Polymer Bulletin 61, 129–135 (2008)
DOI 10.1007/s00289-008-0915-9

Polymer Bulletin

Nanoporous networks derived from functional semi-Interpenetrating Polymer Networks: Preparation and use as ion-exchange chromatographic supports

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Received: 23 November 2007 / Revised version: 31 January 2008 / Accepted: 17 February 2008
Published online: 27 February 2008 –

Summary

The utilization of poly(ε-caprolactone) (PCL)/poly(methyl methacrylate-*co*-methacrylic acid)-based semi-Interpenetrating Polymer Networks as nanostructured precursors provides a straightforward and effective route for engineering COOH-functionalized nanoporous networks. Such functional frameworks can be used as cation-exchange supports in Ion-Exchange Chromatography for the separation of proteins, provided the structures contain a significant initial content of carboxylic acid functions. This investigation illustrates the major role played by the presence of the interconnected pores generated by the oligoester template in the protein retention. The resolution turns out to be better than that obtained with a classically prepared porous support using an organic solvent as a porogen.

Introduction

Over the past few years, porous polymeric materials have been the subject of widespread interest and intense research as they find a large variety of applications in many areas, including separation and filtration techniques, biomolecule immobilization, controlled drug release, tissue engineering, as well as template-assisted synthesis of nanomaterials [1-5]. The design of porous polymers generally involves the introduction of various types of porogens (solvents, gases, small or macro-molecules) within polymer structures, followed by their selective removal. Miscellaneous template-oriented routes, including molecular imprinting [6], supramolecular self-assembly [7], and selective degradation of block copolymers [8], have been developed to create polymeric materials with defined porosity. Our group has recently reported a straightforward and effective approach for generating (meso)porous networks with tunable pore sizes [9,10]. It entails the preparation of semi-Interpenetrating Polymer Networks (semi-IPNs) constituted of oligoester sub-chains entrapped in a poly(methyl methacrylate) (PMMA) sub-network, followed by the extraction of un-cross-linked oligomers. The functionalization of such porous frameworks could provide a great opportunity to broaden their potential applications.

Herein, we report on the preparation of functional porous networks derived from poly(ε-caprolactone) (PCL)/poly(methyl methacrylate-*co*-methacrylic acid) (poly(MMA-*co*-MAA)) semi-IPNs, and their subsequent evaluation as cation-exchange supports in Ion-Exchange Chromatography (IEC). This chromatographic technique is widely used for the separation of ionic and ionizable compounds. Classical ion-exchange techniques have indeed been used for the separation of inorganic cations and anions, amino acids, organic acids, amines, peptides, and proteins [11]. This paper aims at illustrating the retention behavior of model proteins with the COOH-functionalized nanoporous materials engineered through the semi-IPN approach.

Experimental

Materials

Dihydroxy-telechelic PCL oligomer (Aldrich, M_n = 560 g.mol^{-1}, M_w/M_n = 1.2) was used without further purification. Methyl methacrylate (MMA) and methacrylic acid (MAA) were purchased from Aldrich, and distilled under vacuum prior to use. Diurethane dimethacrylate (DUDMA, Aldrich) was used as received. AIBN (Merck) was purified by recrystallization in methanol.

Preparation of porous materials from semi-IPNs

Typically, a PCL/poly(MMA-*co*-MAA) (50/50 wt %) semi-IPN with a MMA/MAA molar composition of 95/5 mol % and a (MMA-MAA)/DUDMA molar composition of 99/1 mol % was prepared as follows. 3.9 g of PCL, 3.5 g ($3.5 \cdot 10^{-2}$ mol) of MMA, 0.158 g ($1.84 \cdot 10^{-3}$ mol) of MAA, and 0.175 g ($3.72 \cdot 10^{-4}$ mol) of DUDMA were mixed in a flask in the presence of AIBN (0.12 g, $7.52 \cdot 10^{-4}$ mol, $[AIBN]_0/([MMA]_0 + [MAA]_0 + 2 \cdot [DUDMA]_0) = 0.02$). This homogeneous mixture was introduced under nitrogen into a mold which consisted of two glass plates clamped together and separated by a 2 mm-thick silicon rubber gasket. The mold was then heated to 65°C for 2 h, and then cured at 110°C for 2 h. Other semi-IPNs were synthesized in a similar manner by changing the MMA/MAA molar ratio.

