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Chromatographic matrix based on hydrogel-coated reticulated polyurethane foams, prepared by gamma irradiation

Mirna L. Sánchez[a], Claudia Y. Giménez[a], Juan F. Delgado[b], Leandro J. Martínez[a], Mariano Grasselli[a,*]

[a] Laboratorio de Materiales Biotecnológicos (LaMaBio), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes-IMBICE-CONICET-CICPBA, Bernal, Argentina

[b] Laboratorio de Obtención, Modificación, Caracterización y Evaluación de Materiales (LOMCEM), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, CONICET, Buenos Aires, Argentina

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ABSTRACT

Novel chromatographic materials for protein purification with high adsorption capacity and fouling resistance are highly demanded to improve downstream processes. Here, we describe a novel adsorptive material based on reticulated polyurethane foam (rPUF) coated with a functional hydrogel layer. rPUF provides physical rigidity through its macroscopic structure, whereas the hydrogel layer provides capacity to adsorb proteins by specific interactions. The hydrogel coating process was performed by the dip-coating method, using a polyvinyl alcohol (PVA) solution. The PVA hydrogel was linked to the rPUF material by using a radiation-induced crosslinking process in aqueous ethanol solution. The ethanol in the solvent mixture allowed a balance between PVA swelling and PVA dissolution during the irradiation step. The resulting material showed higher thermal stability than the non-irradiated one. In addition, a simultaneous radiation-induced grafting polymerization (SRIGP) was done by simple addition of glycidyl methacrylate monomer into the irradiation solution. In a further step, sulfonic ligands were included specifically in the hydrogel layer, which contained around 200% of PVA respect to the original rPUF. Materials were characterized by FT-IR, thermogravimetric analysis, SEM microscopy and EDX analysis. The cation-exchange rPUF material was functionally characterized by the Langmuir isotherm and a dynamic adsorption experiment to analyze the chromatographic properties for protein purification processes.

1. Introduction

Modern bioprocesses depend heavily on the availability of materials that allow obtaining competitive products in terms of quality and costs. Historically, hydrogels have been very important materials in the field of biochemistry. Chromatographic matrices and electrophoresis gels are currently the most successful materials used in protein analysis and purification. In addition, hydrogels have expanded their applications into various biological areas, such as contact lens materials, orthopedic applications, and devices for controlled release of drugs (Hyon et al., 1994; Hoffman et al., 2002; Casolaro et al., 2006; Bae et al., 2006).

Different types of hydrogels can be prepared from natural polymers, as well as from semisynthetic and synthetic polymers, most of which are obtained by crosslinking of their aqueous solutions. As an example, hydrogels have been obtained from polysaccharides and polyacrylamides (Porath and Flodin, 1959; Hjertén and Mosbach, 1962) and chemical crosslinking has been performed to improve their mechanical properties, which are still the main limitation.

From the early days of the radiation processing, radiation-induced crosslinking has been identified as useful to prepare hydrogels (Alexander and Charlesby, 1957; Danno, 1958). At well-defined irradiation doses, PVA hydrogels can be obtained from a PVA solution (Peppas and Merrill, 1976). To introduce some functionality, PVA hydrogels have been grafted by radiation methods with different ligands to prepare adsorptive materials to recover different ions. An example of this is the application of hydrogels in decontamination (Güven et al., 1999).

In the last two decades, one-piece porous polymeric materials (also called monoliths) have been applied to separate macromolecules (Krajnc et al., 2005). These materials add two advantages respect to hydrogels: (i) much higher mechanical resistance and (ii) an internal structure of interconnected channels which facilitate mass transport. Therefore, monolithic columns with these unique structures allow high flow rates at low pressures without losing column efficiency; as a result, a quick separation is achieved (Cabrera et al., 2000).

Porous monolithic materials can be prepared by means of a wide

* Corresponding author.

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variety of techniques (reviewed by Svec (2010)), including the use of radiation-induced polymerization (Grasselli et al., 2001). As mentioned above, solid porous monoliths have good mechanical and hydrodynamic properties. However, they exhibit a very low adsorption capacity. This is associated with the fact that chromatographic ligands are linked to a low specific surface area of these non-hydrogel-based materials.

One particular case is the porous monolithic material called cryogel. This material is prepared by a polymerization reaction of water soluble monomers in freezing state, where the pore structure is obtained by the phase separation of water in frozen state during the polymerization process (Mattiasson et al., 2010). Therefore, the cryogel material is formed by a hydrophilic crosslinked polymer; however, this material has also low adsorption capacity for protein purification (Mattiasson et al., 2010). A recent publication has described an improvement of the adsorptive capacity of this material by simultaneous radiation-induced grafting polymerization (SRIGP) (Bibi et al., 2011). This improvement is due to the grafting modification that occurs in the bulk of the cryogel material. SRIGP is a modification method based on ionizing radiations, which generate radicals onto the base polymer that are responsible for chemical reactions. In this way, the grafting polymerization process occurs in the presence of methacrylate monomers onto a base polymer.

Here, we describe a novel approach to prepare porous monolithic adsorptive materials, based on radiation-crosslinking and functionalization of hydrophilic coating onto reticulated polyurethane foams (rPUFs). These materials were analyzed for chromatographic applications to recover macromolecules such as proteins.

