTP were filtered and washed with water, 0.1 N acetic acid solution and water again. Once the products were purified and dried, the amount of carboxyl groups was determined by back-titration (Table 2) and studied by IR.

### *Chelation of metal ions on modified matrices*

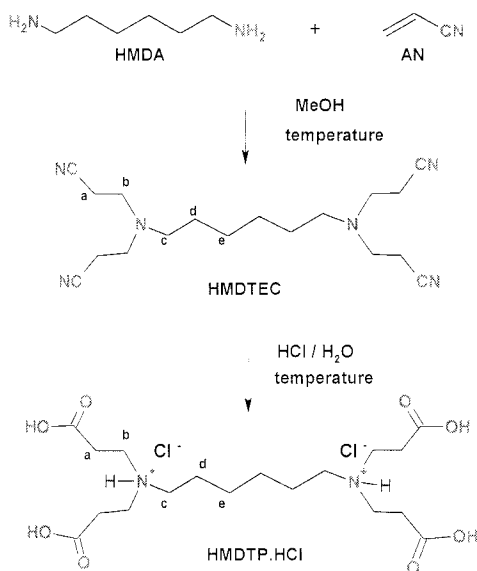
The chelation of metal ions was achieved in the following way: samples of poly(EGDMA-co-HEMA)-BDGE-EDTA or poly(EGDMA-co-HEMA)-BDGE-HMDTP (0.100 g) were put in contact with 3.0 mL aqueous solutions of  $\text{FeCl}_3$  (93 ppm of  $\text{Fe}^{+3}$ , pH 2.0);  $\text{CuCl}_2$  (80 ppm of  $\text{Cu}^{+2}$ , pH 5.0);  $\text{CoCl}_2$  (144 ppm of  $\text{Co}^{+2}$ , pH 6.5);  $\text{NiCl}_2$  (153 ppm of  $\text{Ni}^{+2}$ , pH 6.7) or  $\text{ZnCl}_2$  (123 ppm of  $\text{Zn}^{+2}$ , pH 5.8), during 10 h at room temperature. Before and after chelation, the amount of metal ions in solution was tested by atomic absorption.

## **Results and discussion**

### *Synthesis of HMDTP:*

The general route for the synthesis of HMDTP is shown in Scheme 1. In the first step, a cyanide derivative HMDTEC was obtained from HMDA and AN through a Michael addition reaction [28-30]. The temperature and reagent ratios were varied (Table 1). The reaction yields increased with increasing temperature whereas they were barely

affected by the variation in reagent ratios.



Scheme 1. Synthetic pathway of HMDTP.HCl

On the other hand, the importance in the presence of a proton-giver type solvent (Table 1, reaction 7) due to Michael addition reaction mechanism [30] was evidenced. HMDTEC product was obtained at higher yield (84 %) when 70 °C, 48 h and 1.50 of equivalents ratio of AN / HMDA was used (Table 1, reaction 6). The purified product appeared as a transparent viscous liquid, soluble in organic solvents such as chloroform, ethyl acetate and methanol though it was insoluble in water.

Table 1: Cyanoethylation reaction conditions to obtain HMDTEC

| Reaction       | AN / HMDA<br>(Equivalents ratio) | T<br>(°C) | Yield<br>(%) |
|----------------|----------------------------------|-----------|--------------|
| 1 <sup>a</sup> | 1.00                             | 50        | 47           |
| 2 <sup>a</sup> | 1.00                             | 60        | 64           |
| 3 <sup>b</sup> | 1.50                             | 50        | 26           |
| 4 <sup>a</sup> | 1.50                             | 50        | 33           |
| 5 <sup>a</sup> | 1.50                             | 60        | 64           |
| 6 <sup>a</sup> | <b>1.50</b>                      | <b>70</b> | <b>84</b>    |
| 7 <sup>c</sup> | 1.84                             | 50        | 0            |

