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Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications-model case: Adsorbent for abacavir

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Abstract

This paper reports the development of selective polymers with high binding capacity suitable for large scale solid-phase extraction (SPE), e.g. for industrial applications. The technology of molecular imprinting was employed in the synthesis of selective molecularly imprinted polymers (MIPs). Abacavir, which is a HIV-1 reverse transcriptase inhibitor, was chosen as the target analyte. An already established computational protocol, developed in our group, was employed to select the best monomers leading to polymers with high binding capacity for the target compound. Three different monomer compositions were chosen for the synthesis. The synthesised materials were then tested for the rebinding of abacavir in solid-phase extraction using several different conditions (buffered/non-buffered solutions and in the presence/absence of organic solvents). The best MIP showed a surprisingly high binding capacity, up to 157 mg of drug/g of adsorbent. The high binding capacity could make this polymer suitable for industrial applications to purify and/or concentrate the drug during its production.

Keywords: Molecularly imprinted polymers; Solid-phase extraction; Abacavir; Binding capacity

1. Introduction

Solid-phase extraction is an analytical tool which is continuously growing in importance as a robust analytical method for sample preparation. SPE in various formats is currently a routine technique employed in numerous pharmaceutical, environmental and bioanalytical applications [1]. Separation for most of the current SPE materials is based on physiochemical retention on the functionalised surface. However typical SPE adsorbents lack selectivity and this constitutes a problem when a selective extraction from a complex matrix has to be performed. Therefore, considerable effort is often expended in the search for more selective SPE adsorbents. An enhancement of the molecular selectivity of SPE can be achieved using molecularly imprinted polymers (MIPs). MIPs are a class of smart materials with a pre-determined selectivity. This is achieved by the copolymerisation of a pre-organised complex, formed between functional monomers and the target molecule (the template), with an excess of cross-linker. The polymer selectivity, specificity and affinity are directly related to the strength of this complex. In this study our aim was the development of a polymer for SPE, which would possess high affinity and high binding capacity for the target analyte. A material with these properties would allow the development of a simple and straightforward SPE method, which could be easily scaled-up for industrial applications.

In order to obtain material with very high affinity, functional monomers able to give very strong complexes with the target analyte need to be chosen. In our group we have developed a computational design method, which enable us to select from a virtual library those monomers interacting strongly with the target analyte [2–5]. In this work abacavir was used as a model target analyte. Abacavir (1592U89) is a drug utilised in the treatment of the infection caused by the human immunodeficiency virus HIV-1, the causative agent of the acquired immunodefi-

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ciency syndrome (AIDS). This drug is a novel nucleoside reverse transcriptase inhibitor with antiretroviral activity against HIV-1 [6]. A few groups [7–10] have already reported the development of analytical methods to measure the concentration of abacavir in complex matrices such as urine and blood samples, using high performance liquid chromatography (HPLC) with UV detection. However all the reported methods based on chromatography techniques are expensive, time consuming and unsuitable for industrial applications. By contrast, the availability of MIPs, which could be used in the isolation, purification and pre-concentration of abacavir by SPE would be very appealing and ideally suited to industrial applications. Many reports of the successful application of MIP based SPE for the extraction and pre-concentration of drugs have already appeared in the literature. Most recently MIPs have been used for the extraction of local anaesthetics [1], naproxen [11], atropine [12], metformin [13] and pyrazinamide [14]. In most of these cases the MIP showed specific binding of the target analytes at very low concentrations even at the ppb level [15]. However, to the best of our knowledge, no report has yet demonstrated the development of MIPs which possessed sufficiently high binding capacity for industrial applications, e.g. for the extraction and purification of drugs from concentrated solutions. Whereas it is relatively easy to develop MIPs with good selectivity, the production of materials with very high binding capacity is not so straightforward. One of the pre-requisites to achieve this is by the rational selection of functional monomers strongly interacting with the target analyte. In this paper our computational method was used to develop MIPs with high affinity, which could lead to polymers with high binding capacity for abacavir. The MIPs were then synthesised and characterised in SPE experiments. As expected some of the polymers tested showed very high binding capacity for the target analyte. The best MIP developed here possessed the requirements necessary for industrial applications aimed at the extraction and purification of abacavir.

