Polymer Cookery: Influence of Polymerization Time and Different Initiation Conditions on Performance of Molecularly Imprinted Polymers

Sergey A. Piletsky,* Irene Mijangos, Antonio Guerreiro, Elena V. Piletska, Iva Chianella, Kal Karim, and Anthony P. F. Turner

Institute of BioScience and Technology, Cranfield University, Silsoe, Bedfordshire, MK45 4DT, U.K.

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ABSTRACT: A set of polymers was imprinted with (−)-ephedrine using thermal and UV initiation. The performance of the synthesized materials was studied by HPLC. It was shown that the polymer morphology and enantioselective properties are dependent on the polymerization conditions and time of the reaction. The binding mechanism of synthesized polymers was studied using Van't Hoff analysis. The results of testing strongly indicate that the polymer receptor structure is shaped during the initial phase of polymer gelation. The present study shows the importance of controlling experimental conditions in the MIP synthesis and highlights potential problems expected during scaling up of MIP production.

Introduction

During the past 10 years, substantial progress has been made in understanding the nature of the complex phenomena involved in synthesis and related performance of molecularly imprinted polymers.[1,2] The factors studied include analysis of the type and concentration of the monomers used in polymer preparation,[3,4] role of polymerization temperature,[5,6] pressure[7,8] and solvent.[9,10]

Surprisingly, little is known about the optimal time needed for MIP formation and its relation to the kinetics of polymerization reaction. This could be explained partially by the difficulty in studying the complex processes involved in the formation of highly cross-linked polymers and the monomer−template complexation during polymerization reaction.

We have shown before that small differences in the temperature profile of the initial period of the polymerization reaction causes a profound effect on the polymer morphology and recognition properties.[6] The question which required further attention was the influence of the polymerization time on MIP recognition abilities. It is expected that, irrespective of the initiation protocol (UV or thermopolymerization), the prolonged polymerization time should lead to higher completion of the polymerization reaction with fewer unpolymerizable double bonds remaining in the polymer. Polymers formed during longer periods of time would be more rigid. This should ensure a more defined shape of imprinting cavities and higher specificity of MIPs. On the other hand, more-rigid polymers might have hindered mass exchange and slow binding kinetics. The particular balance of rigidity of imprinting cavities versus polymer flexibility and adaptability (an analogue of induced fit in natural receptors and enzymes) remains poorly understood.

The current study is aimed at the analysis of the role of time of polymerization on MIP recognition abilities. The model system includes a set of hydroxyethyl methacrylate (HEMA)-based polymers, which have been imprinted with (−)-ephedrine using two different initiation protocols: thermal and UV polymerization. The synthesized materials were studied using HPLC, and their morphology, swelling data, and performance were compared.

Results and Discussion

To analyze the effect of polymerization time on the MIP's affinity and specificity, three sets of polymers were imprinted with (−)-ephedrine using different initiation protocols: thermoinitiation at 80 °C, photoinitiation with a "strong" UV lamp (0.163 W/cm^2), and photoinitiation with a "weak" UV lamp (0.016 W/cm^2) at 0 °C. Each set was prepared using different exposure times, which varied from 20 min to 7 days. The polymer's performance was analyzed using chromatographic experiments.

The temperature of the initial period of polymerization reaction was monitored using a thermocouple as described previously (Figure 1).[6] As expected, the real polymerization temperature was significantly higher than the one used for the initiation of the reaction due to the exothermic nature of the process. After the initial 20 min, the reaction temperature monotonically declined with the exception of the "strong" UV lamp initiation protocol, which generated so much heat that the temperature continued to increase further up to 200 °C over the next 3 h. This increase did not result from the reaction itself since the measurement of the temperature of mineral oil exposed to the same conditions gave the same temperature increase (190 °C).

