Recognition Directed Site-Selective Chemical Modification of Molecularly Imprinted Polymers

Robert J. Umpleby II, Gregory T. Rushton, Ripal N. Shah, Andrew M. Rampey, Jessica C. Bradshaw, John K. Berch, Jr., and Ken D. Shimizu*

Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208

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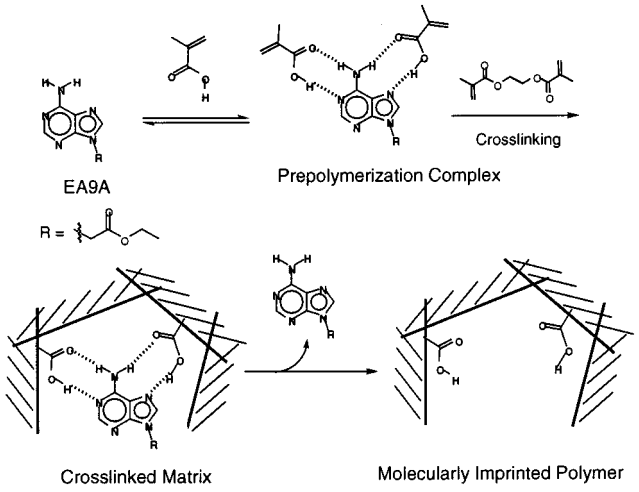
ABSTRACT: Demonstrated is the site-selective chemical modification (SSCM) of molecularly imprinted polymers (MIPs). In this strategy, MIPs are selectively chemically modified to improve the ratio of high-affinity to low-affinity binding sites and therefore the overall binding characteristics of the material. This was accomplished by preferentially eliminating the low-affinity binding sites by esterification with diazomethane or phenyldiazomethane. Selectivity in the esterification reaction was achieved using a guest molecule as an in situ protecting group that preferentially shields the high-affinity sites and leaves the low-affinity sites exposed toward reaction. The corresponding shifts in the populations of high- and low-affinity sites were quantified using affinity distribution analysis, which quantitatively measures the heterogeneous distribution of binding sites in MIPs as number of binding sites (N) with respect to binding affinity (K). Using affinity distribution analysis, the SSCM strategy was shown to improve the percentage of high-affinity sites in a methacrylic acid (MAA)/ethylene glycol dimethacrylate (EGDMA) matrix, imprinted with ethyl adenine-9-acetate (EA9A) in acetonitrile. The effects of different solvents and concentrations of guest molecule on the SSCM also were examined. The greatest improvements due to SSCM were observed when carried out in the imprinting solvent (acetonitrile). The demonstrated SSCM methodology is complementary to existing strategies for improving MIPs and thus can be utilized in tandem to improve the binding characteristics of MIPs.

Introduction

Molecularly imprinted polymers (MIPs) are synthetic materials that can be tailored with affinity and selectivity for a particular guest molecule.[1−4] MIPs are highly cross-linked matrixes formed in the presence of template molecules that are either covalently or noncovalently linked to functional monomers in a prepolymerization complex (Scheme 1). Upon removal of the template, a binding cavity is formed, lined with complementary functionality (usually carboxylic acids). MIPs can be rapidly and inexpensively synthesized and can be tailored with selectivity for almost any molecule of interest. In addition, MIPs demonstrate excellent thermal and chemical stability. These attributes compare favorably with other strategies for producing materials capable of molecular recognition including synthetic antibodies[5] and rationally designed small molecule receptors.[6] Consequently, MIPs have been utilized in a wide range of applications requiring binding and selectivity including as catalysts,[7] enzyme models,[8] chemical sensors,[9] and supports for enantioselective separations.[10]

A characteristic that has limited the utility of MIPs is heterogeneity. MIPs contain binding sites possessing a wide variation in structure, affinity, and selectivity. Thus, MIPs have often been compared to polyclonal antibodies. This heterogeneity diminishes the abilities of MIPs in almost every application, producing nonlinear response curves in sensors[9] and broad asymmetric peaks in chromatographic separations.[11] Binding site heterogeneity in MIPs arises from the low fidelity of the imprinting process which typically yields a much higher percentage of nonselective low-affinity sites as opposed to the desired high-affinity sites. This is particularly problematic for the most common imprinting method in which the prepolymerization complexes are held together by weak noncovalent interactions (Scheme 1). The majority of strategies for reducing heterogeneity have focused on optimizing the imprinting process, leading to marked improvements in the binding characteristics of MIPs.[12,13] However, noncovalent MIPs still display considerable binding-site heterogeneity, On the other hand, only a few of examples have been reported in which the binding properties of MIPs are enhanced by chemical modification.[14−16] We present, herein, an example of this strategy in which MIPs are selectively chemically modified (SSCM) after the polymerization process. The selectivity of the SSCM reaction arises from using the template molecule as an in situ protecting group for the high affinity sites, leaving the low-affinity

Scheme 1. Schematic Representation of the Imprinting Process



* Corresponding author. Tel (803) 777-6523; FAX (803) 777-9521; E-mail shimizu@mail.chem.sc.edu.

