

Influence of Continuous Magnetic Field on the Performance of Molecularly Imprinted Polymers

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Abstract

A set of polymers was imprinted with (-)-ephedrine using UV initiation, under the influence of a constant external magnetic with intensities ranging from 0 to 1.55T. It was shown that polymer morphology and recognition properties are affected by the magnetic field leading to considerable improvements in the performance of newly synthesised materials. Apparently the magnetic field improved the ordering of the polymer structure and facilitated the formation of more uniform imprinting sites.

Introduction

The effect of magnetic field (MF) on the physical and chemical properties of polymers has been under intense investigation (Chiriac and Simionescu, 2000; Chiriac et al., 2000). Polymers synthesised in the presence of a magnetic field often exhibit different properties and polymerisation kinetics when compared to polymers synthesised by traditional procedures (Chiriac et al., 2000). Even weak MF influences chemical kinetics and changes the activation energy and entropy for a chemical

reaction, especially for polar compounds with high diamagnetic susceptibility (Chiriac and Simionescu, 2000; Dibiendu and Maiti, 1998; Vedeneev et al., 1990). Magnetic field effects on the rate of reactions are observed if electronic states with different magnetic properties are involved in a rate determining step. These include singlet and triplet states of initiator radicals (Chiriac and Simionescu, 2000; Chiriac et al., 2000). Magnetic field tends to favour singlet-triplet transitions on the radical pairs and due to 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 longer lifetime of radicals and a reduction of the cage effect (Chiriac and Simionescu, 2000; Chiriac et al., 2000). The catalytic effect of the MF can also be due to distortions of the molecules, increased interactions and modification of angles between bonds (Chiriac and Simionescu, 2000; Chiriac et al., 2000).

Other MF effects include magnetic orientation and concentration, e.g. aligning the molecules and polymer chains. Polymers obtained within a MF present improved thermal stability and lower swelling due to their ordered structure (Chiriac and Simionescu, 2000; Chiriac et al., 2000). For this reason a MF has previously been used to prepare epoxy resins with enhanced properties and to align liquid crystals (Benicewicz et al., 1998; Brostow et al., 1999; Shimoda et al., 1997; Wang and Huang, 2000). Other uses include the preparation of macromolecular compounds with very low conductivity, high transparencies, low elasticity moduli, chemically tuneable properties and good processability (Chiriac et al., 2000).

Here we present the first attempt to apply a magnetic field to improve the performance of molecularly imprinted polymers (MIPs). We have seen from our previous work that physical factors which affect the polymerisation process, such as temperature, pressure and polymerisation time affect both the morphology of

polymers and their recognition properties (Piletsky et al., 2002; Piletsky et al., 2004; Piletsky et al., 2005). In general the decrease in system entropy leads to the improvement in polymer recognition properties. It was expected that an applied MF would enhance the ordering of the structure of synthesised polymer and improve the uniformity of the population of its binding sites.

The model system used to study the influence of a MF on MIPs included a set of hydroxyethyl methacrylate (HEMA) based polymers imprinted with (-)-ephedrine. The polymerisation process was performed in the presence and in the absence of constant magnetic field of different intensities and the resulting polymers were characterised by X-ray diffraction, FTIR, HPLC, surface, porosity and swelling analysis.

Materials and methods

Chemicals. (1*R*,2*S*)-Ephedrine [(-)-ephedrine] and (2*R*,1*S*)-ephedrine [(+)-ephedrine], ethylene glycol dimethacrylate, 2-hydroxyethyl methacrylate, 1,1'-azobis(cyclohexanecarbonitrile), hexamethylenediamine (HMDA) and chloroform were purchased from Aldrich, UK. All chemicals and solvents were of analytical or HPLC grade and used without further purification.

Preparation of molecularly imprinted polymers. Polymer composition was reported elsewhere¹¹. 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 20 mL glass tube, purged with nitrogen for 5 min and sealed. The glass tube with polymerisation mixture was then placed in a homogeneous magnetic field with intensities ranging from 0 to 1.55T at 20 mm from

the electromagnet poles. The electromagnet used consisted of two poles embedded in two magnetic coils, cooled by water and produced by Tochelectropribor, Ukraine. The magnetic field was stabilised by a current source with a relative instability of 0.05% and polymerised under UV radiation for 15 minutes with a mercury quartz lamp of ultrahigh pressure with 120 W, (SVD, Russia). Blank polymer was prepared in the same way but in the absence of template. The bulk polymers were ground in methanol and wet-sieved through 106-25 μm sieves (Endecotts, UK) and sedimented in methanol to remove fines. The polymers were additionally washed with chloroform containing 0.05% HMDA. Spectrophotometric analyses 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 dried under vacuum and used to pack HPLC columns.