The photodecomposition of azo compounds could be substantially faster than their thermodecomposition at 80 °C, the condition traditionally used for MIP preparation. Evidence to support this hypothesis comes from a comparison of previously published data describing the behavior of two similar initiators in acetonitrile and toluene. Thus, for a photoinitiation with a medium-pressure Hg lamp at 25 °C, the half-life ($t_{1/2}$) of azo-bis-(cianovaleric acid) is approximately 140 min.[11] At 80 °C, the thermodecomposition of similar compound, 1,1′-azobis (cyclohexanecarbonitrile) is more than 10 times

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* Author to whom correspondence should be addressed. Tel: +44 (0)1525 863584. Fax: +44 (0)1525 863533. E-mail: S.Piletsky@cranfield.ac.uk.

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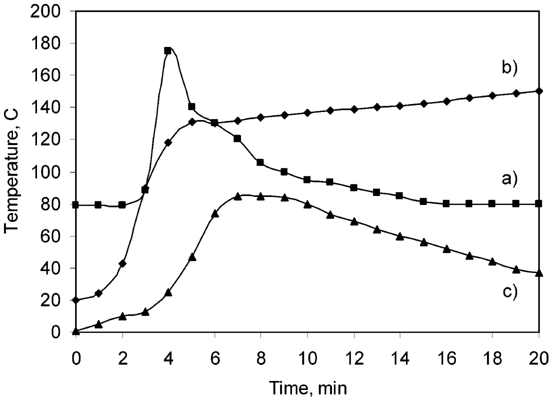

Figure 1. The temperature profile of the polymerization reactions: thermoinitiation (a); "strong" UV lamp (b); and "weak" UV lamp (c). The plots begin 2 min before the start of gelation.

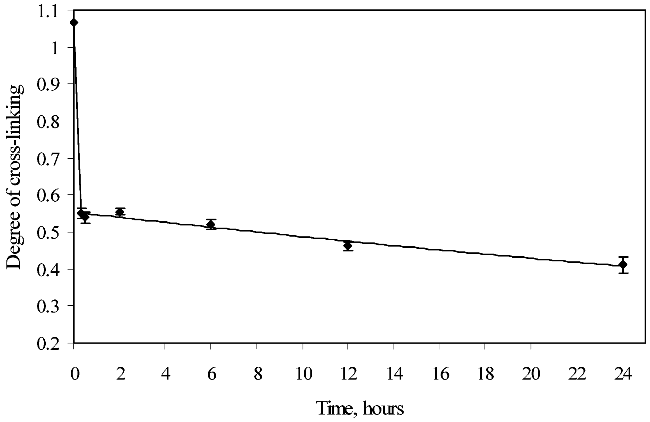

Figure 2. Time dependence of the degree of cross-linking (normalized peak area of C═C stretching) measured by FTIR. Materials were polymerized using the "weak" UV lamp for 0−24 h. The initial "0" point corresponds to the measurement of monomer mixture which does not contain initiator.

slower ($t_{1/2}$ = 1777 min).[12] The thermodecomposition, however, will be significantly faster upon further increase in the polymerization temperature, and above 130 °C, the half-life of this initiator would not exceed 1−2 min.

It would be reasonable to assume that, with the possible exception of "weak" UV lamp initiation, after an initial 15−20 min of the polymerization reaction, most of the initiator in the monomer mixture is decomposed and conversion of double bonds in the polymer is close to completion. This can be seen from the IR spectra of the polymer which show practically no changes in the intensity of the C═C peak at 1634 cm^{-1} after the first 20 min of polymerization (data not shown). In the case of "weak" UV lamp initiation, the polymerization continues further, albeit more slowly, when approximately 10% more double bonds become saturated following 24 h of irradiation (Figure 2). The profiles of time dependence of the degrees of cross-linking for thermoinitiated polymer and the polymer prepared using the "strong" UV lamp were similar. The achieved degrees of cross-linking for "strong" and "weak" UV lamps were also similar (0.4 and 0.41, respectively). The degree of cross-linking for the polymer prepared using thermoinitiation was only 0.7, which indicates that this initiation protocol is less efficient than UV initiation.

