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# Influence of the Polymerization Conditions on the Performance of Molecularly Imprinted Polymers

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ABSTRACT: Although molecular imprinting in organic polymers was introduced by Wulff more than 30 years ago,[1] this promising technology has yet to realize its full commercial potential, partially due to the lack of a clear understanding of how physical factors affect the polymerization process and what basic rules should be applied in order to produce synthetic receptors with the required characteristics. This arises mainly from the complex nature of the polymerization reaction in cross-linked systems which depends on many interrelated factors. Among the most important physical factors affecting the polymerization process, as well as the recognition properties of the materials produced, are temperature, type of initiation, pressure, magnetic field, volume of the polymerization mixture, dielectric constants of monomers and solvent, and polymerization time. This paper was inspired by the urgent need to summarize the knowledge generated concerning how polymerization conditions affect the quality of molecularly imprinted polymers (MIPs) in order to help the imprinting community make rational and effective choices and maximize their chances for success.

## Introduction

During the past 10 years substantial progress has been made in understanding the nature of the complex phenomena involved in the synthesis and related performance of molecularly imprinted polymers (MIPs).[2,3] The factors studied include analyses of the type and concentration of the monomers used in polymer preparation,[4,5] the role of polymerization temperature,[6−10] pressure,[11,12] and solvent.[13,14]

Although numerous attempts to investigate the effects of physical conditions on the recognition properties of the polymeric materials have been made, these studies were not sufficiently systematic or complete. The results could not always therefore be compared due to differences in polymerization formats and polymer composition, which prevented generally applicable conclusions from being drawn.

In an attempt to understand the physical conditions that influence the phenomenon of imprinting, we selected a model system comprising (−)-ephedrine as template, 2-hydroxyethyl methacrylate (HEMA) as functional monomer, and ethylene glycol dimethacrylate (EDMA) as cross-linker. This composition was used in a series of methodical studies, under carefully controlled physical conditions, to determine their effect on polymer performance.[7,12,15] This is a relatively simple system in which the template interacts with two molecules of the neutral monomer HEMA[16] to form at least two hydrogen bonds (Figure 1) which, together with shape complementarity of the imprinted site, will create MIPs capable of chiral recognition. The affinity and specificity of the polymers prepared in these studies were evaluated by HPLC, which provided a quantitative basis for the measurement and analysis of molecular interactions. The separation of two enantiomers is ideally suited for the assessment of the quality of MIPs since it depends on specific interactions taking place in the binding sites created by molecular imprinting. In the present paper we have made an attempt to summarize our findings, combined with supporting evidence provided by other research groups, and to formulate recommendations of the most favorable conditions suitable for the creation of the high-performance imprinted polymers.

The key physical parameters reviewed here include temperature and time of polymerization, the volume of the polymerization mixture, pressure, quantity of initiator, the presence of magnetic field, and the dielectric constants of monomers and solvents. Other parameters such as the type and strength of monomer−template interactions and the role of cross-linker and solvent have been comprehensively discussed elsewhere.[17]

## Temperature

It is generally accepted that a crucial element for the success of the imprinting process is the preservation of the monomer−template complex during the polymerization step. The nature of these equilibria and their effect on the recognition properties of polymers have been modeled by a number of groups,[18−20] and it has also been shown by NMR that observable template−monomer interactions persist, at least during the initial stages of the polymerization.[21,22] The stability of monomer−template complexes, their type, and concentration depend on the polymerization conditions. The formation of complexes is under thermodynamic control, and its energetics can be described by the equation[23]

$$\Delta G_{\mathrm{bind}} = \Delta G_{\mathrm{t+r}} + \Delta G_{\mathrm{r}} + \Delta G_{\mathrm{h}} + \Delta G_{\mathrm{vib}} + \sum \Delta G_{\mathrm{p}} + \Delta G_{\mathrm{conf}} + \Delta G_{\mathrm{vdW}} \tag{1}$$

where the Gibbs free energy changes are: $\Delta G_{\mathrm{bind}}$, complex formation; $\Delta G_{\mathrm{t+r}}$, translational and rotational; $\Delta G_{\mathrm{r}}$, restriction of rotor upon complexation; $\Delta G_{\mathrm{h}}$, hydrophobic interactions; $\Delta G_{\mathrm{vib}}$, residual soft vibrational modes; $\sum \Delta G_{\mathrm{p}}$, sum of interacting polar group contributions; $\Delta G_{\mathrm{conf}}$, adverse conformational changes; and $\Delta G_{\mathrm{vdW}}$, unfavorable van der Waals interactions.