The resulting PCL/poly(MMA-*co*-MAA) semi-IPNs were then extracted for 24 h at 40°C in a Soxhlet apparatus with dichloromethane (solvent reflux). The recovered sol fractions and extracted networks were dried under vacuum prior to further analyses. The extraction of un-cross-linked oligomers thus led to the formation of residual porous methacrylic networks (Figure 1).

Instrumentation

Fourier Transform Infra-Red (FTIR) spectra were recorded between 4,000 and 450 cm^{-1} with a Bruker Tensor 27 DTGS spectrophotometer in Attenuated Total Reflection mode. Solid-state ^{13}C NMR spectra were recorded with a Bruker Avance 300 spectrometer at a resonance frequency of 75 MHz. Scanning Electron Microscopy (SEM) analyses were performed with a LEO 1530 microscope equipped with a high-vacuum (10^{-10} mmHg) Gemini column. The accelerating tensions ranged from 1 to 5 kV. Prior to analyses, the samples were cryo-fractured and coated with a Pd/Au alloy in a Cressington 208 HR sputter-coater.

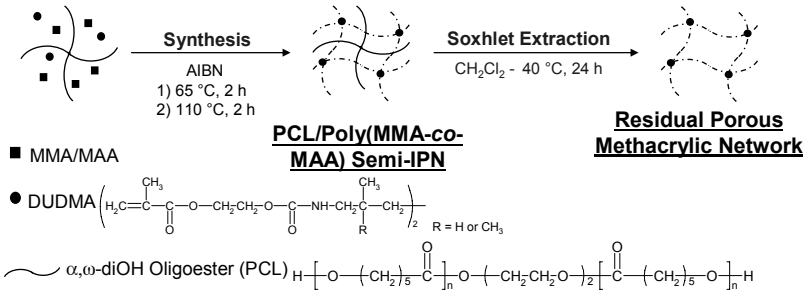

Figure 1. Design of porous methacrylic networks from PCL/poly(MMA-*co*-MAA) semi-IPNs

Ion-exchange chromatography

Porous networks were cryoground under liquid nitrogen using a freezer mill (Spex-CertiPrep 6750 Bioblock) in order to produce powders with a particle diameter of 8-12 µm (as checked by optical microscopy). Particle suspensions in a tris(hydroxymethyl)aminomethane buffer (TRIS-HCl, 2.10^{-2} mol.L^{-1}, pH 7) were pumped through chromatographic columns (10 cm x 4.6 mm, Colochrom-Interchim) thanks to a water pump aspiration. The IEC equipment comprised a Spectra Physics P100 pump, a sample injector (Model 7125 Rheodyne) with a 0.02 mL loop, a column packed as mentioned, and a Spectra Physics 100 UV detector. The ion-exchange capacities of columns were determined using an adsorption/desorption method with benzyltrimethylammonium chloride buffer solutions of varying concentrations (from 1.10^{-3} to 0.1 mol.L^{-1}) at pH 7. The ion-exchange capacity values were measured at the maximum of adsorption, and were calculated from the retention of the elution front using a UV detection at 230 nm [12,13].

The main characteristics of the eluted proteins (Sigma-Aldrich) are given in Table 1 [12-14]. Protein solutions (1 g.L^{-1}) were eluted in TRIS-HCl buffer (2.10^{-2} mol.L^{-1}, pH 7) containing either 0.1 or 0.3 mol.L^{-1} NaCl as an eluent at a flow rate of 1 mL.min^{-1}. Proteins were detected at 280 nm. Their retention factors (k) were calculated using their elution volumes (V_e) and the dead volume (V_d) determined for the elution of a 1 g.L^{-1} $NaNO_3$ solution, as follows:

$$k = \frac{V_e - V_d}{V_d}$$

Table 1. Main characteristics of proteins under study

protein	molar mass (g.mol^{-1})	pI^a	net charge at pH 7	increasing order of hydrophoby^b
ovalbumin	43,000	4.7	–	2
α-chymotrypsin	25,000	8.5	+	3
cytochrome *c*	13,400	9.3	++	1
lysozyme	14,500	11.0	+++	4

[a] p*I*: isoelectric point (pH value for which net charge is 0). [b] The ranking corresponds to the order in which the proteins were eluted through a totally hydrophobic support derived from a PCL/PMMA (50/50 wt %) semi-IPN: cytochrome *c* was the less retained protein (1), while lysozyme was the most retained one (4). Such a ranking matches that reported in reference [14], in the case of protein separation by hydrophobic interaction chromatography.

