Synthesis of macroporous polymers with radical scavenging properties by immobilization of polyphenolic compounds

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ABSTRACT

Solid phase radical scavengers have been prepared by the immobilization of antioxidant (AOX) compounds on macroporous polymers. Poly(glycidylmethacrylate-co-trimethylolpropane trimethacrylate) [poly(GMA–TRIM)] and poly(N-acryloyl-tris(hydroxymethyl)aminomethane-co-glycidylmethacrylate-co-N,N′-methylenebisacrylamide) [poly(NAT–GMA–BIS)] were prepared by free radical polymerization using a mixture of dimethylsulfoxide (DMSO)-poly(ethyleneglycol) 6000 (PEG 6000) as a porogenic solvent. The polymers were aminated with ethylenediamine (EDA) and the linkage of the polyphenolic compounds (gallic and caffeic acids) was carried out by two different approaches: through N,N′-dicyclohexylcarbodiimide/4-dimethylaminepyridine (DCC/DMAP) system (one-step method) or through the previous formation of the acyl chloride of the polyphenolic compounds and subsequent amidation reaction (two-step method). The available phenolic groups on the macroporous polymers were determined using the Folin–Ciocalteu method; the radical scavenging properties of the materials prepared were evaluated using the radical species 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) and 2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation (ABTS$^{+\cdot}$). From the results, higher antiradical capacities were obtained with the polymers in which the immobilization of the antioxidant molecules was performed through the two-step method. The polymeric networks prepared in this work yielded up to 13.2 μmol AOX/g of dry polymer, which allowed a quantitative removal of the radicals tested in less than 30 min.

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1. Introduction

Macroporous polymers (also known as heterogeneous networks) have a rigid porous structure formed during their preparation which remains unaltered in any solvent, and especially in dry state [1]. The inner structure consists of aggregates of microglobules of interconnected polymers by pores whose rigidity results from its high cross-linking degree.

Due to the porous properties commonly found in highly cross-linked macroporous supports, these polymers have attracted increased interest in areas such as solid-phase synthesis, heterogeneous catalysis and chromatographic separation science. Macroporous polymers can be prepared by/through different types of polymerization procedures including suspension polymerization [1], high internal phase emulsion polymerization (polyHIPÉs) [2] and bulk polymerization [3]. The last method leads to the formation of monolithic polymers. In contrast to macroporous beads obtained by suspension polymerization, monolithic polymers are easily prepared within a mold (which determines the form of the support) from a homogeneous polymerization mixture containing the monomers, the radical initiator and the porogenic mixture. The polymer obtained is characterized by having particularly large porous size with homogeneous porous size distribution. Due to such porous properties, these polymers are widely used in processes where high flow rates and low pressures are needed, e.g., stationary phases in different chromatographic modes [3–6], supports for solid phase chemistry (or flow-through reactors) [7,8] and scavengers [9,10]. The main advantage of using porous supports as scavengers is the possibility of purifying a complex mixture by removing an unwanted compound from the solution, due to specific interactions between a specific ligand immobilized on the solid support and the species to be removed or chemically transformed. In this sense, poly(chloromethylstyrene-co-divinylbenzene) monolithic polymers have been used in the flow-through

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depletion of 2-chloroethyl phenyl sulfide (a "mustard gas" simulator), from a mixture of fluorinated solvents [10]. In another study, Tripp et al. used modified poly(styrene-co-divinylbenzene) porous polymers for the scavenging of electrophilic isocyanates [9].

In this work, we extend the concept of using porous polymers as solid-phase radical scavengers by immobilizing gallic acid (GA) and caffeic acid (CA) on poly(GMA–TRIM) and poly(NAT–GMA–BIS) macroporous polymers. The importance of the immobilization of polyphenolic compounds lies in their well-known capacity for the depletion of radical species. Free radicals are involved in the oxidation of organic molecules producing a large variety of undesirable alterations in food, beverages and pharmaceutics. These deleterious agents can also be formed in biological fluids leading to oxidative damage of different target molecules. We have recently reported the immobilization of CA onto 2D polypropylene films [11]. In the present work, the immobilization of antioxidant molecules within the pores of 3D macroporous structures could lead to the preparation of polymers with higher antiradical properties with potential applications in the areas above mentioned as depuration filters avoiding antioxidant dissolution in the medium. Additionally, antioxidants obtained from natural sources have interesting advantages for offering recognized safety when compared to those synthetic like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Phenolic compound reactivity towards these unpaired-electron species strongly depends on both, the number of phenolic groups present in the molecule and the substitution pattern of the aromatic ring [12,13]. Polyhydroxy derivatives of benzoic acid and cinnamic acid such as GA and CA, respectively, have been reported as good free-radical scavengers. Recently, Parisi et al. [14] reported the preparation of cross-linked polymers using ferulic acid (another cinnamic acid derivative), methacrylic acid and ethylene glycoldimethacrylate as monomers. These polymers were prepared both by bulk and precipitation polymerizations. Since the antiradical activity of the support is mainly given by those molecules of polyphenolic compound present on the polymeric surface, a major disadvantage of using ferulic acid as monomer in the polymerization mixture implies that a large percentage of the functional monomer takes part in the polymer bulk, being not accessible to the radical species to be depleted. Therefore, only the monomer present on the polymeric surface is used.

