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Polymer Cookery. 2. Influence of Polymerization Pressure and Polymer Swelling on the Performance of Molecularly Imprinted Polymers

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ABSTRACT: A set of polymers was imprinted with (+)-ephedrine at ambient and 10 bar pressure. The performance of the synthesized materials was studied by HPLC at six different temperatures: 0, 10, 20, 30, 40, and 50 °C. It was shown that the polymer morphology (surface area and porosity) and enantioselective properties are different for these two polymers—an unexpected result which was in contradiction with the theoretical forecasts. A possible mechanism which explains the observed phenomenon is discussed. The contributing factor which influenced the polymer performance was the polymer swelling. This shows that, in addition to temperature, other physical parameters such as pressure and polymer swelling should be analyzed and accounted for in the quality control of MIP synthesis and application.

Introduction

Although molecularly imprinted polymers (MIPs) were discovered more than 30 years ago, they still continue to be in many respects a “black box”, with many unresolved issues related to their design and performance. Due to this, molecular imprinting still remains in its infancy with unfulfilled promises and yet unrealized commercial potential.[1] Thus even today, after more than 1300 papers have been published on different subjects of polymer design and performance, it is practically impossible to suggest a reliable and reproducible protocol for preparation of a polymer with the required properties for a specific type of application. The heart of this problem lies in the poor understanding of the nature of the complex phenomena involved in polymer synthesis and performance which are responsible for its recognition properties. Among the factors which require deeper analysis are the following: (i) the type and concentration of the monomers involved in polymer preparation; (ii) polymerization temperature; (iii) pressure; (vi) cross-linker; (v) solvent; (vi) time of polymerization.

Recently, a major breakthrough has been made regarding the rational choice of monomers for polymer design. Sellergren and Takeuchi independently developed a combinatorial approach where hundreds of polymers were synthesized and screened in a semiautomatic assay in a search for the best performing MIPs.[2] Our group has proposed a computational approach where the screening of monomers is achieved by using molecular modeling software.[3] Both approaches proved to be useful for the fast optimization of polymer composition.

A comprehensive study of the effect of polymerization temperature on polymer−template interactions and thermodynamic origins of MIP recognition properties was also published recently.[4] The results showed that MIPs have a temperature-dependent optimum in their recognition, which is affected by the polymerization temperature. The current study is aimed at the analysis of the role of polymerization pressure and polymer swelling on MIP recognition properties.

Very limited attempts to analyze the role of the pressure on MIP performance have been performed in the past. Sellergren and coauthors have studied MIPs for two templates: atrazine and ametryn made at 1 and 1000 bar in three different solvents.[5] The results were inconclusive, showing that in only one case the MIP made at increased pressure had better affinity than the polymer prepared at ambient pressure. The authors explained the enhanced binding by the positive effect which high pressure has on intramolecular associations in the monomer mixture, which obey eq 1:

$$[\mathrm{d}RT \ln K/\mathrm{d}P]T = -\Delta V^0 \qquad (1)$$

where K is the association constant of the complex, R is the gas constant, T is the absolute temperature, P is the pressure, and ΔV^0 is the difference in the reaction volume.

The summary calculations made in this paper predict that an increase in pressure by 1000 bar will increase the capacity factor by a factor of 1.1−2.2.

It might be reasonable also to assume that, in addition to the influence on intramolecular associations, increased pressure might also affect the kinetics of the polymerization reaction. The influence of temperature and pressure on the rate coefficients of free-radical polymerization can be described by the general eq 2:

$$\mathrm{d}(\ln k_{\mathrm{P}})/\mathrm{d}P = \Delta V^{\ddagger}/RT \qquad (2)$$

Here k_{P} is the propagation rate coefficient and $\Delta V^{\ddagger}$ is the change in activation volume.[6]

Beuermann and Buback have performed extensive analysis of the propagation rate coefficients for different polymeric systems.[6] The conclusion drawn from this work is that for acrylates and methacrylates an increase in pressure by 1000 bar should increase the polymerization rate by a factor less than 1.1. Similar change can also be produced if the polymerization temperature increases by 30 K.

