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Preparation and characterisation of poly(high internal phase emulsion) methacrylate monoliths and their application as separation media

Peter Krajnc[a,*], Nermina Leber[a], Dejan Štefanec[a], Sandra Kontrec[b], Aleš Podgornik[b]

[a] *Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova 17, SI-2000 Maribor, Slovenia*
[b] *BIA Separations d.o.o., Teslova 30, SI-1000 Ljubljana, Slovenia*

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Abstract

Poly(glycidyl methacrylate-co-ethyleneglycol dimethacrylate) monolithic supports were prepared by radical polymerisation of the continuous phase of water in oil high internal phase emulsions. Morphology of monolithic materials was studied by scanning electron microscopy and mercury intrusion porosimetry. The ratio of phase volume and the degree of crosslinking influenced the void size and pore size distribution of resulting polymers. Void sizes between 1 and 10 μm were observed and average pore sizes around 100 nm. Polymers with 60, 75, 80 and 90% pore volume were prepared and even samples with highest pore volume showed good mechanical stability. They were modified to bear weak-anion exchange groups and tested on the separation of standard protein mixture containing myoglobin, conalbumine and trypsin inhibitor. Good separation was obtained in a very short time similar to the separation obtained by commercial methacrylate monoliths. However, higher dispersion was observed. Bovine serum albumin dynamic binding capacity for monolith with 90% porosity was close to 9 mg/ml.

Keywords: Methacrylate monoliths; PolyHIPE; Emulsion polymerisation; Protein separation; Polymer chromatographic supports

1. Introduction

Monolithic chromatographic supports are nowadays used in many different areas, from microchips up to preparative purifications [1]. The main reason is their advantageous features over conventional particle shaped chromatographic supports. Columns filled with bead shaped particles suffer from channeling of the solution and therefore the efficiency of the support can be reduced [2]. This is not the case with the monoliths since they consist of a single block of highly porous material. Besides, convection based transport and very high dynamic porosity are two of the most outstanding properties. Monolithic supports can be prepared by different methods and with various chemistries [1]. Silica based monolithic columns exhibit the highest porosity, over 80%, and are mainly used for RP separation and purification of smaller molecules [3]. On the other hand, methacrylate based monoliths, were applied in a variety of shapes and separation modes for the purification of large molecules like proteins, polynucleotides or even viruses [4]. Usual way of preparing methacrylate monoliths is via bulk polymerisation in the presence of porogenic solvents. In such a manner materials with porosities up to 65% are prepared. Beyond this value, their mechanical stability becomes poor.

An alternative method for the preparation of highly porous monolithic polymer material is polymerisation of the continuous phase of a high internal phase emulsion (HIPE).[1] Typically, the yielding polymer has an open cellular structure with interconnects, which is the result of the internal phase being trapped inside the continuous phase during the polymerisation. After the extraction of internal phase, the porous structure remains. Such monolithic polymers, termed PolyHIPE [6] were initially prepared as styrene/divinylbenzene copolymers and applied as precursors for reactive species [7], as biocatalysts supports [8] and as supports for filtration [9]. With the addition of 4-vinylbenzyl chloride as a monomer,

* Corresponding author. Tel.: +386 2 2294 422; fax: +386 2 2527 774.
E-mail address: peter.krajnc@uni-mb.si (P. Krajnc).

[1] A high internal phase emulsion is one in which the internal phase represents more than 74.05% of total emulsion volume (see [5]).

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a reactive PolyHIPE monolith was produced, functionalized and utilized as a scavenger in a flow through mode [10]. The porosity of such a material can be further enhanced by adding a porogenic solvent to the continuous phase and monoliths with surface area up to 700 m^2/g were prepared in such manner [11].

Open cellular structure of PolyHIPE monolithic materials suggests the possible applications of such monoliths as a separation media. We were therefore intrigued by the possibility of preparing glycidyl methacrylate based PolyHIPE monoliths. Despite the fact that methacrylates posses very attractive chemistry for chromatographic supports, due to their high mechanical and chemical stability, we have found no reports of such a material, while the preparation of poly(glycidyl methacrylate) grafted PolyHIPE material was recently published [12].

