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Evaluation of new selective molecularly imprinted polymers prepared by precipitation polymerisation for the extraction of phenylurea herbicides

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Abstract

Three different molecularly imprinted polymers (MIPs) have been prepared by precipitation polymerisation using linuron (LIN) or isoproturon (IPN) (phenylurea herbicides) as templates and methacrylic acid (MAA) or trifluormethacrylic acid (TFMAA) as functional monomers. The ability of the different polymers to selectively rebind not only the template but also other phenylurea herbicides has been evaluated. In parallel, the influence of the different templates and functional monomers used during polymers synthesis on the performance of the obtained MIPs was also studied through different rebinding experiments. The experimental binding isotherms were fitted to the Langmuir–Freundlich isotherm allowing to describe the kind of binding sites present in the imprinted polymers under study. It was concluded that TFMAA-based polymer using IPN as template presents the best properties to be used as a selective sorbent for the extraction of phenylurea herbicides.
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1. Introduction

Molecular imprinting technology has proved to be very attractive for the scientific community, as reflected by the amount of original papers published in this field during the last years (more than 100 papers per year since 1997) [1]. Molecularly imprinted polymers (MIPs) are synthetic materials able to selectively recognize a guest molecule or related compounds and were introduced by Wulff (covalent approach) [2] and Mosbach (non-covalent approach) [3]. Basically, MIPs are prepared by the polymerisation of a suitable monomer and a cross-linker agent in the presence of a template molecule. After polymerisation, the template is removed from the polymeric matrix leaving cavities complementary in size and shape to the template, and thus the resulting MIP is able to specifically rebind this molecule or related compounds from a complex mixture. Therefore, MIPs have been employed in those fields where a certain degree of

selectivity is required such as catalysis [4], solid-phase extraction [5], sensors [6] and chromatography [7,8].

Usually, MIPs are synthesised by bulk polymerisation making necessary the subsequent crushing and sieving of the obtained polymer. This process is tedious, time consuming and the obtained particles show a random shape and size limiting its applicability. During recent years, new polymerisation strategies have been proposed and recently reviewed [8] dealing with the obtainment of imprinted beads in order to improve the analytical performance of MIPs. Within the different new polymerisation strategies, precipitation polymerisation [9–11] seems to be one of the most simple and well-suited methods to obtain spherical particles with the desired characteristics. Basically, this method consists on the polymerisation of the system (monomer, template and cross-linker) in the presence of a larger amount of porogen than that typically used in the bulk polymerisation method. As a result of this more diluted reaction system, the growing polymer chains are unable to occupy the entire volume of the vessel leading to a dispersion of microgel particles in the solvent. Beside this, it has been reported that the capacity,

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affinity constants and homogeneity of binding sites associated to polymers prepared by precipitation polymerisation are clearly improved compared to those present in MIPs obtained by bulk polymerisation [12,13].

The preparation of a methacrylic acid-based imprinted polymer using isoproturon (a phenylurea herbicide) as template by bulk polymerisation and its subsequent evaluation was reported by our group [14]. In that work, it was concluded that the obtained polymer was able to extract simultaneously several phenylurea herbicides. However, the capacity and selectivity of the imprinted polymer was rather low making difficult the final determination of selected compounds at trace concentration level in real environmental samples. Thus, the aim of this work is the evaluation and characterization of three new imprinted polymers prepared by precipitation polymerisation in order to improve the extraction of phenylurea herbicides. In parallel, the suitability of precipitation polymerisation for the obtainment of polymers with improved recognition capabilities will be demonstrated. Finally, the selectivity of the optimum MIP was evaluated by the molecularly imprinted solid-phase extraction (MISPE) of selected herbicides in corn sample extracts.

2. Experimental

2.1. Reagents

Fenuron (FEN), metoxuron (MXN), chlortoluron (CTN), isoproturon (IPN), metobromuron (MBN), linuron (LIN), carbaryl (CAR) and fenitrothion (FTN) were purchased from Dr. Ehrenstofer (Augsburg, Germany) and the corresponding chemical structures are shown in Fig. 1. Stock standard solutions ($1\,g\,l^{-1}$) were prepared in acetonitrile and stored at -22 °C. Methacrylic acid (MAA), 2-(trifluoromethyl)-acrylic acid (TFMAA), ethylene glycol dimethacrylate (EDMA) and 2,2′-azobis-(methylbutyronitrile) (AIMN) were purchased from Sigma Aldrich (Madrid, Spain). All other chemicals used were of analytical reagent grade obtained from Lab-Scan (Dublin, Ireland). EDMA and MAA were purified by distillation under reduced pressure. AIMN was recrystallised from methanol prior to use. All other chemicals were used as received.

