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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.

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 $DPPH^{\cdot}$ and $ABTS^{+\cdot}$.

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 ($DPPH^{\cdot}$), 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:

$$q_w = \text{swollen mass}/\text{dry mass} \quad (1)$$

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