Results and discussion

Preparation of COOH-functionalized porous networks from semi-IPNs

Various PCL/poly(MMA-*co*-MAA) (50/50 wt %) semi-IPNs were prepared by bulk free-radical copolymerization of MMA, MAA, and DUDMA as a difunctional cross-linker, in the presence of PCL oligomers, with different MMA/MAA molar compositions. The experimental conditions employed were identical to those previously used for similar systems, namely an AIBN-initiated cross-linking reaction at 65°C for 2 h, followed by a 2 h curing process at 110°C [9,10]. Interestingly, all networks were transparent, which is at least indicative of microdomain sizes smaller than about 150 nm, according to Okay [15].

Porous polymer networks were readily obtained by mere extraction of un-cross-linked PCL oligomers from various PCL/poly(MMA-*co*-MAA) (50/50 wt %) semi-IPNs (Figure 1). Regardless of the MMA/MAA molar ratio, it is noteworthy that the extraction was quantitative, as indicated by values close to 50 wt % found for the sol fractions. This quantitativity was confirmed by FTIR and solid-state ^{13}C NMR, as shown by the total disappearance of the characteristic bands of PCL in the spectra of semi-IPNs after extraction. Moreover, the T_g values of extracted semi-IPNs matched those of the corresponding methacrylic single networks (110-160°C). It has to be stressed that PCL removal was performed with a good solvent of oligoesters at a temperature (40°C) far below the T_g of methacrylic networks, which avoided the collapse of the residual porous frameworks. Hence, the SEM micrographs of extracted semi-IPNs revealed highly porous structures with pore diameters ranging from 25 to 75 nm, whatever the MMA/MAA molar ratio (Figure 2 and Table 2).

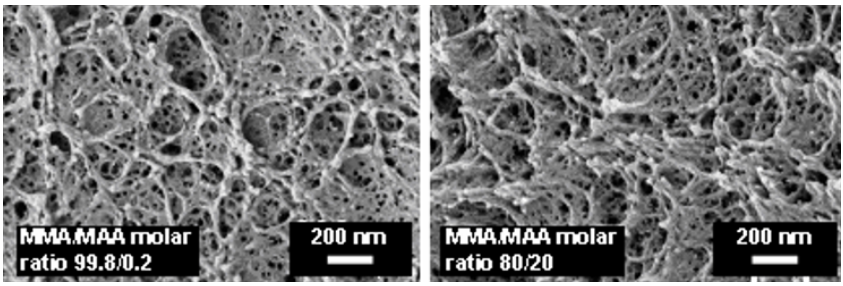

Figure 2. SEM micrographs of extracted semi-IPNs with different MMA/MAA molar ratios

Table 2. Characteristics of porous networks used as ion-exchange chromatographic supports

column	porogen	MMA/MAA molar ratio	$[COOH]_0$[a] (meq.g^{-1})	ion-exchange capacity (meq.g^{-1})	pore diameter[b] (nm)
A	PCL	99.8/0.2	$1.3 \cdot 10^{-2}$	$\leq 10^{-4}$	25 - 75
B	PCL	95/5	0.48	$1.7 \cdot 10^{-2}$	25 - 75
C	PCL	80/20	2.3	$3.6 \cdot 10^{-2}$	25 - 75
D	THF[c]	80/20	2.3	$2.2 \cdot 10^{-2}$	10 - 30

[a] initial carboxylic acid concentration in the as-prepared semi-IPNs after extraction.
[b] determined by SEM. [c] prepared by "classical" porogen solvent technique.

Particle suspensions of porous networks were used to fill chromatographic columns. In order to avoid the collapse of the nanoporous materials during the column filling and IEC experiments, the suspensions were prepared in TRIS-HCl buffer, leading to the ionization of the carboxylic acid functions into carboxylate groups. Such porous materials exhibited ion-exchange capacities up to $3.6 \cdot 10^{-2}$ meq.g^{-1} (Table 2). The ion-exchange capacity values were much lower than the corresponding carboxylic acid concentrations in the as-prepared semi-IPNs after extraction. Such a difference may be explained by a difficult accessibility of carboxylate groups in the porous supports packed in the columns.

