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Novel monolithic materials using poly(ethylene glycol) as porogen for protein separation

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Abstract

Several recipes are described for the preparation of porous polymeric monoliths in the capillary format, using poly(ethylene glycol) (PEG) as porogen as well as constituent in the monomer mixture. Acrylic or methacrylic monomers with a variety of terminal groups, with and without ethylene glycol links of differing lengths in the side chains, have been used in combination with triethylene glycol dimethacrylate (TEGDMA) and trimethylol-propane trimethacrylate (TRIM) as cross-linkers. PEGs of 4–20 kDa molecular weight dissolved in 2-methoxy-ethanol were used as porogens to yield large, biocompatible pores. A number of common solvents have been used as co-porogens for the PEGs, and the surface areas, median pore diameters, and back pressures of the resulting monoliths have been correlated with a number of molecular descriptors by means of chemometrics to describe the results. Photopolymerizations induced by either continuous or pulsed UV light were furthermore compared. Pore size distribution and surface area characterization have been assessed by nitrogen adsorption–desorption and mercury intrusion porosimetry, and scanning electron microscopy (SEM) was used to evaluate the differences in macroporous morphology obtained with the different porogen solutions. Mixtures selected from screening syntheses carried out in vials have been implemented in 100 μm fused silica capillaries and the back pressures measured and cross-validated with the pore information. Some of these capillary columns were finally tested for the separation of proteins using micro-HPLC.

Keywords: Monolith capillary columns for liquid chromatography; Poly(ethylene glycol); Protein separation

1. Introduction

Since, their inception in the late 1980s [1], monoliths have drawn a continuous attention in the chromatographic society. Over time we have reached a better understanding of their high permeability and good mass transfer properties [2,3], as well as their dynamic properties by modeling techniques [4], and they are nowadays one of the more attracting alternatives to packed particle-based columns which have so far dominated the separation material domain [5,6]. The large variety in attainable properties [7] has made monoliths versatile media for high performance liquid chromatography [8]. The low cost and swiftness of their synthesis, as well as the possibility of direct use after in situ polymerization make monoliths appreciated for capillary liquid chromatography [9,10] and electrochromatography [11]. Tuning of the pore domain is still one of the most challenging issues concerning these materials. While work has been done to obtain pores structurally arranged with templating agents [12] or the pore walls stereochemically oriented as in molecularly imprinted polymers [13], a major remaining issue in protein separation is still to establish biocompatible stationary phases. Protein unfolding and denaturation is in fact as dependent on the stationary phase as it is on the mobile phase [14,15]. The importance of the monomers has already been demonstrated, and acrylate monomers have proven to be among the best in order to arrive at surfaces with low denaturing propensity [16]. However, it has also been proven that the porogens are of great importance to establish 'protein-friendly' pores, and for instance, water, has been used for that purpose [17].

Poly(ethylene glycol) (commonly named PEG-M, with M replaced by its molecular weight average) is a non-toxic flexible polyether [18] with chemical structure HO–(CH_2–CH_2–O)$_n$–H. It was initially studied and characterized by Laurenço in the 1860s [19,20] and is still considered to be unique polymer [18]. PEG has hydrophilic heads comprised of hydroxyl groups providing a good water-solubility at low temperature [21], while the polyoxyethylene chain allows it to be dissolved in most organic solvents. It has a unique behaviour

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in aqueous environments due to its constantly moving chains and has been extensively investigated as a material for prevention of biofouling [22]. PEG has been widely used in a variety of pharmaceutical and biological applications, as well as in many commercial products [23]. For instance, drug delivery research [24,25] has gained from its singular pharmacokinetics and biodistribution [26]. The possibility to conjugate PEG to lipids and macromolecules by various methods [27] allows it to release medications or proteins very slowly and makes it one of the most common molecules in this area.

We hypothesized that these properties should provide biocompatible surfaces if PEG is used as porogen, since the PEG will establish pores of large dimensions with a 'protein-friendly' surface. Moreover, PEGs are available in a wide range of well defined molecular weights, allowing a study of their sterical effect when forming the pores in the material. We report in this study the successful use of PEG as porogen and linker of the functional group in syntheses of various organic monolithic materials for application in protein separation science and especially in hydrophobic interaction chromatography (HIC) [28,29] mode.

2. Experimental section

2.1. Materials

2,3-Epoxypropyl methacrylate (GMA), trimethylolpropane trimethacrylate (TRIM), poly(ethylene glycol) phenyl ether acrylate with average molecular weights of 236 and 324 (PEGPEA-236 and PEGPEA-324, respectively), PEG-1500, activated acidic aluminum oxide, and 2-methoxyethanol were obtained from Aldrich Chemical (Schnelldorf, Germany). Benzoin methyl ether (BME), triethylene glycol dimethacrylate (TEGDMA), PEG-4000, PEG-10,000, PEG-20,000, γ-methacryloxypropyl-trimethoxysilane (γ-MAPS), and toluene were purchased from Fluka (Buchs, Switzerland). Methanol was obtained from Baker (Phillipsburg, NJ). All co-porogens were of at least analytical grade, from various companies. TEGDMA and PEGPEA were deinhibited prior to use by passage through a column of aluminum oxide. TRIM was used directly as received, while GMA was deinhibited and distilled under vacuum. Protein test probes cytochrome C, lysozyme, ovalbumin, trypsin inhibitor, α-chymotrypsinogen, and bovine albumin serum (BSA) were purchased from Sigma (St Louis, MO).