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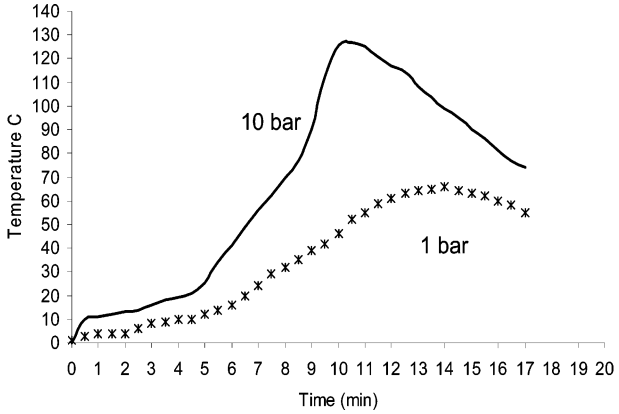

Figure 1. Temperature profile of the polymerization reaction. The plots begin 2 min before the start of gelation.

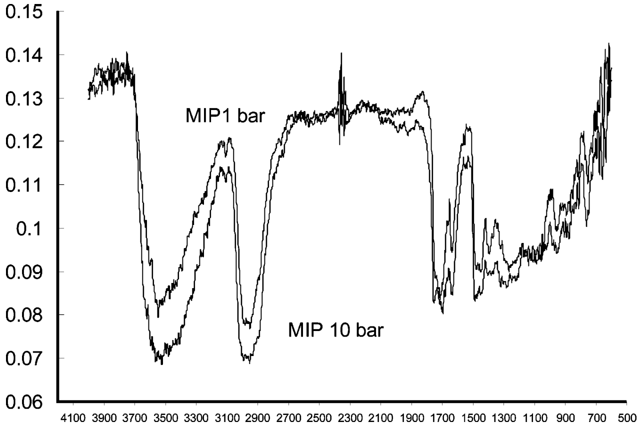

Figure 2. FTIR spectra of polymers MIP 1 bar and MIP 10 bar.

From these calculations it was clear that neither the molecules' association nor the kinetics of the polymerization reaction and polymer morphology should be affected by small changes in pressure with a magnitude below 100 bar (normally generated during bulk polymerization in closed vessels). To confirm this, a set of polymers has been imprinted with (+)-ephedrine at ambient and 10 bar pressure. The performance of the synthesized materials was studied by HPLC and their morphology studied by nitrogen sorption porosimetry.

Results and Discussion

This work is an extension of our earlier paper focused on the analysis of the temperature impact on MIP performance.[4]

To analyze the impact of pressure on the MIP's affinity and specificity, two polymers, imprinted with (+)-ephedrine at 0 °C, were prepared at ambient and 10 bar pressure using the same monomer composition (see Experimental Section). The polymer's performance was analyzed using chromatographic experiments.

The temperature of the polymerization mixture was monitored using a thermocouple (Figure 1).

As was shown previously,[4] 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 (Table 1). After an initial 17 min reaction, temperature monotonically declined until it reached 7 °C after 3 h. What was not anticipated that the polymerization reaction generated more heat at high rather than at ambient pressure.

The same experiment was repeated three times giving fairly reproducible results. This difference appeared to be related to the number of nonreacted double bonds, since the infrared spectra showed that the intensity of C=C stretch at 1631 cm^{-1} is higher for the polymer made at ambient pressure as compared with polymer made at 10 bar (Figure 2). Accordingly to the calibration curve, MIP made at ambient pressure contains 12% and MIP made at 10 bar contains 5.8% nonpolymerized double bonds. Another contributing factor is the kinetics of the polymerization reaction which is seemingly faster at 10 bar as compared with ambient pressure.

In addition it appeared that high pressure also assisted in the production of optically transparent gellike materials with a decreased surface area and pore volume and increased density (Table 1). According to the previously cited calculations, it should be impossible for such an effect to originate from the small variations in propagation rate coefficients induced by a 10 bar difference in applied pressure.[6]

In theory, greater heat generated by polymerization at high pressures should lead to the formation of a larger number of free radicals and a larger number of small globules formed during phase separation. The polymer composed of smaller globules has a larger number of smaller pores and a larger surface area.[4,7] The reverse effect observed in our case obviously originates from the different mechanism involved in determining polymer morphology.

