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Evaluation of 2,3-epoxypropyl groups and functionalization yield in glycidyl methacrylate monoliths using gas chromatography

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ABSTRACT

Poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (poly(GMA-*co*-EDMA)) is most frequently used as parent monolith to obtain stationary phases with a variety of surface chemistries for liquid chromatography and capillary electrochromatography. Functionalization is performed by opening the accessible 2,3-epoxypropyl groups of the monolith with a suitable reagent. The number of 2,3-epoxypropyl groups which are accessible before and after the functionalization reaction, and the grafting yield, are important parameters, required both to optimize functionalization and to interpret the chromatographic performance of functionalized monoliths. In this work, a method capable of providing this information for parent and functionalized poly(GMA-*co*-EDMA) monoliths prepared both *in silica* capillaries and in other supports is proposed. First, sulfuric acid and lithium aluminium hydride ($LiAlH_4$) are sequentially used to release the 2,3-epoxypropyl groups as glycerol, which is subsequently determined by GC. About 6.0 mmol of 2,3-epoxypropyl groups per gram of monolith was found in this work for the parent monoliths prepared *in silica* capillaries using UV-initiation. The monoliths were also functionalized using ammonia (NH_3), diethylamine (DEA) and epinephrine, and the amount of residual 2,3-epoxypropyl groups, and hence the functionalization yield, were established by also measuring the GC peak of glycerol. The amounts of 2,3-epoxypropyl groups and the derivatization yields were established with RSDs of 1.7 and 3.4%, respectively. The proposed method was also applied to the characterization of poly(GMA-*co*-EDMA) monoliths prepared in glass vials. Significant differences with respect to those prepared in 100 μm I.D. silica capillaries were evidenced.

1. Introduction

Organic monolithic stationary phases, which emerged in the early 90s of the past century, are still attracting much interest. Their easy *in situ* preparation, high permeability and biocompatibility, stability along wide pH-ranges, and readily modifiable surface chemistries make these materials to be a competitive alternative to the conventional particle-based stationary phases [1–3]. In contrast to the single-step copolymerization approaches, the functionalization of the monolith after polymerization is easily performed, offering a much wider range of possibilities. Surface functionalization allows the independent tuning of both surface chemistry and the mechanical and flow-through porous properties of the parent monolith. Also, functionalization provides an excellent way of sequentially using reagents that could be incompatible with polymerization mixtures for solubility or redox reasons. In particular, functionalization at the 2,3-epoxypropyl groups of poly(GMA-*co*-EDMA) by ring opening is the most frequently used methodology for monolith surface modification [4–23]. A wide variety of chromatographic properties (ion-exchange, hydrophobic/hydrophilic, chiral, *etc.*) can be achieved and finely tuned. Thus, poly(GMA-co-EDMA) based monoliths have been modified by using amines [5,7–9], amino acids [10,11], polymers [9,12], sodium sulfite [13], sodium hydrogen sulfide [9,14], sulfuric acid [15,16], a variety of chiral ligands [9,17–22] and nanoparticles [11,23].

An important aspect of monolith functionalization is the evaluation of the initial number of 2,3-epoxypropyl groups, and after functionalization, the evaluation of the derivatization yield. During functionalization, part of the 2,3-epoxypropyl groups could remained unreacted, particularly if the functionalization reagent exhibits steric hindrance to access some of them. In addition, part of the 2,3-epoxypropyl groups may have been hydrolyzed to yield 2,3-dihydroxypropyl groups, particularly under harsh

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functionalization conditions. Then, the functionalization yield should be established by evaluating the joint concentration of both the 2,3-epoxypropyl and 2,3-dihydroxypropyl groups that are still present after functionalization. Hence, quantitative information about the amount of ligand actually bonded to the monolith can be indirectly derived. This information is relevant both to optimize functionalization procedures and to interpret the chromatographic performance of the functionalized monoliths. However, a literature survey reveals that this relevant information is rarely available. To our knowledge, procedures to determine the functionalization yield of the monolith have been provided and applied in a few reports [8,14,15,22]. Further, the functionalization yield has been generally established in monoliths prepared as bulk probes (as in glass vials), and only rarely inside the capillary columns which have been prepared for liquid chromatography (LC) or capillary electrochromatography (CEC) separations [8,14]. The material of the bulk probes is generally analyzed by a number of techniques, including FTIR and Raman spectroscopies, differential scanning calorimetry and elemental analysis. These techniques provide useful information about the monolith structure and composition, but the information related to the surface functionalization is frequently missed. Also, when the amount of grafted groups is a minor mass fraction of the monolith, methods addressed to determine the groups by analyzing the whole material are susceptible to be affected by large systematic and random errors. Therefore, the development of a universal procedure which could be employed to determine the amounts of 2,3-epoxypropyl groups and the functionalization yield, independently from the functionalization procedure and grafted reagents, in monoliths prepared in any format, including capillaries, is nowadays a demanding requirement. On the other hand, monoliths prepared as bulk materials may significantly differ from those obtained in capillary format. However, as far as we know, the possible structural and functional differences between monoliths obtained in these largely different formats have not been investigated. A procedure capable of measuring the amount of accessible reactive group with the required accuracy in both capillary monoliths and bulk materials would be also useful to investigate differences between them.