The effect of temperature on monomer−template and polymer−template interactions is not straightforward. Lower temperatures

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should favor complexation due to a reduction in the influence of residual vibrational modes ($\Delta G_{\mathrm{vib}}$) and an increase in the strength of polar interactions ($\sum \Delta G_{\mathrm{p}}$) (eq 1).[23−25] At the same time lower temperatures may increase the adverse contributions of conformational ($\Delta G_{\mathrm{conf}}$) and van der Waals ($\Delta G_{\mathrm{vdW}}$) terms in eq 1.

Analyses of the role of the polymerization temperature on complexation and polymer recognition properties have been performed in several studies.[18,26,27] Thus, Sellegren et al. presented NMR experiments which demonstrated that lowering the polymerization temperature results in an increase in the concentration of the prepolymerization complex.[18] The consequence should be an increase in the number of specific binding sites created during polymerization compared with the same polymer prepared at a higher temperature. O'Shannessy et al.[6] and later Lin et al.[28] compared sets of MIPs, prepared at temperatures ranging from 0 to 60 °C, using different initiators and showed that polymers prepared at low temperature have better specificity compared to those prepared at higher temperature. Other studies have reported similar results;[9,10] however, one study[8] was exceptional in showing a greater imprinting effect for polymers prepared at 70 °C than the equivalent material prepared at 0 °C. This result may be explained by the unusual nature of the system where the template, anthracene, was imprinted in an EDMA polymer without additional functional monomer. Under these circumstances the balance of terms in eq 1 will be different than for more polar or charged templates and monomers, with terms with a significant entropic contribution dominating.

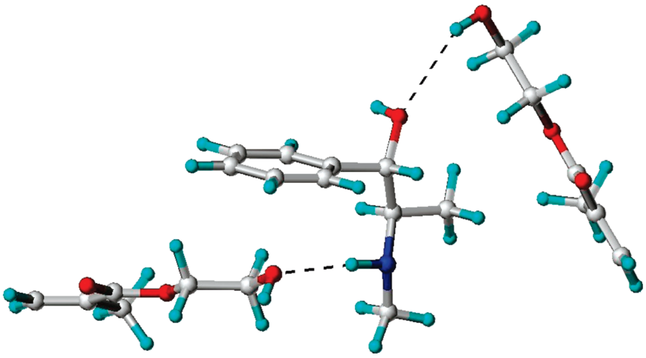

**Figure 1.** Template (−)-ephedrine and possible minimized structure of its complex with 2-hydroxyethyl methacrylate, modeled using SYBYL 7.6.[5]

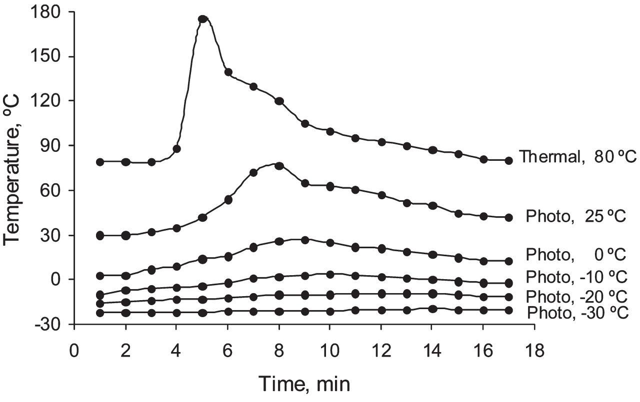

**Figure 2.** Temperature profile of the polymerization reaction. The plots begin 2 min before the start of gelation. Adapted from Piletsky et al.[7]

To analyze thoroughly the impact of polymerization temperature on the affinity and specificity of MIPs, we prepared a set of polymers, imprinted with (−)-ephedrine at six different temperatures which varied from −30 to 80 °C.[7] The temperature within the polymerization mixture was monitored using a thermocouple (Figure 2). As one might expect, the exothermic nature of the polymerization process resulted in recorded (real) temperatures that were significantly higher than the external (initiation) temperatures (Table 1, rows 1−8). The real polymerization temperatures for polymers MIP1 and MIP2, initiated at 80 and 25 °C, reached the relatively high values of 187 and 78 °C, respectively. At lower temperatures the rate of the polymerization reaction was reduced. Polymers prepared at low temperature were homogeneous, gel-like materials with decreased surface areas and lower pore volumes (Table 1, rows 1−8). While many of these polymers show low surface areas in the dry state, as measured by nitrogen porisimetry, it does not detract from their ability to perform in HPLC mode. Indeed, a similar observation was made by Sellergren and Shea,[3] who noted that "a gel-like non-porous polymer performed similarly to a mesoporous polymer".