2.2. Polymers preparation

Template molecule (LIN or IPN, 1 mmol), functional monomer (MAA or TFMAA, 4 mmol) and 20 ml of dry toluene were placed into a 25 ml round-bottomed flask and the mixture was left in contact for 10 min. Subsequently, EDMA (20 mmol) and AIMN (0.2 mmol) were added. The flask was sealed and the mixture was purged with nitrogen for 15 min. Polymerisation took place in a water bath at 67 °C for 24 h. Finally, the template was removed by Soxhlet extraction with methanol for 16 h. The combinations of template:monomer used, leading to three different polymers,

were: IPN-MAA (MIP1); IPN-TFMAA (MIP2) and LIN-TFMAA (MIP3). The corresponding control polymers (CP-MAA and CP-TFMAA) were prepared as described above but without the addition of template.

2.3. Rebinding experiments

Polymer particles (100 mg) were placed in an empty solid-phase extraction cartridge and, after conditioning with 10 ml of toluene, 1 ml of standard solution of each herbicide independently or a mixture of all of them at concentrations ranging from 0.1 to $500\,\mu g\,ml^{-1}$ was loaded into the cartridge at room temperature. In order to remove phenylureas non-specifically bound to the polymeric matrix, the cartridge was washed with 5×1 ml of toluene. After drying, analytes were quantitatively eluted with 5×1 ml of methanol. The obtained fraction was evaporated to dryness and redissolved in 1 ml of acetonitrile. Analyte concentrations in this solution, representing the amount of analyte bound to the polymer (B) were determined by HPLC–UV as described below. The amount of unbound analyte to the polymer (F) was obtained by subtracting B from that of the initial analyte loaded to the polymer.

2.4. Data analysis

Langmuir–Freundlich (LF) adsorption isotherm was fit to the log plot (log B versus log F) of the experimental adsorption isotherms obtained, according to the rebinding experiments described above. This was accomplished using the solver function in Microsoft Excel by varying the fitting parameters to reach a value of 1 for the coefficient of determination (R^2) as described by Umpleby et al. [15].

2.5. Corn sample preparation

A volume of 40 ml of acetonitrile was added to 10 g of dry corn sample and, after manual shacking during 10 min, the mixture was centrifuged for 30 min. The supernatant was filtered through a 0.45 μm filter, and evaporated to dryness. The dried extract was redissolved in 1 ml of toluene containing a mixture of all the phenylureas used in this study at a concentration level of $1\,mg\,l^{-1}$.