In another approach to preparing porous polymers with covalently bound polyphenolic compounds, Maeda et al. reported the immobilization of phenolic compounds on cross-linked beads obtained by suspension polymerization [15,16]. Although the polymers exhibited radical scavenging activity, they reached their highest activity after more than 8 h. Such slow kinetic could be ascribed to the low mass transfer of the process due to the presence of small pores in the polymer beads.

In view of this, this study explores the covalent immobilization of GA and CA on poly(GMA–TRIM) and poly(NAT–GMA–BIS) macroporous polymers. Firstly, in order to yield the amine functionality on the polymeric surface, the epoxy groups presented in the supports were reacted with EDA. Secondly, the immobilization of the phenolic compounds was carried out using two different approaches. Finally, the available phenolic groups on the porous materials were determined using the Folin–Ciocalteu method; the antiradical activities of the polymers obtained were evaluated against two different radical species DPPH· and ABTS⁺·.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA), N-acryloyl-tris(hydroxymethyl)aminomethane (NAT), trimethylolpropane trimethacrylate (TRIM), N,N′-methylenebisacrylamide (BIS), N,N′-dicyclohexylcarbodiimide (DCC), triethylamine, 2,2′- azobisisobutyronitrile (AIBN), thionyl chloride, ethylenediamine (EDA), Folin–Ciocalteu reagent, gallic acid (99%) and caffeic acid (99%) were purchased from Sigma–Aldrich (Buenos Aires, Argentina). 4-dimethylaminepyridine (DMAP) 99%, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and poly(ethylenglycol) 6000 (PEG 6000) were purchased from Fluka (Buenos Aires, Argentina). All chemicals were used as received without further purification. THF was freshly distilled over benzophenone.

2.2. Instrumentation

The pore-size distribution of the monolithic materials was determined by mercury intrusion porosimetry using an Autopore II 9220 Micromeritics (Norcross, USA). Their surface morphology was studied by Scanning Electron Microscopy (SEM) using Philips XL-30 equipment (Eindhoven, Netherlands). UV–Vis absorption measurements were carried out using a spectrophotometer UNICAM UV2 (UNICAM, Cambridge, United Kingdom).

2.3. Polymer preparation

The synthesis of poly(NAT–GMA–BIS) macroporous polymer was carried out as previously reported [17]. Briefly, NAT (11 wt.%), GMA (9 wt.%) and BIS (14 wt.%) were dissolved in a porogenic mixture of DMSO (49 wt.%) and PEG 6000 (17 wt.%). After the dissolution of monomers, the mixture was purged with nitrogen for 10 min and AIBN was then added (1 wt.% with respect to monomers). Subsequently, the polymerization mixture was placed into polypropylene syringes for 24 h at 60 °C. For the synthesis of poly(GMA–TRIM), a similar approach was used and the polymerization mixture consisted of GMA (19 wt.%), TRIM (19 wt.%), DMSO (45 wt.%) and PEG 6000 (17 wt.%). After the polymerization reactions, the plastic syringes were cut in one of the extremes by a lathe and the polymers were removed by pushing the plunger down to empty the syringe. The polymer obtained as a solid cylinder was ground afterwards. These polymers were purified with methanol in a Soxhlet apparatus for 24 h to eliminate the unreacted reagents, and dried under vacuum to constant weight. Their porous properties were subsequently analyzed. The dry polymers were ground in a mortar and sieved to collect the 63–125 μm particle size fractions. The amount of available epoxy groups in each polymer was determined in duplicate by using the pyridinium chloride method [18].

2.4. Swelling measurement

Dried samples were placed in distilled water or methanol and kept at room temperature in order to determine their swelling behavior. After 24 h, the swollen samples were removed from the solvent, superficially dried with tissue paper and weighed. The equilibrium weight swelling ratio q_w was calculated as:

$$q_w = \text{swollen mass/dry mass} \tag{1}$$

2.5. Reaction of amination of poly(NAT–GMA–BIS) and poly(GMA–TRIM)

Macroporous polymers (1.00 g) were immersed in 25 mL of 0.5 M phosphate buffer pH 8.00 containing 1.6 and 1.8 mL of EDA for poly(NAT–GMA–BIS) and poly(GMA–TRIM), respectively. The amination reactions were allowed to proceed under stirring at 60 °C for 24 h. Then, the amine-containing polymers were thoroughly washed with the coupling buffer, water and ethanol to

remove the unreacted compounds. Finally, the amine-containing polymers [poly(NAT–GMA–BIS)–EDA or poly(GMA–TRIM)–EDA] were dried under vacuum and the amount of amine groups was determined in duplicate using the HCl-titration method [19].