The exothermic nature of the polymerization reaction has a direct impact on the selection of polymerization conditions suitable for upscaling and manufacture. Thus, for the same relative reactor geometry used in laboratory-scale monolith production, increasing the volume of the monomer mixture results in pronounced increases in the temperature generated within the reaction vessel. The peak temperature reached inside a 1 mL-scale polymerization was 116 °C, for a 50 mL reaction this increased to 176 °C, and just doubling the volume again to 100 mL resulted in a peak temperature as high as 300 °C, which will cause most solvents to boil and significantly increases the risk of pressure explosion. In practical terms, therefore, in the absence of efficient heat exchange the volume of monomer mixture used in MIP-monolith preparation should not exceed 50 mL.

**Table 1. Polymerization Conditions and Properties of the Imprinted Polymers Prepared at Different Temperatures[7] and Pressures[15]**

| polymer | $T_{\mathrm{init}}$, °C | $T_{\mathrm{max}}$, °C | BET surface area, $m^2\ g^{-1}$ | total pore volume, $cm^3\ g^{-1}$ | average pore diameter, nm | density, $g\ mL^{-1}$ |
|---|---|---|---|---|---|---|
| MIP1 | +80 | +187 | 96.2 | 0.22 | 8.3 | |
| MIP2 | +25 | +78 | 15.8 | 0.028 | 6.9 | |
| MIP3 | 0 | +27 | 1.9 | 0.004 | 7.2 | |
| MIP4 | −10 | +4 | 1.4 | 0.004 | 11.2 | |
| MIP5 | −20 | −10 | 0.9 | 0.002 | 10.8 | |
| MIP6 | −30 | −19 | 1.5 | 0.004 | 10.0 | |
| Blank1 | +80 | +190 | 91.3 | 0.26 | 11.3 | |
| Blank6 | −30 | −20 | 2.8 | 0.01 | 12.5 | |
| MIP(1 bar) | 0 | +66 | 1.67 | 0.0027 | 1.9 | 0.58 |
| MIP(10 bar) | 0 | +127 | 0.18 | 0.0008 | 8.9 | 0.62 |

Chromatographic evaluation of the performance of polymers at different temperatures was performed at 8 column temperatures: −10, 0, 10, 20, 30, 40, 50, and 55 °C. As expected, for each column temperature, capacity factors ($k'$) and separation factors ($\alpha$) both increased with decreases in polymerization temperature. The effect on $k'$ was more pronounced for the template, (−)-ephedrine, than for its enantiomer, (+)-ephedrine, which resulted in a large increase in the highest observed separation factor: from $\alpha = 1.53$ for MIP1 prepared at +80 °C to $\alpha = 4.04$ for MIP5 prepared at −20 °C.

Based on the chromatographic data, Van't Hoff plots[29] were built for the MIPs (Figure 3).[7] The major change in the slopes of Van't Hoff plots observed at 20−30 °C is thought to be associated with a change in the mechanism of binding, from exothermic to endothermic, at this temperature.

In contrast to the general belief, polymers prepared at high temperature performed better at high temperature and polymers prepared at lower temperature performed better at low temperatures. The present results indicate that polymerization temperature, and the optimum temperature used in rebinding experiment, are related, both playing a crucial role in the performance of the synthesized materials. The affinity and specificity of polymers in general could be significantly improved by decreasing the polymerization temperature. Liu et al.[30] have shown that redox initiation of methacrylic acid-based (−)-ephedrine-imprinted polymers gave superior results in terms of polymer affinity to photopolymerized material. The redox-initiated polymers, which also exhibited a higher surface area than their photoinitiated counterparts, showed a more even cure, and the rate of polymerization could be controlled by the concentrations of the initiator components. While this initiation system is not so well studied in MIP synthesis, it may be a viable alternative to photopolymerization to make high-performance MIPs.

## Pressure

There have been very few attempts to analyze the role of pressure on MIP performance. Sellergren et al. studied MIPs for two templates: atrazine and ametryn, made at 1 and 1000 bar, in three different solvents.[11] The authors noticed that MIPs made at high pressure had higher affinities compared with the corresponding polymers prepared at ambient pressure. The enhanced binding was explained by the positive effect of high pressure on intramolecular associations in the monomer mixture, which obey eq 2:

$$\left[\frac{\mathrm{d}RT\ln K}{\mathrm{d}P}\right]T = -\Delta V^0 \tag{2}$$

where $K$ is the association constant of the complex, $R$ the gas constant, $T$ the absolute temperature, $P$ the pressure, and $\Delta V^0$ the