Table 1. Polymerization Conditions and Properties of the Imprinted Polymers

initiation[a]	polymerization time	BET surface area, $\text{m}^2\ \text{g}^{-1}$	total pore volume, cc g^{-1}	av pore diameter, nm
thermo	1 hour	70.84	0.304	8.6
	3 hours	69.20	0.240	7.0
	24 hours	59.91	0.217	7.2
	72 hours	59.34	0.210	7.1
	102 hours	58.22	0.154	5.3
	192 hours	56.94	0.155	5.5
UV-S	10 min	37.48	0.076	4.1
	20 min	33.03	0.065	3.9
	30 min	33.79	0.072	4.3
	45 min	39.45	0.097	4.4
	60 min	36.99	0.065	3.5
	120 min	37.83	0.074	3.9
UV-W	20 min	24.54	0.027	2.2
	2 hour	29.15	0.03	2.1
	6 hour	39.98	0.04	2.1
	12 hour	54.10	0.058	2.2
	24 hour	25.8	0.028	2.2
annealed polymer	24 hours	26.7	0.025	1.9

[a] UV-S, "strong" UV lamp; UV-W, "weak" UV lamp, 0 °C; thermo, 80 °C.

It is clear that the polymerization process does not stop after this initial 20 min period. The continuing process involves transformation of the radicals bound to the polymer chains.[13] The midchain radicals in particular are less reactive than the propagating radical of the monomers or oligomers because of significant sterical congestion. Reinitiation by the midchain radicals, which will be considerably slower, will contribute to the continuing increase in polymer cross-linking during the later stages of polymerization.[14] Continuing exposure of the polymer to heat or UV radiation will affect polymer morphology and produce more-rigid materials. If the monomer−template complexes "survived" during the initial critical period (15−20 min), which should have a major impact on MIP recognition properties, very little changes would be expected in their concentration or strength during continuing exposure of the polymer to heat or UV irradiation.

The effect of polymerization time on polymer morphology is shown in Table 1. It can be seen that three different initiation protocols produced materials with different surface area and porosity, which are higher for the thermopolymerized MIPs, lower for the polymers made with the "strong" UV lamp, and lowest for the polymers made with the "weak" UV lamp, a fact most likely related to the temperature of polymerization.[6] The porosity and surface area of thermopolymerized polymers continued to decrease upon an increase in polymerization time; however, this tendency seems to be reversed for the materials polymerized using the "weak" UV lamp. The polymers prepared using the "strong" UV lamp had hybrid behavior without a clear change in their porosity and surface area.

Polymers prepared with the "weak" UV lamp had increased optical transparency which could indicate a higher degree of homogeneity of synthesized materials.

To analyze the influence of polymerization time on MIP recognition properties, the synthesized materials were packed in chromatographic columns and tested by HPLC in chloroform with hexamethylenediamine (HMDA) for their ability to interact with (+)- and (−)-ephedrine. The results of this evaluation, expressed in terms of time dependence of separation factors for ephedrine enantiomers, are presented in Figures 3−5.

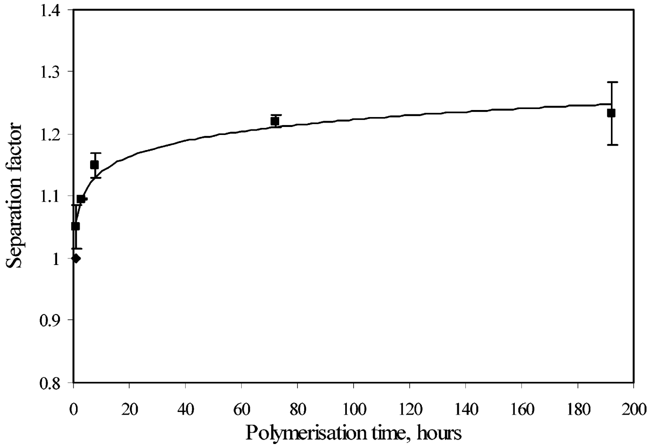

Figure 3. Influence of the polymerization time on separation factor (α) for polymers prepared at 80 °C and measured at 20 °C. Flow-rate, 1 mL min^{-1}; mobile phase, 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