2.2. Pre-treatment of the capillary

UV transparent fused silica capillaries (100 μm i.d.) obtained from Polymicro Technology were treated as described elsewhere [30], with the procedure being selected on the basis of a previous study [31]. Briefly, the capillaries were washed with acetone and deionized water, then a 1 M NaOH solution was flushed through the capillary for approx. 5 min, where after the capillary was sealed in the filled state. It was heated at 100 °C for 2 h, then cooled to room temperature and washed in sequence with deionized water and acetone (15 min each), and finally dried by a flow of dry nitrogen for at least 1 h. A silanization reaction to introduce methacrylic anchoring groups on the surface was carried out by filling the capillary with a degassed mixture of 10% of γ-MAPS in toluene, sealing it in the filled state and keeping it at room temperature for 2 h. Finally, the capillary was washed with toluene and dried with nitrogen flow for at least 1 h.

2.3. Preparation of the monoliths

Stock mixtures were prepared to minimize the errors due to weighing in minute quantities of reagents. Porogen stocks were prepared by dissolving 20 g of each PEG in 100 g of 2-methoxyethanol at 50 °C. Before these mixtures were used, particular care was taken to ascertain that solutions were transparent; if not, slight heating was applied until the solution appeared clear. In experiments requiring a co-porogen, the appropriate solvent was directly added to the porogen mixture. Respective monomers and cross-linkers were added in the ratios described in Table 1 and solutions were stored at −15 °C without being stirred. Equimolar proportions of cross-linker and monomer were always used. Prior to polymerization (only one sample at a time was taken out of the freezer), the solution was quickly heated to room temperature using 40 °C water and thoroughly mixed to ensure that the solutions were completely homogeneous. It was then degassed with nitrogen gas for 10 min before adding the photoinitiator BME (1% w/w with respect to monomer) to the solution. The mixtures were briefly shaken and degassed again during 3 min before being pipetted into four 1.6 mL GC-vials. The vials were sealed with PTFE-lined cap and 6 cm pieces of pre-treated fused silica capillary were introduced in two of the vials by pushing the ends through pre-pierced septa. Capillary force and a slight overpressure from the capping caused a slight flow and after two drops of monolith mixture had exited from the capillary, the end was sealed by piercing it into a GC rubber septum. Special care was taken to ascertain that no air bubbles were trapped in the capillary, or in the GC-vial close to the capillary ends. The two vials fitted with the capillaries were polymerized along with the two remaining vials, in either of two photopolymerization units. The continuous light polymerization unit was a Spectrolinker XL-1500 UV (Spectronics, Westbury, NY) fitted with six 15 W BLB blacklight tubes, emitting UV-A radiation predominantly at 365 nm. Prior to use this unit had been warmed up during 3 h to ascertain constant temperature and intensity during the course of the 60 min polymerization period. The pulsed light reactor was a high power strobo lamp (D.T.S., Misano Adriatico, Italy), constructed from an XOP 15 O/F 1500 W linear low pressure nonozone-producing xenon discharge lamp, ignited by a 5 μF capacitor. Once triggered, energy supporting the discharge is supplied directly from the mains through a full bridge rectifier. The energy contained in each pulse can be adjusted by triggering the lamp during different phases of the mains cycle. The polymerization was done at maximum triggering rate and intensity, yielding pulses of ~3 ms duration at 12.5 Hz repetition rate. Special care was

Table 1
Experiments and corresponding ratios or conditions of polymerization

Monolith	Monomer[a]	Main porogen[b]	Co-porogen	Mixture ratio[c]	UV source[d]
M1	GMA	10,000	None	40/60	1
M2	PEGPEA	10,000	None	40/60	1
M3	GMA	4000	None	40/60	1
M4	GMA	1500	None	40/60	1
M5	GMA	20,000	None	40/60	1
M6	GMA	10,000	1,4-Dioxane	30/60/10	1
M7	GMA	10,000	Tetrahydrofuran	30/60/10	1
M8	GMA	10,000	Ethyl acetate	30/60/10	1
M9	GMA	10,000	*N*-Methyl pyrrolidone	30/60/10	1
M10	GMA	10,000	Chloroform	30/60/10	1
M11	GMA	10,000	Acetonitrile	30/60/10	1
M12	GMA	10,000	Cyclohexanol	30/60/10	1
M13	GMA	10,000	1,4-Butanediol	30/60/10	1
M14	GMA	10,000	1-Decanol	30/60/10	1
M15	GMA	10,000	1-Dodecanol	30/60/10	1
M16	GMA	10,000	Methylbenzene	30/60/10	1
M17	GMA	10,000	*N*,*N*-Dimethyl formamide	30/60/10	1
M18	GMA	10,000	Methyl *t*-butyl ether	30/60/10	1
M19	GMA	10,000	Cyclohexane	30/60/10	1
M20	GMA	10,000	Dichloromethane	30/60/10	1
M21	GMA	10,000	None	30/70	1
M22	GMA	10,000	*N*-Methyl pyrrolidone	30/60/10	2