Surface area. The determinations of specific surface areas were performed using a NOVA 1000 E Series Gas Sorption Analyser (Quantachrome), based on the nitrogen BET. All reported data represent the results of 3-5 concordant experiments, with standard deviation below 5%.

X-ray diffractometry. The semi-transparent polymer was studied by X-ray diffractometry. X-ray diffractometry was performed using a DRON-3M (Burevestnik, Russia), with Cu K_α radiation. The x-ray scattering intensity as a function of the diffraction angle Θ was determined in directions parallel and perpendicular to the magnetic field force lines in the specimens. Peaks were calculated according to the formula:

$$2d \sin\Theta = n\lambda \quad (1)$$

where d is the interplane distance, Θ the angle of scattering and λ the wavelength (0.154 nm for Cu K_α). Mean orientation factors (k) were calculated as the ratio

between intensity of crystalline maximums for samples treated in magnetic field and untreated analogues. All reported crystallographic data represent the results of 3-5 concordant experiments, with standard deviation below 5%.

HPLC analyses. For the analysis of MIP recognition properties, the polymer particles were suspended in methanol and packed in stainless steel HPLC columns (10 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 Agilent HPLC system (USA). 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 20 µl of a 1 mg mL⁻¹ sample. The retention of ephedrine enantiomers was examined at 20 °C. Acetone was employed as a void marker. Capacity factors (*K'*) were determined from

$$K' = (t - t_0)/t_0 \quad (2)$$

where *t* is the retention time of a given species and *t*₀ is the retention time of the void marker (acetone). Effective enantioseparation factors (*α*) were calculated from the relationship:

$$\alpha = K'(+)/K'(-) \quad (3)$$

where *K'*(+) and *K'*(-) are the capacity factors of the (+)- and (-)-ephedrine, respectively. All reported chromatographic data represent the results of 3-5 concordant experiments.

Frontal chromatography. Varying concentrations of (-)-ephedrine (*A*₀ = 0.025-16 mM) were injected onto a 100×4.6 mm HPLC column packed with polymer. The eluent was 0.05% hexamethylenediamine in chloroform at a constant flow of 1 mL min⁻¹. The results were plotted as follows: 1/{[*A*₀]/(*V*-*V*₀)} versus

$1/[A_0]$, where V and V_0 are the elution volume of (-)-ephedrine and the void marker (acetone). The number of binding sites was calculated from the intercept on the ordinate ($1/B_t$) and the dissociation constant from the intercept on the ordinate ($-1/K_{diss}$)¹⁹. All reported frontal chromatography data represent the results of 3-5 concordant experiments. The standard deviation of the measurements was below 5%.

IR Spectroscopy. The FT-IR spectra were recorded using Thermo Nicolet Avatar 370 FT-IR (USA) in absorbance mode between 1630 and 1680 cm^{-1} , and the net area was normalized against a net reference with integration limits between 990 and 920 cm^{-1} (Piletsky et al., 2005).

Swelling Analysis. Swelling experiments were performed as described previously (Piletsky et al., 2002). Three hundred milligrams of the polymer particles with the mesh size 25-106 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:

$$\text{Sr} = (m_s - m_0) / m_0 \quad (4)$$

Where m_s is the mass of the swollen polymer and m_0 is the mass of dry polymer.

Results and discussion

To analyse the effect of a continuous external magnetic field on the performance of imprinted polymers, monomer mixture was placed between two electromagnets and polymerised with UV radiation under seven different magnetic field intensities (0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.55 T) for 15 minutes. It should be noted