In this work, the preparation of poly(glycidyl methacrylate–co-ethyleneglycol dimethacrylate) PolyHIPE monolithic material, its characterization and the application as a chromatographic support for protein separation are described.

2. Experimental

2.1. Polymerisation and modification

2.1.1. Chemicals

Glycidyl methacrylate (GMA; Aldrich, Steinheim, Germany) and ethylene glycol dimethacrylate (EGDMA; Aldrich) were washed with 5% $NaOH_{aq}$ to remove the inhibitors. Potassium persulfate (Fluka), calcium chloride hexahydrate (Merck, Darmstadt, Germany), the surfactant Synperonic PEL 121 (ICI Chemical, London, UK) and diethylamine (DEA; Fluka, Buchs, Switzerland) were used as received.

2.1.2. Polymerisation of GMA/EGDMA PolyHIPE

Organic phase, consisting of 14.51 g of GMA, 6.76 g of EGDMA and 4.28 g of synperonic PEL 121 was placed in a three necked flask and the mixture was stirred with an overhead stirrer at 350 rpm. To this aqueous phase (90 ml in the case of 90% pore volume, 80 ml in the case of 80% pore volume, 75 ml in the case of 75% pore volume and 60 ml in the case of 60% pore volume consisting of 0.2% potassium persulfate and 2% calcium chloride hexahydrate in deionised water) was added dropwise. The emulsion was transferred to the mold (PET container) and cured at 55 °C for 48 h. Monoliths were purified via Soxhlet extraction with water (24 h) and ethanol (24 h) and dried in vacuo at 50 °C.

Monoliths with different degrees of crosslinking (EGDMA) were prepared in the same manner.

2.1.3. Modification of epoxy groups

Three PolyHIPE monolithic disks of a diameter 12 mm and thickness 3 mm were immersed in 50 ml of DEA for 20 h at 32 °C. Monoliths were extensively washed with ethanol and distilled water before usage.

2.2. Structural characterisation of the monoliths

FT-IR spectra were recorded on a Perkin-Elmer FT-IR 1650 spectrometer (Fremont, USA) and scanning electron microscopy pictures were taken on a Jeol JSM-840A (Tokyo, Japan).

The pore size distribution was determined using mercury porosimetry Pascal 440 (ThermoQuest Italia, Rodano, Italy) in the range of 14–20 000 nm. A piece from the monolith was cut to an approximate weight of 0.1 g and completely dried before the measurement.

Porosity of the PolyHIPE monolith was determined by immersing dried sample of disk geometry into distilled water. Dimensions and masses of dry and wetted monolith were measured and the porosity was calculated according to equations:

$$\varepsilon = \frac{(\Delta m/\rho) - \Delta V}{V_d} \quad (1)$$

$$\Delta V = V_w - V_d \quad (2)$$

where ε: porosity (/); Δm: mass difference between the wet and the dry state (g); V_w: volume of the wet monolith (ml); V_d: volume of the dry monolith (ml); ρ: density of the wetting liquid (g/ml).

2.3. Chromatographic characterisation of the monoliths

2.3.1. Equipment

Chromatographic experiments were performed on a gradient HPLC system built of two 64 Pumps, an injection valve with a 20 μl SS sample loop, a variable wavelength monitor with a 10 mm optical path set to 280 nm with a 10 μl volume flow-cell, the response time was set to 0.1 s and the HPLC hardware/software (data acquisition and control station), were all from Knauer (Berlin, Germany).