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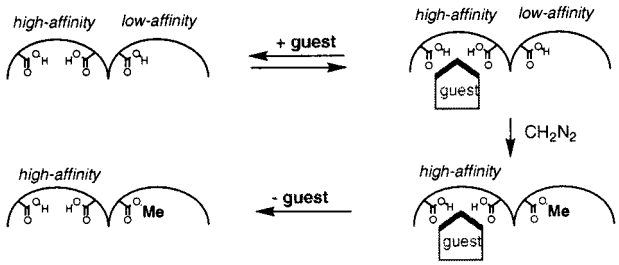
Scheme 2. Schematic Representation of the Site-Selective Chemical Modification Strategy[a]



[a] The guest molecule acts as a protecting group for highest affinity binding sites, enabling specific esterification and inactivation of the lower affinity sites.

sites exposed for chemical modification which "knocks out" their binding affinity.

The SSCM strategy is outlined below (Scheme 2). A heterogeneous MIP is first equilibrated with guest molecules that preferentially occupy the high-affinity sites. The exposed low-affinity sites are then selectively inactivated by esterification of the hydrogen-bonding carboxylic acid functionalities. The resulting polymer should have a higher percentage of high-affinity sites and thus improved recognition characteristics in terms of both heterogeneity and average binding affinity. The overall process is akin to a kinetic resolution process in which the high- and low-affinity sites are differentiated on the basis of differences in reactivity.

Despite the potential of site-selective chemical modification and its complementarity to traditional approaches,[3] only a single report has emerged.[15] The difficulty lies not in the application of SSCM but in the ability to observe the expected changes in the distribution of binding sites. Most analyses have modeled MIPs as homogeneous surfaces, grouping all the binding sites into one (Langmuir) or two (bi-Langmuir) general classes.[17,18] These homogeneous models do not readily allow comparison of changes in the distributions that are expected from the SSCM strategy. For example, the most commonly applied analysis of MIPs is the limiting slopes analysis of curved Scatchard plots, which is a form of the bi-Langmuir model.[12,19] This analysis is highly concentration dependent, yielding a different set of binding properties (association constants (K) and numbers of binding sites (N)) for each concentration window examined.[20] Hence, the comparison of binding properties of different or even of the same MIP by homogeneous models is difficult. [20,21]

A possible solution presented itself in our recent measurements of affinity distributions of MIPs. This analysis treats MIPs as heterogeneous surfaces composed of infinite classes of binding sites.[20] For example, a noncovalently imprinted polymer was shown to have an overall exponentially decaying affinity distribution (Figure 1), with the undesirable low-affinity sites being the most prevalent.[22] This quantitative distribution is consistent with other measures of heterogeneity in MIPs[19,23−25] and helps explain the detrimental effects of heterogeneity on the binding properties of MIPs. The small percentage of high-affinity sites becomes quickly saturated, and the binding properties of MIPs become dominated by the more prevalent low-affinity sites. Demonstrated, herein, is that the affinity distribution is an efficient quantitative analytical tool that enables the optimization of the binding properties of MIPs via the site-selective chemical modification strategy.

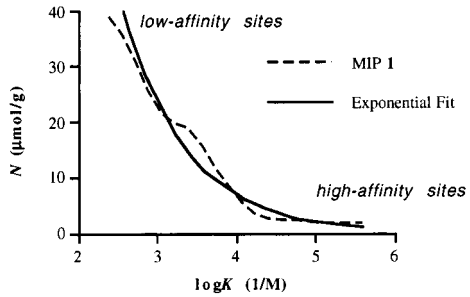


Figure 1. Affinity distribution for MIP1 with respect to EA9A in acetonitrile.

Experimental Section

Instrumentation. UV measurements were taken on a Beckman DU-7 spectrophotometer.

General Procedures. Ethyl adenine-9-acetate (EA9A) was utilized as obtained from Aldrich Chemicals. Ethylene glycol dimethacrylate (EGDMA, Aldrich) was dissolved in ether and washed twice with aqueous 1 M NaOH solution and once with aqueous saturated NaCl solution to remove the inhibitor. After drying with anhydrous $MgSO_4$, evaporation of the ether afforded the pure monomer. Methacrylic acid (MAA, Aldrich) was distilled (5 mmHg, 0 °C). Azo(isobutryonitrile) (AIBN, Aldrich) was recrystallized from methanol. Acetonitrile was obtained from Fischer Scientific. Reaction mixtures were degassed using a Branson ultrasonic cleaner. Particles were sized with standard testing sieves (VWR Scientific). Solutions of diazomethane[26] in diethyl ether and phenyldiazomethane[27] in toluene were synthesized as previously described. (CAUTION: diazomethane and phenyldiazomethane are shock-sensitive materials that should be handled with care. For safe handling procedures see ref 26.)

General Procedure for the Preparation of MIP1. The synthesis of MIP1 was adapted from Shea et al.[12,20] To a solution of EA9A (354 mg, 1.6 mmol) and AIBN (263 mg, 1.6 mmol) in acetonitrile (37.2 mL) was added MAA (1.6 mL, 19.2 mmol) and EGDMA (25.7 mL, 136 mmol). The reaction mixture was sonicated under nitrogen for 15 min to remove dissolved oxygen gas. The polymerization was initiated photochemically by a Hanovia medium-pressure 450 W mercury arc UV lamp at 0° and allowed to proceed for 8 h. The polymerization chamber was turned 180° after 20 min, 40 min, and 4 h of reaction time. The polymer was ground with a Braun coffee grinder, and particles in the size range 38−150 μm were collected. To further remove the fine particulates, the particles were suspended in methanol. Particles not settling within 5 min were discarded. The polymers were Soxhlet extracted with methanol/acetonitrile (1:4) and dried under vacuum.