however that such short polymerisation time is not ideal for the preparation of MIPs (Piletsky et al., 2005), but due to technical limitations it was difficult to sustain high magnetic field intensities for a longer period of time. The results are only intended to be a demonstration of the influence of continuous magnetic field applied during the polymerisation of MIPs. The effect of MF on the fine structure of the polymer was analysed by X-ray diffractometry. The X-ray scattering of polymer samples as a function of the diffraction angle Θ was determined in directions parallel and perpendicular to the magnetic field force lines in the specimens. Figure 1 represents the x-ray scattering intensity as a function of the diffraction angle Θ determined in directions perpendicular and parallel to the magnetic field force lines in the specimens (MIP 1.55T). In the direction perpendicular to the force lines there are two crystal maxima with $d_1 = 2,85$ nm and $d_2 = 2,15$ nm (Figure 1a). At larger angles it is possible to see a large feebly marked amorphous halo, characteristic for this type of polymer (Figure 1b). The diffraction curve of the same samples, received in a direction parallel to the direction of magnetic field force lines in the specimens, shows the same maxima are poorly distinguished from the background (Figure 1c). The diffraction curve of a sample, polymerised in the absence of magnetic field has only one crystal reflex with $d = 2,85$ nm and a weak shoulder conforming to the second reflex (Figure 2). The diffraction curves for both MIP and blank polymers were practically identical (data not shown).

Although it is difficult to assign these peaks precisely to the corresponding structural elements, it is believed that these maximums correspond to the repetition distance along the packed polymer chains (Plate et al., 1980). The results indicate that the magnetic field induced a certain degree of orientation in the polymer, and materials prepared at higher MF intensities possess a higher mean orientation factor

(Figure 3). Orientation in the magnetic field is caused by the anisotropy of a diamagnetic susceptibility of the growing macromolecules and crystallites. As a consequence, the structure formed in the presence of a MF differs from the one generated in its absence.

As previously mentioned, the MF can also exert a catalytic effect on polymerisation reactions (Benicewitz et al., 1998). Then, it would be reasonable to assume that with increased MF intensity, less pendant vinyl groups remained on the polymer. This was confirmed by infrared spectroscopy, a technique commonly used for fine quantification of functional groups during chemical reactions (Piletsky et al., 2005; Vernooij et al., 2002; Mosnáček et al., 2004; Duffy et al., 2001; Burcham et al., 2001; Guilde et al., 2001). The degree of cross-linking (normalised area of the C=C stretching peak) for polymer synthesised at 1.55T is approximately 45% smaller than that of polymers synthesised without the influence of MF, Figure 4 and Table 1. This confirms that more double bonds became saturated when a MF is applied during polymerisation. Due to the effect of the MF on macromolecular chains, termination by recombination is favoured instead of disproportionation (Chiriac et al., 2000). This allows a more efficient use of generated radicals and leads to higher level of cross linking. Together with the ordered structure, these differences affected polymer morphology and produced more rigid materials, as confirmed by measuring polymer swelling in chloroform (Table 1). The surface areas for the polymers were calculated by the nitrogen BET (Piletsky et al., 2005). Results indicate a decrease in surface area with increasing MF intensities (Table 1). Polymers prepared under the influence of MF appear to be more homogeneous gel-like materials with low surface area and reduced swelling (Table 1).

To analyse the influence of the MF on MIP recognition properties, the synthesised materials were packed in chromatographic columns and tested using HPLC, as described previously (Piletsky et al., 2002), for their ability to discriminate between (+)- and (-)-ephedrine (template). The result of this testing performed in chloroform with 0.05% HMDA is presented in Table 1.

There was a clear effect of the MF on the performance of the imprinted polymers, with a clear increase in separation factors (α) (see Figure 5 and Table 1). The highest α was obtained when polymer was prepared with a MF of 0.4T. Subsequent increases in MF intensity actually resulted in a decrease of α , although polymers still displayed a separation factor superior to those prepared in the absence of MF. This can be attributed to the “stretching” effect of the MF force lines on the growing polymer chains, which interfered with the tri-dimensional arrangement of monomers around the template. In this way, for materials synthesised at higher MF intensities, this effect can overcome the benefits resulting from the structural ordering induced by the magnetic field. Blank polymers (prepared without template but under the influence of MF) were unable to discriminate between (+) and (-)-ephedrine (data not shown). The shape of the peaks on the HPLC chromatograms was also improved (Figure 6), with an asymmetry factor of $3.00 \pm 1.3\%$ (MIP prepared with a MF of 0.4T) as compared to $6.25 \pm 4.8\%$ of polymer prepared in the absence of MF. Asymmetry factors higher than 1 indicate a tailing peak (Majors and Carr, 2001). The improvement in asymmetry factor by a factor of 2, as a result of an applied MF, indicates a fundamental change which could improve the chances for practical application of the MIPs developed.