2. Materials and methods

2.1. Materials

rPUF from Eurofoam Deutschland GmbH was kindly donated by Prof. Marcelo Fernandez Lahore (Jacobs University). rPUF type Filtren TM 60, with a pore size of about 250 μm, was used as base material without purification. The material was cut into small cylinders of 0.7 cm in diameter and 2.8 cm in height.

PVA Mowiol 10–98, M.W. 61,000 (Sigma-Aldrich, catalog number 10852) hydrolysis grade 98–98.8%, Hydroxyethyl cellulose (HEC), M.W. 720,000 (Sigma-Aldrich code number 9004-62-0) and Agarose (Biodynamics, Molecular biology, low electroendosmosis grade) were used. Glycidyl methacrylate (GMA), dimethyl acrylamide (DMAA) and Lysozyme were from Sigma Chemical Co. (USA). Acetone, sodium sulphite anhydrous, isopropanol, ethanol, and other chemical reagents were purchased from Anedra (Buenos Aires, Argentina). All chemicals used were analytical grade.

2.2. Hydrogel coating preparation

The preparation of the material was divided in four main steps: (i) foam coating; (ii) crosslinking and (iii) grafting procedure and (iv) ligand functionalization reaction.

2.2.1. Foam coating

rPUF was coated with different water-soluble polymer solutions. Each polymer solution was prepared taking into account the maximum dissolution of each polymer in water. The concentrations of each polymer solution are listed in Table 1.

rPUF cylinders were coated with hydrophilic polymer solutions by the dip-immersion method. Pieces of the porous material were submerged in a selected polymer solution at a given temperature for ten seconds and further squeezed to remove the polymer in excess. Immediately, they were immersed in isopropanol at 85 °C for ten seconds, squeezed and then dried in an oven at 55 °C until constant weight. The coating degree (*CD%)* was calculated as the percentage of increase in dried weight, as follows (Eq. (1)):

Table 1
Polymer solutions and conditions of the coating process.

Polymer	Concentration (w/v)	pH	Temperature (°C)
Agarose	8	7	85
HEC (90 kDa)	1.4	7	R.T.
PVA (61 kDa)	10	7	85

$$CD\% = 100 \bullet \left[\frac{W_1 - W_o}{W_0} \right] \quad (1)$$

where W_0 and W_1 are the initial and final weights of the material, respectively.

2.2.2. Grafting/crosslinking process

Radiation-induced crosslinking and grafting polymerization was performed by irradiation of polymer samples with a gamma source. Coated rPUF materials, soaked in an irradiation solution, were sealed in a sample container. Then, 0.4 g of coated and dried material (10 cylinders) was soaked in 100 mL of ethanol:water 1:1 v:v. To perform simultaneous crosslinking and SRIGP, GMA or GMA and DMMA monomers were added to the irradiation solution. The following conditions were studied: 2%v/v, 4%v/v, and 6%v/v of GMA, 5%v/v of DMAA, 5%v/v of DMAA + 1%v/v of GMA, and 5%v/v of DMAA + 4% v/v of GMA. Solvents were previously degassed for 15 min by nitrogen gas bubbling.

The samples in the containers were irradiated in a ^{60}Co irradiation source (PISI semi-industrial source, Comisión Nacional de Energía Atómica, Ezeiza, Argentina) with a 10 kGy irradiation dose at a dose rate of 1 kGy h^{-1} (if not specified) and room temperature. Dosimetry was performed with Red Perspex type dosimeters. After irradiation, materials were washed with ethanol:water 1:1 v:v and subsequently with 96% ethanol several times until all the homopolymer trapped inside the porous structure was washed out. Materials were dried at 55 °C in an oven until constant weight. The grafting degree (*GD%*) was calculated as the percentage of increase in weight, as follows (Eq. (2)):

$$GD\% = 100 \bullet \left[\frac{W_2 - W_1}{W_1} \right] \quad (2)$$

where W_1 and W_2 are the weights of the coated and the grafted material, respectively.

2.2.3. Hydrogel functionalization

Strong cation-exchange ligand was immobilized onto the hydrogel based on the ring-opening reaction of the epoxy groups of polyGMA with sodium sulphite (Camperi et al., 1999). Then, 0.2 g of grafted rPUF was incubated with 20 mL of a mixture of sodium sulphite/isopropanol/water (10:15:75 w/w/w) at 37 °C, overnight under agitation.

After extensive washing with water, the remaining epoxy groups to diol groups were hydrolyzed by incubation with a H_2SO_4 solution 0.5 M at 80 °C for 2 h (Camperi et al., 1999). After extensive washing with water, the cation-exchange rPUF (SP-rPUFs) were dried at 55 °C in an oven overnight.

The sulfonic groups were quantified by Inductively Coupled Plasma Spectroscopy with Atomic Emission Spectroscopy (ICP-AES) (INCAPE-CENACA, Universidad Nacional de la Plata, La Plata, Buenos Aires, Argentina).

2.3. Material characterization

2.3.1. Swelling degree

Swelling experiments were performed by placing 0.04 g of the material in distilled water overnight. After the incubation, the material was removed from the water, quickly and carefully dried with a filter paper, and its weight gain measured. The swelling degree was