General Procedure for Batch Rebinding Studies. Ten different solutions of EA9A in acetonitrile spanning the concentration range 0.06−2.5 mM were prepared. An aliquot of 5 mL of each solution was added to 125 mg of polymer in screw-cap vials. The vials were shaken for 2 h and centrifuged. UV measurements were taken at 255 and 276 nm on the supernatants. From the observed absorbance values was subtracted the absorbance value for a blank sample, which was prepared by shaking a solution of acetonitrile (5 mL) containing no EA9A with the polymer (125 g). This absorbance (A_F) corresponds to the absorbance of free (unbound) EA9A in solution. The free concentration (F) was calculated as $F = A_F T/A_T$, where T and A_T are the concentration and absorbance of the stock solution, respectively. The bound concentration (B) of EA9A was calculated as $B = T - F$.

Typical Procedure for the Chemical Modification of MIP1. To a 250 mL round-bottom flask containing MIP1 (2.0 g) and 73 mg (0.33 mmol) of ethyl adenine-9-acetate in acetonitrile (110 mL) was added either diazomethane in diethyl ether (1.02 mL of a 0.64 M solution), phenyldiazomethane in toluene (3.2 mL of a 2.0 M solution), or TBAF (1.9 mL, 1.0 M solution in THF) and MeI (58 μL, 0.92 mmol).

The mixture was shaken for 2 h on a Burrell wrist action shaker. In the case of the esterifications with MeI, the reagents were removed by extraction with 5% AcOH in MeOH and then with MeOH (five times). In the case of the diazomethane esterifications, polymer was extracted six times with MeOH. Evaporation in vacuo yielded the derivatized polymer. The procedure was repeated with the above esterification conditions in the absence of the template molecule to yield the control polymers.

Measurement of Splitting Yields. The splitting yield for MIP1 was measured by grinding a known amount of polymer synthesized as described above in a mortar and pestle. The entire amount was transferred to a Soxhlet thimble and extracted with a methanol/acetonitrile (1:4) overnight. The solution was then filtered, concentrated down under vacuum, and taken up in 50 mL of acetonitrile. The concentration of the solution was measured using HPLC (silica column, ^{i}PrOH/diethylamine/chloroform (19.8:0.2:80)) which was calibrated using EA9A stock solutions.

Affinity Distribution Analysis. Binding isotherms (bound (B) vs free (F) guest concentrations) were measured for batch rebinding studies of the MIPs by equilibrating varying concentrations of EA9A (0.05−2.5 mM) with the MIP (125 mg) in acetonitrile (5.0 mL). The MIP was settled by centrifugation, and the free concentration of EA9A in solution was measured by UV spectroscopy (at 255 and 276 nm). From the experimental binding isotherms, the affinity distribution was extracted utilizing a numerical approximation method (eq 1) described by Hunston et al. and described previously for MIPs.[20,28]

$$N = \left| \frac{B_1 - B_2}{2 \log a} - \frac{a((B_3 - B_4) - 2(B_1 - B_2))}{2(a-1)^2 \log a} \right| \qquad (1)$$

where N = number of binding sites, a = constant $10^{0.2}$, B_1 = interpolation of B at $F = a/K$, B_2 = interpolation of B at $F = 1/aK$, B_3 = interpolation of B at $F = a^2/K$, and B_4 = interpolation of B at $F = 1/a^2K$. Equation 1 calculates the number of binding sites (N) for each class of sites having association constant K. This requires the interpolated values for B (i.e., B_1, B_2, B_3, and B_4) from the experimental binding isotherm at specific values of F that are calculated on the basis of the particular value of K (see legend for eq 1 above). The above approximation is applied by selecting a series of values for K within the range $K = 1/F_{min}$ to $1/F_{max}$. Any set of values for K can be chosen to map out the relationship between N and K. Typically, 20 values of K were selected that are spaced out logarithmically over the K range. Values of N are then calculated for each value of K.

For the MIPs in this study, interpolations of the experimental binding isotherms were greatly accelerated by fitting them to a Freundlich isotherm.[29] The Freundlich isotherm relates the concentration of bound guest [B] to the concentration of free guest [F] by the equation ($[B] = a[F]^m$), where a and m are fitting coefficients. The suitability of the Freundlich isotherm has been previously demonstrated for MIP1 and was also confirmed for esterified polymers by linear log−log plots of the binding isotherms.[22,30]

Results

A well-studied adenine selective system (MIP1) was chosen in which to evaluate the site-selective chemical modification (SSCM) strategy. MIP1 is comprised of the most common MIP matrix of methacrylic acid (MAA)/ethylene glycol dimethacrylate (EGDMA). Hence, successful implementation of the SSCM strategy on MIP1 would have the potential for improving the majority of MIPs. MIP1 is analogous to an adenine selective system optimized by Shea et al. and extensively characterized.[12] We have substituted ethyl adenine-9-acetate (EA9A) for 9-ethyladenine as the template molecule.