[a] Equimolar proportions of monomer and cross-linker were always used. Cross-linker mixtures were composed of 1/3 w/w TRIM/TEGDMA.
[b] Average molecular weight of the PEG used in the porogen mixture which contained 1/5 w/w of PEG in 2-methoxyethanol.
[c] The weight ratio of either monomer/porogen or monomer/porogen/co-porogen.
[d] For the same integrated radiation energy and the same temperature in a Spectrolinker XL-1500 UV (1) or in a custom made UV-reactor equipped with a pulsed xenon lamp (2).

taken to ascertain that approximately the same total amount of energy and the same temperature was used in both procedures. The light intensity was thus measured and integrated by an International Light (Newburyport, MA) Model IL1400 radiometer with a model XRL140B probe. The continuous light polymerizations followed a protocol developed previously and in the pulsed polymerization experiments, the distance from the xenon source to the items being polymerized was used to adjust the intensity. The integrated radiation between 326–401 nm, as measured by the IL1400/XRL140B, was 10 ± 1 J/cm^2. A flow of nitrogen gas was adjusted to maintain a constant temperature of ~ 35 °C in the reactor to ensure that the PEG remained dissolved. After polymerization, the capillaries were removed, trimmed on both sides to a final length of 30 mm, and kept in deionized water until tested. The vials polymerized along with the capillaries were broken and the polymers cut in small cubic pieces (~ 1 mm^3) and submersed for at least 2 h in hot (70 °C) water/methanol 1/1 in order to remove most of the PEG and non-reacted chemicals. They were then transferred to a Soxhlet extractor and extracted with THF for at least 24 h. Drying took place in an oven at 100 °C and samples were kept in dry vials before characterization.

2.4. Surface area measurements

Nitrogen adsorption–desorption was performed on a Micromeritics (Norcross, GA) Flowsorb II 2300. Around 0.5 g of polymer was degassed carefully at 120 °C under nitrogen gas for 1–2 h to remove all water or solvent contained after extraction. The surface areas were recorded and related to the mass of the sample. Two samples were measured in triplicate for each polymerization mixture. Values presented in Table 2 are thus averages of the six measurements.

2.5. Mercury intrusion porosimetry

The porous properties of the materials were determined by mercury intrusion porosimetry using a Micromeritics Autopore II 9220 instrument. Prior to analysis the samples were dried in vacuum at 40 °C over night. Calculations on the data obtained are based on the Washburn [32] equation, in its applicable form:

$$D = \frac{1}{P} 4\gamma \cos \varphi \quad (1)$$

where D is the pore diameter, P the applied pressure, γ is the surface tension of mercury, and ϕ the contact angle between the mercury and the sample. It is quite obvious that the pores of these materials are not cylindrical, but this is a limitation of the only widely available technique for macropore characterization.

2.6. SEM

Scanning electron microscopy samples were placed on sticky carbon foils (used to increase conductivity), attached to standard aluminum specimen stubs, and coated with a ~ 20 nm thick gold layer by using a combination of sputter coating by an Edwards (Crawley, UK) model S150A sputter coating unit, and

Table 2
Back pressures, surface areas, and median pore diameters measured for all samples

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22
Back pressure[a] (MPa)	31.4	14.7	>150[b]	>150[b]	9.4	6.1	5.4	4.6	22.3	3.1	6.0	3.4	2.1	2.0	1.8	4.0	26.1	4.4	2.8	2.9	2.8	5.1
Surface area[c] (m^2/g)	5.2	3.6	18.1	48.3	3.7	3.8	4.2	3.9	7.3	3.5	4.5	4.0	3.5	3.3	3.4	3.8	7.3	3.8	3.5	3.3	3.7	5.0
Median pore diameter (μm)	0.67	0.73	0.21	0.02	1.05	1.17	1.21	1.36	0.74	1.45	1.15	1.34	1.51	1.64	1.72	1.45	0.74	1.36	1.67	1.66	1.56	1.14

[a] Back pressure recorded at 3 μL/min on a 30 mm long capillary column.
[b] These columns showed a back pressure of more than 25 MPa for a 5 mm long capillary column segment.
[c] Average of at least three desorption measurement on two samples.

evaporation by a modified Edwards E14 vacuum coating unit, incorporating an automatic tilting and rotation device. Microscopic analysis of all samples was carried out in an S-360 iXP SEM (Leica Cambridge Ltd, Cambridge, UK) operated at 10 kV, 100 pA probe current, and 0° tilt angle. Final images were recorded from randomly chosen areas at the magnification indicated in each SEM.

2.7. Capillary testing

The 30 mm long capillaries were connected to a Shimadzu (Kyoto, Japan) LC-10ADVP pump. After the connection had been made; the capillary was submersed in a water bath maintained at 70 °C and a washing flow of pure methanol started at 3.0 μL/min. The pressure during the washing step was recorded using a Clarity chromatographic data acquisition system (Data Apex, Prague, Czech Republic) to monitor the removal of viscous PEG residues from the column. When the pressure had stabilized, the capillary was allowed to cool to room temperature and the back pressure was recorded as a function of flow rate. In those cases where the capillaries caused very high back-pressure (>10 MPa at 3.0 μL/min), 5 mm long capillary pieces were used and the pressure was recalculated proportionally to their size [33], assuming a linear pressure drop along the capillary. For each monolithic material, two capillaries polymerized in different GC-vials were measured and the averages of the values are given in Table 2.