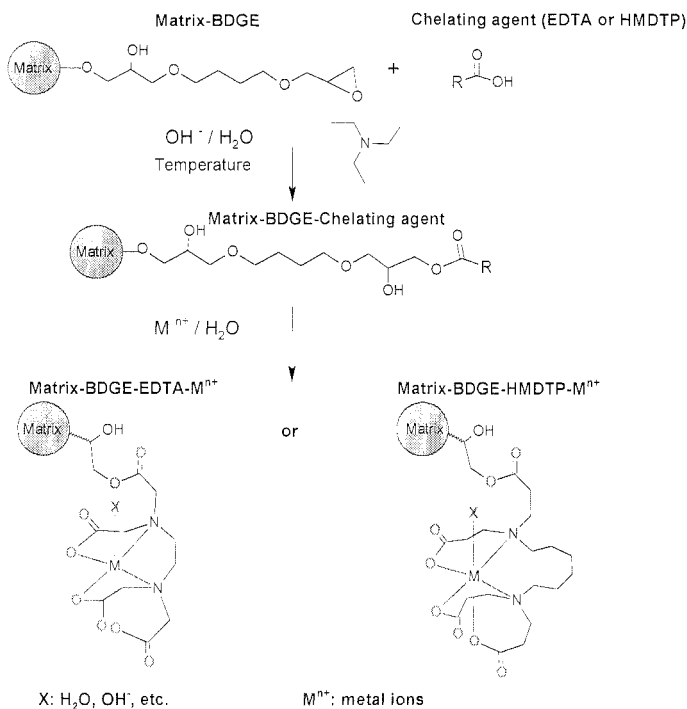
a-Time of reaction: 48 h

b- AN was added to HMDA in three steps: one third at the beginning of reaction, one third after two hours and one third after eighteen hours. Total time of reaction: 48 h

c- Bulk reaction. Time of reaction: 30 h

The cyanide groups were submitted to acid hydrolysis reaction and the evolution of their conversion to carboxyl groups was monitored by FTIR. Thereby the appearance of bands corresponding to carboxyl groups at 1705 cm<sup>-1</sup> ( $\nu$  C=O) and disappearance of signal attributed to cyanide groups at 2247 cm<sup>-1</sup> ( $\nu$  C≡N), were observed. Then, an excess of water was added to eliminate the chlorhydric acid by simple distillation (b.p. of azeotrope HCl/water:108°C). The undistilled aqueous solution was crystallized at

room temperature. The yield of the white solid HMDTP product was 80 %.



Scheme 2. Esterification and chelation reactions on modified matrix

Table 2: Coupling reaction conditions on poly(EGDMA-co-HEMA)-BDGE

| Reaction  | Chelating agent | Carboxyl/ epoxy/ base (equivalents ratio) | Carboxyl groups (meq / g) | Esterification yield* (%) |
|-----------|-----------------|---|---------------------------|---------------------------|
| 8         | EDTA            | 5:1:2.50                                  | 0.130                     | 31                        |
| 9         | EDTA            | 5:1:1.25                                  | 0.150                     | 36                        |
| <b>10</b> | <b>EDTA</b>     | <b>10:1:2.50</b>                          | <b>0.200</b>              | <b>48</b>                 |
| 11        | EDTA            | 10:1:1.25                                 | 0.096                     | 23                        |
| 12        | EDTA            | 50:1:2.50                                 | 0.110                     | 26                        |
| 13        | HMDTP           | 5:1:2.50                                  | 0.128                     | 30                        |
| 14        | HMDTP           | 5:1:1.25                                  | 0.125                     | 30                        |
| <b>15</b> | <b>HMDTP</b>    | <b>10:1:2.50</b>                          | <b>0.160</b>              | <b>38</b>                 |

\* Theoretical esterification yield (%), considering one carboxyl group reacted for each chelating agent: (carboxyl / epoxy groups)  $1/3 \times 100$ .

#### Matrix: characterization and modification

Poly(EGDMA-co-HEMA) obtained from suspension polymerization [25-26] constitutes a highly porous crosslinked network. It was ratified by its total porosity value (%P: 86), defined as  $\%P = V_{p, \text{total}} / d_0 \times 100$  [24]. The total specific pore volume was 3.69 mL/g; the total specific surface area was 113.1 m<sup>2</sup>/g and the sample apparent

density ( $d_0$ ) was 0.234 g/mL. These data were obtained from mercury intrusion porosimetry method (pore diameter between 4 – 4.10<sup>5</sup> nm). Poly(EGDMA-co-HEMA) containing 5.0 meq of hydroxyl groups per gram of dry polymer [26], was submitted to activation procedures using BDGE. The amount of epoxy equivalents found for poly(EGDMA-co-HEMA)-BDGE product was 0.140 meq/g.