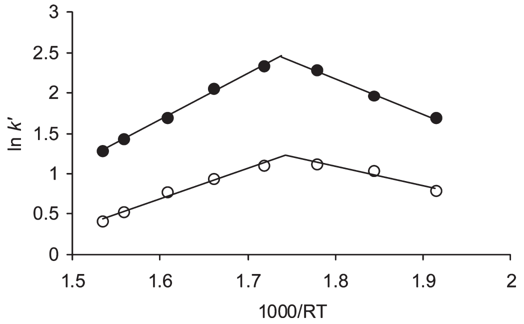

**Figure 3.** Van't Hoff plots of the capacity factors ($k'$) for 48.5 nmol of ephedrine enantiomers on MIP synthesized at −30 °C. Flow rate: 1 mL min$^{-1}$; mobile phase: 0.05% HMDA in chloroform; (●) (−)-ephedrine; (○) (+)-ephedrine. Adapted from Piletsky et al.[7]

difference in reaction volume. The summary calculations made in this paper[11] predict that an increase in pressure by 1000 bar will increase the capacity factor by a factor of 1.1−2.2.

In addition to the influence on intramolecular associations, pressure also affects the kinetics of the polymerization reaction. The influence of temperature and pressure on the rate coefficients of free-radical polymerization can be described by eq 3:

$$\frac{d(\ln k_p)}{dP} = \frac{\Delta V^{\#}}{RT} \qquad (3)$$

Here $k_p$ is the propagation rate coefficient and $\Delta V^{\#}$ is the change in activation volume.[31] Beuermann and Buback have performed an extensive analysis of the propagation rate coefficients for different polymeric systems.[32] The conclusion drawn from this work was that for acrylates and methacrylates an increase in pressure by 1000 bar should increase the polymerization rate by a factor less than 1.1. A change of a similar magnitude can also be produced by an increase in the polymerization temperature of 30 K.

From these calculations it was clear that the association behavior, kinetics of the polymerization reaction, or polymer morphology should not be greatly affected by small changes in pressure with a magnitude below 100 bar (normally generated during bulk polymerization in closed vessels). Practical testing in our hands however provided evidence that this is not always the case, since the affinity of a polymer prepared at 10 bar was substantially higher that polymer prepared at ambient pressure.[15] The morphology of polymers synthesized at different pressures also varied (Table 1, rows 9 and 10). This difference appeared to be related to the temperature generated during polymerization. The temperature developed during the exothermic polymerization reaction at ambient pressure (66 °C) was above the boiling temperature for chloroform (solvent used in this polymerization mixture). In contrast, the temperature developed at 10 bar (127 °C) was comfortably below the boiling point of chloroform at this pressure. It has been suggested that boiling of the solvent affected the polymerization kinetics, overall polymerization rate, and final degree of conversion due to variations in the initiation, chain growth, and termination rates.[33,15] This confirms that the pressure within the polymerization vessel should be analyzed and accounted for when considering quality control in MIP synthesis.

## Polymerization Time

It has been accepted that, irrespective of the initiation protocol, prolonging the polymerization time should lead to greater degree of conversion in the polymerization reaction with fewer unpolymerized double bonds remaining in the polymer. Polymers formed over longer reaction periods would therefore be more rigid. More rigid polymers should consequently contain imprinting cavities of a better defined shape, resulting in MIPs with higher specificity. On other hand, more rigid polymers may suffer from hindered mass transfer and slow binding kinetics. The particular balance of rigidity of imprinting cavities versus polymer flexibility and adaptability (analogous to induced fit in natural receptors and enzymes) remains poorly understood.