Characterization of the resulting MIP1 gave results analogous to those reported by Shea for a 9-ethyladenine imprinted polymer. The imprinting effect was verified by comparing the rebinding of the template (EA9A) in acetonitrile of MIP1 with a blank polymer (BP1) synthesized under identical conditions but in the absence of template (see Table 1). An overlay of the two binding isotherms demonstrates the greater affinity and capacity of the imprinted matrix (Figure 2). A Scatchard plot of the binding isotherm of MIP1 yielded the expected upwardly curved Scatchard plot, confirming both the binding ability and the heterogeneity of the polymer. Finally, splitting yields were measured to find the amount of template retained in the MIP during the polymerization process. Exhaustive extraction of the polymer with MeOH/acetonitrile recovered 95% of the template molecule used in the polymerization process. Again, this value is identical to that reported by Shea for the similar 9-ethyladenine imprinted polymer.

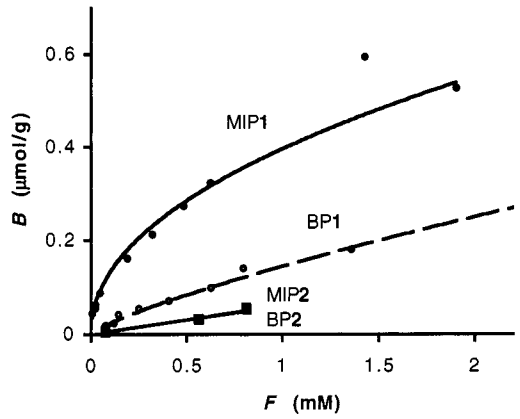


Figure 2. Overlays of the binding isotherms with respect to EA9A in CH_3CN for methacrylic acid (MAA) MIP1 and the corresponding blank polymer (BP1) in comparison to an MIP synthesized using methyl methacrylate (MIP2) and the corresponding blank polymer (BP2). The isotherms for MIP2 and BP2 are indistinguishable on the above graph.

Table 1. Synthesis Conditions of Imprinted and Blank Polymers

polymer	template	polymer matrix
MIP1	EA9A	MAA/EGDMA
BP1		MAA/EGDMA
MIP2	EA9A	MMA/EGDMA
BP2		MMA/EGDMA

Affinity distributions calculated from the binding isotherm of MIP1 showed an exponentially decaying population of binding sites with respect to binding affinity (Figure 1). This suggested that the corresponding binding isotherm could be fit to a Freundlich isotherm which is known to characterize the binding behavior of systems that have an exponential distribution of binding sites.[29,31,32] Indeed, it was found that the binding isotherm (bound (B) vs free (F) guest) of MIP1 and all the subsequent chemically modified variants of MIP1 could be fit to a Freundlich isotherm. The use of the exponential model significantly accelerated the calculation of the corresponding affinity distributions (see Experimental Section).[22]

Binding sites in MIP1 were inactivated via esterification of the carboxylic acids in the MAA/EGDMA matrix.[33] Esterification eliminates the hydrogen-bonding interactions between the polymer matrix and the template molecule. The effectiveness of esterification in eliminating binding sites was assessed first by comparing the binding abilities of an MIP containing methacrylic acid (MIP1) as a functional monomer with a

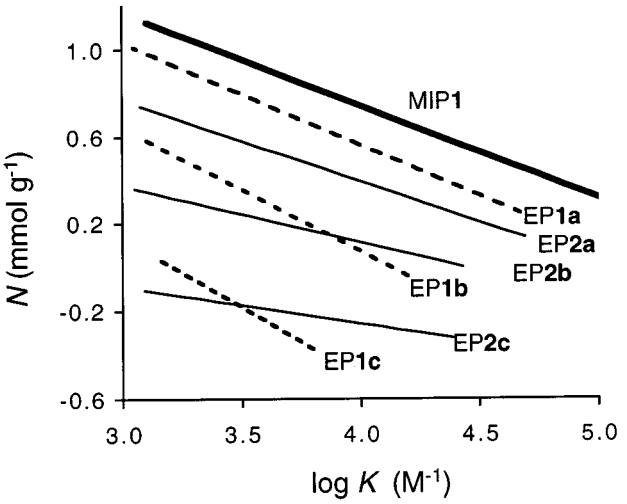


Figure 3. Comparisons of the affinity distribution spectra with respect to EA9A in acetonitrile for MIP1, and polymers esterified with increasing concentrations of diazomethane in the absence (EP1a, b, and c) and presence (EP2a, b, and c) of 3 mM EA9A.