2.6. MISPE of sample extracts

An amount of 100 mg of imprinted polymer (MIP3) were placed onto a solid-phase extraction cartridge and conditioned with 10 ml of toluene. Next, sample extract in toluene was loaded and washed with 5 ml of toluene. Finally, after drying the cartridge, analytes were eluted with 5×1 ml of methanol. This fraction was evaporated to dryness and redissolved in 1 ml of acetonitrile for final analysis by HPLC–UV. The conditioning step used between samples consisted of 5 ml of methanol, 5 ml of acetonitrile and 5 ml of toluene.
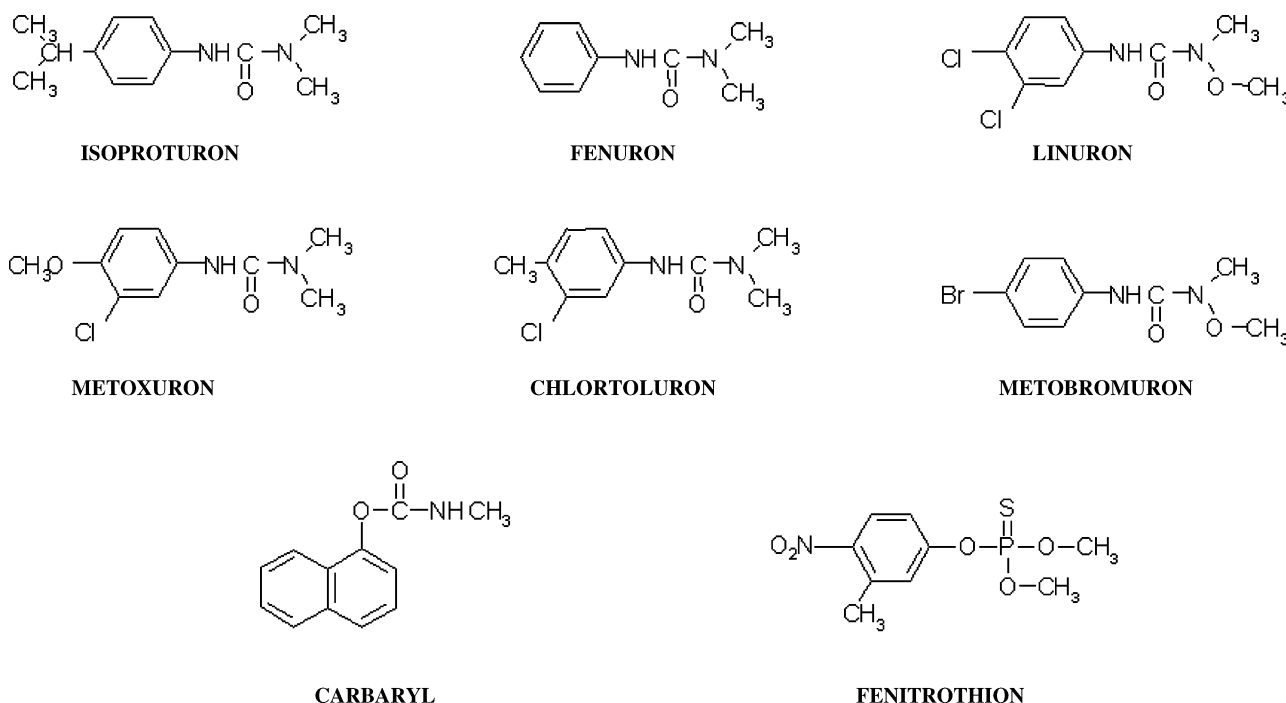
Fig. 1. Chemical structures of selected pesticides.

2.7. Chromatographic analysis

HPLC measurements were made in a Hewlett-Packard 1100 Series HPLC instrument equipped with a quaternary high-pressure pump and a photo diode-array detection (DAD) system. A Rheodyne 7725i injection valve with a 20 μl injection sample loop and a Kromasil 5 ODS (150 mm × 4.6 mm i.d.) analytical column were used. Chromatographic separation was carried out in gradient mode at a flow rate of 1 ml min^{-1}. The gradient elution was performed as follows: from 65% water (A) and 35% acetonitrile (B) to 35% A and 65% B in 10 min and returning to initial conditions in 5 min. In those experiments where each phenylurea was analysed independently, isocratic elution using two different mobile phases (70% A:30% B for FEN and MXN and 50% A:50% B for CTN, IPN, MBN and LIN) was used. Phenylurea herbicides were monitored at 244 nm and quantified by external calibration using peak area measurements.

3. Results and discussion

As stated in the Introduction, precipitation polymerisation seems to be one of the best suited methods for the synthesis of imprinted beads with high yields. However, it has been reported that the selected template affects polymer morphology even hindering the obtainment of imprinted beads [13,16]. Fig. 2 shows the scanning electronic micrographs of the imprinted polymers studied in the present work. It is clear, for the three imprinted polymers, that agglomerates of nano-particles of different sizes were obtained. This result prevents the further use of the obtained polymers as stationary
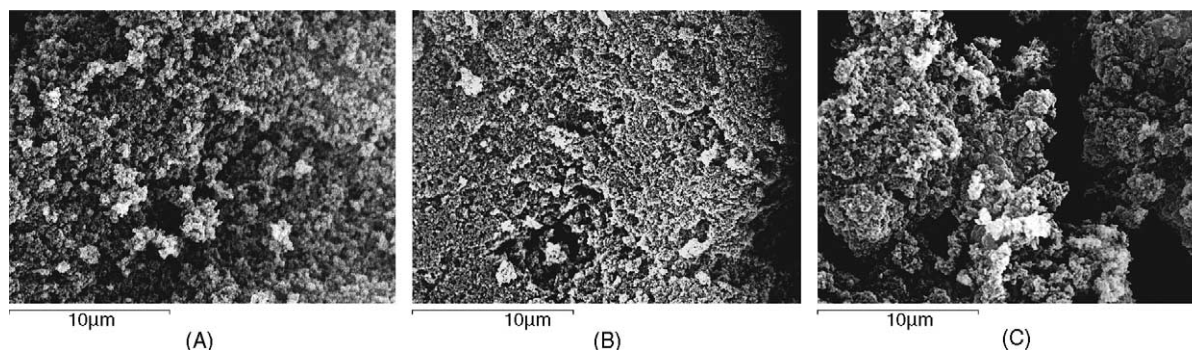

