Multivariate Analysis and Experimental Design in the Screening of Combinatorial Libraries of Molecular Imprinted Polymers

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The combination of an experimental design/multivariate analysis method and a high-throughput technique was developed in the screening and evaluation of molecular imprinted polymers (MIPs). Combinatorial libraries of MIPs were prepared automatically at a reduced scale and screened for high affinity with regard to six experimental factors with a large impact in the MIP synthesis: the amounts of the functional monomer, cross-linker and template; the volume of the porogen, the amount of the initiator and the types of initiation. With the objective of optimizing a polymer imprinted with the non-steroidal anti-inflammatory drug piroxicam, different polymer libraries were prepared by varying the six factors according to a two-level fractional factorial design of resolution VI. This optimization resulted in polymers showing high affinity as a result, mainly, of reduced binding to the non-imprinted polymers. The type of cross-linker showed a strong influence on the specific binding of the template when assessed by equilibrium rebinding experiments. The cross-linking monomer divinylbenzene gave rise to an enhancement in the binding capacity of the MIP that may be attributed to its similar structure with the functional monomer considered (4-vinylpyridine). The binding capacity of the MIP and blank polymers was related to the six experimental factors using a partial least squares regression (PLS) method. The regression models of the polymer libraries showed the same trend in the MIP and blank polymers, suggesting a higher specific binding capacity when the polymerization was photoinitiated at low temperature; the amounts of the functional monomer and the cross-linker were similar. The proposed procedure is time and cost effective and can be used as a general tool for preparing MIPs for different analytes. The proposed method provides new insight into the influence and interaction of the main factors that affect the MIP performance that could facilitate improvements in the MIP process design in the future.

Molecular imprinting is a rapidly developing technique to prepare polymers with high recognition properties.[1,2] This technique leads to highly stable synthetic polymers that possess selective molecular recognition properties because of recognition sites within the polymer matrix that are complementary to the analyte in the shape and positioning of functional groups. Molecular imprinted polymers (MIPs) have been adapted to different practical applications, given their characteristics, such as ease of preparation and mechanical and chemical robustness.[3] MIPs have been used as chiral stationary phases for separation purposes,[4,5] as recognition components in chemical sensors,[6,7] in immunoassays,[8,9] and as affinity matrices in solid-phase extraction (SPE).[10–12] Recently, an article that was focused on new developments and recent advances in the field of molecularly imprinted materials has been published.[13]

The success of the imprinting process relies on an adequate selection of the chemical parameters that influence the molecular recognition properties of the synthesized polymers throughout their structure and morphology. The physical factors governing the formation of template-monomer interactions and ligand-polymer binding have been described in order to rationally design MIP systems.[14] Nevertheless, because both the morphology of the polymer and the chemical microenvironment of the binding site are critical to the overall performance of the MIP, the number of experimental factors and their possible interactions that influence these two variables are large. Therefore, imprinted polymers are suitable samples for combinatorial synthesis,[15] allowing the main factors to be rapidly screened.

Chemometric tools have been suggested as methods for the screening and optimization of MIPs.[16,17] There are two aspects of any experimental problem that should be considered: the design of the experiment and the statistical analysis of the data. These two subjects are closely related because the analysis method depends directly on the design employed. The statistical design of experiments refers to the process of planning experiments so that appropriate data that can be analyzed by statistical methods will be collected, resulting in valid and objective conclusions. The paramount goal is to create representative and informative experiments with the minimum number of experiments. Multivariate regression analysis gives a model relating the changes in the factors to changes in the responses. The model will indicate which factors are important, how they combine to influence the responses method, recognize patterns in the data and suggest predicted optimums.[18]

Another suggested approach has been computational methods to search for the optimal imprinting conditions using molecular modeling software.[19,20] With the aim of this theoretical approach, a virtual library of functional monomers was designed and screened against a desired template for the optimal binding interactions. The best monomers were then virtually

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annealed to the template to optimize the arrangement of the functional monomers with energy minimization iterations.