2.6. Immobilization of antioxidants (GA or CA) on poly(NAT–GMA–BIS)–EDA and poly(GMA–TRIM)–EDA

Fig. 1 shows the chemical reactions used to immobilize polyphenolic compounds on the porous polymers. As shown in this scheme, the covalent binding of GA and CA to the amine-modified polymers to yield amide groups was carried out through two different approaches: (a) using the DCC/DMAP system, and (b) reaching in a previous step the acyl chloride of the polyphenolic compounds and the further amidation reaction.

2.6.1. Immobilization of antioxidants using the DCC/DMAP system
This reaction was performed using poly(GMA–TRIM)–EDA macroporous supports. Each polyphenolic compound (0.65 g) and the amine-modified support (0.40 g) were mixed in a three-necked flask containing 25 mL of anhydrous THF and stirred for 20 min at room temperature under nitrogen atmosphere. DCC (0.75 g) and DMAP (0.04 g) were then added and the coupling reaction was allowed to proceed for 8 h at 60 °C. The modified porous polymers [poly(GMA–TRIM)–EDA–GA-2 and poly(GMA–TRIM)–EDA–CA-4] were purified using methanol in a Soxhlet apparatus for 24 h in order to eliminate the unreacted free antioxidant molecules. Then, the samples were dried under vacuum until constant weight.

2.6.2. Immobilization of the antioxidants through their acyl chloride derivatives
This reaction was performed using both poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA macroporous polymers under anhydrous conditions using nitrogen flow. Firstly, the acyl chloride of each polyphenolic compound was formed by adding 0.6 g of the antioxidant (GA or CA) and 1.3 mL of SOCl₂ in a three-necked flask containing 25 mL of anhydrous THF. Each reaction was allowed to
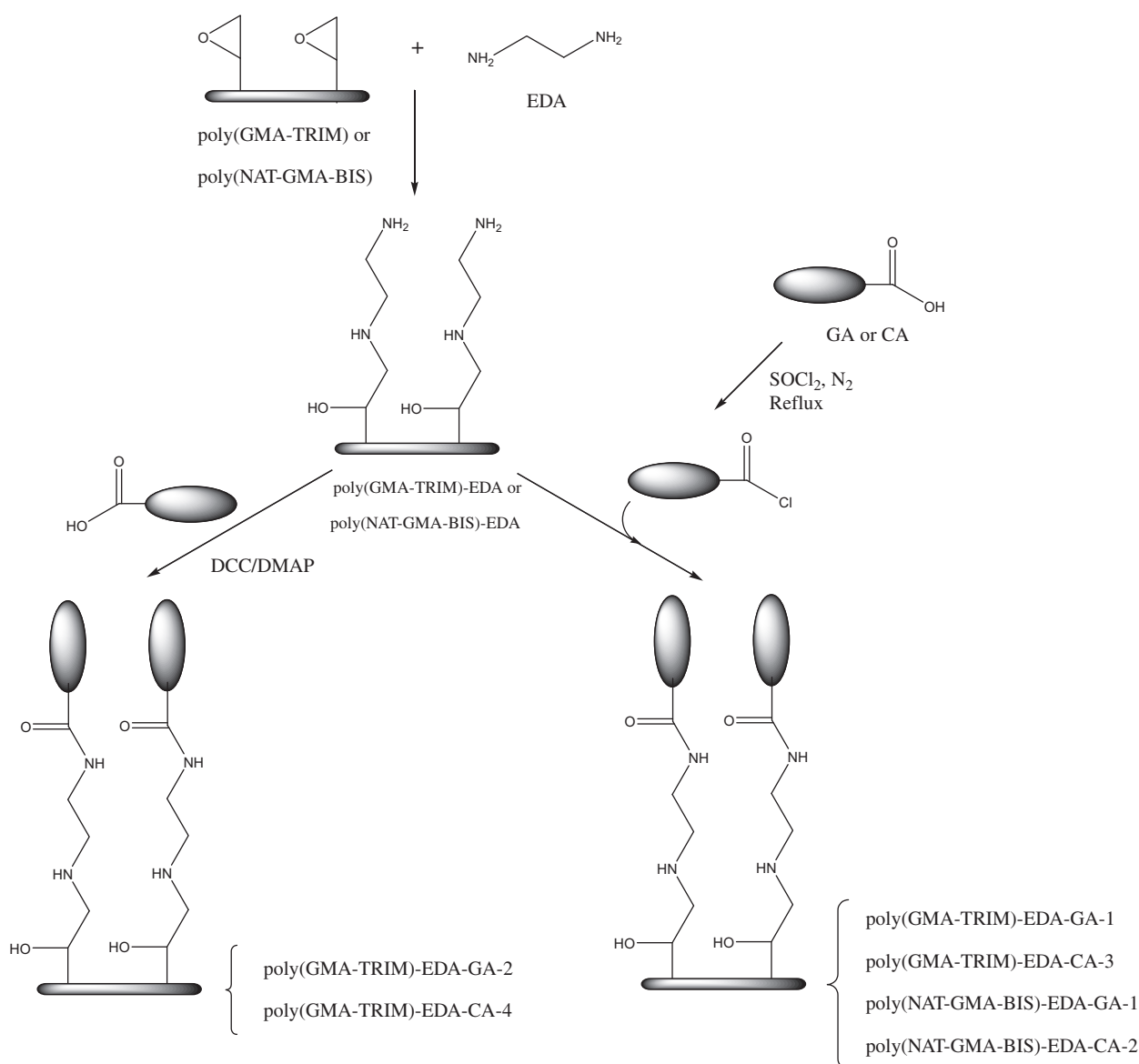