Fig. 2. Scanning electron micrographs (×5000 magnification) of imprinted polymers prepared by precipitation polymerisation. (A) MIP1 (IPN-MAA); (B) MIP2 (IPN-TFMAA) and (C) MIP3 (LIN-TFMAA).

phases in HPLC. However, from our point of view, just the simplicity of the experimental procedure used and the elimination of crushing and sieving steps justify the use of this methodology for the preparation of imprinted polymers with high yields. In fact, in the present work, after removing the template molecules and non-reactive monomers, the polymer yields obtained were 89, 91 and 94% for MIP 1, MIP 2 and MIP 3, which are rather higher than those obtained by bulk polymerisation (typically < 60%). In addition, the obtained polymers might be employed in other areas where strict size and shape requirements are not so necessary (i.e. solid-phase extraction).

Apart from these practical considerations, it is important to make an estimation of the binding properties of the system (i.e. capacity, affinity constants) as it will provide insights on how molecular recognition takes place in MIPs and also will help to select the right polymer for a certain application. Thus, the obtained MIPs were evaluated by rebinding experiments and the experimental adsorption isotherms were fit to three mathematical models: Langmuir, Freundlich and Langmuir–Freundlich isotherms.

The Langmuir (L) adsorption isotherm is able to model homogeneous systems (Eq. (1)) while the Freundlich (F) adsorption isotherm successfully models non-homogeneous systems (Eq. (2)).

$$B = \frac{N_t a F}{1 + a F} \tag{1}$$

$$B = 1 + a F^m \tag{2}$$

On the other hand, Umpleby et al. [15,17] have recently demonstrated that the Langmuir–Freundlich isotherm (LF) is able to address the heterogeneity of MIPs in both covalent and non-covalent approaches and in sub-saturated and saturated zones. The LF isotherm describes a relationship between the concentration of bound (B) and free (F) guests in heterogeneous systems according to Eq. (3):

$$B = \frac{N_t a F^m}{1 + a F^m} \tag{3}$$

where N_t is the total number of binding sites (capacity), a is related to the median binding affinity constant K_0 ($K_0 = a^{1/m}$) and m is the heterogeneity index, which will take values between 0 and 1. When m is closer to 1, the material presents a more homogeneous binding site distribution.

It is important to point out that the conclusions derived from a rebinding experiment may only be considered accurate if the studied system is under equilibrium conditions in order to minimize the kinetic aspects involved in the guest-host interactions [12,18], and if the analytes are only specifically bound to the polymer. Therefore, the washing step was optimised first, in order to minimize non-specific interactions between target analytes and the polymeric matrix. Toluene, acetonitrile and methanol were tested and it was found that a washing step consisting of 5 × 1 ml of toluene was enough to elute IPN and LIN from both control polymers (CP-MAA

and CP-TFMAA) whereas they remained bound to the corresponding imprinted polymers. Besides this, in order to confirm the presence of imprinted sites, carbaryl and fenitrothion, two commonly used pesticides but with a completely different structure (Fig. 1), were also evaluated in a separate experiment and, in this case, both carbaryl and fenitrothion were not retained by any of the MIPs under study. These experiments confirm the selectivity of the imprinted polymers and the presence of specific binding sites in its structure, making it suitable for performing rebinding experiments.

After minimizing non-specific interactions and in order to assess equilibrium, the molecularly imprinted solid-phased extraction (MISPE) process was carried out for each analyte by loading 1 ml of a solution containing 10 μg of the analyte under study and was kept in contact with the polymer for 15, 30, 60 and 120 min in different experiments. After this incubation, the polymer was washed with 5 × 1 ml of toluene and the analytes were quantitatively eluted and B calculated as described in Section 2. A parallel experiment loading the toluene solution containing analytes at 10–15 ml min⁻¹ (incubation time = 0) was also performed. The obtained results were compared each other and not significant differences were found between the B values obtained for the different periods of incubation. This result indicates that mass transfer was fast and therefore equilibrium was immediately reached in the three imprinted polymers under study.