2.3.2. Protein separation

Protein separation on PolyHIPE methacrylate monoliths was performed using standard protein solution. Myoglobin (Sigma, St. Louis, MO, USA), conalbumin (Sigma) and soybean trypsin inhibitor (Fluka) were dissolved in 20 mM Tris–HCl, pH 7.4 (binding buffer) to the following concentrations: 1, 3 and 4 mg of protein/ml. The eluting buffer was 20 mM Tris–HCl, pH 7.4 containing 1 M NaCl. A linear gradient from 0 to 70% of elution buffer in 53 s was applied. Flow rate was 4 ml/min. As a reference a convective interactive media (CIM) DEAE monolithic disk was used (BIA Separations, Ljubljana, Slovenia).

2.3.3. Dynamic capacity measurement

The bovine serum albumin (BSA; Fluka) was dissolved in a binding buffer (20 mM Tris–HCl, pH 7.4) to the concentra-

tion of 1 mg/ml. The solution was pumped through the monolithic column at a flow rate of 3 ml/min and the absorbency, set at 280 nm, of the outlet was measured. The capacity of the PolyHIPE monolithic column was calculated on 50% of the final absorbance value of the break-through curve.

3. Results and discussion

While preparation of monolithic polymers via bulk polymerisation requires use of porogenic solvents to achieve permanent porosity, emulsions offer another way of porosity templation. However, emulsions are thermodynamically unstable systems and the addition of a surfactant is necessary for an emulsion to survive heating needed for the initiation of polymerisation. Most PolyHIPE materials so far have been prepared from hydrophobic monomers and a surfactant with an a hydrophilicity–lipophilicity balance (HLB) [13] value of around 4, such as sorbitan monooleate, which successfully stabilized an emulsion. The use of more hydrophilic monomers, however, represents a bigger challenge for the application of emulsion polymerisation. With the specific application of emulsion derived monoliths in mind, i.e. separation of proteins, we chose the already proven glycidyl methacrylate chemistry. Appropriate hydrophilicity, stability and the possibility of chemical functionalisation via epoxy groups are the advantages of this type of resins. Surfactants suitable for styrene and 4-vinylbenzyl chloride based emulsions, namely sorbitan monooleate and sorbitan trioleate, did not prove appropriate for the glycidyl methacrylate based emulsions. After careful optimization it was discovered that a surfactant with as low HLB value as 0.5 must be applied. Once the emulsion stability problem solved, the influence of the water to oil phase ratio on the monolith morphology was investigated. A series of polymers, with pore volume of 60, 75, 80, and 90% were therefore prepared (see Table 1).

Volume of added aqueous phase was calculated in a way to match with the expected monolith porosity. Therefore, we estimated actual porosity using Eq. (1). Results presented in Table 1 are in good agreement with the theoretical predictions. It means that no collapse or significant shrinkage of the skeleton occurred during the polymerisation or during the extraction of internal phase.

As seen from Table 1 and Figs. 1 and 2, polymers with lower pore volume have smaller pores and smaller voids. For the sample with 60% pore volume an average pore size of 104 nm was found, while for the sample with 90% pore volume an average pore size of 501 nm was determined by mercury intrusion porosimetry. The effect of crosslinking degree

Table 1
Structural properties of the methacrylate PolyHIPE monoliths

Sample	Crosslinking degree (%)[a]	Volume of aqueous phase (ml)	Porosity (%)	Average pore size[b] (nm)
1	25	60	57.0	104
2	25	75	75.6	229
3	25	80	83.9	278
4	25	90	90.2	501
5	40	75	76.1	42

[a] Crosslinking degree in mol% of EGDMA.
[b] From mercury intrusion porosimetry.