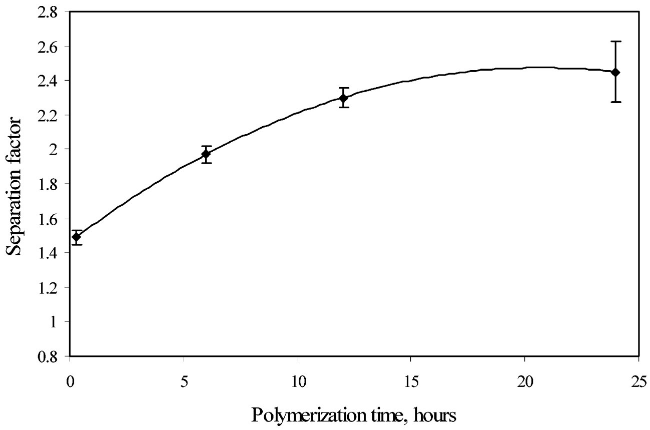

Figure 4. Influence of the polymerization time on separation factor (α) for polymers prepared using the "weak" UV lamp and measured at 20 °C. Flow-rate, 1 mL min^{-1}; mobile phase, 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

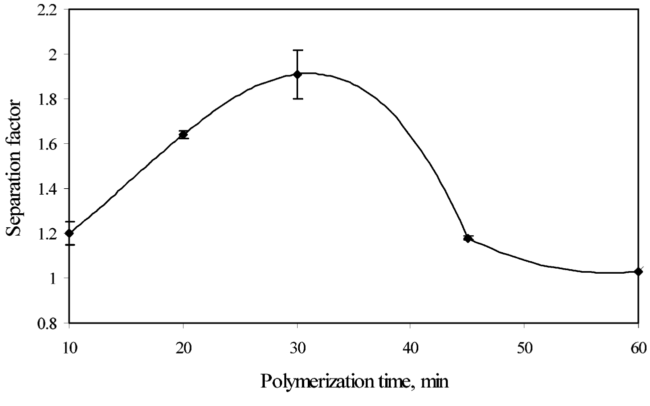

Figure 5. Influence of the polymerization time on separation factor (α) for polymers prepared using the "strong" UV lamp and measured at 20 °C. Flow-rate, 1 mL min^{-1}; mobile phase, 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

Apparently, an increase in polymerization time increased polymer selectivity, with separation factors reaching saturation at 72 h for thermopolymerized MIPs, 20−25 h for MIPs polymerized using the "weak" UV lamp, and only 30 min for MIPs polymerized using the "strong" UV lamp. The achieved separation factors were significantly higher for the polymers prepared using the "weak" UV lamp, as compared with the "strong" UV lamp and thermopolymerized MIPs, which had the poorest selectivity. This difference could be attributed to the fact that a lower polymerization temperature is beneficial for creating highly selective MIPs.[6]

Prolonged exposure to strong UV irradiation decreased binding of both enantiomers to the polymer and MIP selectivity. Intense UV irradiation and heat created by the "strong" UV lamp caused degradation of the polymer structure. As was shown before, poly(HEMA) heated above 195 °C decomposes, forming HEMA as a main product and also 2-isopropenyloxyethyl methacrylate and ethylene glycol.[15] This degradation process could be an important issue for engineers considering the scaling-up of MIP production. It would be possible for the exothermic polymerization reaction, performed in a large volume, to generate enough heat to greatly reduce polymer selectivity.