Influence of semi-IPN ion-exchange capacity

As expected for anionic matrices, the positively charged proteins were retained, while the negatively charged one (ovalbumin) was eluted at the dead volume. The porous semi-IPN prepared with a MMA/MAA molar ratio of 99.8/0.2 (column **A**) did not exhibit any cation-exchange behavior, even at low NaCl concentration, probably due to a low ion-exchange capacity ($\leq 10^{-4}$ meq.g^{-1}). Indeed, the cationic proteins were eluted according to their hydrophoby solely, demonstrating the hydrophobic character of the matrix (Table 3). For the 95/5 MMA/MAA molar ratio (column **B**), a competition between ion-exchange and hydrophobic adsorption occurred. Actually, at low NaCl concentration, the cationic proteins were adsorbed onto the column in agreement with their total charge (Table 3), while their hydrophoby was predominant at higher NaCl concentration (Table 4). When increasing the MMA/MAA molar ratio to 80/20 (column **C**), true cation-exchange supports were obtained as proteins were eluted in accordance with their respective global charge (Table 3). Moreover, their retention factors (k values) decreased when NaCl concentration was increased to 0.3 mol.L^{-1} (Table 4).

Table 3. Values of k as determined from the elution of each protein through different columns with [NaCl] = 0.1 mol.L^{-1}

	column			
	A	**B**	**C**	**D**
ovalbumin	0	0	0	0
α-chymotrypsin	0.11	8.85	14	4.45
cytochrome *c*	0	9.85	NE[a]	7.65
lysozyme	0.32	NE[a]	NE[a]	NE[a]

[a] non eluted.

Table 4. Values of k as determined from the elution of each protein through different columns with [NaCl] = 0.3 mol.L^{-1}

	column		
	B	**C**	**D**
ovalbumin	0	0	0
α-chymotrypsin	1.30	0.17	0.11
cytochrome *c*	0.50	0.62	0.11
lysozyme	5.50	NE[a]	6.80

[a] non eluted.

Influence of porogen nature

A porous network was also prepared by using THF as a porogen solvent, the latter being incorporated in a similar amount than that of PCL oligomers in semi-IPN precursors (50 wt %). The porogen solvent technique led to a porous material with pore sizes ranging from 10 to 30 nm (Table 2). As expected, the retention factors of proteins with this support (column **D**) were lower than those previously determined with the corresponding extracted semi-IPN (column **C**, Tables 3 and 4), as the ion-exchange capacity was found to be smaller for the former network (Table 2). In addition, despite possessing a higher ion-exchange capacity, the proteins were eluted more quickly through the classical porous support than through the semi-IPN-derivatized system with a MMA/MAA molar ratio of 95/5 (column **B**, Tables 3 and 4). Interestingly, Sherrington has reported an increase of the surface area due to an improvement of the pore connectivity through the creation of interconnected channels, when adding oligomers as co-porogens in the generation of porous poly(divinylbenzene) resins by the porogen solvent route [16]. Consequently, in the case of the support prepared with THF as a porogen solvent (column **D**), it is plausible that macromolecules such as proteins could not access the total surface bearing the carboxylate ions, because of the occurrence of closed pores. On the contrary, the semi-IPN approach led to the formation of porous networks with high pore connectivity [9], providing a higher accessibility of the carboxylate groups. The pore interconnectivity was supported by density measurements. The true density values of extracted semi-IPNs –as determined by helium pycnometry at 25°C– were indeed very close to those of corresponding non-porous single networks (around 1.15-1.20). This strongly suggested that the nanoporous networks observed by SEM were characterized by open pores or interconnected channels through which a fluid (helium or water) could flow.

Conclusions

The extraction of un-cross-linked PCL oligomers from functional semi-IPNs provides a straightforward and versatile strategy for preparing nanoporous polymer networks with interconnected pores and/or channels. Such frameworks behave as cation-exchange supports with retention properties dependent on the p*I* value of proteins, provided the structures contain a significant initial content of carboxylic acid functions. This investigation illustrates the major role played by the presence of the interconnected pores generated by the oligoester template in the protein retention. The resolution turns out to be better than that obtained with a classically prepared porous support using an organic solvent as a porogen.
The *in situ* synthesis of a porous monolithic material in the column through the semi-IPN approach could represent a valuable alternative for advanced chromatographic techniques, such as electrochromatography.

Acknowledgements. Financial support of the French Ministry of Research is gratefully acknowledged. The authors are indebted to Prof. Ph. Guérin, Prof. F. Lauprêtre, and Dr. S. Boileau for helpful discussions. They are also thankful to Dr. C. Karakasyan and M. Guerrouache for their kind assistance in IEC experiments.

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