The only other parameter which is seriously affected by an increase in pressure is boiling temperature of the solvent, used as a porogen in the polymerization reaction. For chloroform an increase in pressure by 10 bar means an increase in boiling temperature from 61 to 184 °C.[8] The temperature developed during the exothermic polymerization reaction at ambient pressure (66 °C) is above the boiling temperature for chloroform. In contrast, the temperature developed at 10 bar (127 °C) is comfortably below the chloroform boiling temperature at this pressure. The fact that chloroform was boiling in the monomer mixture during polymerization at ambient temperature was proven by measuring the change in weight of the synthesized polymer which was around 0.7% (1.4% of chloroform). A very small change in weight (<0.3%) was detected for the polymer polymerized at 10 bar. The boiling solvent by itself can reduce the temperature in reaction mixture although judging from the small quantity of evaporated solvent this effect should not exceed 1 °C. It seems reasonable that the boiling solvent could affect the polymerization kinetics, overall polymerization rate, and final degree of conversion due to varying initiation, chain growth, and termination rates.[9]

To check the influence of variations in the polymers morphology on their enantiorecognition properties, the synthesized materials were packed in chromatographic

Table 1. Polymerization Conditions and Properties of the Imprinted Polymers

polym	T init, °C	T max., °C	BET surf area, $m^2\ g^{-1}$	tot. pore vol, $mm^3\ g^{-1}$	av pore diameter, nm	density, g/mL
MIP1 bar	0	+66	1.67	2.7	1.9	0.58
MIP10 bar	0	+127	0.18	0.82	8.9	0.62

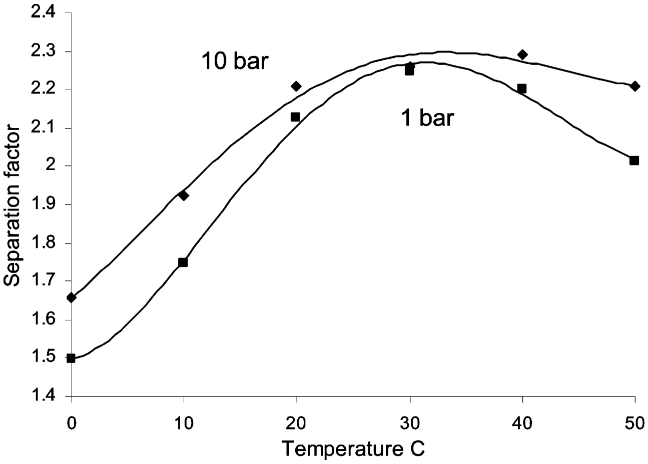

Figure 3. Influence of the polymerization temperature on separation factors (α) for MIP 1 bar and MIP 10 bar measured at different temperatures: flow rate, 1 mL·min^{-1}; mobile phase, 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

columns. The chromatographic evaluation of the synthesized polymers was performed at 6 temperatures: 0, 10, 20, 30, 40, and 50 °C. The results of this evaluation, expressed in terms of temperature dependence of separation factors for ephedrine enantiomers, are presented in Figure 3.

As expected from previous results,[4] temperature had a complex effect on enantioseparation for both polymers. Upon increase in temperature the value of separation factors (α) initially increased, reaching a maximum at 30 °C and followed by a decrease at higher temperature (Figure 3). Both polymers had similar chromatographic behavior with broad peaks characteristic for MIPs (Figure 4).