In order to speed up the optimization of MIP formulations, several authors[21–23] have proposed different high-throughput semi-automated approaches to synthesize and screen large libraries of MIPs in small quantities and with different compositions, as a prior step to the preparation of larger amounts of selected candidates.[24] Although this technique has been used mainly to find optimal functional monomers for various molecules, it can also be used to screen for other MIP factors, such as cross-linking monomers, porogens, initiators, or types of polymerization.[25]

This paper describes an approach for preparing and screening a library of various imprinted polymers through the application of an experimental design and the use of the multivariate analysis methodology to optimize the selected significant factors in the preparation of MIPs with high affinity. Polymers were prepared in a small-scale using a semi-automatic procedure using a programmable liquid-handling equipment.[21] Their template rebinding ability, assessed by discrete binding tests, was evaluated and correlated to the MIP and the corresponding blank polymers composition. Six factors that have an important influence on the analytical performance of a MIP were screened using a two-level fractional factorial design. These factors were the amount of functional monomer, the amount and type of cross-linker, the amount of the template and initiator, the volume of porogen (acetonitrile), and the polymerization procedure (thermal or photochemical). The binding capacity of each library was fitted using the partial least square (PLS) regression method,[26,27] in order to obtain a multivariate model that identified the main factors that determine the recognition properties of the MIPs. With the objective of optimizing a polymer imprinted with the non-steroidal anti-inflammatory drug piroxicam (Fig. 1), polymer libraries were prepared using 4-vinylpyridine as a functional monomer and varying the MIP composition according to an experimental design.

Experimental

Materials. Piroxicam, 4-vinylpyridine (4-vpy), ethylene glycol dimethacrylate (EDMA), trimethylolpropane trimethacrylate (TRIM), divinylbenzene (DVB), bisphenol A dimethacrylate (BPADMA), and 2,2′-azobisisobutyronitrile (AIBN) were purchased form Wako Pure Chemical Industries (Osaka, Japan). The monomers were purified prior to use as follows: 4-vpy was distilled under reduced pressure; EDMA, TRIM, and DVB were passed through a column filled with an inhibitor–remover packing from Aldrich. AIBN and BPADMA were used without further purification. Acetonitrile and acetic acid were of HPLC grade. Glass vials (2 mL) with silicone septa were used as polymerization reactors. The water used in the HPLC was obtained from a water-purification system (Eyela, Still Ace SA-27E1N).

HPLC Analysis. Quantification of piroxicam was performed with a HPLC system consisting of a HPLC pump (Hitachi, L-6320), a UV–vis detector (Hitachi, L-4200), an automatic sampling injector (Gilson, 231XL), and a controller-data processor Unipoint (Gilson). For HPLC evaluation, 20 µL samples were injected and analyzed using a reversed-phase Puresil (4.6 mm × 150 mm, 5 µm) C18 column, with the mobile phase acetonitrile/20 mM phosphoric acid (60/40, v/v) at a flow rate of 1.0 mL min^{-1} and UV detection at 350 nm.

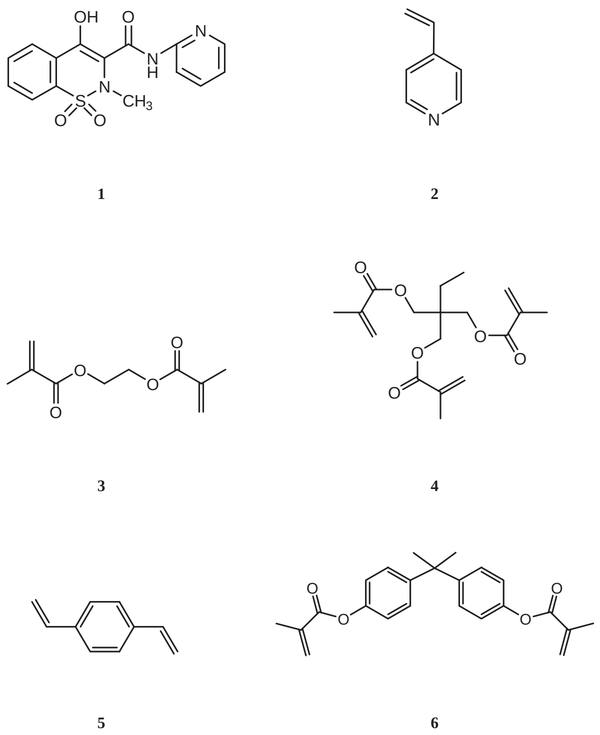

Fig. 1. Structures of the template molecule, functional monomer, and cross-linkers: 1, piroxicam; 2, 4-vinylpyridine; 3, ethylene glycol dimethacrylate; 4, trimethylolpropane trimethacrylate; 5, divinylbenzene; 6, bisphenol A dimethacrylate.