Table 2. Reaction Conditions for Esterified Polymers (EP) Synthesized from MIP1

polymer	reagent(s)	template	solvent
EP1	CH_2N_2		CH_3CN
EP2	CH_2N_2	EA9A (3 mM)	CH_3CN
EP3	$PhCHN_2$		CH_3CN
EP4	$PhCHN_2$	EA9A (3 mM)	CH_3CN
EP5	$PhCHN_2$		$CHCl_3$
EP6	$PhCHN_2$	EA9A (3 mM)	$CHCl_3$
EP7	$PhCHN_2$		Et_2O
EP8	$PhCHN_2$	EA9A (3 mM)	Et_2O
EP9	$PhCHN_2$	EA9A (12 mM)	CH_3CN
EP10	MeI/TBAF		CH_3CN
EP11	MeI/TBAF	EA9A (3 mM)	CH_3CN

methyl methacrylate containing MIP2 (Figure 2). The methyl methacrylate containing polymer has significantly lower affinity for the guest molecule (EA9A) as shown by the much lower uptake of EA9A. In addition, the MMA matrix is unable to retain an imprint of the template molecule (EA9A), as the binding isotherms of the polymers synthesized in the presence (MIP2) and absence (BP2) of template were experimentally indistinguishable.

Esterifications of MIP1 with diazomethane had a similar effect of eliminating binding sites in the polymer. For example, treatment of MIP1 with increasing concentrations of diazomethane in acetonitrile yielded esterified polymers, EP1a, EP1b, and EP1c, respectively (see Table 2). Comparisons of the affinity distributions of the esterified polymers demonstrated a diminishing affinity for EA9A with increasing levels of esterification (Figure 3). Selectivity in the esterification reaction was achieved by performing the reaction in the presence of the template molecule (3 mM, EA9A), yielding EP2a, EP2b, and EP2c. The precise amounts of diazomethane added to each of these polymers were difficult to estimate due to the inaccuracies in estimating the concentration of the diazomethane solutions in ether, the volatility of diazomethane, and the inhibition of the reaction by the guest. In addition, it was found from control reactions that diazomethane will react slowly with EA9A. Therefore, the degree of esterification in these polymers was better assessed by measuring their binding capacities with the guest molecules. Polymers

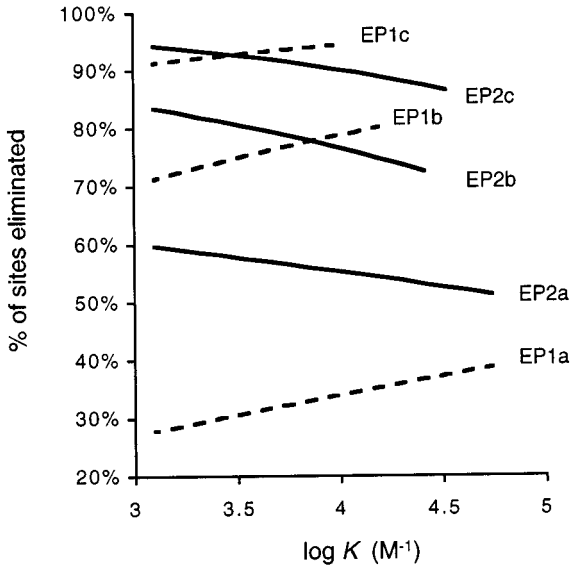


Figure 4. Percentages of binding sites eliminated by esterification relative to the unesterified MIP1, for esterification with diazomethane in the absence (EP1a, b, and c) and presence (EP2a, b, and c) of 3 mM EA9A.

with higher degrees of esterification showed lower affinity for the guest and vice versa.

The resulting affinity distributions with and without guest (EP1 and EP2) were overlaid for comparison (Figure 3). The straight lines in the log N vs log K format of the affinity distributions are a consequence of the underlying exponential distribution of binding sites in these polymers. This format is particularly informative because the relative slopes of the distribution curves can be correlated to the selectivity of the esterification reaction. For example, parallel lines would describe a situation in which the esterification reaction occurred with similar selectivity at each association constant. In contrast, convergent lines correspond to a selective reaction in which the classes of sites in the region where the lines converge have been preferentially protected.

Comparison of the affinity distributions for control polymers EP1 with MIP1 demonstrated that treatment with increasing concentrations of diazomethane effectively eliminated increasing numbers of binding sites, thereby reducing the overall binding ability of the resulting polymers (Figure 3). This is apparent from the diminishing areas under the affinity distribution curves from EP1a to EP1c.[33] The selectivity of the esterification reaction was similar for all three control polymers synthesized in the absence of template molecule. The affinity distributions show a slight degree of divergence at higher association constants, suggesting that the control reaction preferentially eliminates the more desirable higher affinity sites. Qualitative conformation is provided by calculation of the percent of sites eliminated with respect to binding affinity (Figure 4).[34] Surprisingly, diazomethane appears to be approximately 10% more effective at eliminating high-affinity sites than low-affinity sites. One explanation is that the esterification reaction is not intrinsically selective for the high-affinity sites. Instead, the apparent selectivity arises from a shift in the distribution of sites toward lower affinity. Esterification of high-affinity sites not only eliminates high-affinity sites but also generates new low-affinity sites. This leads to an overall population shift to lower affinity.