2.8. Chromatographic evaluation

A Shimadzu micro gradient system using two LC-10ADVP pumps and an SPD-10AVP UV-detector fitted with a 35 nL capillary flow cell from LC Packings (Amsterdam, The Netherlands) was operated at 214 nm and utilized for the chromatographic evaluation of the columns. Analytes were injected at room temperature in the starting eluent by an electrically actuated Upchurch (Oak Harbor, WA) micro-injector with a 35 nL external fused silica capillary loop. The separatation column was monolith M2, prepared as above, but with a length of 60 mm to accommodate Upchurch microfittings at both ends. A linear gradient from 10 mM phosphate buffer pH = 7.0 with 2 M ammonium sulfate to buffer only was run in 10 min. The Clarity system was used for data acquisition and evaluation of chromatographic parameters.

3. Results and discussion

The reference monolith (M1) was chosen as starting point after numerous mixtures had been tested in scouting experiments. First of all, the choice of cross-linker was made on the basis of biocompatibility, but an important factor was also flexibility, to avoid material failure due to internal stress during polymerization, or osmotic shock when using gradients with large spans in salt strength and organic solvent contents. TEGDMA is a high reactivity cross-linker based on a relatively short PEG chain which grants it a good flexibility. Poly(ethylene glycol) dimethacrylate $M_n \sim 550$ was also tested

(based on the same molar concentration) but the length of its PEG chain was apparently too long and too similar to the porogen, resulting in the creation of very small pores and a material that collapsed onto itself after drying creating a glassy monolith lacking flow-through properties. We subsequently discovered that the polymer structure was too weak to resist high flows, and the capillaries were getting blocked by disintegration of the material. TRIM was, therefore, added as a co-crosslinker to impart strength to the monolith. The ratio of monomer to porogen was fixed at 2/3, also after a screening. With such mixtures, homogeneous distribution of pores (according to SEMs and porosimetry measurements) and a facile removal of the porogen were obtained. Another important parameter is the polymerization time, which was fixed at 1 h in the continuous light mode to enable comparison with previous work [34].

A central composite circumscribed, CCC, design was generated to investigate the role of the two ratios (monomer/porogen 70/30 $\pm$ 5 and co-porogen/porogen 14.3/85.7 $\pm$ 5) in the mixture as well as the time of photo-polymerization (a change of 10 min around a center point of 45 min). The design was then fitted with MLR (multiple linear regression) when introducing the surface area as response factor. As the box-cox plot was presenting a lambda max at 0.5, it was decided to transform the response to square root. The results showed that both ratios were important. Polymerization time was of lower significance, and previous experiments had shown that a minimum of 30 min was required. It was, therefore, set at 1 h for a complete polymerization.

3.1. Choice of monomers

The functional monomers are of great importance in separation materials. Five monomers [GMA, PEGPEA with two chain lengths, poly(ethylene glycol) methacrylate, and poly(ethylene glycol) behenyl ether methacrylate] were initially selected, based on their supposed bio-compatibility. In the following screening, only GMA and PEGPEA were found usable, based on assessments of mechanical strength, surface area, and behaviour after cleaning and drying of the material. The remaining monomers produced glassy gels that lacked flow-through properties. The length of the PEG chain in the PEGPEA (2 or 4 repeating units) did not influence the mechanical strength nor the surface areas of the formed materials significantly. The GMA was kept for the possibilities of functionalization that can be done after polymerization in order to achieve, for example, anion exchange or cation exchange materials. The monoliths synthesized using the small glycidyl group of the GMA had a very different structure compared to the one obtained from the bulky PEGPEA chains. Micrographs (Fig. 1, the four SEMs on the left) show the GMA materials with distinct boundaries between the aggregated particles, as commonly seen in organic monoliths. The PEGPEA monolith has a similar structure on the macropore level, but the skeleton was largely fused into a continuous structure and appeared to have a much smoother surface. Preliminary evaluations to be reported elsewhere indicate that these materials have very good behaviour in chromatography.

3.2. Variation in the PEG porogen chain length

From the wide range of PEGs available, we selected four different molecular weights based on an initial screening, to gain insight into the mechanism of pore formation when this atypical molecule is used as porogen. With respect to the possibility of porogen removal, as well as the appearance of the generated material, PEG-1500 (equivalent to 33 repeating units) was chosen as smallest member of the series. At the other end of the scale, PEG-20,000 (equivalent to 453 repeating units) appeared to be the upper limit for creation of very large pores. At higher molecular weights marginal effects were seen on the macroporous structure, and problems arose from limited solubility and the difficulties of removing the porogen from the monolith. Two additional, intermediate chain lengths were selected to establish a harmonic series with essentially doubled molecular weight between each member. As can be seen in the Fig. 2, longer