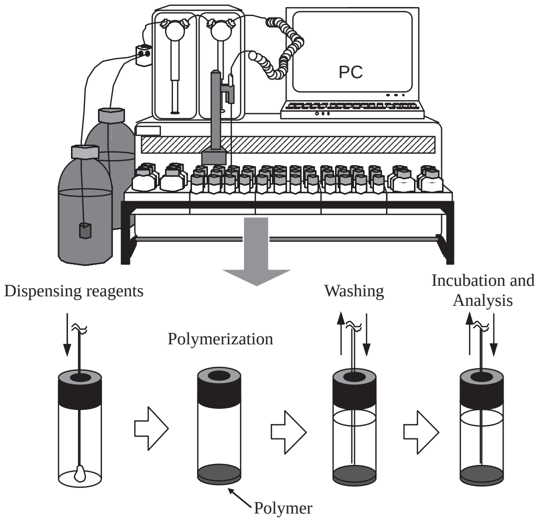

Fig. 2. Scheme showing the high-throughput preparation and washing of the polymers using the liquid handler equipment.

Semi-Automated Polymer Preparation. The polymers were prepared as a thin layer in the bottom inner surface of glass vials (2.0 mL). Reagents for the molecular imprinting, piroxicam as a template, 4-vpy as a functional monomer, a cross-linker, AIBN as a polymerization initiator, and acetonitrile as a pore former were dispensed into the vials by a programmed liquid handler (Gilson, model 232XL) (Fig. 2). All the reagents, except for the cross-linkers (EDMA, TRIM, DVB, and BPADMA), were dis-

Table 1. Settings of the Factors on the Combinatorial Libraries

Factor		Settings		
		low	center	high
4-vpy	(μmol)	1	5.5	10
Cross-linker	(μmol)	50	100	150
Piroxicam	(μmol)	0.2	0.6	1
AIBN	(μmol)	2	6	10
Acetonitrile	(μL)	60	110	160
Initiation		photochemical (UV), thermal		

solved in acetonitrile for the dispensation. Twelve stock solutions were prepared for the synthesis of each MIP library: three stock solutions of 4-vpy dissolved in acetonitrile at its low, high, and center levels and nine stock solutions of piroxicam and AIBN dissolved in acetonitrile at different ratios according to the settings specified in Table 1. For preparing non-imprinted polymers, initiator stock solutions were prepared without piroxicam. The volume of acetonitrile was set by dispensing 10 μL of 4-vpy stock solutions and 50, 100, or 150 μL of the piroxicam/AIBN stock solutions so that the mole ratio of the mixture components in each vial was according to the experimental design. The vials were sealed with a rubber septum, purged with nitrogen for 2 min at room temperature and placed under UV irradiation (Toshiba, FL15BLB) at 5 °C (photochemical polymerization) or in a thermostatic water bath at 60 °C (Eyela, UA-100) (thermal polymerization). Samples were allowed to polymerize for 24 h.

Extraction and Rebinding Experiments. Into each vial, 1 mL of a washing solvent, acetonitrile–acetic acid (1:1), was dispensed and removed after incubation for 2 hours. This washing procedure was repeated 5 times, and followed by washing with 1 mL of acetonitrile (5 times). Under this washing protocol, no piroxicam was detected in the supernatant solution using the HPLC analysis method. During these procedures the polymers were not ground or sieved. Finally, the polymers were dried overnight at room temperature and the amount of dried polymer was calculated.

The rebinding experiments were performed by incubating each polymer with 1 mL of 0.5 mM piroxicam in acetonitrile (HPLC grade) for 24 h, under mechanical shaking, at room temperature. The concentration of the free (unbound) piroxicam was determined by HPLC (duplicate injections) using the method described above. The amounts of bound piroxicam were obtained by subtracting the free piroxicam from the initial amount.

Experimental Design and Data Processing. The polymer design and modeling were carried out using MODDE 6.0 software (Umetrics, Sweden) and six factors in the experimental design. The selected factors were (a) the amount of the functional monomer; (b) the amount of the cross-linker; (c) the amount of the initiator (AIBN); (d) the amount of the template (piroxicam); (e) the volume of porogen (acetonitrile) and, (f) the initiation procedure (photochemical or thermal).

A two-level fractional factorial experimental design of resolution VI was considered for each 4-vpy/cross-linker combination. The settings for each factor are given in the Table 2. These settings were chosen while considering the criteria for stabilizing the monomer-template complexes and the imprinted sites. The numbers of samples for each MIP library was 38 (2^{6-1} plus 6 center points) and 22 for the non-imprinted polymer libraries (2^{5-1} plus 6 center points). These samples for each polymer library were used as a training set to obtain empirical multivariate models.