The polymer made at ambient pressure was more susceptible to the temperature than the polymer made at 10 bar. Thus, the plot of the temperature dependence of the separation factor was steeper for MIP 1 bar as compared with MIP 10 bar, even if the maximum value in α was approximately the same for both these polymers (Figure 3). The separation factor was approximately 10% higher (at 0 °C) for polymer made at 10 bar as compared with 1 bar. This difference, although not very significant, in the magnitude substantially exceeds theoretical forecasts. Thus, from the logarithmic dependence of association constant (K) on pressure the affinity of the polymer should increase negligibly (by a factor of 1.001) upon increase in pressure from ambient to 10 bar.[5]

The reason for this lies in the higher rigidity of the polymer made at higher pressure, which contains a smaller number of nonpolymerized double bonds. The swelling of the more rigid MIP 10 bar should be less affected by temperature variations thus having a less detrimental effect on polymer recognition properties.

In principle, the effect of swelling of imprinted polymers on its recognition properties remains unclear. It is expected that the distance between polymer functional groups and their orientation in the binding cavity can be affected by a swelling process and due to this the MIP can loose its specificity when immersed in the "wrong" solvent or exposed to the "wrong" temperature.

To study this, the swelling of both polymers was measured in chloroform. As anticipated, the swelling of MIP 1 bar was more pronounced than swelling of MIP 10 bar (Table 2).

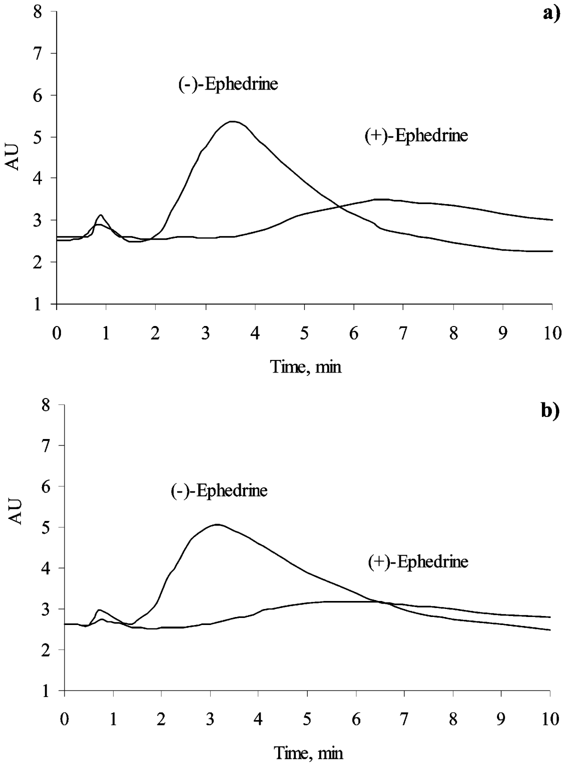

Figure 4. Chromatograms of (+)-ephedrine and (−)-ephedrine on MIP 1 bar (a) and MIP 10 bar (b): flow rate, 1 mL·min^{-1}; mobile phase, 0.05% HMDA in chloroform; temperature, +40 °C. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

Table 2. Swelling Ratios[a] of the Polymers

polym	swelling ratio in chloroform
MIP1 bar	1.88 ± 0.2
MIP10 bar	1.5 ± 0.15

[a] Swelling ratio = volume of the wet polymer/volume of the dry polymer.

To study the magnitude of the swelling effect on the MIP recognition properties we repeated consecutive injections of ephedrine enantiomers into a column packed with polymer dried in a vacuum. As solvent was run through the column and the polymer started swelling, we observed a sharp initial increase in the values of the separation factor, which after 2 h continued to slowly increase for at least 20 h (Figure 5).

This change correlated with change in particles size of the polymer (Figure 6). The first point on this plot represents the size of dry particles measured with an optical microscope. We had to change the measuring setup since fast measurement or measurement of dry samples was impossible with available equipment. Nevertheless measured data were statistically correct and were in agreement with swelling data presented in Table 2.

The same profile was observed for polymers MIP 10 bar and MIP 1 bar (data not shown). This swelling affected mostly the template recognition, since binding of the opposite enantiomer was practically unaffected. The capacity factor K' for (−)-ephedrine remained basically unchanged during all this period of time, which shows that the polymer transport properties in this experiment were not affected by the swelling process.