3.1. Rebinding experiments

To perform this study, independent solutions of each phenylurea herbicide (see Fig. 1 for chemical structures) or a mixture of all of them at a concentration level ranging from 0.1 to 500 μg ml⁻¹ were loaded onto the different cartridges containing MIP 1, MIP 2 or MIP 3, and B and F were calculated as described above. The experimental data were fit to Langmuir, Freundlich and Langmuir–Freundlich (LF) isotherms. The best R^2 coefficients were obtained when the LF isotherm was used confirming the suitability of this isotherm to model the interactions taking place in MIPs. It is important to stress that both Langmuir and Freundlich models were able to yield a good approximation of the binding behaviour of the studied systems, but the LF isotherm gave a better overall fit to the adsorption isotherm because of its ability to model both saturation and sub-saturation concentration regions.

3.2. Recognition of each compound independently

The LF isotherm is able to produce a direct measurement of the binding properties through the calculation of the fitting coefficients N_t, m and a. N_t yields a direct measurement of the binding sites, m is the heterogeneity index and a is related to the association constant, as described above. The use of these coefficients allows to compare the behaviour of different MIPs and of different compounds to the same material. Table 1 shows the calculated values of the fitting coefficients

Table 1
Fitting parameters for the LF fit to the experimental adsorption isotherms of studied phenylureas loaded independently on different imprinted polymers

	MIP1			MIP2			MIP3		
	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m
FEN	0.999	6.18 (0.17–11.25)	0.926	1.947	32.83 (0.17–656.81)	0.945	1.201	30.37 (0.34–16420.36)	0.470
MXN	0.729	20.01 (1.25–114.34)	0.911	1.115	30.59 (1.31–571.69)	0.936	0.786	51.01 (8.89–22867.60)	0.462
CTN	0.467	7.91 (1.12–147.40)	1	0.462	17.55 (1.12–81.57)	0.814	0.461	6.42 (2.37–288.81)	0.957
IPN	2.260	33.39 (0.05–1031.56)	0.980	2.787	15.91 (0.05–1375.42)	0.904	0.394	250.08 (4.93–20631.32)	0.466
LIN	0.193	7.00 (1.28–57.67)	1	0.095	6.21 (1.26–51.47)	1	0.257	23.24 (10.35–279.59)	0.961
MBN	–	–	–	0.075	16.61 (1.36–55.70)	1	0.115	10.14 (11.07–284.62)	1

[a] Calculated from the experimental maximum and minimum free analyte concentration (F_{max} and F_{min}) by the relationships $K_{min} = 1/F_{max}$ and $K_{max} = 1/F_{min}$.

for MIP 1 (IPN-MAA based polymer), MIP 2 (IPN-TFMAA based polymer) and MIP 3 (LIN-TFMAA based polymer) for each phenylurea herbicide. The accuracy of these values must be evaluated with respect to the concentration window in which they were measured and the corresponding isotherms must cover saturation and sub-saturation regions. This was assessed by proving that K_o falls between the limits $1/F_{max}$ and $1/F_{min}$ [15], and by the low relative standard error obtained for the fitting analysis (around 6% in all cases).

From the experimental data obtained from each compound loaded independently (Table 1) several conclusions may be derived. First at all, it can be observed that MIP 1 presents the highest capacity for IPN, the template molecule, regarding the rest of assayed compounds confirming that well-defined binding sites were obtained during imprinting process. It is also interesting to observe that the capacity reached for LIN was the lowest of all retained compounds, and the fact that MBN was not retained at all by this polymer. Both compounds, LIN and MBN, contain a methoxy group near to the urea group (see Fig. 1), which is likely the responsible of the monomer:template interaction through hydrogen bonding during the prearrangement step. Thus, it seems clear that the presence of –OCH$_3$ groups disrupts the interaction of this compounds with imprinted sites in certain manner either by steric repulsion or by unspecific interactions inside the imprinted cavities (i.e. methoxy group may also interact by hydrogen bonding with the acidic moieties inside the cavities) as discussed below. On the other hand, the N_t value obtained for FEN, which is the smallest of all the phenylureas used in this study, is the highest among those obtained for the studied compounds, being only lower than the N_t value obtained for IPN (template molecule). This might indicate the ability of FEN to rebind to the polymer using the smaller binding sites. Phenylureas (CTN and MXN) containing halogenated heteroatoms and/or methoxy groups on its structure, but not close to the urea functional group, show lower capacities than that exhibited by FEN (the smallest) and IPN (template molecule). This fact might be attributed to the difficulty to accommodate its bigger structures inside the imprinted cavities although it does not prevent recognition to take place. In fact, a high median affinity constant was obtained for MXN (only below the K_o value obtained for IPN) suggesting that the kind and position of groups in the aromatic ring might have a certain influence on the strength of the interaction. Finally, the m values obtained are very close to 1 indicating a highly homogeneous binding site distribution in MIP1. However, it is important to stress that it does not mean that only one kind of binding site exist but several different sites with rather similar size, shape and affinity for the studied compounds.