The ratio between the amount of template bounded and the total amount of polymer was considered to be the response in the multivariate analysis. The binding capacity values obtained from each polymer library (4-vpy/cross-linker combination) were related with the factors considered in the experimental design by an empirical model obtained using a partial least squares (PLS) regression method.[27] The data were log-transformed in order to approach normality and, in addition, centered and scaled to unit variance before the analysis. The principal components of the PLS method were determined on the basis of a non-linear iterative partial least squares (NIPALS) algorithm; also the number of significant principal components was determined by the cross-validation method. The optimal number was selected so that it yielded the minimum prediction error, calculated as the predictive residual sum of squares (PRESS) for each model dimension. The goodness of fit and the prediction ability of the models were assessed by the R^2 and Q^2 parameters, where R^2 is the fraction of the variation of the response explained by the model and Q^2 is the fraction of the variation of the response that can be predicted by the model. The MIP and blank polymer libraries were modelled independently.

An interpretation of each calibration model was carried out using the regression coefficients of the model and the variable influence on the projection parameter (VIP).[28] In the coefficients plots, the relative sizes and signs of the coefficients related to the center and scaled factors indicate the influence of each model term, and reflects the underlying structure of the data.[29] On the other hand, the VIP represents a condensed summary of the importance of the factors with respect to the response variables, allows ranking all factors in order of decreasing importance in fitting the model.

Results and Discussion

In molecular imprinting, many factors influence the properties of the MIPs, and because of the absence of a clear understanding of the mutual effect between these factors there are no well-developed rules to follow for designing polymers exhibiting the desired recognition properties. Due to the complexity of the imprinting process, the use of a combinatorial approach, through the application of an experimental design, allows one to screen the factors in order to assess their influence on the affinity properties of the MIPs, and hence optimize the polymer composition. The continuous factors chosen were the amount of the functional monomer (4-vpy), the amount of the cross-linker (EDMA, TRIM, DVB, and BPADMA), the amount of the template (piroxicam), the amount of the initiator (AIBN), and the volume of porogen (acetonitrile). Finally, the type of initiation was chosen as a discrete factor (photochemical, thermal). Samples of each library were prepared according to a two-level fractional factorial design of resolution VI (2^{k-1}) with six center points, where k is the number of factors.

The obtained polymers showed different physical characteristics, ranging from hard to much softer polymers containing lower cross-linking ratios. The amount of cross-linker influences the amount of polymer obtained being higher when the amount of the cross-linker was increased. The weights of the polymers were in the order 10–34 mg, 15–46 mg, 8–30 mg, and 10–31 mg when EDMA, TRIM, DVB, and BPADMA were used as cross-linkers, respectively. The small difference between the binding values of the replicas indicates that the polymers can be reproducibly prepared using the semi-automatic method. The reproducibility was higher than 96% in

Table 2. Polymer Composition of the Piroxicam-MIP Library According to a Two-Level Fractional Factorial Design of Resolution VI and Six Center Points

Sample	4-vpy (μmol)	Cross-linker (μmol)	Piroxicam (μmol)	AIBN (μmol)	Acetonitrile (μL)	Initiation
1	1	50	0.2	2	60	UV
2	10	50	0.2	2	60	thermal
3	1	150	0.2	2	60	thermal
4	10	150	0.2	2	60	UV
5	1	50	1	2	60	thermal
6	10	50	1	2	60	UV
7	1	150	1	2	60	UV
8	10	150	1	2	60	thermal
9	1	50	0.2	10	60	thermal
10	10	50	0.2	10	60	UV
11	1	150	0.2	10	60	UV
12	10	150	0.2	10	60	thermal
13	1	50	1	10	60	UV
14	10	50	1	10	60	thermal
15	1	150	1	10	60	thermal
16	10	150	1	10	60	UV
17	1	50	0.2	2	160	thermal
18	10	50	0.2	2	160	UV
19	1	150	0.2	2	160	UV
20	10	150	0.2	2	160	thermal
21	1	50	1	2	160	UV
22	10	50	1	2	160	thermal
23	1	150	1	2	160	thermal
24	10	150	1	2	160	UV
25	1	50	0.2	10	160	UV
26	10	50	0.2	10	160	thermal
27	1	150	0.2	10	160	thermal
28	10	150	0.2	10	160	UV
29	1	50	1	10	160	thermal
30	10	50	1	10	160	UV
31	1	150	1	10	160	UV
32	10	150	1	10	160	thermal
33	5.5	100	0.6	6	110	UV
34	5.5	100	0.6	6	110	UV
35	5.5	100	0.6	6	110	UV
36	5.5	100	0.6	6	110	thermal
37	5.5	100	0.6	6	110	thermal
38	5.5	100	0.6	6	110	thermal

all of the polymer libraries when the binding capacity calculated under the same experimental conditions (centre points) was compared to the total variation of the binding capacity in each polymer library.