This improvement can be attributed to the creation of more ordered and rigid structure of the polymers as a result of the application of a MF. The improvement in

enantioseparation, together with the improvement of peak shape, serve as an indication of the creation in the polymer of a population of more uniform binding sites. The detailed testing of the quantity and quality of accessible binding sites was performed by frontal chromatography (Andersson et al., 1996). For this study we selected the MIP with the higher separation factor (MIP 0.4T) and control polymer synthesised without MF. Template concentrations ranged from 16 mM (90% saturation) down to 0.025 mM. Results indicate a considerable increase in the uniformity of the binding site population for polymer prepared under 0.4T MF, when compared with polymers made at 0T. The Scatchard plot for MIP 0.4T (Figure 7a) indicates that there is an apparent uniform population of high-affinity binding sites (K_{diss} of 67.90 μ M) for a broad range of template concentrations (0.025 to 1 mM). There is also a second population of low affinity binding sites (K_{diss} of 1.79 mM) for template concentrations ranging from 2 to 16 mM. Although the dissociation constant for MIP prepared at 0T is lower (K_{diss} of 43.30 μ M), its linear range is much reduced (0.025 to 0.2 mM), see Figure 7b. In addition there are two other discrete populations of low affinity binding sites (K_{diss} of 0.26 mM for template concentrations ranging from 0.3 to 1 mM) and K_{diss} of 1.07 mM for the concentration range 2 to 16 mM, Figure 7b and 7c. The number of high affinity binding sites (B_h) for the imprinted polymer prepared at 0.4T is 1.7 times higher (8.47 μ Mol/g) than that of MIP 0T (4.96 μ Mol/g). Remaining lower affinity binding sites for MIP 0.4T are 22.66 μ Mol/g; for MIP 0T the numbers are 12.19 μ Mol/g (K_{diss} 0.26 mM) and 1.44 μ Mol/g (K_{diss} 1.07 mM). For blank polymer synthesised under 0.4T MF the K_{diss} and total number of binding sites B_t are, respectively 8.95mM and 0.019mM/g.

Conclusion

We have shown that the magnetic field plays an important role in determining the morphology and performance of synthesised imprinted polymers. A continuous external magnetic field applied during polymerisation created more rigid and ordered polymer with a higher level of cross-linking. This effect was responsible for substantial improvement in polymer performance in enantioseparation. Additionally, the high heterogeneity of the population of binding sites, a common problem affecting MIPs, was also reduced when polymers were prepared under the influence of magnetic field. This technique can be used in the future as a generic method to improve the recognition properties of imprinted polymers.

Acknowledgement

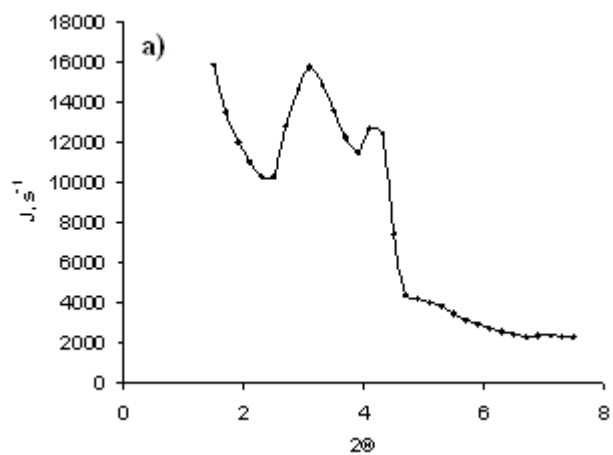
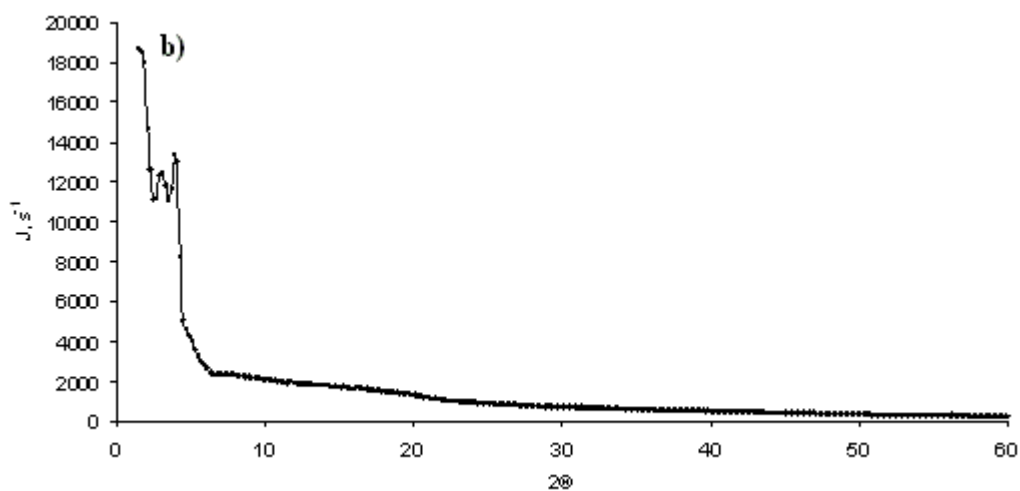
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Caption to figures