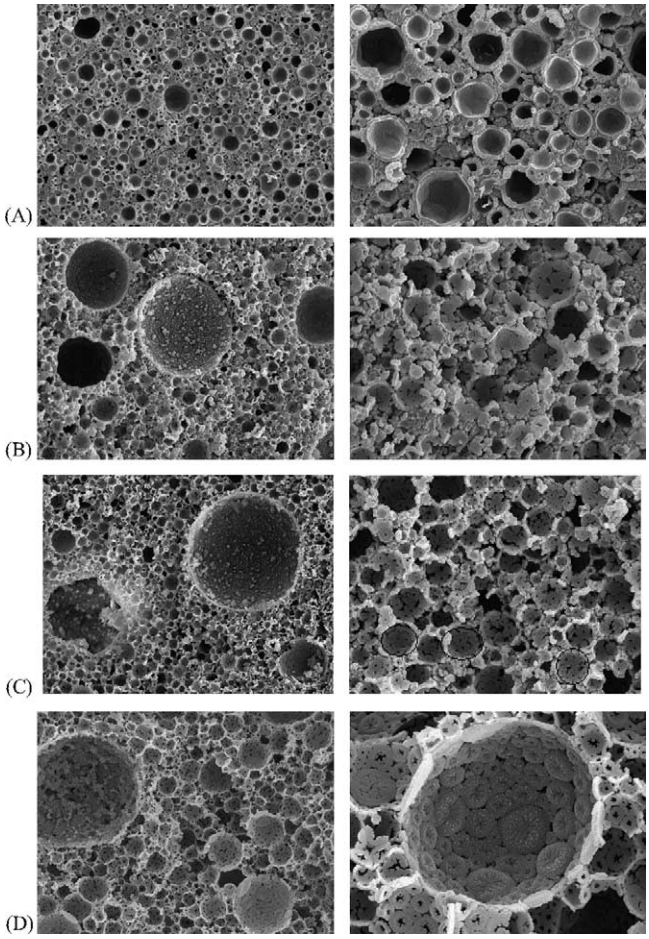

Fig. 1. SEM image of GMA/EGDMA PolyHIPE at 1000 (left column) and 3000 (right column) magnification. For all monoliths 25% of crosslinker was used. (A) 60% pore volume, (B) 75% pore volume, (C) 80% pore volume, (D) 90% pore volume. On the section (C) circles denote what is termed as "voids".

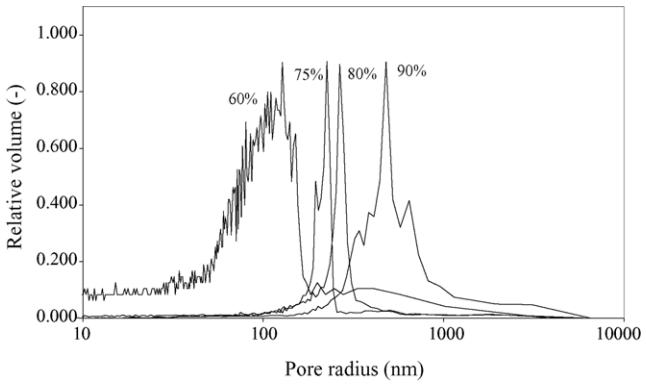

Fig. 2. Pore size distribution data for PolyHIPE methacrylate monoliths. Peak heights have been normalised for easier comparison. Numbers near the peak represent monolith porosity.

(EGDMA level) was further examined. Polymers with 25 and 40% of EGDMA were prepared. Higher crosslinking degree resulted in smaller pore size. 25% crosslinked monolith exhibited average pore size of 230 nm, while 40% crosslinked polymer showed an average pore size of only 42 nm. The same trend was observed also with the methacrylate monoliths prepared via bulk polymerisation [14].