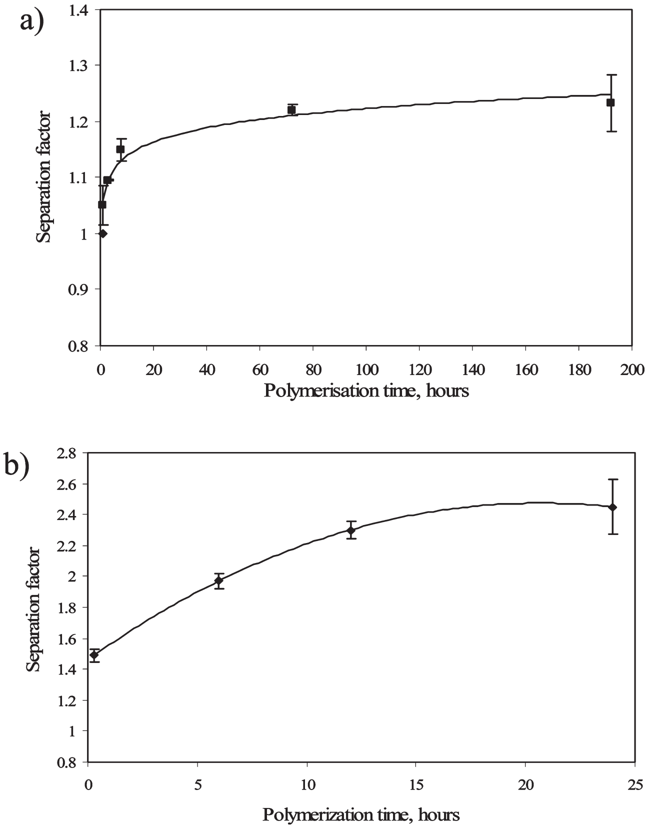

**Figure 4.** Influence of polymerization time on the separation factor (α) for polymers prepared using thermal polymerization at 80 °C (a) and UV polymerization (b) and measured at 20 °C. Flow rate: 1 mL min$^{-1}$; mobile phase: 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in a 40 μL injection volume.[15]

To analyze the effect of polymerization time on the affinity and specificity of MIPs, three sets of polymers were imprinted with (−)-ephedrine, either thermally and using photoinitiation.[34] Each set was prepared using different reaction or exposure times, which varied from 20 min to 7 days. As before, the real polymerization temperature was significantly higher than that used for initiation due to the exothermic nature of the process.

The performance of polymers was analyzed in chromatographic experiments. Apparently an increase in polymerization time increased polymer selectivity, with separation factors (α) reaching saturation after 72 h for thermally polymerized MIPs and 20−25 h for MIPs polymerized by UV irradiation. The separation factors were significantly higher for the photopolymerized materials (Figure 4).[34]

This tendency could be explained by the chemical properties of the initiator 1,1′-azo-bis(cyclohexanecarbonitrile). The photodecomposition of azo compounds can be substantially faster than decomposition induced thermally 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 photoinitiation with a medium-pressure Hg lamp at 25 °C, the half-life ($t_{1/2}$) of azo-bis(cyanovaleric acid) is ≈140 min.[35] At 80 °C the thermal decomposition of a similar compound, 1,1′-azo-bis(cyclohexanecarbonitrile) is more than 10 times slower ($t_{1/2}$ = 1777 min).[36] Thermal decomposition, however, will be significantly faster at higher polymerization temperatures, and above 130 °C the half-life of this initiator would not exceed 1−2 min.

It would be reasonable to assume that in thermal initiation after an initial reaction period of 15−20 min most of the initiator in the monomer mixture is decomposed, and conversion of double bonds in the polymer will be close to completion. In the case of UV initiation the polymerization continues for longer, albeit more slowly, such that ≈10% more double bonds become saturated during the following 24 h of irradiation.

The level of cross-linking in polymers synthesized by UV initiation was much higher than for the polymer prepared using thermal initiation, which indicated that the latter initiation protocol is less efficient than UV. An increase in MIP selectivity upon an increase in polymerization time seemed to be directly related to the increased level of cross-linking and to increases in polymer rigidity.[34,37]

This confirmed that the polymerization time plays an important role in determining the morphology and performance of the synthesized materials. Increased polymerization time increases the rigidity of the polymer structure and facilitates the formation of imprinted cavities with better defined shapes.

### Quantity of Initiator

Polymers formed with higher percentages of initiator would in theory be more rigid, which should ensure that imprinting cavities with better defined shape and higher specificity would be formed. On the other hand, lower amounts of initiator would decrease the peak temperature reached inside the polymerization mixture, which is crucial for the formation of good quality imprinting cavities. In order to assess the impact that the type and quantity of the initiator have on the recognition properties of MIPs, a collection of polymers were imprinted with (−)-ephedrine using varying amount of two different initiators: 1,1′-azo-bis(cyclohexane-1-carbonitrile) (ACC) and 2,2-dimethoxy-2-phenylacetophenone (DMPA).[37] Interestingly, the type of initiator has relatively little impact on polymer performance compared with other factors, in particular temperature, type of initiation, and polymerization time. A chemometric approach was used to relate several parameters such as the concentration of initiator and the polymerization time with the separation factors of the synthesized polymers (Figure 5). It was found that the most rigid materials prepared, using high concentrations of initiator and long polymerization times, were less selective than less rigid MIPs polymerized using low concentrations of initiator. Apparently the main factor determining the quality of polymers was once again the temperature reached during polymerization. High temperatures produced with large quantities of initiator outweighed (in negative terms) the positive impact of polymer rigidity. The general conclusion made from this work is that in order to achieve the best performance in enantioseparation (and possibly other applications as well), MIPs should be synthesized, where possible, for long polymerization times using low concentrations of initiator and at low temperatures.