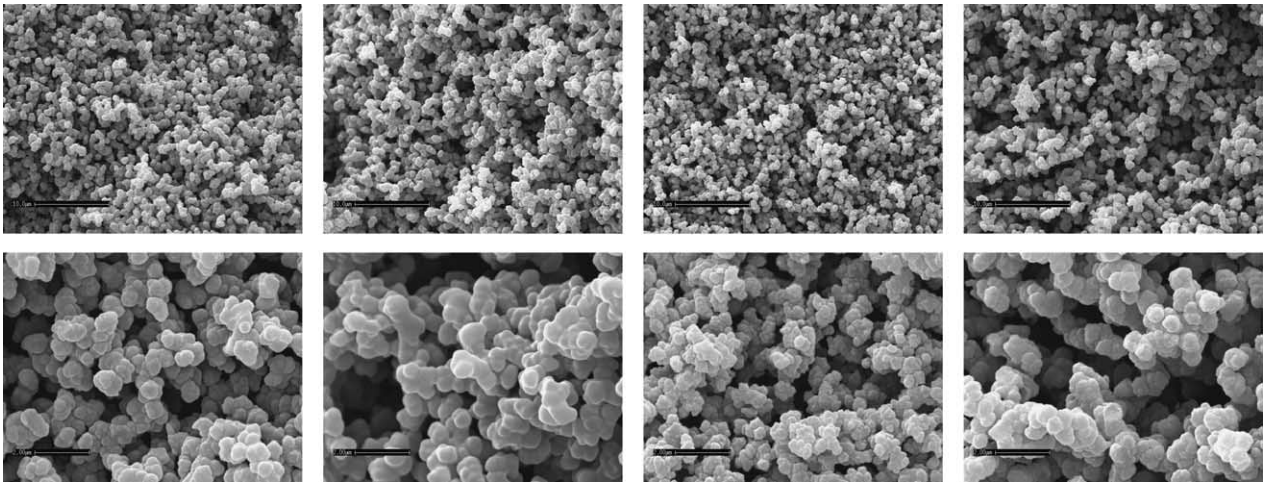

Fig. 1. Scanning electron micrographs of some of the monoliths (from left to right) M1, M2, M9 and M22 (Table 1) using a magnification of 3000 (top) and 11,000 (bottom).

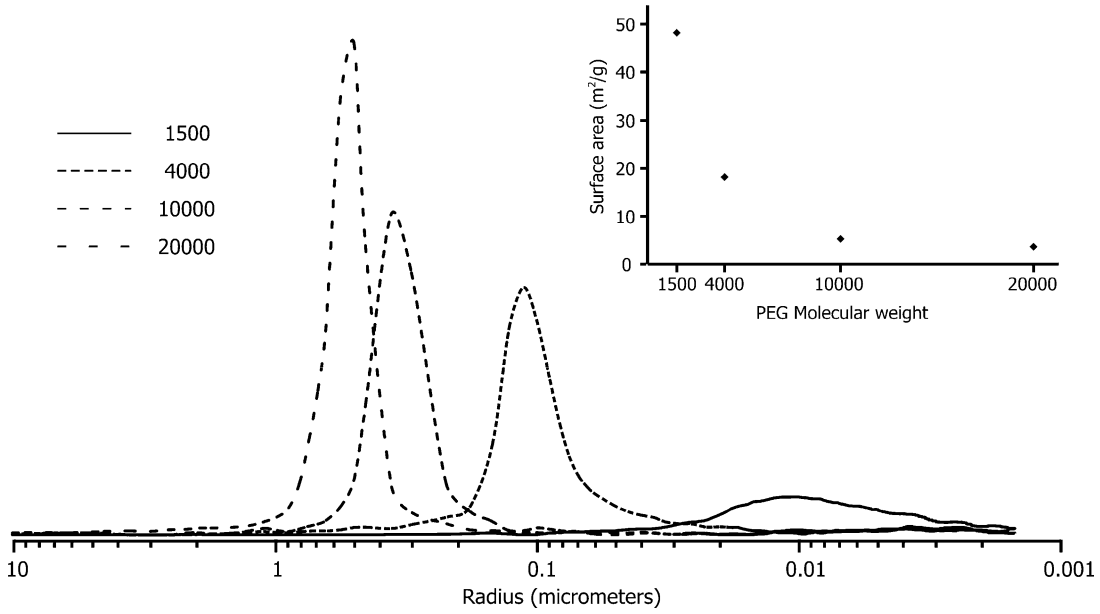

Fig. 2. Median pore diameter of the monoliths synthesized using PEGs of different molecular weight. The insert in the top-right corner shows surface area vs. molecular weight of the PEG in the porogen.

chains produced pores of larger diameter, while the surface area became lower. This is not surprising in view of the inverse relationship between pore diameter and surface area. It can be emphasized that the relationship between chain length and surface area (or pore diameter) is not linear, and therefore, doubling the chain length from 10,000 to 20,000 Da led to monoliths with almost identical back pressures after cleaning. Mixtures using 5–20% of PEG-1500 or PEG-4000 added to the PEG-10,000 or PEG-20,000 were used in an attempt to obtain a bimodal structure of the pores. However, almost no change (order of 0.2 m^2/g increase for 20% of PEG 4000 replacing PEG 10,000) was observed. We believe that the behaviour of PEG in 2-methoxyethanol is similar for different chain lengths, and therefore, increasing the chain length just leads to a solvated system with higher sterical hindrance, and therefore, larger pores. Adding smaller chains to longer ones will not make a separate solvated systems, but merely reduce the viscosity of the original one by dilution. Future attempts in this direction will, therefore, involve mixtures of intermediate size polymers of different kind, which are prone to phase segregate in solution.