Pore size distribution of the monoliths having same degree of crosslinking but different porosities are presented in Fig. 2. As we can see, monolith with the lowest porosity has the broadest pore size distribution, followed by the monolith with the highest porosity. Obtained data can hardly be concluded from the scanning electron microscopy (SEM) pictures of the monolith (Fig. 1). In Fig. 1 pores of at least 5000 nm can be clearly seen. However, no such pores are detected with mercury porosimetry. To properly interpret pore size distribution data, the measuring principle should be understood. Pore size distribution is obtained by the intrusion of the mercury into the pores of certain size at a defined applied pressure. The pore diameter into which the mercury can penetrate is inversely proportional to the applied pressure: smaller the pores, higher pressure has to be applied. Looking carefully into the structure of the methacrylate PolyHIPE monoliths we can see that each large pore is surrounded by the wall containing small pores. Large pores are therefore connected only via small pores and this is also the only way how the mercury can penetrate into the large pores. Once the high enough pressure of the mercury is achieved, the mercury is able to penetrate through the small pores and it fills the entire void of the large pore. Therefore, the entire volume of the large pores is assigned to the pore diameter of the small pores present in the wall. Structure of the PolyHIPE methacrylate monoliths is a very nice example, how the pore size distribution data obtained by mercury porosimetry might be misleading not knowing the real structure, especially its connectivity. On the other hand, obtained data are still useful since they provide information of the maximal size of the molecules, which can penetrate into such a structure.

PolyHIPE methacrylate monoliths were further characterised using FT-IR spectroscopy on all samples to confirm their chemical structure (see Fig. 3). Acrylate carbonyl group is clearly evident as the peak at 1730 cm^{-1}. In addition, the peak around 900 cm^{-1} confirms the presence of epoxy groups, which remain unreacted during the polymerisation and can be used for further transformation into other groups, in our case into weak anion-exchange groups DEAE. This finding is very important since it is not obvious that epoxy groups would remain intact during the polymerisation when a new polymerisation procedure for the preparation of methacrylate monoliths is applied. In fact, the FT-IR data are very similar to the methacrylate monoliths prepared via bulk polymerisation [15] confirming similarity in the chemical composition.

To investigate chromatographic properties of obtained monoliths, samples with highest porosity (80 and 90%) were modified to introduce DEAE groups. These two monolith types were selected since it is not possible to prepare mechanical stable monolith of such a high porosity with conventional bulk polymerisation. Besides, they exhibit the largest pores among the prepared monoliths. To make the comparison with the commercially available methacrylate monolithic supports most representative, PolyHIPE monolith dimension was adjusted to a standard disk format with a diameter of 12 mm and the thickness of 3 mm. Their chromatographic evaluation was performed with a standard protein mixture containing myoglobin, conalbumine and trypsin inhibitor. Separation data are presented in Fig. 4. It can be seen that the dispersion of PolyHIPE monoliths is slightly higher but separation is still acceptable. The peak of myoglobin, which is not retained under applied conditions, shifts toward longer retention times as a consequence of higher porosity of PolyHIPE monoliths. From the peak shape it can be concluded that the structure of the monolith is uniform and as such suitable for chro-

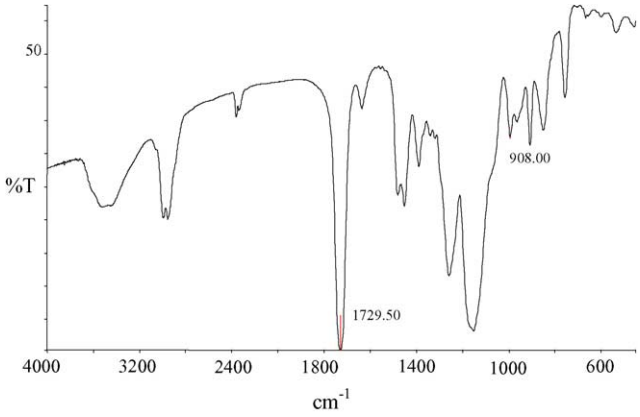

Fig. 3. FT-IR spectrum of GMA/EGDMA PolyHIPE. Peak at around 1730 cm^{-1} confirms presence of acrylate carbonyl group and peak at 908 cm^{-1} indicate presence of epoxy groups.