Fig. 1. Representation of the sequential reactions steps performed for the covalent immobilization of antioxidants (GA or CA) on porous poly(GMA–TRIM) and poly(NAT–GMA–BIS).

proceed at reflux for 6 h under stirring. After that, the THF and thionyl chloride in excess were evaporated under vacuum. A brown solid was obtained as the acyl chloride of the polyphenolic compound. Then, triethylamine (0.1 mL), each amine-containing polymers (0.40 g) and 25 mL of anhydrous THF were mixed in the reaction flask containing each acyl chloride. The coupling reactions were carried out at reflux for 10 h at 60 °C under stirring. Once the reactions finished, the modified polymers [poly(GMA–TRIM)–EDA–GA-1; poly(GMA–TRIM)–EDA–CA-3; poly(NAT–GMA–BIS)–EDA–GA-1 and poly(NAT–GMA–BIS)–EDA–CA-2] were purified with methanol in a Soxhlet apparatus for 24 h and dried under vacuum.

2.7. Determination of antiradical activity of the products

The radical scavenging properties of the porous polymers containing antioxidants in their structure were evaluated against two different radicals using their well-known bleaching methods: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·) [20] and 2,2′-azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation (ABTS$^{+·}$) [21]. All determinations were performed in triplicate.

2.7.1. DPPH· bleaching method

Approximately 17 mg of dried modified polymers were placed in a flask with 15 mL of DPPH· solution in methanol (initial absorbance at 515 nm c.a. 1.00 ± 0.01 AU). The mixture was stirred and the remaining DPPH· was measured by monitoring the absorbance changes at 515 nm every 15 min for 1 h. For each experiment, the suspension was centrifuged at 1500 rpm for 4 min and the absorbance of the supernatant was monitored. All determinations were performed in triplicate. The antiradical activity (ARA%) of the different polymers was calculated according to the following equation:

$$ARA\% = 100 \times (1 - A_{ss}/A_0) \qquad (2)$$

where A_{SS} is the absorbance of the solution in the steady state and A_0 is the absorbance of DPPH· solution before adding the polymer. The absorbance of the system in the steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software. The antioxidant capacities of the polymeric supports were expressed as μmol of the respective antioxidant equivalents per g of dry polymer. In this sense, calibration curves were prepared with series of standard solutions of CA and GA. Aliquots of 1 mL of each standard solution were added to 25 mL of DPPH· solution in methanol to reach final concentrations between 5 and 25 μM. Absorbance changes were monitored at 515 nm for 30 min. The percentage of the radical disappearance was then determined according to Eq. (2) and a calibration curve was obtained plotting ARA% vs. antioxidant concentration. The correlation coefficients (r) of the calibration curves for CA and GA were 0.9896 and 0.9924, respectively. Control reactions were performed using poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA macroporous polymers in order to quantify the unspecific antioxidant depletion from the polymeric supports without coupled antioxidant.

2.7.2. ABTS$^{+·}$ bleaching method

ABTS was dissolved in distilled water to yield a 7 mM solution. Radical cation solution was prepared by incubating 1 mL ABTS solution with 3.42 mL (2.45 mM) potassium persulphate solution in the dark for 16 h at room temperature. Subsequently, the colored radical solution was diluted with water to reach a value of absorbance of 1.00 ± 0.01 AU at 734 nm. In order to determine the antiradical capacity of the antioxidant-containing polymers, approximately 10 mg of dried supports were placed in a flask containing 15 mL of ABTS$^{+·}$ solution. The absorbance decrease at 734 nm was evaluated every 15 min for 1 h. The experimental de-sign was the same as that used for the DPPH· bleaching method. All determinations were performed in triplicate. The ARA% for ABTS$^{+·}$ was calculated according to Eq. (2) and the radical scavenging activities of the modified polymers were expressed as μmol antioxidant equivalents/g dry polymer. The calibration curve was prepared with standard solutions of CA and GA. Aliquots of 1 mL of each standard solution were added to 25 mL of ABTS$^{+·}$ solution in methanol to reach the final concentrations between 0.1 and 1.5 μM. The absorbance of the system was monitored at 734 nm for 30 min; subsequently, the percentage of the radical disappearance was calculated according to Eq. (2) and a calibration curve was obtained plotting ARA% vs. antioxidant concentration. The correlation coefficients (r) of the calibration curves for CA and GA were 0.9949 and 0.9881, respectively. Control reactions were performed using poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA supports.