Table1. Polymer properties.

Polymer	Polymerisation degree ^a	Swelling ratio ^b	Separation factor ^c	Surface area, m ² g ⁻¹
MIP 0T	0.62± 3.2%	1.80±0.55%	1.29±0.77%	7.22
MIP 0.2T	0.54±1.8%	1.79±1.1%	1.49±6.5%	5.04
MIP 0.4T	0.53±3.7%	1.77±1.7%	1.86±5.3%	1.68
MIP 0.6T	0.52±1.9%	1.76±0.12%	1.63±1.8%	0.81
MIP 0.8T	0.50±2.0%	1.74±1.1%	1.56±0.64%	1.32
MIP 1T	0.48±2.0%	1.71±0.58%	1.57±0.36%	0.61
MIP 1.2T	0.37±2.7%	1.68±0.59%	1.50±4.6%	0.50
MIP 1.55T	0.34±2.9%	1.50±0.66%	1.51±5.2%	0.025

^a Normalized area of the C=C stretching peak. ^b Swelling ratio = amount of solvent adsorbed/dry weight polymer. ^c Separation factor for (-)-ephedrine (template) and (+)-ephedrine.



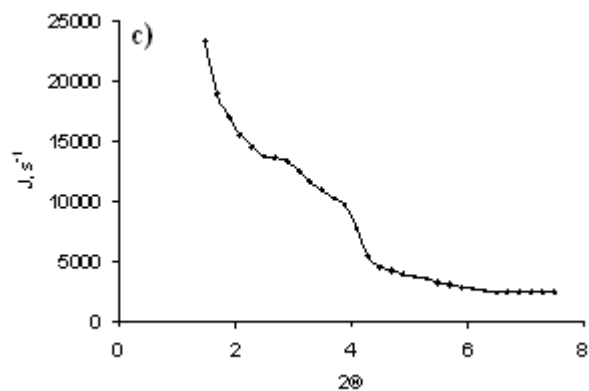


Figure 1. Scattering intensity J as a function of the diffraction angle Θ obtained with $\text{Cu K}\alpha$ radiation of polymer prepared in the presence of magnetic field (1.55T). Measurements were made in a direction perpendicular (a and b) and parallel (c) to the magnetic field force lines in the specimens. The scanning step of the x-ray patterns was 0.2° . The time of impulse collection at each step was 10 s.

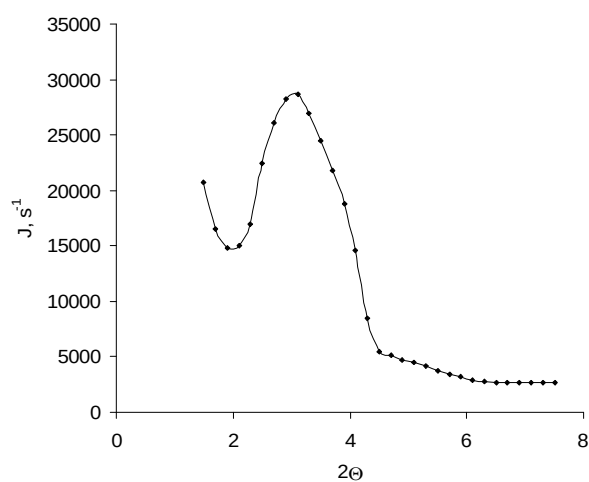


Figure 2. Scattering intensity J as a function of the diffraction angle Θ obtained with $\text{Cu K}\alpha$ radiation of polymer prepared in the absence of magnetic field.