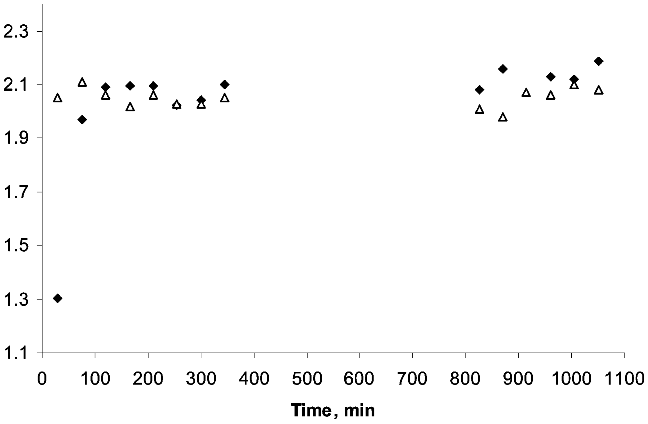

Figure 5. Separation factor α (◆) measured for consecutive injections of ephedrine enantiomers and capacity factor K' (△) for (−)-ephedrine determined for a column packed with MIP 10 bar: flow rate, 1 mL·min^{-1}; mobile phase, 0.05% HMDA in chloroform; temperature, +20 °C. Injection amounts were 8 μg (48.5 nmol) in 40 μL of injection volume.

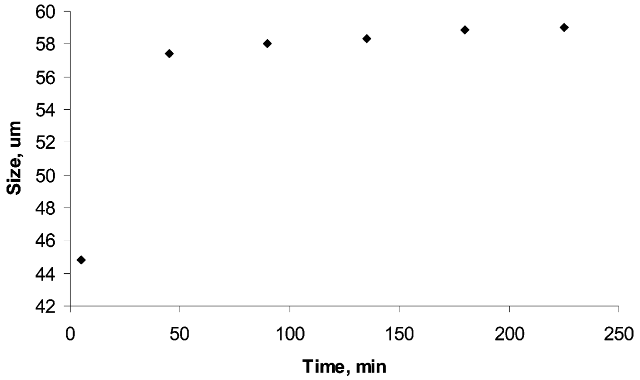

Figure 6. Average size of the polymer particles (MIP 10 bar) measured in chloroform continuously over 6 h. The first point on the plot representing dry particles was measured using optical microscope. The variation coefficient for the measurements was below 5%.

This finding clearly indicates that polymer swelling plays an important role in MIP recognition. Thus, for better performance it might be necessary to control polymer flexibility and provide binding conditions where the template can be adequately accommodated by corresponding binding cavities.

For a future work it might be useful to apply fluorescent labels to measure the microscopic swelling effect, which might differ from the macroscopic swelling. Macroscopic swelling depends more on the less cross-linked intradomain region in the polymer, but it is the swelling in highly cross-linked globules which is more important for MIP performance since it affects the structure of imprinting cavities and their interaction with the template.

The previously unaccountable effects of polymerization pressure and polymer swelling might be responsible for poor reproducibility and occasionally weak performance of the polymers synthesized in different laboratories under different experimental conditions.

Conclusion

The present results indicate that polymerization pressure plays an important, although indirect, role in the morphology and performance of the synthesized materials. The mechanism of the pressure effect appears to be linked with its influence on the boiling temperature of the solvent used for polymer preparation. It was also shown that MIP performance is influenced by polymer swelling. This confirms that, in addition to temperature, other physical parameters such as pressure and polymer swelling should be analyzed and accounted for in the quality control of MIP synthesis and application.