3.3. Effect of co-porogens addition

The solvent used for dilution of the PEGs was in all cases 2-methoxyethanol. It was selected because of its ability to dissolve PEG, and it should also be kept in mind that this is in principle the shortest member of the PEG series, with methoxy termination. A series of solvents were chosen as co-porogens and included in the monolith mixtures. The aim of using these co-porogens was to assist in the creation of a bimodal pore structure (with the smaller pore family still the size of biomacromolecules) that would afford a somewhat higher surface area with good bulk flow properties, thus increasing the capacity of the material with maintained mass transfer properties. A PLS model was established to reveal the relationship between the response variables and the physicochemical properties of the co-porogens. Among solvents commonly used as porogens in preparation of porous polymers, we chose a set of 15 with varying properties (Table 1). Two materials were chosen as references in this study, considering either the co-porogen as a part of the porogen (reference M21) or as an extra substance within the mixture (reference M1) reducing the amount of monomer. We assumed that a co-porogen with properties similar to PEG would fit in this scale.

A set of 12 physicochemical properties [melting point, refractive index, density (20 °C), boiling point, molecular weight, minimum energy conformation, log*P*, molar refractivity, standard connectivity indexes of orders 0–2, and dipole moment] were calculated using CAChe Worksystem Version 6.1 (Fujitsu/Oxford Molecular) with structures entered using the CAChe graphical editor (CAChe Editor). For each structure, geometry optimisation by a conjugate gradient method was carried out using the augmented MM2 [35] force field with a convergence value of $4–10^{-2}$ I/mol and a maximum updates for the iteration control of 3000. Structures were input into CAChe ProjectLeader and a number of descriptors were computed such as log*P* [35], molar refractivity [35], connectivity index (orders 0, 1 and 2) and dipole moment. This latter descriptor was calculated using the AM1 semi-empirical method [35], part of MOPAC 2002 [35] or were compiled from the supplier of the chemicals. The three evaluation parameters (back pressure on a 30 mm column, surface area, and median pore diameter) were then introduced as responses in a PLS (partial least square) computational model. A first model was drawn with SIMCA-P+ software (Umetrics, Sweden) without transforming the variables, but it was recognized that the distribution of the data was skewed for the flow and the surface area. Therefore, a logarithmic

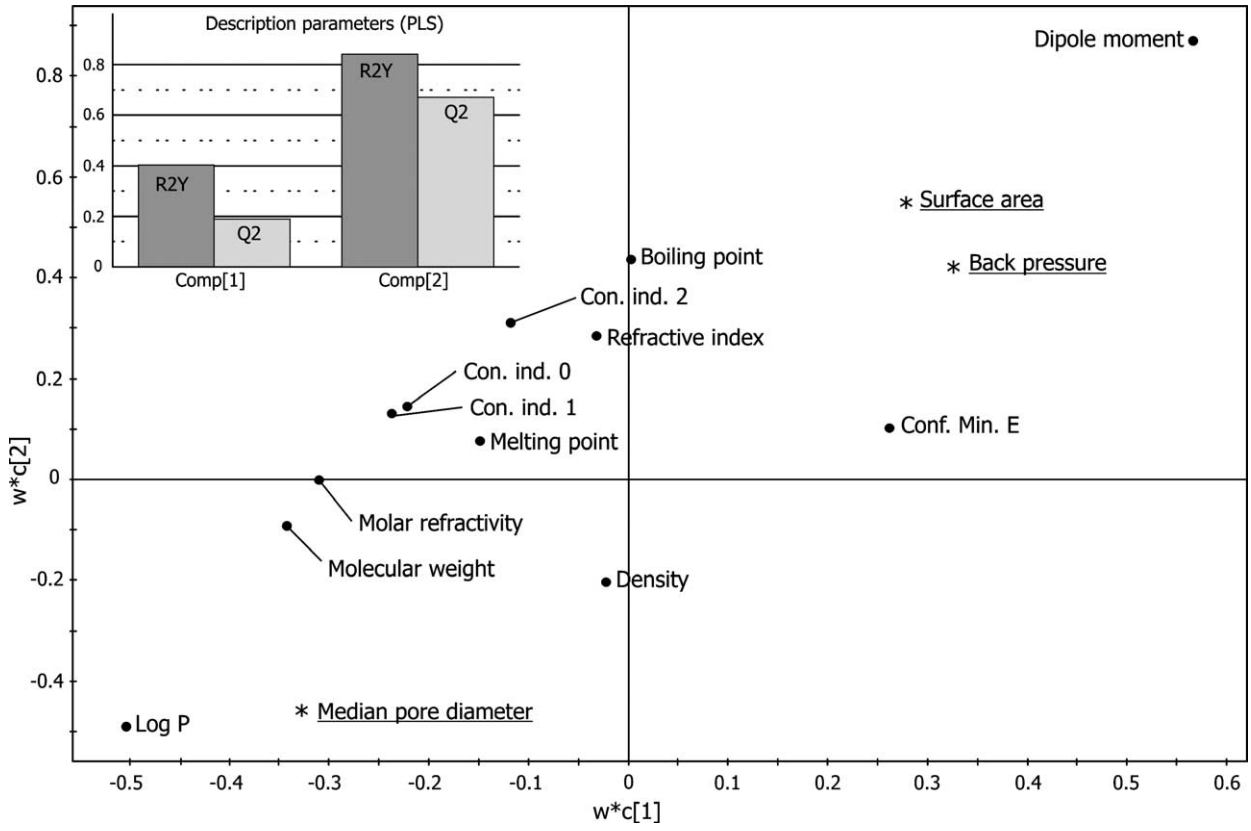

Fig. 3. Loading scatter plot of the partial least square model for various descriptors (log *P*, molecular weight, density, configuration minimal energy, density, refractive index, melting and boiling points, molar refractivity, dipole moment, connectivity index order 0, 1 and 2) related to the three response factor (surface area, median pore diameter, back pressure of the monolith).