2.8. Determination of available phenolic groups on the macroporous supports

The content of active phenolic groups on the macroporous solids was determined using the Folin–Ciocalteu method [22] with some modifications. For this, 50 mg of modified polymer were added to 1 mL of Folin–Ciocalteu reagent and 4 mL of distilled water. The mixture was stirred at room temperature. After 3 min, aliquots of 1 mL of 2% Na$_2$CO$_3$ and 3 mL of water were added. The reaction was allowed to stand for 3 h at room temperature. After that, the suspension was centrifuged at 1500 rpm for 15 min and the absorbance of the supernatant was measured at 760 nm. In order to determine the total phenolic content of the polymers, calibration curves were prepared using standard solutions of CA and GA with a concentration range between 5 and 25 μM. Control reactions were performed with the amine-containing polymers to analyse active groups. The correlation coefficients (r) of the calibration curves for CA and GA were 0.9783 and 0.9842, respectively. The results were expressed as μmol phenolic equivalents/g dry polymer. All determinations were performed in triplicate.

3. Results and discussion

3.1. Preparation of poly(GMA–TRIM) and poly(NAT–GMA–BIS) macroporous polymers

Macroporous polymers prepared by bulk polymerization generally present large porous sizes and considerable specific surface areas. According to such properties, these polymers may be interesting materials to be used as catalysts, solid phase chemicals or pollutant scavengers in view of the possible high mass transfer reached by convective transport. We have reported the preparation of poly(NAT–GMA–BIS) monoliths to be used as affinity supports in the purification of antithrombin III (AT-III) [17]. Due to the porous properties yielded using PEG 6000 as a porogenic solvent, poly(NAT–GMA–BIS) and poly(GMA–TRIM) were prepared as an epoxide-containing base material. Several studies reported the use of polymeric diluents as porogens in the synthesis of porous polymers [17,23–25]. The use of polymeric porogen in the polymerization mixture results in a phase separation at an early stage of the polymerization reaction, leading to products with macroporous structures. Table 1 shows the porous properties of poly(GMA–TRIM) and poly(NAT–GMA–BIS). These results indicate that poly(GMA–TRIM) had higher porosity (53.7%), the pore size of 685 nm being at the highest peak in the distribution profile curve, a specific surface area of 6.52 m^2/g (considering pore sizes in the range of 100–1000 nm) and a total pore volume of 1.085 mL/g.

Table 1
Porous properties and swelling characteristics of poly(NAT–GMA–BIS) and poly(GMA–TRIM) macroporous polymers.

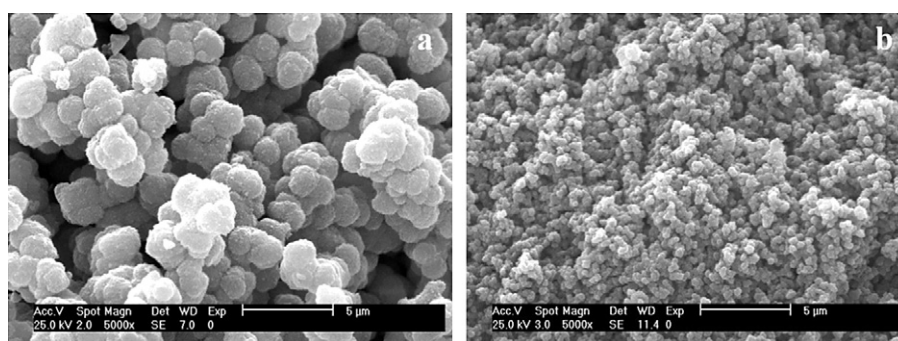| Sample	$D_{p,max}$[a] (nm)	V_p[b] (mL/g)	S_s[c] (m²/g)	Porosity (%)	q_w Water	Methanol
poly(GMA–TRIM)	684.9	1.085	52.8	53.7	2.0	2.2
poly(NAT–GMA–BIS)	304.6	1.012	61.5	54.0	2.6	2.3

[a] Pore size at the highest peak in the pore size distribution profile.
[b] Total pore volume.
[c] Specific surface area.

Fig. 2. Scanning electron micrographs (5000×) of porous poly(GMA–TRIM): (a) and poly(NAT–GMA–BIS) (b).

Fig. 2 shows the SEM image of poly(GMA–TRIM). This polymer showed a heterogeneous surface consisting of large microglobules aggregated to clusters. As shown, poly(GMA–TRIM) presented larger size clusters and consequently higher porous size than poly(NAT–GMA–BIS).

From Table 1, it is possible to confirm that poly(NAT–GMA–BIS) absorbed more water than poly(GMA–TRIM). The presence of hydrophilic monomers as NAT and BIS in the terpolymer allows it to have a more hydrophilic structure.

3.2. Surface modification of macroporous polymers

Glycidyl methacrylate is a monomer commonly used in the preparation of macroporous networks since the epoxide groups present in their structures can be easily modified to yield polymers with desired functionalities. In this work, this monomer was used in the preparation of both polymers. The content of epoxide groups in poly(GMA–TRIM) and poly(NAT–GMA–BIS) was 1.33 and 1.23 mmol epoxy/g of dry polymer, respectively.