In this work, a method based on two quick reactions, addressed to release the 2,3-epoxypropyl and 2,3-dihydroxypropyl groups as glycerol, followed by its determination by gas chromatography with flame ionization detection (GC-FID), is proposed. The method was applied both to monoliths prepared in capillaries and glass vials, in both cases before and after opening of the epoxy rings with NH_3, diethylamine (DEA) and epinephrine. The amount of these ligands that were grafted on the monoliths was first established from the difference between the amount of glycerol found before and after functionalization. Hence, the functionalization yield was calculated as the ratio between the amount of grafted ligands and that of the reactive groups found in the parent monoliths. The results obtained using capillary columns were compared with those obtained using bulk monoliths prepared in glass vials, which provided evidence about their differences.

2. Materials and methods

2.1. Reagents and other materials

Glycidyl methacrylate (GMA), ethylene dimethacrylate (EDMA), 3-(trimethoxysilyl)propyl methacrylate, diethyl amine (DEA), ammonia (NH_3), glycerol, 1-amino-2,3-propanediol, 3-(dimethylamino)-1,2-propanediol, ethylene glycol, diethylene glycol and lithium aluminium hydride ($LiAlH_4$) were from Aldrich (Milwaukee, WI, USA). Methanol (MeOH), HPLC-grade diethyl ether (Et_2O) and hexane were from Scharlab (Barcelona, Spain). Azobisisobutyronitrile (AIBN) was from Fluka (Buchs, Switzerland). Uncoated fused-silica capillaries of 10 cm total length and 375 μm O.D. × 100 μm I.D. with UV-transparent external coating (Polymicro Technologies, Phoenix, AZ, USA) were used.

2.2. Instrumentation

To introduce the functionalizing reagents into the monolithic capillary columns, a syringe pump (Model 100, KD Scientific, New Hope, PA, USA) was employed. The washing steps used for monoliths in capillaries were performed with an HPLC pump (1100 Series, Agilent Technologies, Waldbronn, Germany). A 7890A GC system, equipped with a G4513A autosampler and a FID (Agilent Technologies), and provided with a Zebron ZB-WAX plus column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) from Phenomenex (Aschaffenburg, Germany), was employed. Nitrogen was used as carrier.

2.3. Preparation of poly(GMA-co-EDMA) monoliths

For the preparation of monoliths in capillary format, and prior to polymerization, the inner wall of the fused-silica capillaries was modified to provide covalent attachment of the monolith. For this purpose, wall modification with 3-(trimethoxysilyl)propyl methacrylate was performed as described [24,25]. The polymerization mixtures were prepared by weighing GMA (20 wt%), EDMA (5 wt%), and a binary pore-forming solvent constituted by cyclohexanol (70 wt%) and 1-dodecanol (5 wt%). AIBN (1 wt% with respect to the monomers) was added as initiator [8,9]. To obtain a clear solution, sonication for 10 min followed by purging with nitrogen for 10 more min was applied. The preconditioned capillary was filled with the polymerization mixture up to a length of 10 cm. Photopolymerization was accomplished by irradiation of the capillaries within an UV chamber at 0.9 J cm^{-2} for 30 min. Then, an HPLC pump was used to flush the columns for 30 min with MeOH to remove the pore-forming solvents and remaining unreacted monomers.

Bulk monoliths were also prepared in 1.5 cm I.D. glass vials by using 0.5 mL of polymerization mixture. The vials were located opened inside the UV chamber to be irradiated in the same conditions as those used for the capillaries, although silanization of their inner surface was not performed. After polymerization, the vials were broken and the bulk material was separated from the glass pieces. This material was repeatedly washed with MeOH on a Büchner funnel to remove the pore-forming solvents and unreacted monomers and dried at 80 °C for 6 h. The bulk polymer was ground into a very fine powder with mortar and pestle.

2.4. Functionalization of poly(GMA-co-EDMA) monoliths with amine compounds

The reaction schemes for the functionalization of poly(GMA-*co*-EDMA) monoliths with amine compounds are illustrated in Fig. 1. First, the capillary columns were flushed with MeOH, followed by flushing with an aqueous solution of either NH_3, DEA or epinephrine. For NH_3 and DEA, 4.5 M aqueous solutions at 60 °C were passed for 2 h at 60 μL h^{-1} [9]. For epinephrine, a 10 mM solution in 100 mM aqueous sodium tetraborate of pH 8.0 at 60 °C was passed for 2 h at 240 μL h^{-1} [8]. Then, the capillaries were washed with MeOH until reaching pH 7 at the outlet.

The bulk monolithic materials (obtained from glass vials) were functionalized under similar preparation conditions as described above for the capillaries. Thus, powdered bulk material (5 mg) was mixed with the derivatization solutions, and the mixtures were stirred at 60 °C for 2 h. In particular, *ca*. 0.7 mL and 1.3 mL