The scanning step of the x-ray patterns was 0.2° . The time of impulse collection at each step was 10 s.

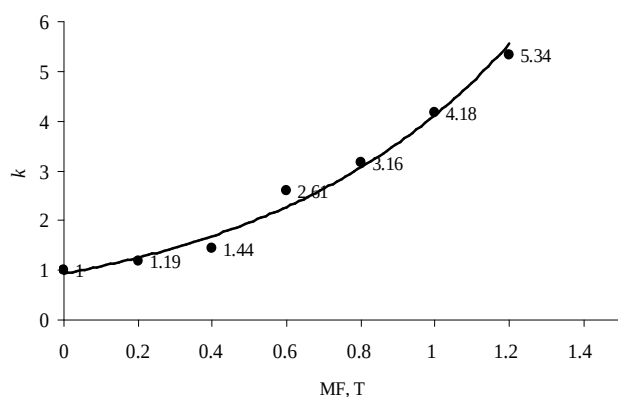


Figure 3. Influence of the magnetic field strength on the mean orientation factor (k) of imprinted polymers. Measurements were made by X-ray scattering and k is the ratio between the intensity of crystalline maximums for polymer samples prepared in the presence and in the absence of MF.

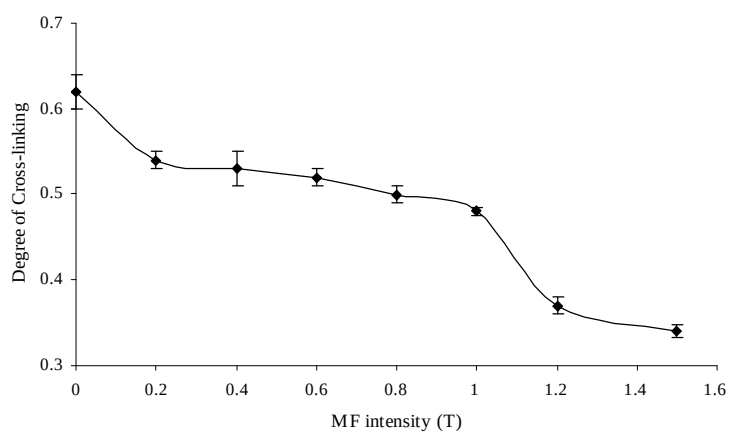


Figure 4. Dependence of the degree of cross-linking (normalized area of the C=C stretching) on the magnetic field applied during polymerisation. Measurements were made by FTIR as described earlier (Piletsky et al., 2005).

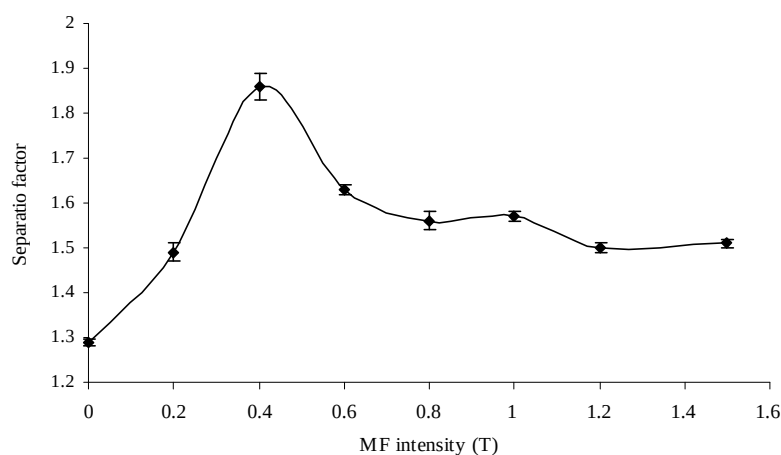


Figure 5. Influence of the MF on the separation factor, measured on HPLC mode at 20 °C. Flow rate 1mL min⁻¹; mobile phase chloroform with 0.05% HMDA. Injection amounts were 20μl of a 1 mg mL⁻¹ sample.