The reaction yields of the synthesis of solid supports poly (GMA–TRIM) and poly (NAT–BIS–GMA) were gravimetrically determined, in all cases, close to 100%. Nevertheless, according to the epoxide content in poly(GMA–TRIM) and poly(NAT–GMA–BIS), only 97.8% and 64.9% of these epoxides derived from GMA would be accessible.

After the preparation of the porous polymers, the epoxide groups reacted at basic pH with EDA in order to obtain amine groups on the polymeric surface. The content of amine groups for the modified polymers poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA was 0.92 and 0.52 mmol amine/g of dry polymer, respectively. According to this quantification, the reaction for the incorporation of EDA yielded 70% and 45% for poly (GMA–TRIM)–EDA and poly (NAT–GMA–BIS)–EDA, respectively. These materials containing free amine groups in their structure were used for the covalent immobilization of the different antioxidants through amidation reactions. In this sense, two different approaches were used: (a) a single-step method consisting of a direct amidation reaction between the amine groups present in poly(GMA–TRIM)–EDA and the carboxylic groups of the polyphenolic compounds (GA and CA) using DCC/DMAP, and (b) a two-step method consisting in the formation of the acyl chlorides of the polyphenolic compound (GA and CA) and their subsequent amidation reaction with the amine groups present in poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA. Table 2 shows the total phenolic content for all the antioxidant-containing polymers.

Concerning the results of the quantitative determinations for phenolic moieties linked to the polymer matrix, we can conclude that the nucleophilic substitution reactions had low yields: 1% and 4% for poly (GMA–TRIM)–EDA and 2% and 6% for poly (NAT–BIS–GMA)–EDA for gallic and caffeic acids, respectively.

Table 2
Total phenolic contents and radical scavenging capacities against DPPH· and ABTS⁺· (µmol antioxidant/g dry polymer) of the synthesized polymers.

Polymer sample	Antioxidant immobilization approach	Phenolic content	Antiradical capacity DPPH·	ABTS⁺·
Poly(GMA–TRIM)–EDA	–	1.3 ± 0.3	−0.15 ± 0.02	1.5 ± 0.5
Poly(GMA–TRIM)–EDA–GA-1	SOCl₂	5.7 ± 0.1	10.8 ± 0.9	12 ± 2
Poly(GMA–TRIM)–EDA–GA-2	DCC/DMAP	8.7 ± 0.2	5.2 ± 0.4	7.6 ± 0.4
Poly(GMA–TRIM)–EDA–CA-3	SOCl₂	9 ± 1	35 ± 4	16 ± 1
Poly(GMA–TRIM)–EDA–CA-4	DCC/DMAP	7.5 ± 0.4	13.7 ± 0.2	14 ± 2
Poly(NAT–GMA–BIS)–EDA	–	0.7 ± 0.3	0.365 ± 0.004	4 ± 1
Poly(NAT–GMA–BIS)–EDA–GA-1	SOCl₂	10.1 ± 0.7	13.0 ± 0.3	20 ± 3
Poly(NAT–GMA–BIS)–EDA–CA-2	SOCl₂	13.2 ± 0.6	30.4 ± 0.3	33 ± 2

Comparing the two different strategies, the immobilization of CA on poly(GMA–TRIM)–EDA was more efficient using the two-step approach. However, in the case of GA, the amount of polyphenolic compound immobilized on the same polymeric scaffold was higher through the DCC/DMAP method. Despite such differences, the radical scavenging properties reached with the polymers in which the antioxidants were immobilized by the acyl chloride method were higher.

Comparing both polymeric supports in which the same antioxidant was immobilized by the two-step method, the extent of coupling was higher for poly(NAT–GMA–BIS)–EDA, in all cases. As an example, poly(NAT–GMA–BIS)–EDA–GA-1 and poly(GMA–TRIM)–EDA–GA-1 contained (10.1 ± 0.7) and (5.7 ± 0.1) μmol GA/g dry polymer, respectively. A similar trend was observed for the polymers with CA in their structure. These differences could be accounted for the larger specific area found in poly(NAT–GMA–BIS)–EDA (Table 1), allowing major availability of amine groups to react with the antioxidant molecules.

In our previous research [11] reporting UV-grafted films as polymeric supports, the covalent binding of the polyphenolic moiety to the films was accomplished by forming an ester functional group. In order to improve the stability of the linkage between the polymer and the antioxidant, and to extend the potential application of the macroporous polymer to a wider pH range in more aggressive conditions, we propose immobilization through the formation of an amide group as an important improvement from the viewpoint of the polymer robustness.