transform was applied to these two variables, and the resulting model was found to be more accurate with only two components. A plot of the loadings for these two components is described in Fig. 3 and allowed us to draw some conclusions concerning the effects of the porogens on the monolith structure. First of all, surface area and back pressure are well correlated (87%) and both are negatively correlated to the median pore diameter (93 and 107%), which is reasonable if we consider that higher median pore diameter causes lower back pressure as well as lower surface area. It should then be kept in mind, that the closer to the origin the observations are, the less important they are in the model. Therefore, we can consider that the dipole moment and log *P* are the molecular properties that best describe this model. It can also be predicted that porogens that exhibit high dipole moment or low log *P* value are likely to produce monoliths of low median pore diameter (and, therefore, also a high back pressure and a high surface area). These properties are not the only ones that contribute to the porogen effect in this monomer system, but are most significant and should be considered when choosing a co-porogen for systems similar to those investigated here.

From the score plot of this model (Fig. 4), four distinct categories of co-porogens were revealed with no outliers. The long chain alcohols (dodecanol and decanol) that have linear structures similar to PEG and should, therefore, not interact much in the process of pore formation. The two amides (DMF and NMP) have very high dipole moments and appear to be efficient in disturbing the equilibrium in the porogen/monomer solution. Finally, acetonitrile is one of the strongest hydrogen bond acceptors. This apparently gives acetonitrile unitary properties and it can, therefore, not be classified in one of the previous groups or in the 'center group' alongside porogens with low impact on the pore dimensions.

The changes in surface area and median pore diameter for the new materials were not really confirming the targeted properties. However, since mercury intrusion porosimetry is not particularly reliable for pores below 8 nm, we made multipoint BET measurements (Micromeritics ASAP 2000) for some of the materials (M2 and M22) to get a more reliable picture of the pore distribution. From these measurements, it is clear that very few smaller pores have been created in the material, and therefore, the shift in the surface area and related back pressure are just due to the decrease of the median pore diameter and not to a bimodal pore distribution.

3.4. Continuous vs. pulsed UV polymerization

After a screening of several UV radiation systems (from a 400 W high pressure mercury curing stage to a low intensity Xe stroboscope) a radiation chamber based on six 15 W 365 nm black-light tubes and a high intensity xenon stroboscope were selected to represent a continuous and a pulsed light source. Results presented in Fig. 2 (the four SEMs on the right) and in Table 2 (M9 and M22) show the difference between polymers

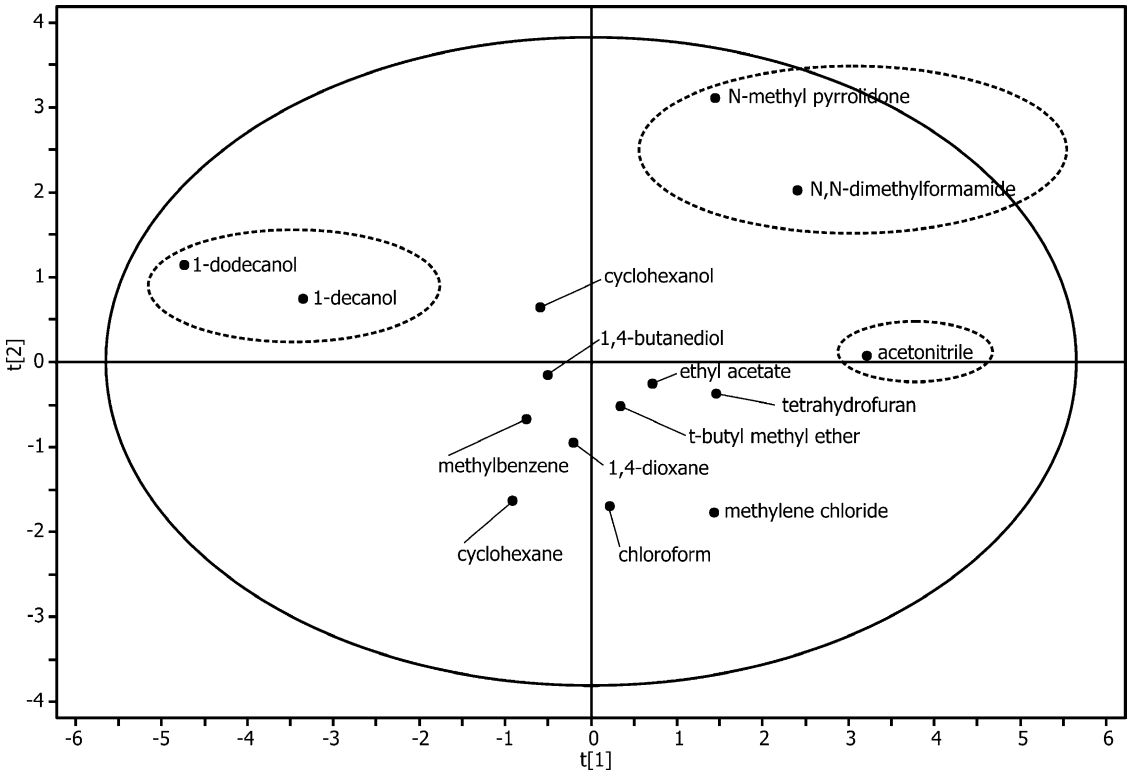

Fig. 4. Score plot of the partial least square model with two components.