Experimental Section

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

Preparation of Molecularly Imprinted Polymers. Two polymers were synthesized as described previously[4] at 0 °C at two different pressures: 1 and 10 bar (Table 2). A 10:1 molar ratio of functional monomers to template was used to saturate all functional binding sites in the template. 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 bottles were placed in a Calipso pressure chamber (AG Thomas Ltd, U.K.) filled with ice and equilibrated for 20 min at 0 °C. The polymerization was initiated photochemically using a fiber optic light source with a 300 W CERMAX xenon arc lamp (Perkin-Elmer Optoelectronics, Inc.) for 3 h duration of exposure. The bulk polymers were ground in methanol with an electrical mortar SL2 (Silverson, U.K.) and mechanically wet-sieved through 38 μm sieves (Endecotts, U.K.) 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 “e” surface area and pore size analyzer (Quantachrome) on the basis of 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 × 4.6 mm) at 1000 bar pressure using 1666 HPLC column slurry packer (Alltech, U.K.). The evaluation experiments were carried out using an HPLC system, which included a ConstaMetric-3200 solvent delivery system (LDC Analytical, U.K.), Perkin-Elmer ISS-100 automatic injection system, and a Waters Lambda-Max model 481 LC detector (U.K.). The temperature of the column and eluent was maintained constant within ±0.1 °C using a thermostat (LTD20G, Grand Instruments, Cambridge, U.K.). 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 UV detector at 260 nm. Injection amounts were 8 μg (48.5 nmol) in 40 μL injection volume. The affinity of ephedrine enantiomers was examined at the temperature values of 0, 10, 20, 30, 40, and 50 °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. A 4.5 mg amount of finely ground polymer or corresponding aliquots of EGDMA were mixed with 60 mg of KBr powder. This mixture was pressed into small disks, and their FT-IR spectra were recorded on an IRScope II instrument (Bruker) using reflectance mode. The net peak area of a C=C stretch was determined between the integration limits of 1660 and 1580 cm^{-1}, and this net area was compared with standards containing different concentrations of EGDMA.

Particle Size Measurement. The average size of polymer particles was measured by a Mastersizer X particles size analyzer (Malvern). The dry samples were measured with optical microscope. Images of 200 particles were captured, and their average diameter was calculated. All measurements were made at 20 °C.

Swelling Analysis. Swelling experiments were performed as described previously.[5] Dry polymer was packed in graduated micropipet (400 μL volume) and sealed loosely with a Whatman filter frit. Excess solvent was then added and volume of the polymer measured. The swelling is given as volume of swollen polymer/volume of dry polymer. Both tested fractions had average size of particles 43 μm (80% of particles). Measurements were made at 20 °C before and after exposing polymer to chloroform for 6 h.

Polymer Density Study. Dry polymer (1 mL, 38−100 μm) was placed in a calibrated graduated glass cylinder and weighed. This gave the apparent dry density of the polymer.[5]

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References and Notes

(1) Piletsky, S. A.; Alcock, S.; Turner, A. P. F. *TIBTECH* **2001**, *19*, 9−12.
(2) (a) Takeuchi, T.; Fukuma, D.; Matsui, J. *Anal. Chem.* **1999**, *71*, 285−290. (b) Lanza F.; Sellergren, B. *Anal. Chem.* **1999**, *71*, 2092−2096.
(3) (a) Piletsky, S. A.; Day, R. M.; Chen, B.; Subrahmanyam, S.; Piletska, O.; Turner, A. P. F. PCT/GB01/00324, 2001. (b) Piletsky, S. A.; Karim, K.; Piletska, E. V.; Day, C. J.; Freebairn, K. W.; Legge, C.; Turner, A. P. F. *Analyst* **2001**, *126*, 1826−1830.
(4) Piletsky, S. A.; Piletska, E. V.; Karim, K.; Freebairn, K. W.; Legge, C. H.; Turner A. P. F. *Macromolecules* **2002**, *35*, 7499−7504.
(5) Sellergren, B.; Dauwe, C.; Schneider T. *Macromolecules* **1997**, *30*, 2454−2459.
(6) Beuermann, S.; Buback, M. *Prog. Polym. Sci.* **2002**, *27*, 191−254.
(7) Viklund, C.; Svec, F.; Frechet, J. M. J. *Chem. Mater.* **1996**, *8*, 744−750.
(8) http://www.chemsoc.org/exemplarchem/entries/pkirby/exemchem/Nomograph/Nomograph.html.
(9) Beuermann, S.; Buback, M.; Schmaltz, C. *Macromolecules* **1998**, *31*, 8069−8074.

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