3.3. Antiradical activity of macroporous polymers containing immobilized phenolic compounds

The radical scavenging capacity of the polymers containing GA and CA was evaluated by the DPPH· and ABTS$^{+·}$ bleaching methods recognized for their simplicity and versatility. These methods are based on the disappearance of colored synthetic radicals due to the reaction with the antioxidant by hydrogen atom or electron donation. In this work, phenolic molecules covalently bound on the polymeric surfaces demonstrated to be able to produce the bleaching of both colored radical solutions. Table 2 shows the free-radical scavenging ability of polymers containing different polyphenolic compounds (immobilized by both methods). Antiradical activities were expressed in μmol antioxidant/g of dry polymer. The DPPH· and ABTS$^{+·}$ assays were also performed with control polymers (without antioxidant in their structures). The low antiradical activity observed in the DPPH· and ABTS$^{+·}$ assays with the control polymers poly(GMA–TRIM)–EDA and poly(NAT–

GMA–BIS)–EDA clearly indicates that the radical scavenger properties of the polymers studied in this work can be ascribed to the polyphenolic compounds immobilized on the polymeric support surfaces.

By comparing poly(GMA–TRIM)–EDA–GA-1 and poly(GMA–TRIM)–EDA–GA-2 with both DPPH· and ABTS$^{+·}$ methods, the anti-radical activity proved to be higher in those final products obtained using thionyl chloride. These differences are clearly seen in Fig. 3a and b, which show the kinetic depletion of DPPH· and ABTS$^{+·}$ radical species measured for the GA-containing polymers. The radical scavenging activities measured in the DPPH· and ABTS$^{+·}$ tests for poly(GMA–TRIM)–EDA–GA-1 (prepared using SOCl$_2$ in the two-step method) were (10.8 ± 0.9) and (12 ± 2) μmol GA/g of dry polymer, respectively. These values were higher than those for poly(GMA–TRIM)–EDA–GA-2 (prepared using DCC/DMAP in the single-step method), which were (5.2 ± 0.4) and (7.6 ± 0.4) μmol GA/g of dry polymer, respectively.

Fig. 4 shows a similar trend for poly(GMA–TRIM)–EDA–CA-3 and poly(GMA–TRIM)–EDA–CA-4 prepared by the two approaches. The antiradical scavenging capacity reached in the DPPH· and ABTS$^{+·}$ assays for poly(GMA–TRIM)–EDA–CA-3 (prepared using SOCl$_2$) was (35 ± 4) and (16 ± 1) μmol CA/g of dry polymer, respectively. However, the results obtained for poly(GMA–TRIM)–EDA–CA-4 (prepared using DCC/DMAP) for the DPPH· and ABTS$^{+·}$ tests were (13.7 ± 0.2) and (14 ± 2) μmol CA/g of dry polymer, respec-

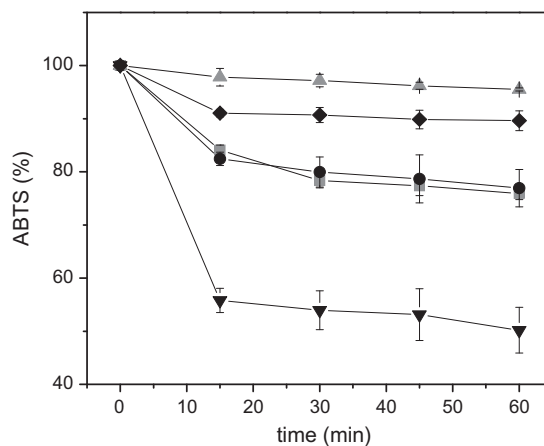**Fig. 4.** Kinetic behavior of ABTS$^{+·}$ solutions after additions of poly(GMA–TRIM)–EDA (▲), poly(GMA–TRIM)–EDA–CA-4 (via DCC/DMAP) (●), poly(GMA–TRIM)–EDA–CA-3 (via SOCl$_2$) (■), poly(NAT–GMA–BIS)–EDA (♦) and poly(NAT–GMA–BIS)–EDA–CA-2 (via SOCl$_2$) (▼) macroporous polymers.
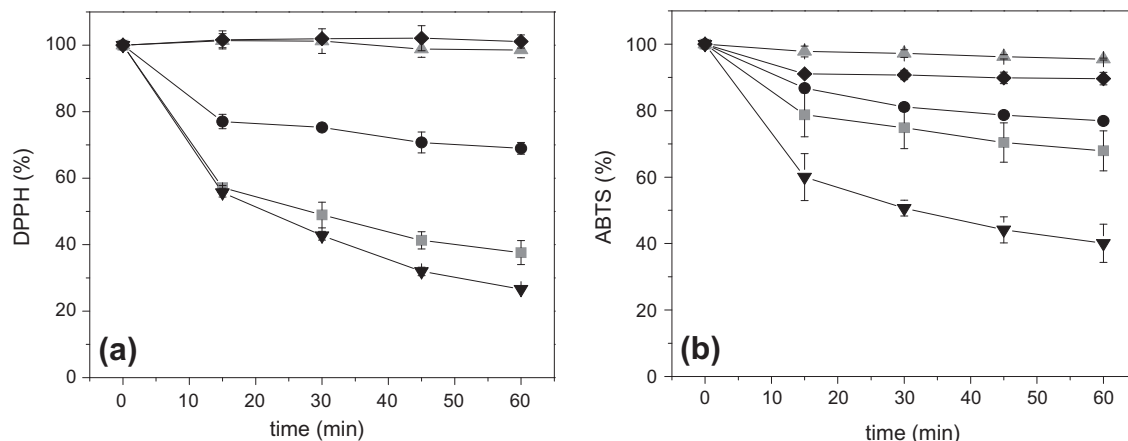
Fig. 3. Kinetic behavior of (a) DPPH· and (b) ABTS$^{+·}$ solutions after additions of poly(GMA–TRIM)–EDA (▲), poly(GMA–TRIM)–EDA–GA-2 (via DCC/DMAP) (●), poly(GMA–TRIM)–EDA–GA-1 (via SOCl$_2$) (■), poly(NAT–GMA–BIS)–EDA (♦) and poly(NAT–GMA–BIS)–EDA–GA-1 (via SOCl$_2$) (▼) macroporous polymers.