In the next experiment, we intended to determine to what extent the initial period of polymerization affects the properties of the synthesized material. For this, we started polymerization at 0 °C using a "weak" UV lamp, and after the gelation started (5 min on the plot in Figure 1), the polymer was transferred to an oil bath where it was kept for 24 h at 80 °C. Apparently, the annealing process had little effect on polymer morphology (Table 1). Since temperature has a major impact on polymer selectivity, we used chromatographic retention data from variable-temperature runs to evaluate the thermodynamic factors underlying polymer−template interactions governing MIP selectivity.[16]

The relationship between the retention factor (K') for an analyte in a chromatographic system can be expressed in term of Van't Hoff equation:

$$\ln K' = -(\Delta H°/RT) + (\Delta S°/R) + \ln \phi \qquad (1)$$

K' (capacity factors) were determined from $K' = (t - t_0)/t_0$, where t is the retention time of a given species and t_0 is the retention time of the void marker (acetone). $\Delta H°$ is the enthalpy and $\Delta S°$ is the entropy of transfer of the solute from the mobile phase to the stationary phase, T is the temperature, R is the gas constant, and ϕ is the phase ratio (volume of the stationary phase divided by volume of mobile phase). The dependence of the separation factor on temperature can be expressed by the equation:

$$\ln \alpha = -(\Delta(-,+)\Delta H°/RT) + (\Delta(-,+)\Delta S°/R) + \ln \phi \qquad (2)$$

ln α vs $1/RT$ (Van't Hoff plot of α) should be linear if $\Delta(-,+)\Delta H°$ and $\Delta(-,+)\Delta S°$ are independent of the temperature. A nonlinear plot would be observed if the binding mechanism changes over the temperature range.[17]

The Van't Hoff plots for the separation factor are shown in Figure 6. For all polymers measured at 0−40 °C, an increase in temperature resulted in an increase in the selectivity of separation. The transition period at ~40 °C for MIPs made with the "weak" UV lamp is characteristic for a change of the separation mechanism from entropy-controlled to enthalpy-controlled.[6] The possible interpretation of these results would be that entropic factors in selective recognition for present MIP systems outweigh enthalpic factors. The

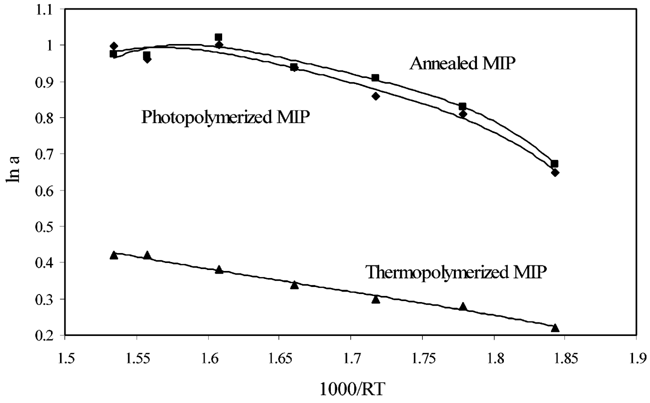

Figure 6. Van't Hoff plots of the separation factor (α) for 48.5 nmol of ephedrine enantiomers on columns with synthesized polymers. Flow-rate, 1 mL min^{-1}; mobile phase, 0.05% HMDA in chloroform.

structure of the synthesized materials contains hydroxyethyl groups capable of forming hydrogen bonds. The network of these bonds formed after the extraction of the template could be broken again by a readsorbed template molecule. This process would lead to the imprints assuming a more-open configuration and greater freedom of movement in the polymer functional groups. The increase in entropy will be responsible for an energy gain which will outweigh unfavorable enthalpy changes. This effect must be stronger for the template as compared with the opposite enantiomer. This type of behavior where selective binding is entropy-driven has been frequently observed for natural and synthetic receptors.[18]

These plots showed that, although very little difference exists in the performance of the polymers synthesized with the "weak" UV lamp (with and without annealing), they both behaved differently from the thermopolymerized MIP.

From the fact that the behavior of the annealed polymer resembled more that of the polymer made at 0 °C rather than the polymer made at 80 °C, it is possible to conclude that the gelation phase is the period which determines the creation of selective imprints.