EDTA and HMDTP reagents were covalently bound on poly (EGDMA-co-HEMA)-BDGE matrix through esterification reactions (Scheme 2) under different experimental conditions (Table 2). In general, the yields were high even though this reaction type is developed in a heterogeneous system. Besides, these reactions were favored by the presence of TEA used as catalyst, which confers specificity to the epoxy-carboxyl reaction and eliminates side reactions between oxirane and hydroxyl groups in the matrix or those in formation. For both chelating agents, EDTA or HMDTP, the equivalent ratio that permitted the highest esterification yield was reached using 10: 1: 2.50 equivalent ratio of carboxyl/ epoxy/ base, respectively. This could be attributed to the occurrence of a major proportion of carboxylate groups as effective nucleophile, taking into account the acid-base equilibrium. Besides, it can be noticed that using the same experimental conditions but varying the chelating agent (Table 2, reactions 8 and 13; 9 and 14; 10 and 15), the amount of carboxyl groups in final products was higher when EDTA (of minor molecular size) was used. It was attributed to the effect of the accessible surface area on the coupling reactions yield. In general, in FTIR spectra of chelating agent-containing matrices, a new band of carbonyl group appears at 1610-1550 cm<sup>-1</sup> assigned to carboxylate groups.

Table 3: Metal ion amounts adsorbed on chelating agent-containing matrices

| Chelating agent | Fe <sup>3+</sup><br>( $\mu\text{mol} / \text{g}$ ) | Co <sup>2+</sup><br>( $\mu\text{mol} / \text{g}$ ) | Cu <sup>2+</sup><br>( $\mu\text{mol} / \text{g}$ ) | Ni <sup>2+</sup><br>( $\mu\text{mol} / \text{g}$ ) | Zn <sup>2+</sup><br>( $\mu\text{mol} / \text{g}$ ) |
|-----------------|--|--|--|--|--|
| EDTA            | 11-17  | 4-8  | 2-8  | 2-6  | 10-15  |
| HMDTP           | 12-15  | 2-5  | 2-4  | 2-6  | 3-4  |

The chelation of metal ions on chelating agent-containing matrices [poly(EGDMA-co-HEMA)-BDGE-HMDTP and poly(EGDMA-co-HEMA)-BDGE-EDTA] yielded colored products. The amounts of chelated metal ions on matrices are listed in Table 3. The more efficient chelation of divalent metal ions on pentadentate chelators was reached with Zn<sup>2+</sup> on poly(EGDMA-co-HEMA)-BDGE-EDTA, whereas the chelation yield of Fe<sup>3+</sup> was high for both chelating agent-containing matrices as consequence of the metal electronic structures. This behavior showed a high specificity of the poly(EGDMA-co-HEMA)-BDGE-HMDTP to form complex with Fe<sup>3+</sup> metal ions. According to metal-ion concept based on the polarizability of interacting ions, Fe<sup>3+</sup> can be considered to be a "hard" Lewis acid. Ni<sup>2+</sup> (also Co<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>) are in on the borderline for "soft" acids. Accordingly, Fe<sup>3+</sup> may associate more strongly with oxygen as the ligand atom, whereas Ni<sup>2+</sup> has preference for nitrogen and sulfur [10-11, 16].

## Conclusions

A complexing agent of metal ions, HMDTP, was synthesized in a high yield (80 %) and characterized. Then, the optimization of coupling from chelating agents (HMDTP or EDTA) on poly(EGDMA-co-HEMA)-BDGE matrix was carried out by

esterification. For both chelating agents, the reagents ratio (in equivalents) that permitted the major amount of carboxyl groups on modified matrices was 10: 1: 2.50 of carboxyl/ epoxy/ base, respectively. Additionally, the readiness of an efficient and simple coupling method by esterification from epoxy and carboxyl groups should be highlighted as an alternative for modification reactions of activated matrices in aqueous solutions.

The highest chelation on poly(EGDMA-co-HEMA)-BDGE-EDTA was found for  $\text{Fe}^{3+}$  and  $\text{Zn}^{2+}$ , whereas the same behavior on poly(EGDMA-co-HEMA)-BDGE-HMDTP was reached only with  $\text{Fe}^{3+}$ , showing so each adsorbents its specificity by different metal ions.

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