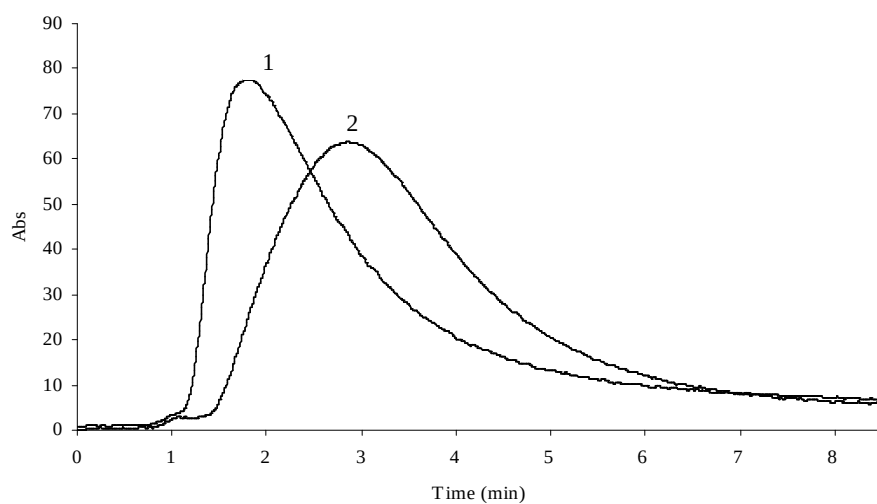


Figure 6. Chromatograms for (-)-ephedrine on MIP prepared without MF (1) and MIP prepared under the influence of a MF of 0.4T (2). Flow rate 1 mL min^{-1} ; mobile phase chloroform with 0.05% HMDA. Injection amounts were $20\mu\text{L}$ of a 1 mg mL^{-1} sample, at $20\text{ }^{\circ}\text{C}$.

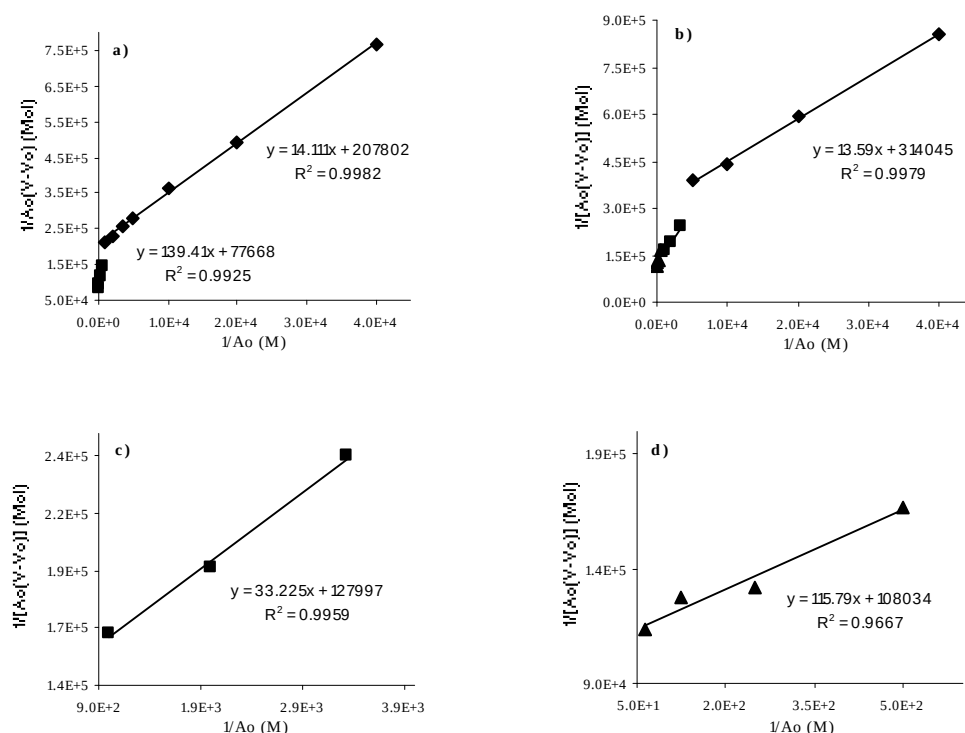


Figure 7. Scatchard plots for imprinted polymers prepared under a magnetic field of 0.4T (a), and 0T (b). Figure c and d represent in more detail the two series corresponding to discrete populations of low affinity binding sites for MIP prepared without MF. These two series are represented at the original scale in figure b. Flow rate = 1 mL min^{-1} , mobile phase = 0.05% HMDA in chloroform, (-)-ephedrine concentrations ranged from 16 to 0.025 mM .

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