In order to improve the retention of LIN and MBN, a second imprinted polymer (MIP2) using TFMAA as functional monomer was prepared and evaluated in the same manner than MIP1. It is known that TFMAA is able to interact more strongly through hydrogen bonding and thus theoretically this new polymer should be able to retain quantitatively in a

wider concentration range all the tested analytes. In general, according to the fitting parameters shown in Table 1, not only the N_t values for all the analytes were increased in this new polymer but also MIP2 possesses the ability to rebind LIN and MBN. However, the capacity obtained is still rather low suggesting again that –OCH$_3$ group has a negative influence in the recognition mechanism. This fact is also clearly indicated by the K_o values obtained for LIN and MBN, being the lower values of all of them. Besides this, the high values obtained for the m index indicates a high degree of homogeneity in this polymer too, confirming the existence of well-defined binding sites.

The highly homogeneous binding site distribution obtained both in MIP1 and MIP2 confirms the suitability of precipitation polymerisation to prepare imprinted polymers with improved characteristics compared to polymers prepared by bulk polymerisation as has been previously suggested by our group [12,13]. Apparently, precipitation polymerisation prevents the formation of complexes of different template:monomer stoichiometry (one of the reported reasons for the observed heterogeneity of non-covalent MIPs) during prearrangement step, since this step is carried out in a very diluted system.

Finally, a third polymer (MIP 3) was synthesised using TFMAA as functional monomer and with LIN as template in order to study the influence of the template in the formation of imprinting cavities. In this case (Table 1), the capacity to selectively rebind the template molecule (LIN) and MBN (the most structural related phenylurea), both with a methoxy group in its structure, increased considerable compared to the N_t values obtained for MIP1 and MIP2. However, it is important to stress that the m values obtained in this case are rather below 1 representing clearly a heterogeneous binding site distribution. Accordingly, the existence of a methoxy group in the template molecule (LIN) affects negatively the template:monomer interaction during pre-polymerisation step and likely several complexes involving different groups and/or different stoichiometry might be formed, leading to a material with a more heterogeneous binding site distribution. This fact confirms that the poor interaction of LIN and MBN with MIP1 and MIP2 cannot be attributed to a steric effect, due to its bigger size, but to a different kind of interaction as suggested above.

3.3. Competition for the binding sites

The objective of this study was to evaluate the competition for the binding sites that can take place when all the phenylureas were loaded simultaneously onto the MIPs in order to select the most suitable polymer to be used in solid-phase extraction process of phenylurea herbicides. This study was carried out by loading 1 ml of mixtures of phenylureas in toluene according to the procedure described in Section 2. The experimental adsorption isotherms obtained were fit to the LF isotherm (Fig. 3) and the obtained fitting coefficients are shown in Table 2. Firstly, it is clear that the capacities ob-

Table 2
Fitting parameters for the LF fit to the experimental adsorption isotherms of studied phenylureas loaded all together in a mixture on different imprinted polymers

	MIP1			MIP2			MIP3		
	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m	N_t (μmol g^{-1})	K_o ($K_{min} - K_{max}$) (mM^{-1})[a]	m
FEN	0.304	441.99 (0.84–8210.12)	0.973	0.408	3168.62 (8.95–82101.81)	0.988	0.606	2279.64 (1642.04–821018.1)	0.745
MXN	0.236	82.62 (11.18–3266.80)	0.916	0.267	127.27 (11.71–4573.52)	1	0.446	723.58 (601.78–228676)	0.766
CTN	0.201	258.32 (1.09–5343.02)	1	0.127	92.75 (4.50–2249.69)	0.824	0.267	392.29 (10.67–106860.4)	0.716
IPN	0.270	3017.67 (1.06–68,771.06)	0.970	0.175	345.86 (1.05–4126.26)	1	0.297	433.10 (2.20–68771.06)	0.812
LIN	0.106	4.37 (1.26–257.54)	1	0.018	309.57 (1.25–716.96)	0.799	0.081	17.85 (5.18–3700.82)	0.823
MBN	–	–	–	0.037	52.55 (1.30–700.00)	0.736	0.075	99.37 (2.64–8633.34)	0.716