that received the same amount of energy by either continuous or pulsed light. The amount of energy irradiated onto the growing polymer was chosen instead of a time as a comparison parameter because initiation in UV polymerization is driven by photolytic cleavage. However, the difference was still not substantial (1 h polymerization for the continuous light and 20 min more for the pulsed one), which means that each pulse had a much higher intensity than the continuous light. Considering the speed of preparation, we can argue for a pulsed polymerization, which also gave a larger macropore diameter and hence provides a new option for controlling the pore formation process. Furthermore, the materials obtained from the pulsed polymerization experiments were more reproducible in their porous properties than the ones obtained with continuous light (data not shown).

3.5. Chromatographic evaluation

The column M2 was used to demonstrate the chromatographic possibilities of the monoliths described here. A set of

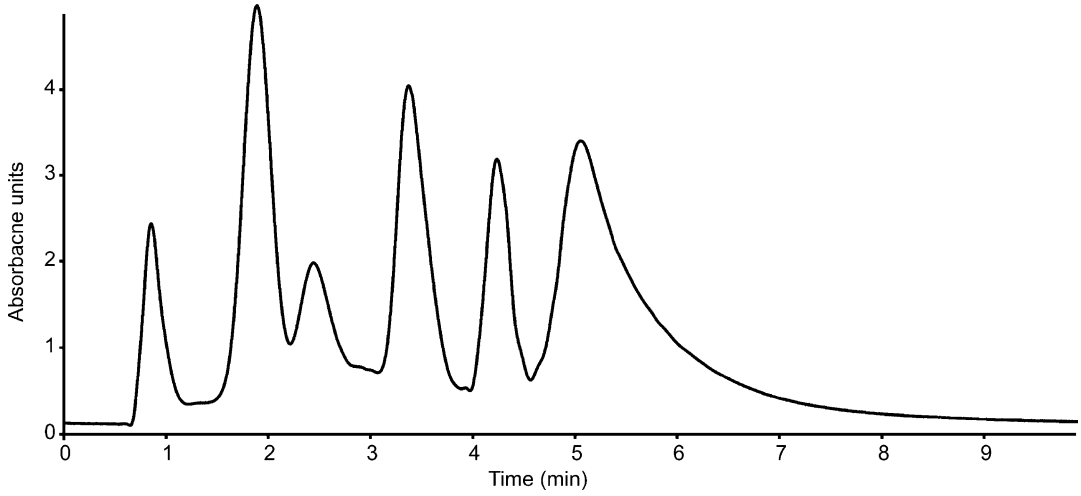

Fig. 5. Chromatogram from the separation of (in order of elution) cytochrome C, lysozyme, ovalbumin, trypsin inhibitor, α-chymotrypsinogen and BSA when using hydrophobic interaction chromatography (linear gradient from 2 M ammonium sulfate in phosphate buffer 10 mM pH 7 to the buffer only over 10 min) at room temperature. Separation was done on a 60 mm long and 100 μm i.d. monolithic column M2. UV detection, 214 nm. The gradient background signal, based on 10 consecutive blank runs, has been subtracted.

six proteins was chosen for their differences in hydrophobicity (GRAVY: Grand average of hydropathicity) and in size; cytochrome C (GRAVY = −0.902, M_w = 11.7 kDa); lysozyme (−0.150, 16.2 kDa); ovalbumin (−0.006, 42.8 kDa); trypsin inhibitor (0.039, 22.5 kDa); α-chymotrypsinogen (0.051, 25.7 kDa); BSA (0.429, 69.3 kDa). Hydrophobic interaction chromatography was of main interest because of the amphipilic surface of this particular column, comprised of aromatic groups on PEG spacers. As shown in Fig. 5, we were able to separate these six proteins in 6 min. The retention was mainly driven by the hydrophobic character of the proteins (from cytochrome C to α-chymotrypsinogen), but also by the size of a relatively large protein such as BSA. At least four consecutive injections were made and the peaks observed were highly reproducible. Moreover, peak asymmetries (calculated at 10% height) were all in the range of 1.3–1.8, except BSA which tailed more (asymmetry factor of 3). These are good indicators that the proteins were not excessively denaturated and this may, therefore, be considered as an indication of the creation of protein-friendly material. Separations on the 60 mm column can be made faster when non-linear gradients are used, and a more complete chromatographic study is currently carried out.

4. Conclusions

By varying the molecular weight of linear poly(ethylene glycol) dissolved in the porogen mixture, we have shown that it is possible to tune the pore dimensions in one more way. The use of various co-porogens allows an even wider range of possibilities as soon as the properties of the co-porogen are well characterized. It has been shown that the light source is also an important parameter, and that pulsed photopolymerization is fast and has intriguing possibilities. The possibility to synthesize these monoliths in capillaries and the facile removal of the polymeric porogen predicts interesting future chromatographic uses to this material. Moreover, the biocompatibility of the PEG seems to provide attractive properties towards proteins during separation, creating large pores that seem to cause little denaturing even for a large protein such as BSA in hydrophobic interaction mode.

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