### Magnetic Field

Polymerization reactions are affected by the presence of a magnetic field.[38−40] Magnetic fields (MF) tend to favor singlet−triplet transitions of radical pairs. For energetic reasons radicals in the triplet state are less prone to geminate recombination. As a result, initiation efficiency, reaction rate, and yield are improved due to the increased lifetimes of radicals and a reduction of the cage effect. The "catalytic" effect of MFs can also arise from distortions of the molecules, increased interactions, and modification of bond angles. Other MF effects include magnetic orientation and concentration, e.g., alignment of the molecules and polymer chains along magnetic lines of force. Polymers obtained within a MF exhibit improved thermal stability and reduced swelling due to their ordered structures.[38,39]

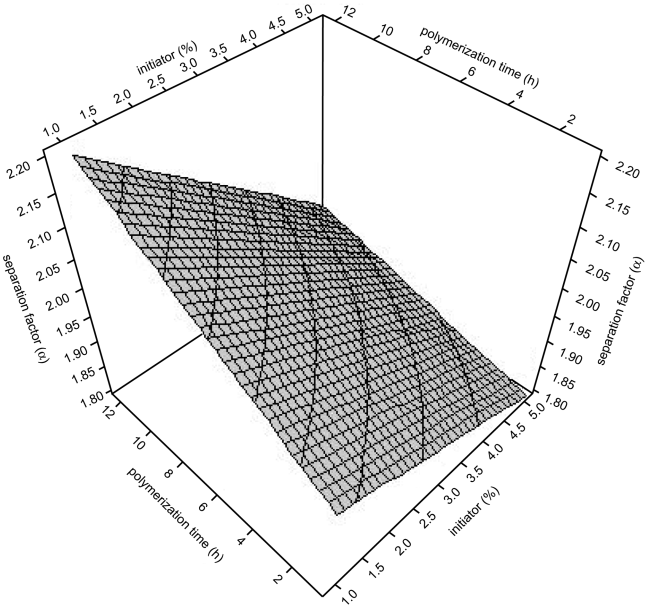

**Figure 5.** Influence of the polymerization time and concentration of initiator (1,1′-azo-bis(cyclohexane-1-carbonitrile)) on the separation factor (α) for polymers prepared using UV polymerization and measured at 20 °C. Flow rate: 1 mL min$^{-1}$; mobile phase: 0.05% HMDA in chloroform. Injection amounts were 8 μg (48.5 nmol) in a 40 μL injection volume. Adapted from Mijangos et al.[37]

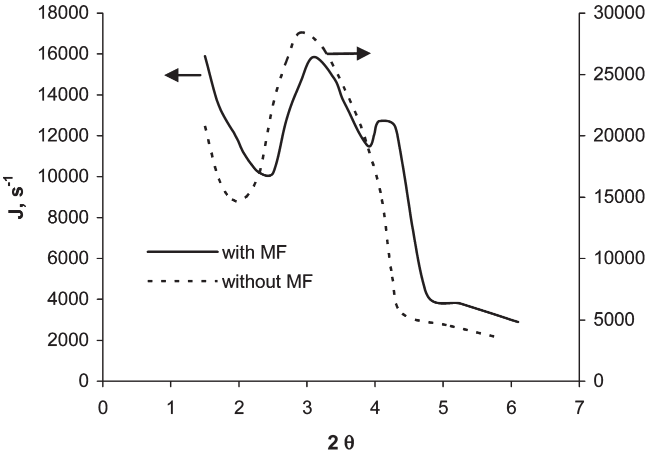

**Figure 6.** Scattering intensity $J$ as a function of the diffraction angle Θ, obtained with Cu Kα radiation, for polymer prepared in the presence of magnetic field (solid line) and in the absence of magnetic field (dotted line). Measurements were made in a direction perpendicular to the magnetic field force lines in the specimens. Adapted from Guerreiro et al.[41]

It was expected that a magnetic field applied during the synthesis of MIPs will impose a higher degree of order on the polymer structure and improve the uniformity of the binding site population. To prove this, we synthesized a set of polymers imprinted with (−)-ephedrine.[41] The polymerization process was performed in the absence and in the presence of a constant magnetic field of high intensity (up to 1.55 T). The resulting polymers were characterized by HPLC, FTIR, X-ray diffraction,

**Table 2. Physical and Recognition Properties of MIPs Prepared with and without Magnetic Field**

| polymer | swelling ratio | mercury porosimetry surface area, $m^2\ g^{-1}$ | total porosity, % | density,[a] $g\ cm^3$ | separation factor (α) | asymmetry factor[b] |
|---|---|---|---|---|---|---|
| with MF | 1.41 | 0.1628 | 18.45 | 0.61 | 2.31 | 1.17 |
| without MF | 1.83 | 0.1607 | 24.09 | 0.57 | 1.65 | 2.92 |

[a] Apparent density of powdered polymer. [b] Peak asymmetry factor is the ratio between the difference in retention volume from the peak maximum to the trailing edge of the peak and the difference in retention volume from the leading edge of the peak to the peak maximum, both measured at 10% of peak height.[42]

and analysis of their surface areas, porosities, and swelling properties.