[a] Calculated from the experimental maximum and minimum free analyte concentration (F_{max} and F_{min}) by the relationships $K_{min} = 1/F_{max}$ and $K_{max} = 1/F_{min}$.
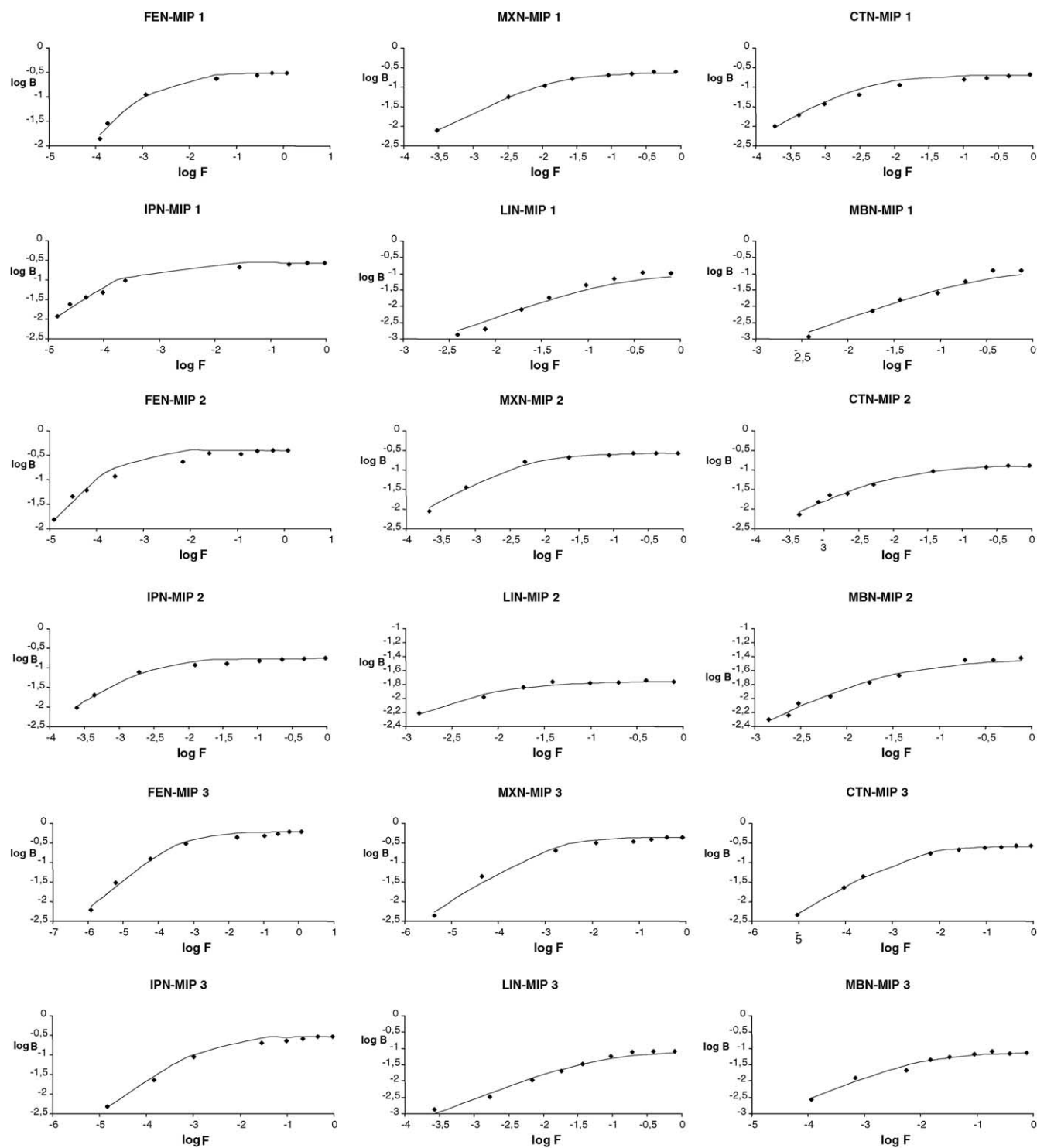
Fig. 3. Log plots of the adsorption isotherms for studied analytes loaded all together in a mixture on different MIP. The experimental data (♦) were fit to the Langmuir–Freundlich (solid line) isotherm.