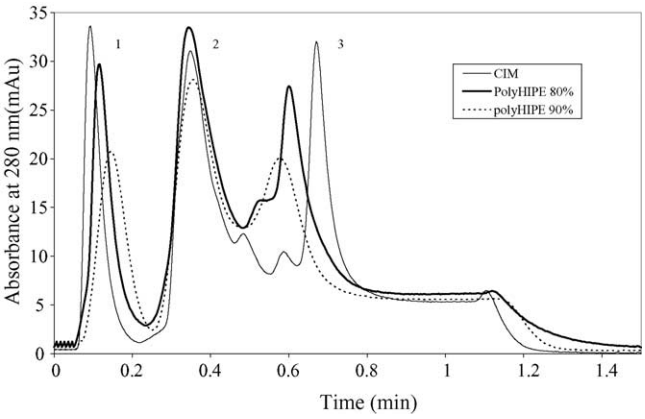

Fig. 4. Gradient separation of a protein mixture on PolyHIPE and CIM methacrylate monolithic columns. Conditions: mobile phase: buffer A: 20 mM Tris–HCl buffer, pH 7.4; buffer B: 20 mM Tris–HCl buffer + 1 M NaCl, pH 7.4; flow rate: 4 ml/min; gradient: 0–70% buffer B in 53 s; sample: 1 mg/ml of myoglobin (peak 1), 3 mg/ml of conalbumin (peak 2) and 4 mg/ml of soybean trypsin inhibitor (peak 3) dissolved in buffer A; injection volume: 20 μl; detection: UV at 280 nm.

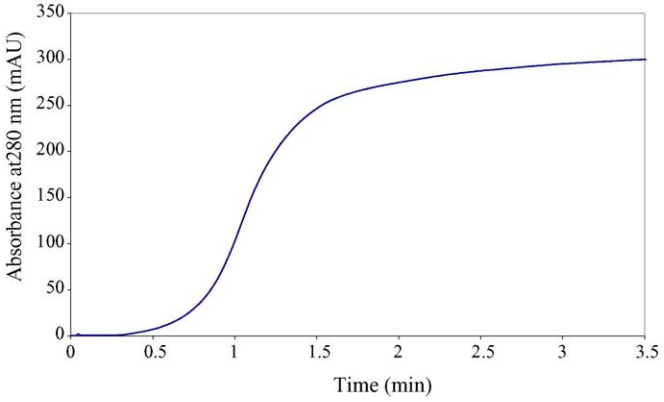

Fig. 5. Dynamic binding capacity of PolyHIPE methacrylate monolith with 90% porosity; flow rate: 3 ml/min; sample: 1 mg/ml of BSA in a 20 mM Tris–HCl buffer, pH 7.4; detection: UV at 280 nm.

matographic separations. Higher dispersion in comparison to methacrylate monoliths prepared via bulk polymerisation can be speculated already from their structure (see Fig. 1). One can imagine that large voids with a diameter of several micrometers act as small mixing reactors. Therefore, liquid passing through the monolith should behave like passing through a series of mixing reactors, which cause higher dispersion in comparison to the straight pores.

To investigate suitability of PolyHIPE methacrylate monoliths for purification, a protein capacity was measured using BSA as a reference. We tested the monolith with 90% porosity having the largest pores and consequently the lowest surface area. A breakthrough curve is shown in Fig. 5. We can see that acceptable break-through occurs and that the capacity is around 9 mg/ml of support measured at 50% of absorbance. This is much lower than the commercially available CIM supports, but we have to take into account that probably neither polymerisation neither modification conditions were optimized. Furthermore, higher capacities are expected with PolyHIPE monoliths of lower porosities. Therefore, the presented data indicate that PolyHIPE monoliths can be an interesting novel stationary phase for chromatography but further studies are required to investigate possible benefits over the existing monolithic chromatographic supports.

4. Conclusions

Emulsion polymerisation of glycidyl methacrylate showed good prospects as an alternative method for preparing highly porous monolithic supports for separation. Good mechanical properties of polymers with porosity as high as 90% is an important feature in the view of applications of porous monoliths, while epoxy groups in the polymer matrix offer possibilities of chemical modifications. Further experiments regarding the use of novel polymer supports as separation media but also as supports for solid phase synthesis and polymer assisted solution phase chemistry are currently under progress.

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