Figure 6 depicts the X-ray scattering intensity as a function of the diffraction angle, Θ, determined in the direction perpendicular to the magnetic field force lines in the specimens. In the direction perpendicular to the force lines there are two crystal maxima with $d_1 = 2.85 \pm 0.25$ nm and $d_2 = 2.15 \pm 0.25$ nm, corresponding to the repeat distance along the packed polymer chains. The structure formed in the presence of a MF differs from that generated in its absence (Figure 6b). The results of this study indicated that the magnetic field induced a certain degree of orientation in the polymer. Orientation is caused by the anisotropic effect of the magnetic field on the magnetically susceptible macromolecular radicals and on whole polymeric domains during phase separation.

The normalized area for the C═C peak at 1637 $cm^{-1}$ in the FT-IR spectrum was 0.53 for polymer synthesized in a magnetic field and 0.59 for the material synthesized in the absence of magnetic field. This confirmed that fewer unreacted double bonds remain when a MF was applied during polymerization. Because of the effect of the MF on macromolecular chains, termination by recombination is favored over disproportionation.[39] This allows for a more efficient use of the radicals generated and leads to higher levels of cross-linking. Together with the ordered structure, these differences affected polymer morphology and produced more rigid materials. The polymers synthesized in a MF had lower swelling ratios and lower surface areas, while being denser (Table 2).

There was also a clear effect of the MF on the performance of the imprinted polymers in HPLC. Separation factors were substantially higher and the shape of the peaks was also improved, with an asymmetry factor of 1.17 as compared to 2.92. The improvement in asymmetry factor by a factor of 2.5, as a result of an applied MF, indicates a fundamental change which could improve the chances for realizing practical applications of MIPs. This improvement can be attributed to the creation of more ordered and rigid structures within the polymers as a result of the application of a MF. MIPs synthesized in MF also exhibited higher affinity for the template with a dissociation constant, $K_{\text{diss}}$, of 1.6 mM, compared to 2.14 mM for the MIP prepared in the absence of a MF. The equivalent figure for the nonimprinted polymer synthesized in the MF was $K_{\text{diss}} = 8.95$ mM.

It has been shown that the application of a magnetic field during synthesis plays an important role in determining the morphology and performance of imprinted polymers. A high magnetic field created more rigid and ordered polymers with higher levels of cross-linking. This effect was responsible for a substantial improvement in polymer performance in enantioseparation. Magnetic field can be used in the future as a generic technique to improve the recognition properties of imprinted polymers.

## Dielectric Constant of the Components of Monomer Mixture

The dielectric properties of a monomer mixture will affect the entropy and enthalpy of the monomer−template complexation ($\sum\Delta G_{\text{p}}$ and $\Delta G_{\text{h}}$ in eq 1). Solvent could also improve unfavorable van der Waals interactions ($\Delta G_{\text{vdW}}$) through an effective solvation process. This is particularly important for monomers that form ion pairs with the template. During polymerization a polyelectrolyte is formed where the properties of neighboring groups provide an additional impact on the properties of each separate functional group. In apolar media, represented by solvent, monomers and partially formed oligomeric and polymeric chains, the template ions condense with the corresponding counterions, forming ion pairs. Ion pairs tend to aggregate in multiplets due to dipole−dipole interactions,[43] providing an additional cross-linking effect and accelerating phase separation.[44] Lowering the polarity of the solvent increases significantly the process of conformational transition.[45] In relation to MIPs these effects would suggest that parameters such as the dielectric constants of the monomer mixture and its components would influence the morphology of the synthesized polymer as well as the ionization properties of functional groups. The ionization of monomers by template would have an impact on the distribution of functional groups and phase separation during the early stage of polymer formation.