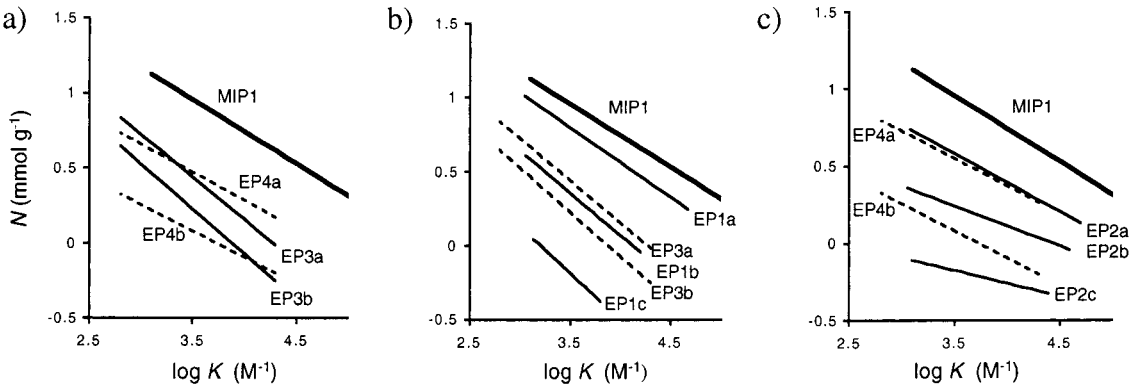


Figure 5. Comparisons of the affinity distribution with respect to EA9A in CH_3CN of MIP1 esterified in acetonitrile (a) with phenyldiazomethane in the presence (EP4) and absence (EP3) of 3 mM EA9A, (b) with phenyldiazomethane (EP3) and diazomethane (EP1) in the absence of EA9A, and (c) with phenyldiazomethane (EP4) and diazomethane (EP2) in the presence of 3 mM EA9A.

The esterification reactions carried out in saturated solutions of template molecule (EA9A, 3.0 mM) yielded EP2. Approximately 10−50% more diazomethane was required to achieve the same levels of esterification as in the control experiments, which was suggestive of the ability of the template molecule to protect binding sites. The presence of EA9A clearly changes the selectivity pattern of the esterification reaction when compared to control EP1 (Figure 3). All three template protected polymers (EP2a, b, and c) have very different slopes from the corresponding control polymers (EP1a, b, and c). The flatter slopes of EP2 imply that a greater percentage of high-affinity sites have been retained in the presence of template, while at the same time, more low-affinity sites were eliminated. Again, these observations are supported by the calculation of the percent of sites eliminated with respect to binding affinity (Figure 4). Therefore, the apparent selectivity of the esterification reaction for the high-affinity sites in the control polymers (EP1) is reversed in the presence of guest molecule (EP2).

The above changes in selectivity are consistent with the ability of the template molecule to act as a noncovalent protecting group for the high-affinity sites. The observed changes in selectivity, though modest, are significantly outside of the error margin of the affinity distribution analysis, which was estimated as ±10−20% in the number of sites based on multiple measurements on the same polymer. The magnitude of the selectivity difference is perhaps not surprising considering that the SSCM strategy runs counter to statistics. The template molecule must find and protect the few high-affinity sites among an exponential excess of low-affinity sites.

The SSCM procedure was next repeated using phenyldiazomethane. Identical trends were observed for SSCM carried out in phenyldiazomethane (EP3 and EP4) and compared with polymers synthesized with diazomethane (Figure 5a). Again, in the absence of template molecule (EP3), the benzylation reaction proceeds with similar selectivity to the methylation reaction. In contrast, in the presence of template molecule (EP4), the esterification reaction proceeds selectively such that the lower affinity sites are eliminated in preference to the higher affinity sites.

The selectivity of the esterification reaction with phenyldiazomethane was compared with that of diazomethane in the absence and presence of template (Figure 5b,c). In the absence of template molecule (Figure 5b), the esterification reactions using either reagent proceeded with similar selectivity as seen by the similar slopes of EP3 and EP1. The two reagents are almost identical in reactivity despite the much lower reactivity of phenyldiazomethane as found from control reactions. Apparently, the kinetic and size difference between the diazomethane and phenyldiazomethane are insufficient to significantly alter the selectivity of the reagent for the MIP matrix as both are equally able to access a similar range of binding sites.

Similarly, in the presence of template molecule (Figure 5c) both the phenyldiazomethane (EP4) and diazomethane (EP2) esterified polymers yield similar selectivities. The only deviation is at high levels of esterification, where the slope of the diazomethane polymer (EP2c) is flatter than that of the corresponding phenyldiazomethane polymer (EP4). However, overall the esterifications carried out with phenyldiazomethane gave very similar trends to that initially observed with diazomethane. The subsequent reactions were all carried out using phenyldiazomethane, as it is much easier to synthesize, handle, and store. Solutions of phenyldiazomethane in ether could be stored for several weeks in the freezer, whereas diazomethane rapidly decomposed and had to be constantly remade for each study. In addition, phenyldiazomethane is considerably less shock sensitive and hence was much easier and safer to handle.

Next, the solvent dependence of the SSCM reaction was studied. Comparisons were made between esterification reactions with phenyldiazomethane carried out in three different solvents: acetonitrile (EP3 and EP4), chloroform (EP5 and EP6), and diethyl ether (EP7 and EP8). We expected that the esterification reaction in the presence of template molecule would become more selective in the less polar solvents chloroform and diethyl ether.