tively. Therefore, the two-step strategy led to polymers with higher antiradical activity. A possible drawback to using this last approach is that it needs the previous formation of the acyl chloride. Concerning the use of carbodiimide as a coupling agent, its main disadvantage involves the difficulties posed to remove the urea byproduct from the polymeric structure due to its limited solubility in most organic solvents [26]. From these results, GA and CA were more conveniently bound to poly(NAT–GMA–BIS)–EDA by the acyl chloride method.

Comparing the two different polymeric scaffolds, the antiradical capacities of the final products in all cases were higher when poly(NAT–GMA–BIS) porous polymer was used (Figs. 3a and b and 4). For instance, the antiradical activity shown by poly(NAT–GMA–BIS)–EDA–GA-1 for the DPPH$\cdot$ and ABTS$^{+\cdot}$ methods was (13.0 ± 0.3) and (20 ± 3) µmol GA/g of dry polymer, respectively. The same parameters for poly(GMA–TRIM)–EDA–GA-1 were (10.8 ± 0.9) and (12 ± 2) µmol GA/g of dry polymer, respectively. The higher radical scavenging properties reached with poly(NAT–GMA–BIS) supports could be related to both, the higher specific surface and the higher hydrophilicity of this polymer, mainly attributed to the presence of NAT monomer in its structure. On the other hand, the swelling of poly(NAT–GMA–BIS) (Table 1) is larger than that of poly(GMA–TRIM) in the presence of the polar solvents used for the DPPH$\cdot$ and ABTS$^{+\cdot}$ assays (methanol and water, respectively); therefore, the availability of the antioxidant active groups to react with the radical species is higher.

In relation to the two phenolic compounds analyzed in this work, in both polymeric supports, CA showed higher radical scavenging activity than that of GA. This behavior could be ascribed to the presence of the electron-donating group —CH=CH—CO- found in the CA structure, which could stabilize the radical formed after abstraction of an H-atom [27].

The values of ARA% yielded in this study are in the same order as those reported for porous polymeric beads containing different antioxidants. However, the polymers obtained in this work improved considerably the kinetic aspect of the scavenging reaction, and, therefore, enhanced their potential as antiradical membranes. Maeda et al. [15] immobilized different polyphenolic compounds on porous poly(chloromethylstyrene-co-tetraethyleneglycol dimethacrylate) beads prepared by suspension polymerization. This polymer exhibited porous size of 50 nm and specific surface of 30 m^2/g. The DPPH$\cdot$ depletion reached with that support ranged between 10% and 90%. However, in all cases, the steady states reached were above 500 min. As can be seen from Figs. 3a and b and 4, the radical scavenging activities obtained with the polymers presented in this work reached their higher activity in less than 30 min. These differences are mainly related to the open porous structure found in poly(GMA–TRIM) and poly(NAT–GMA–BIS) which increases the mass transfer between the radical species and the antioxidant groups immobilized on the polymeric surfaces. Moreover, the use of porous structures yielded supports with higher antiradical activities than those reached for polymeric films containing CA [11]. The radical scavenging properties displayed by the polymers poly(GMA–TRIM)–EDA–CA-3 and poly(NAT–GMA–BIS)–EDA–CA-2 obtained in this work were over 5 times higher than the activities previously reported for polypropylene films [11].

4. Conclusion

In this work we reported the successful immobilization of polyphenolic compounds on aminated poly(GMA–TRIM)–EDA and poly(NAT–GMA–BIS)–EDA macroporous polymers. The radical scavenging properties reached using these porous materials were higher than those previously reported by using polypropylene films as polymeric supports. Due to the open-porous structure obtained with these polymeric scaffolds, the modified polymers showed fast and efficient antiradical activity towards DPPH$\cdot$ and ABTS$^{+\cdot}$ radical species. From these results, it can be concluded that the radical scavenging properties attained with these materials were related to the hydrophilicity of the polymeric scaffold, the type of polyphenolic compound, and the immobilization strategy used for the covalent binding of the antioxidant. Although the radical scavenging properties of the modified polymers were tested in batch mode, a similar synthetic strategy could be used to immobilize polyphenolic compounds on monolithic columns, and these materials could be therefore used in flow-through applications.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.reactfunctpolym.2012.07.017.

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