**Table 3. Influence of Solvent Polarity on the Quantity (Quantity of Groups Available for Titration) and Quality of Carboxylic Groups in Acrylic Acid Polymers (Adapted from Piletska et al.[46])**

| | | acrylic acid polymer | |
|---|---|---|---|
| solvent | solvent dielectric constant | groups, $10^{-5}$ mol $g^{-1}$ | $pK_a$ polymer |
| DMF | 38.3 | 39.3 ± 0.6 | 6.6 ± 0.1 |
| ethanol | 25.0 | 115 ± 5.2 | 7.9 ± 0.1 |
| chloroform | 4.8 | 159 ± 4.4 | 7.8 ± 0.1 |
| toluene | 2.4 | 189 ± 7.7 | 7.0 ± 0.1 |

To study these effects, polymers imprinted with (−)-ephedrine were prepared using 10 popular functional monomers in solvents with different polarity.[46] An extensive potentiometric titration of the corresponding imprinted and blank polymers was conducted in order to evaluate the effective p*K* of functional groups of functional monomers in the polymer environment and determine the level of inclusion of monomers into the final polymers. The inclusion and availability of functional monomers appeared to be connected to the dielectric constant of the solvent used for polymer preparation (Table 3). Polymers made with hydrophobic solvents such as chloroform and toluene revealed a notable increase in the amount of ionizable groups, which demonstrates a better inclusion of functional monomer. The mechanism of this effect could be related to phase separation. Polar functional monomers can be concentrated either inside or outside growing polymeric globules. If the solvent is more polar than the cross-linker, then polar and ionized monomer molecules would tend to concentrate outside the polymer globules. If the solvent is less polar, then they will be repelled into the interior of the polymer as it phase separates. Another effect that can account for the increased inclusion of acidic monomers is the formation of carboxylic acid dimers via hydrogen bonding, which is favored in solvents of low polarity. These dimers can also be formed between monomer and carboxylic groups already included in the polymer chains,[47,48] increasing the local concentration of monomers, with a consequent increase in the reaction rate and inclusion of these molecules in the growing polymer. In this way reasonably small variations in solvent polarity could have a dramatic effect on the inclusion of monomers and consequently on polymer performance. Titration can also be used to infer information about the ordering of carboxyl groups in the presence or absence of template; thus, Sellergren and Shea[49] related differences in the titration behavior of MIP and blank to the charge density on the polymer, which resulted in a lower apparent $pK_a$ for the MIP. This subtle effect was ascribed to the effect of template (phenylalanine anilide) on the distribution of methacrylic acid residues.

Apart from a significant increase in the amount of monomer available for the interaction with the template, reducing the dielectric constant of the solvent increases polymer specificity and affinity.[46]

The practical conclusion that can be drawn from this part of the work is that in order to enhance the performance of imprinted polymers that rely on ionic interactions, the dielectric constant of the solvent used for polymer preparation should be lower than the dielectric constant of the cross-linker.

## Conclusion

In summary, it has been confirmed that polymerization temperature affects the affinity and specificity of MIPs, both of which could be significantly improved by selecting a lower polymerization temperature. The optimum temperature with respect to polymer performance depends on the temperature used in polymer preparation. Thus, polymers synthesized at high temperature will perform better at higher temperature and similarly polymers prepared at low temperature perform better at low temperature. Because of the exothermic nature of free radical polymerization, the practical limit for bulk polymerization is around 50 mL. The reaction volume should not exceed this figure.

It was found that photoinitiated polymerization produced polymers with higher degrees of cross-linking and greater specificity, which is also related to the lower temperature achieved during polymerization.

Moderate applied pressure affects the performance of the polymer indirectly through the prevention of solvent boiling in the monomer mixture. Polymerization at higher pressure generates more rigid polymers with better defined shapes of imprinting cavities and therefore higher specificity.

Increasing the polymerization time leads to more rigid polymers and consequently improves the specificity of imprinted materials.

The specificity of the polymer could also be improved by the introduction of a magnetic field. The effect of the magnetic field is related to its influence on the initiation efficiency, reaction rate, and yield of the polymerization reaction, brought about by extending the lifetime of radicals in the system. The orientational effect of the magnetic field on the molecules and polymeric chains in the polymerization mixture is evident in the MIPs which have more uniform binding sites with enhanced affinity and specificity.

In order to enhance the performance of imprinted polymers that rely on ionic interactions, the dielectric constant of the solvent used for polymer preparation should be lower than the dielectric constant of the cross-linker.

Of all the parameters listed, the polymerization temperature and polymerization rate are the most important to control. These factors are primarily influenced by the nature of initiation and initiator concentration; however, other polymer properties, in particular morphological factors such as porosity, are also affected. The effect of other parameters (for example pressure) can also be linked to temperature effects, emphasizing that temperature control is the most important influence over polymer properties. In selecting the best conditions for preparing materials suited to a particular application, some compromise may have to be made between performance and the desired property. If it becomes necessary to optimize a material for a critical application where the effects of polymerization parameters (such as temperature) work in opposing directions (for example, where a high surface area is essential as well good affinity), a more systematic approach, such as a chemometric-based experimental design,[37] would be recommended.

Finally, it should be noted that the polymerization reaction is very complex process which could be affected by many physical factors. To produce high-performance MIPs, all factors should be thoroughly considered and controlled appropriately.

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