The binding capacity of the polymers was very different, depending on the polymer composition and cross-linker used in their synthesis (Table 3). The MIPs obtained with DVB as a cross-linker showed the highest binding capacity in the order of 1.7–78.5 μmol piroxicam g^{-1} polymer, whereas, the MIPs obtained with TRIM as a cross-linker showed the lowest binding capacity in the order of 0.4–4.4 μmol piroxicam g^{-1} polymer. In the entire polymer libraries, the calculated PLS models were quite good, explaining more than 92% of the response variance, i.e., the models showed a strong, quantitative relationship between the factors considered in the experimental design and the binding capacity of the samples. The PLS models representing blank polymers showed a better goodness of fit (R^2) than that corresponding to the MIPs. Regarding the Q^2 parameter, all of the PLS models showed a prediction ability higher than 75% of the response, indicating that predictions about the influence of each factor in the binding capacity of the polymers should be reasonably accurate. The PLS models of the MIP libraries showed a better prediction ability than the models of the blank polymers, except when TRIM was used as a cross-linker. However, since the difference between R^2 and Q^2 is less than 0.3, the models can be considered to be statistically significant.

To interpret the PLS models, the variable influence on projection (VIP) and the regression coefficients for each factor were considered. The VIP values of the factors for each polymer library are shown in Fig. 3. In the MIP libraries the most influential factors in the models are the amount of 4-vpy and the amount of cross-linker used in their synthesis. This is not surprising, since the association between the functional mono-

Table 3. Binding Range and Summary of Fit of the PLS Models for the Piroxicam-MIP and Blank Polymer Libraries

Cross-linker	R^2 (%)[a]		Q^2 (%)[b]		R.S.D. (%)[c]		Binding (μmol piroxicam g^{-1} polymer)	
	MIP	Blank polymer	MIP	Blank polymer	MIP	Blank polymer	MIP	Blank polymer
EDMA	92.2	95.8	86.4	75.1	0.11	0.10	1.8–10.3	0.5–11.8
TRIM	92.4	96.2	82.0	85.8	0.07	0.09	0.4–4.4	0.3–3.1
DVB	93.7	94.9	78.8	80.9	0.13	0.11	1.7–78.5	0.4–18.7
BPADMA	94.0	94.5	83.1	75.7	0.08	0.09	0.7–7.4	0.9–5.8

a) R^2: fraction of variation of the response explained by the model. b) Q^2: fraction of variation of the response that can be predicted by the model. c) R.S.D.: relative standard deviation of the model.

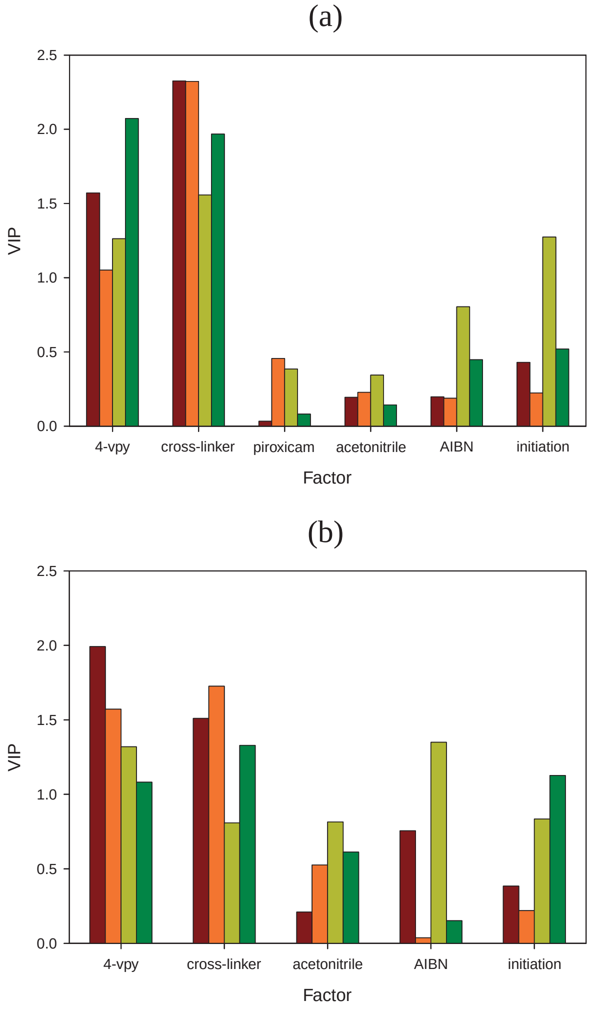

Fig. 3. Variable influence on projection (VIP) of each factor for the (a) MIP and (b) blank polymer libraries. 🟥 EDMA; 🟧 TRIM; 🟨 DVB; 🟩 BPADMA.