tained in this study for all the analytes in all the three polymers are lower than those obtained when the phenylureas were loaded independently. This result is easily explained taking into account that the tested compounds compete each other for a limited number of binding sites present in the polymeric matrix. However, the observed diminishment on the capacities did not occur in the same rate for all the phenylureas which suggests that each compound selects an appropriated kind of binding site. For instance, the highest value for the fitting coefficient N_t in the three polymers correspond to FEN,

which indicates that FEN is able to interact with high affinity with the smaller binding sites, where the others phenylureas cannot access. In addition, the *m* index increased compared to that obtained in the previous experiments loading phenylureas independently. This result proves that, in the presence of related compounds able to interact with the polymer and to compete for the binding sites, each analyte selects a specific binding site according to its size and affinity. Nevertheless, the *m* indexes obtained with MIP2 for LIN and MBN are not in accordance with this statement. However, it is important to stress that in this case the measured concentration levels are very low, as can be observed from the capacity obtained, and therefore the associated error to these measurements was significant (∼30%). Finally, it is important to point out that both LIN and MBN are clearly displaced by the other analytes in the competition experiments in the three tested polymers and are able to interact with a very small number of binding sites. This result confirms that, as suggested above, the presence of a –OCH$_3$ group near to the urea moiety disrupts the interaction of LIN and MBN with the imprinted cavities, even with those presents in MIP3 which were formed using LIN as template.

3.4. MISPE of phenylurea from corn sample extracts

Apart from these theoretical considerations, and according to the obtained results, MIP3 seems to be able to retain selectively all the tested analytes with high enough capacity and affinity. Thus, a preliminary evaluation on the use of this polymer in MISPE of selected herbicides in a real sample (corn) was carried out. Fig. 4 shows the chroma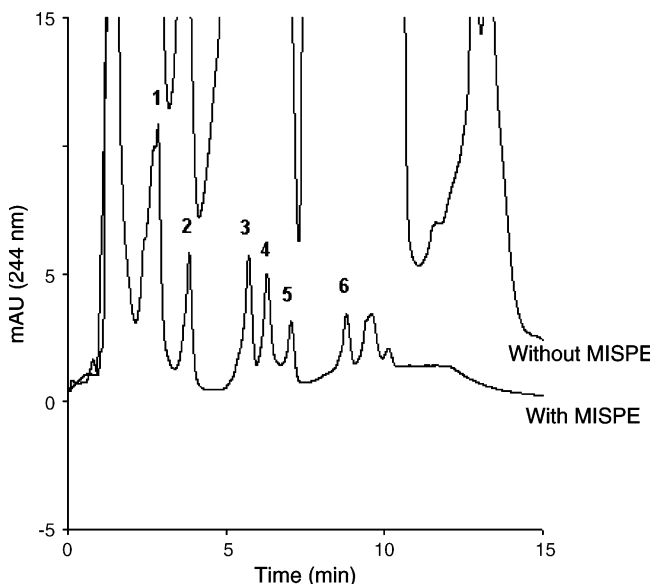tograms obtained with and without MISPE of blank and spiked (100 ng g^{-1} concentration level) corn sample extracts. As can be observed, the quantification of phenylureas without clean-up is not possible due to interferences appearing in the chromatograms whereas they can be easily detected after cleaning sample extracts by the proposed MISPE procedure. This preliminary evaluation is very promising and thus both MIP2 and MIP3 polymers are under further evaluation in our laboratory for the development of molecularly imprinted solid-phase extraction methods for the determination of phenylurea herbicides from environmental and food samples.

4. Conclusions

According to the obtained results, precipitation polymerisation is a powerful strategy for the preparation of MIPs with improved characteristics. The degree of homogeneity obtained, comparable to that reported in MIPs prepared using the covalent approach, makes this polymerisation methodology a clear alternative to the traditional bulk polymerisation process. However, the influence of template during polymerisation, preventing the obtainment of imprinted beads in some cases, makes questionable its use for the preparation of stationary phases to be used in HPLC and further research should be done.

On the other hand, as expected, the use of TFMAA as functional monomer leads to the synthesis of polymers with higher capacities and affinity constants making possible the simultaneous extraction of several phenylurea herbicides since each compound is able to interact with specific binding sites in the presence of related compounds. Besides this, it has been confirmed that the selection of the template molecule used during polymerisation strongly affects the capabilities of the obtained polymer for rebinding not only the template molecule but also related compounds. Finally, the LF isotherm confirms to be a powerful tool to be used either to study the adsorption of different compounds to the same polymer or for comparison between different MIPs.

Fig. 4. Chromatograms obtained at 244 nm without and with MISPE of corn sample extracts spiked with phenylureas (100 ng g^{-1}). Peak numbers: (1) FEN; (2) MXN; (3) CTN; (4) IPN; (5) MBN; (6) LIN. Chromatographic conditions: see Section 2.

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