To prove this point further, a "reverse" experiment was made. For this, we started polymerization using thermoinitiation at 80 °C, and after the gelation started, the polymer was transferred into the ice bath, where it was treated for 24 h at 0 °C using the "weak" UV lamp. The chromatographic behavior of the resulting polymer was very similar to the polymer prepared using thermoinitiation. Both UV-annealed polymer and the polymer prepared by thermoinitiation had similar separation factors: α = 1.17 and α = 1.23, respectively. This confirmed that the initiation during the gelation phase determines to a large degree the quality of imprinted polymers.

An increase in MIP selectivity upon increase in polymerization time seems to be related to an increase in polymer rigidity. To prove this, the swelling of the polymers synthesized using thermoinitiation and a "weak" UV lamp was measured in chloroform. The experiments showed a proportional decrease in polymer swelling upon increase in polymerization time, which also correlates with increase in polymer selectivity (Figure 7). Apparently, the changes in polymer swelling are minimal after the initial 8−10 h period of polymerization for both types of initiation.

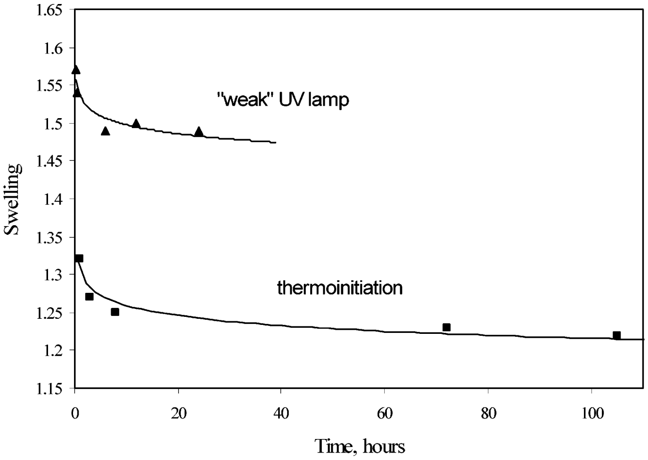

Figure 7. Dependence of the polymer swelling in chloroform on the polymerization time.

The variation in polymer rigidity might also affect transport and binding kinetics. This effect can be quantified using Van Deemter analysis.[19] The detailed analysis, however, required measurements to be performed at different flow rates, which was difficult to achieve due to excessive broadening of the peak shape at flow rates below 0.5 mL/min and too high back pressure at flow rates above 2 mL/min.

Conclusion

The present results indicate that polymerization time plays an important role in determining morphology and performance of synthesized materials. Increase in polymerization time increases the rigidity of polymer structure and facilitates the formation of imprinting cavities with better defined shape. The selectivity of binding seems to be determined by the entropic factors resulting from the change in polymer conformation produced by the template. The initial period (gelation phase), when selective cavities are formed, appeared to be the most critical in formation of imprinting cavities and determining MIP selectivity.

Experimental Section

Chemicals. 1R,2S-Ephedrine ((−)-ephedrine) and 2R,1S-ephedrine ((+)-ephedrine) were supplied by Chemical Development, GlaxoSmithKline R&D, UK. Ethylene glycol dimethacrylate (EGDMA), 2-hydroxyethyl methacrylate (HEM), 1,1′-azobis(cyclohexanecarbonitrile), hexamethylenediamine (HMDA), and chloroform were purchased from Aldrich (UK). All chemicals and solvents were analytical or HPLC grade and were used without further purification.