mer and the template prior to polymerisation will affect the number and shape of binding sites in the polymer. The fact that the 4-vpy (functional monomer) showed a high influence in the models is a strong indication of its interaction with piroxicam (template). This was demonstrated using other monomers, such as methacrylic acid and styrene, which gave rise to lower VIP values for this factor and poor binding results. The fact that methacrylic acid gave rise to poor results may not be expected, considering the basic sites on the target molecule. Nevertheless, this molecule adopts a closed conformation through an intramolecular hydrogen bond in a six-member ring in aprotic solvents, which is very stable and insensitive to micro-environmental changes,[30] thus hindering the interaction with the methacrylic acid. The amount of cross-linker influences the rigidity of the cavity structure and the polymer flexibility; therefore, it is not surprising that the variation of this factor showed a high influence on the binding affinity of the MIPs. Regarding the amount of piroxicam, the influence of this factor showed a different behavior, depending on the type of cross-linker used to synthesize the polymers. The template showed a significant influence when TRIM or DVB was used as cross-linkers, whereas in the case of EDMA and BPADMA this factor showed no influence on the PLS models. On the other hand, the volume of acetonitrile in the considered range showed practically the same influence on the binding capacity for all of the MIPs prepared, regardless of the cross-linker used in their synthesis. The amount of AIBN and the type of polymerization showed a significant influence only when DVB was used as a cross-linker to synthesize the polymers. However, concerning the blank polymers none of the factors showed a significant influence on the binding capacity of the polymers. Therefore, the presence of a template and its interaction with a suitable functional monomer makes the functional monomer/cross-linker ratio in the pre-polymerization mixture to have a significant influence on the binding capacity of the MIP.

Regarding how each factor influences in the models, the sizes and signs of the regression coefficients of the models relating to centered and scaled factors were considered to evaluate the sensitivity of the binding capacity to changes of each factor (Fig. 4). In general, all of the factors showed the same type of influence on the MIP and blank polymer libraries. The most prominent value of each regression coefficient was obtained when the MIPs were obtained with DVB as a cross-linker. Taking into account the two factors that display the highest influence in the MIPs (4-vpy and cross-linker), the binding capacity improves when the amount of functional monomer increases and the amount of the cross-linker decreases. This behavior has been observed by other authors, which showed the best results when the amounts of the functional monomer and cross-linker is similar and their ratio approached one.[16,17,25,31] This may be due to the synthesized MIP using such a ratio that would have less structural rigidity compared to a MIP prepared with a higher amount of cross-linker. Providing the degree of crosslinking is low, sufficient swelling oc-

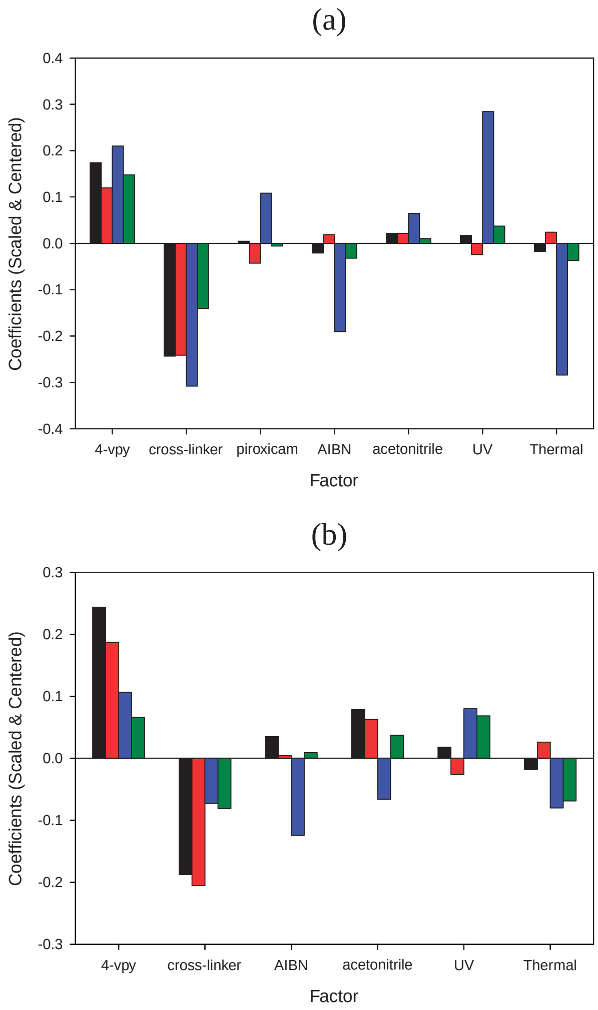

Fig. 4. PLS regression coefficients of the models for the (a) MIP and (b) blank polymer libraries. ⬛ EDMA; 🟥 TRIM; 🟦 DVB; 🟩 BPADMA.