First, the polymer modified in chloroform was examined (Figure 6). Again, the polymers esterified in the presence of template (EP6) show enhanced affinity for EA9A as compared to a polymer esterified in the absence (EP5) of template (Figure 6a). The selectivity of the esterification reaction in chloroform was compared to that in acetonitrile (Figure 6b). In the absence of template, the esterification reactions in chloroform (EP5) were found to be comparable to those in acetonitrile (EP3), as the affinity distributions of both sets of polymers had similar slopes. Similarly, in the presence of template molecule (Figure 6c), the esterifications in chloroform (EP6) displayed similar selectivity as polymers synthesized under similar conditions in acetonitrile (EP4). Overall, the MIP modified in chloroform was

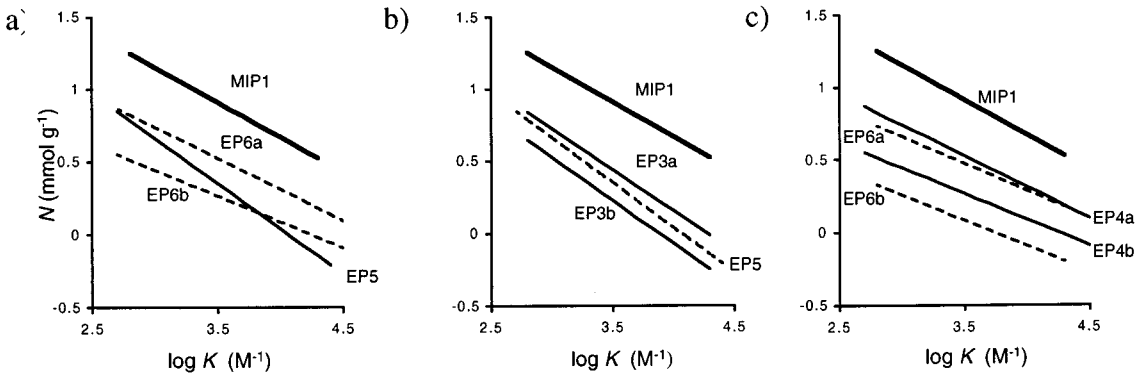


Figure 6. Comparisons of the affinity distributions with respect to EA9A in CH_3CN of polymers synthesized by the esterification of MIP1 with phenyldiazomethane: (a) in $CHCl_3$ in the absence (EP5) and presence (EP6) of 3 mM EA9A, (b) in absence of template molecule in CH_3CN (EP3) and in $CHCl_3$ (EP5), and (c) in the presence of 3 mM EA9A in CH_3CN (EP4) and in $CHCl_3$ (EP6).

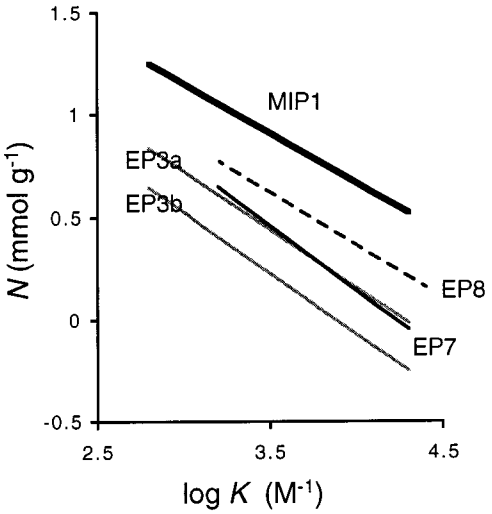


Figure 7. Comparisons of the affinity distribution spectra with respect to EA9A in CH_3CN of MIP1 esterified with phenyldiazomethane in diethyl ether in the presence (EP8) and absence (EP7) of 3 mM EA9A and polymers esterified in the CH_3CN in the absence of template (EP3).

within experimental error identical to the acetonitrile polymer.

The next solvent examined was diethyl ether. The esterification of MIP1 was carried out in the absence (EP7) and presence (EP8) of template using phenyldiazomethane in diethyl ether (Figure 7). Unlike the polymers synthesized in chloroform and acetonitrile, the effect of the template molecule on the selectivity of the esterification reaction was not readily discernible as evidenced by the similar slopes of the affinity distributions of EP7 and EP8. Overall, the reactions in diethyl ether yielded no significant improvement in selectivity in comparison to the original unesterified polymer MIP1. This can be seen by the similarity in affinity distributions of both EP7 and EP8 to those of polymer synthesized in CH_3CN in the absence of template (EP3). Therefore, the reactions in diethyl ether were not explored any further.

The observed trend (acetonitrile $\approx$ chloroform $\gg$ diethyl ether) is rationalized in two ways. In the case of MIP1, the primary interaction between the polymer matrix and the guest are hydrogen-bonding interactions; thus, the ability of different solvents to augment or disrupt these interactions is of interest. Indeed, solvent polarity is often not the best predictor of the affinity of guests for MIPs.[33] In the case of SSCM, better correlation is seen with the Kamlet and Taft solvatochromic parameter β which is a measure of the hydrogen-bond donor ability of a solvent.[35] Diethyl ether has the best hydrogen-bonding ability ($\beta = 0.47$) and thus disrupts the interactions of the template with the carboxylic acid groups of the MIP, minimizing the effect of the template on the selectivity of the SSCM reaction. The Kamlet–Taft parameters predict that acetonitrile will be considerably better than chloroform ($\beta = 0.31$ vs $\beta = 0.00$). However, our studies find that acetonitrile and chloroform yield very similar results. This can be rationalized in light of a similar trend observed in MIPs in which imprinted polymers show the highest selectivity when measured in the same solvent in which it was imprinted. This "common solvent effect" has often been found to overcome the normal polarity trends.[12,36] In the case of MIP1, the polymer was imprinted in acetonitrile, and thus the highest selectivity for the SSCM reaction is also expected in acetonitrile.