Preparation of Molecularly Imprinted Polymers. Three sets of imprinted polymers were synthesized as described previously.[6] To a solution of (+)-ephedrine (1.21 mmol, 0.2 g) in chloroform (8.82 g) was added 2-hydroxyethyl methacrylate (12.1 mmol, 1.57 g), ethylene glycol dimethacrylate (35.9 mmol, 7.1 g) and 1,1′-azobis (cyclohexanecarbonitrile) (0.18 g). The monomer mixture was placed into a 50 mL glass tube and purged with nitrogen for 5 min. The polymerization was initiated using (i) a fiber optic light source with a 0.016 W/cm^2 intensity CERMAX Xenon Arc Lamp (PerkinElmer Optoelectronics, Inc., USA) in an ice bath with NaCl at 0 °C, (ii) an UVAPRINT 100 CVI UV source with 0.163 W/cm^2 intensity (Dr. Hönle); and (iii) using a mineral oil bath at 80 °C. It was impossible to control temperature during UV irradiation with the UVAPRINT 100 CVI UV source due to the high heat generated by this lamp. The bulk polymers were ground in methanol with an electrical mortar SL2 (Silverson, UK) and mechanically wet-sieved through 38 μm sieves (Endecotts, UK) and sedimented in methanol to remove fines. The polymers were additionally washed out with chloroform containing 0.05% hexamethylenediamine. Spectrophotometric analysis of ephedrine concentration in washing solutions, performed at 260 nm, indicated that 94−95% of the template was removed successfully from the polymer. Polymer particles were collected, dried under vacuum, and used for packing HPLC columns.

The determinations of specific surface area were performed using NOVA 1000 E Series High-Speed Gas Sorption Analyzer (Quantachrome) based on the nitrogen BET.

HPLC Analysis. For the analysis of MIP recognition properties, the polymer particles were suspended in methanol and packed in stainless steel HPLC columns (150 mm × 4.6 mm) at 1000 bar pressure using 1666 HPLC column Slurry Packer model 1666 (Alltech, UK). The evaluation experiments were carried out using an HPLC system, which included a ConstaMetric-3200 solvent delivery system (LDC Analytical, UK), a PerkinElmer ISS-100 automatic injection system, and a Waters Lambda-Max model 481 LC detector (UK). The temperature of the column and eluent was maintained constant within ±0.1 °C using a thermostat (LTD20G, Grand Instruments, Cambridge, UK). Columns were washed with 0.05% hexamethylenediamine in chloroform at a constant flow (1 mL min^{-1}) until a stable baseline was achieved. HPLC analysis was performed at a flow-rate of 1.0 mL min^{-1} and monitored by an UV detector at 260 nm. Injection amounts were 8 μg (48.5 nmol) in 40 μL injection volumes. The retention of ephedrine enantiomers was examined at temperature values of 0, 10, 20, 30, 40, and 55 °C. The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. All reported chromatographic data represent the results of 3−5 concordant experiments. The standard deviation of the measurements was below 5%.

Capacity and Separation Factors. Capacity factors (K') were determined from $K' = (t - t_o)/t_o$, where t is the retention time of a given species and t_o is the retention time of the void marker (acetone). Effective enantioseparation factors (α) were calculated from the relationship: $\alpha = K'(+)/K'(-)$, where $K'(+)$ and $K'(-)$ are the capacity factors of the (+)- and (−)-ephedrine, respectively.

IR Spectroscopy. The FT-IR spectra were recorded on an IRScope II instrument (Bruker). The net peak area of a C=C stretching was determined in the absorbance mode between 1630 and 1680 cm^{-1}, and this net area was normalized against a net reference with integration limits between 990 and 920 cm^{-1}.[20]

Swelling Analysis. Swelling experiments were performed as described previously.[6] Three hundred milligrams of the polymer particles with the mesh size 38−67 μm were packed in 1 mL solid-phase extraction cartridges (Supelco, UK). Cartridges were filled with 1 mL of chloroform. After 6 h equilibration at 20 °C, the excess of solvent was removed from the polymer by applying reduced pressure for 1 min and the weight of the swollen polymer was measured. The swelling ratio (Sr) of the polymers was calculated from the following equation:

$$\mathrm{Sr} = (m_s - m_o)/m_o \qquad (3)$$

Where m_s is the mass of the swollen polymer and m_o is the mass of dry polymer.

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