curs in appropriate solvents to allow the entire network to be penetrated.[32] On the other hand, the association step prior to the polymerization step will be shifted towards complex formation in the presence of an excess of functional monomer relative to the template. This fact would play a major role in the molecular recognition properties of the MIP. Nevertheless, a large excess of functional monomer could give rise to non-selective binding to functional groups randomly distributed in the polymer, and a decrease of the number of high-affinity binding sites.[33]

Regarding the other four factors, the highest specific binding capacity according to the sign of the coefficients of the PLS models was observed when the amounts of the template and the volume of acetonitrile were set at a high level, the amount of AIBN was set at a low level and using photochemical initiation to obtain the MIPs. The amount of template affects the equilibrium of the complex formation with the functional monomer that is postulated to be directly responsible for the number of high-affinity binding sites formed in the MIPs.[34] In this sense, the number of specific recognition sites generated in the MIP will increase as the amount of template increases. However, at some maximum value, it has been reported that the number of specific binding sites decreases.[35] This may be due to a loss of high-performance sites, which are replaced with inferior sites,[36] or the loss of site integrity due to coalescence of the binding sites, which is related to the extent of template self-association. The highest influence of this factor was observed when DVB was used in the synthesis of the MIPs possible due to the similar structure of this cross-linker with the functional monomer selected in their synthesis. The porogen affects both the porosity of the polymers and the swelling properties. The high volume of porogen makes the total pore volume of the polymers increase and, consequently, a decrease of their surface area occurs. Nevertheless, in this particular case, this factor showed a low influence on the binding capacity of these polymers according to the VIP value. Regarding the type of initiation, the photochemically initiated polymers at low temperature showed a significantly higher binding capacity than the thermal initiated polymers, which is in agreement with previous published work.[37] The photopolymers probably show a more open pore structure, giving the template more rapid access to the sites, which are confined to a smaller surface area. Therefore, under optimal experimental conditions the polymers should have a high content of macropores and high polymer swelling due to the lower amount of cross-linkers, which allows the template to diffuse freely to the high-affinity sites of the MIP.[34]

The response surface for each model showing the influence of the amount of 4-vpy and cross-linker on the binding results of the MIPs and blank polymers, as estimated by the models, are showed in Fig. 5, where the upper surfaces correspond to the MIP libraries and the lower surfaces to the blank polymer libraries. In these plots the level of the other factors were set in order to obtain the highest binding values in the MIP library. All of the response surfaces showed the same trend in the MIP and blank libraries, and the binding capacity of the polymers increases when the amount of 4-vpy is at a high level and the amount of cross-linker is at a low level, as pointed out above. The highest specific binding capacity of the MIPs, i.e., the difference between the binding capacity of the MIP and the blank polymer at the same conditions, was obtained when the MIPs were obtained using DVB as a cross-linker (68.8 μmol piroxicam g^{-1} polymer). This enhancement in the binding capacity may be simply attributed to a higher functional group density by the polymers in the vicinity of the template, since this cross-linker has a similar structure to the functional monomer (4-vpy), which may provide more positive π–π interactions with the template to afford molecular recognition.[38] Nevertheless, the shape of the binding site would play an important role in the performance of the polymers obtained, because when BPADMA was used as a cross-linker, the specific binding capacity of the MIPs was not improved. On the other hand, the polymers obtained with TRIM showed the lowest specific binding capacity (0.6 μmol piroxicam g^{-1} polymer) perhaps due to the high cross-linked polymers obtained with this cross-linker, which does not allow the template to diffuse to the high-affinity binding sites of the MIP.

Conclusion

The binding capacity of non-covalently imprinted polymers as a function of the main polymerization factors that influence