Next, the effects of the concentration of template molecule present during the SSCM reaction were examined. We expected that the esterification reaction might be more selective when carried out under higher concentrations of template molecule because the binding cavities would have a higher occupancy and would be better protected from esterification. Therefore, MIP1 was esterified with phenyldiazomethane in the presence of 12 mM EA9A in acetonitrile (EP9) and compared to the 3 mM EA9A (EP4) in the previous studies. Indeed, in the presence of 12 mM EA9A, the esterification reaction proceeded to a lesser extent as measured by the rebinding experiments with EA9A. However, the selectivity as judged by the slope of the corresponding affinity distribution (not shown) of the resulting polymer (EP9) was within experimental error of the polymer esterified in the presence of 3 mM EA9A (EP4). The lack of change in the selectivity of the esterification reaction on increasing the template concentration was particularly surprising as 3 mM and even 12 mM EA9A are far from the saturation conditions for MIP1. A possible explanation is that only a subset of the binding sites in MIP1, most likely the higher affinity sites, are influenced by the presence of the template molecule during the esterification reaction, and at 3 mM EA9A these sites are already saturated and the addition of further EA9A serves to further protect more sites but not selectively.

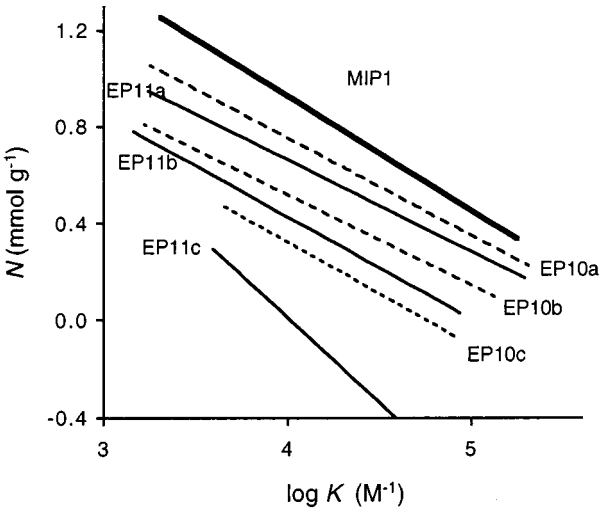


Figure 8. Comparisons of the affinity distribution spectra with respect to EA9A in acetonitrile for polymers esterified with increasing concentrations of MeI/TBAF in the absence (EP10a, b, and c) and presence (EP11a, b, and c) of 3 mM EA9A.

Finally, the importance of the interactions of the template (EA9A) with the polymer in inducing selectivity was provided by examination of polymers synthesized using a more polar set of esterification reagents (MeI/TBAF). Esterification of MIP1 with increasing concentrations of MeI/TBAF in the absence (EP10) of template again led to the successive elimination of binding sites (Figure 8). However, unlike the diazomethane polymers, esterifications with MeI/TBAF carried out in the presence of EA9A (EP11) show no changes in selectivity. The affinity distributions for EP10 and EP11 are roughly parallel and hence have similar ratios of high- and low-affinity sites. Possibly, the highly polar and basic TBAF salt effectively competes with EA9A for the carboxylic acid binding sites, diminishing the ability of the template to act as a selective protecting group.[37] Thus, the inability of the template molecule to change the selectivity of the reaction performed under more competitive conditions is consistent with the necessity of good template/polymer interactions. Interestingly, these esterification conditions (MeI, TBAF) are similar to those successfully used by McNiven et al. (MeI, DBU) to selectively chemically modify MIPs and enhance binding selectivity in their steroid binding MIPs.[15] The differences in the success of these conditions may be due to the different systems studied or in the methods used to analyze the subsequent changes in the binding properties.

Conclusion

We have demonstrated that MIPs can be site-selectively chemically modified, leading to polymers with a higher percentage of high-affinity sites and therefore enhanced overall affinity. The strategy seeks to selectively eliminate the less desirable low-affinity sites by selectively protecting the high-affinity sites with the template molecule. Treatment of the matrix with diazomethane or phenyldiazomethane then preferentially reacts with the low-affinity sites, knocking out their binding ability. The application and optimization of this strategy are enabled by the ability to quantitatively visualize the heterogeneity present in MIPs and measure changes in the number of high- and low-affinity binding sites. The SSCM strategy is complementary to existing imprinting methodologies and serves to further enhance the binding properties of MIPs. The overall chemical modification process is easily and rapidly executed and coupled with molecular imprinting is a particularly efficient route to tailored receptors or selective supports, especially when compared with other methodologies such as eliciting antibodies or designing synthetic molecular receptors.

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