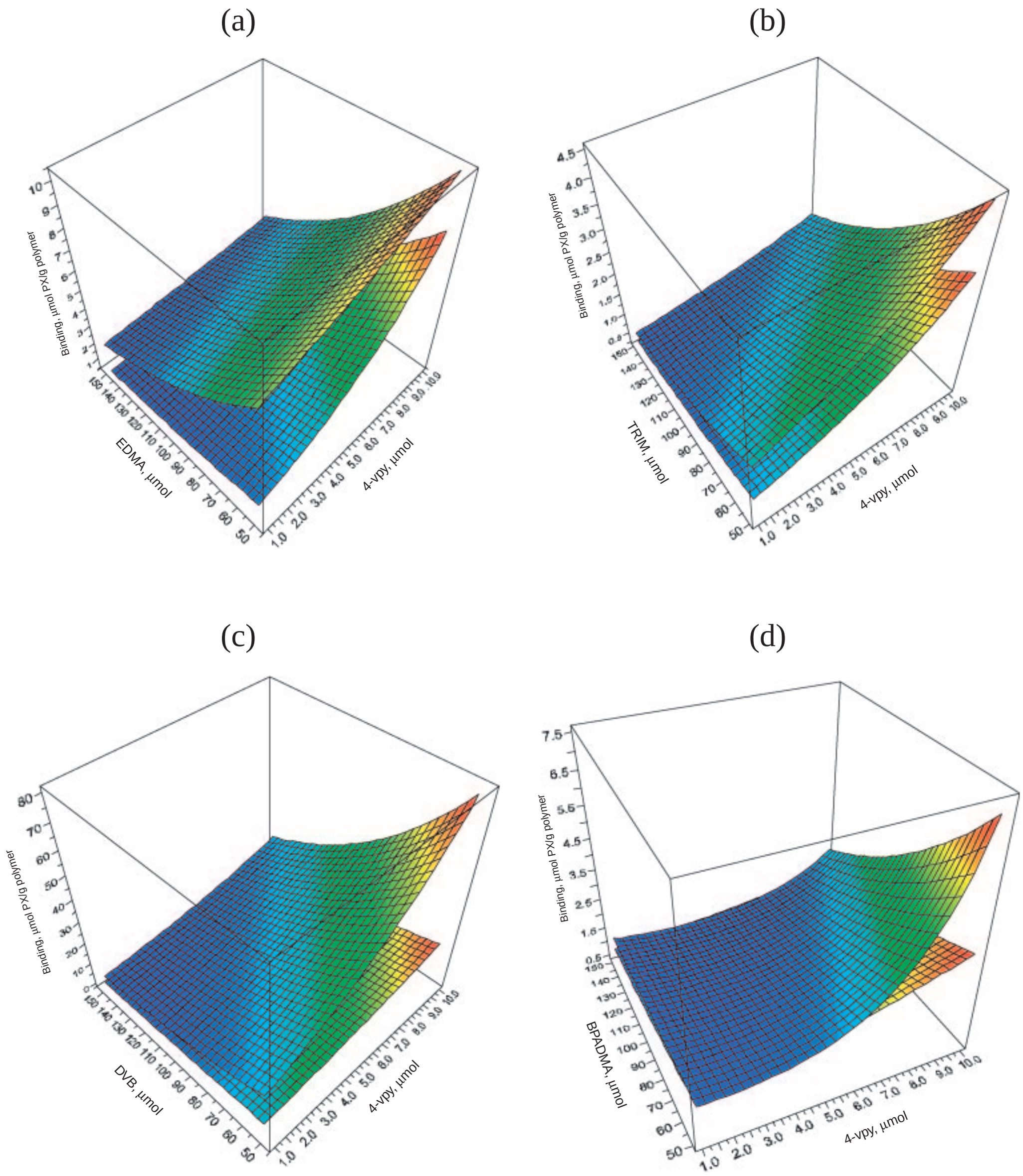

Fig. 5. Surface plots showing the multivariate models for each library. (a) 4-vpy/EDMA; (b) 4-vpy/TRIM; (c) 4-vpy/DVB; (d) 4-vpy/BPADMA.

the imprinting process was evaluated using an experimental design and multivariate analysis for several polymer libraries. The results were represented in terms of an empirical model, which facilitated their interpretation. The multivariate models show a strong correlation between the factors selected in the experimental design and the binding capacity values of the MIPs and blank polymers. All of the factors considered in the experimental design have the same influence both on the MIP and the blank polymers. The amount of 4-vpy and the cross-linker showed a large influence on the polymer capacity for the template, being the highest when these factors have similar values. The type of cross-linker showed a significant influence on the specific binding capacity of the polymers; DVB was the cross-linker with the highest uptake of the template. An enhancement in the binding capacity with this cross-linker may be attributed to the structural similarity with the functional monomer (4-vpy), which can promote positive π–π interactions with the template to afford molecular recognition. Regarding the other factors considered in the experimental design, they show only a small influence on the binding capacity of the polymers, except in the 4-vpy/DVB MIP library.

The combination of an in situ molecular imprinting employing a liquid-handling instrument and the chemometric procedure has been shown to be a useful tool to speed up and reproduce the preparation of the MIPs while providing some insight on the interactions between the factors that have an important effect in the MIP performance. Taking into account the large number of parameters that influence the selectivity, affinity, capacity, and kinetics of the template rebinding to the polymers, the described procedure allows a rapid identification of the important factors for preparing MIPs with high specific binding for a particular target compound, and it will be a valuable general tool for future developments of MIP materials.

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