2. Material and methods

2.1. Material

Abacavir hemi-sulphate was kindly provided by Glaxo-SmithKline (Manufacturing Operation, Cork, Ireland). The monomers itaconic acid (IA), acrylamide and *N*,*N*-methylene-bisacrylamide (bisacrylamide), the cross-linker ethylene glycol dimethacrylate (EGDMA), the initiator 1,1′-azobis(cyclohexane-carbonitrile), the porogen *N*,*N*-dimethylformamide (DMF) and isopropanol (IPA) were purchased from Aldrich (Poole, Dorset, U.K.). The empty cartridges, filters and vacuum unit used for SPE experiments were from Supelco (Poole, Dorset, U.K.). All solvents were of analytical or HPLC grade and were used as received.

2.2. Computational design of high affinity polymers for abacavir

The computational design performed to optimise the composition of MIPs has been already extensively described elsewhere [2–5]. The workstation used to simulate monomer–template interactions was a Silicon Graphics Octane running IRIX 6.5 operating system. The workstation was configured with two 195 MHz reduced instruction set processors, 1GB memory and a 20GB fixed drive. The system was used to execute the software packages SYBYL 6.9™ (Tripos Inc., 1699 South Hanley Road, St. Louis, Missouri 63144, USA). Regarding the procedure, briefly, the structure of abacavir was drawn and its energy minimised both in vacuum (dielectric constant = 1) and in water (dielectric constant = 80) to get stable conformations in such extremely different environments. A virtual library containing 21 of the most commonly used monomers in molecular imprinting was also constructed and minimised. The LEAPFROG™ algorithm was used to screen the library of functional monomers for their possible interactions with the template. The monomers giving the strongest interactions with the template, minimised both in water and in vacuum, were identified and used for the synthesis of polymers using the general template–functional monomer molar ratio of 1:4. This ratio is a rather commonly used in non-covalent molecular imprinting [16].

2.3. Polymer synthesis

Due to solubility problems in most organic solvents the abacavir hemi-sulphate was converted into the free base by chloroform extraction of an aqueous solution after addition of NaOH. The resulting abacavir free base was then used as a template for the synthesis of MIPs. Three MIPs were synthesised in DMF. Acrylamide and itaconic acid, which were identified as the two best monomers, were used to synthesise two polymers (MIP2 and MIP3, respectively) with template–acrylamide and template–itaconic acid ratios of 1:4. The third MIP (MIP1), containing both acrylamide and bisacrylamide, was synthesised using a molar ratio of template–acrylamide–bisacrylamide of 1:2:2. The composition of the polymerisation mixtures of these three MIPs is reported in Table 1. A corresponding blank polymer, BP3 was also prepared from the same composition using the procedure employed for the MIP3, but in absence of template. For the synthesis of the polymers the oxygen was removed by purging the polymerisation mixtures with nitrogen and the polymerisation carried out by photochemical irradiation for

Table 1
Polymerisation mixtures of synthesised MIPs using abacavir free base as the template

Reagents	Quantity (mg)		
	MIP1	MIP2	MIP3
Abacavir free base	250	250	250
Bisacrylamide	135	–	–
Acrylamide	124	248	–
Itaconic acid	–	–	454
Cross-linker[a]	4490	4500	4300
Initiator[b]	50	50	50
DMF	5000	5000	5000

[a] Ethylene glycol dimethacrylate.
[b] 1,1′-azobis(cyclohexane-carbonitrile).

20 min using a Hönle 100 UV lamp (intensity 0.157 W cm^{-2}) (Hönle UV, UK). The polymers were ground and wet-sieved with methanol to obtain particles of 63–106 μm.

2.4. Solid-phase extraction experiments

SPE cartridges were packed with 100 mg of MIPs or blank particles having a size of 63–106 μm. All the SPE experiments were then performed using a vacuum manifold [17]. At the outset and between each experiment, MIP1 and MIP2 cartridges were washed using 0.25 M HCl with 50% of methanol, 0.1 M NaOH with 50% of methanol and water and the MIP3 cartridges were washed using only 0.25 M HCl with 50% of methanol and water. Then the capability of the different polymers to bind 10 mg l^{-1} of abacavir hemi-sulphate was assessed by the following general protocol. A volume (1 ml) of abacavir hemi-sulphate solution was loaded into the SPE cartridges. The filtrates were collected and analysed using a spectrophotometer at 254 nm to evaluate the concentration of the unbound abacavir. Then the abacavir was recovered from the cartridges by using 1 ml of 0.25 M HCl in 50% of methanol. The filtrates were again collected and tested at the spectrophotometer to quantify the recovered analyte. These experiments were performed using solutions of abacavir hemi-sulphate (10 mg l^{-1}) in different solvents: water, 50 mM Na-phosphate buffer (pH 8.0 and 6.0) and 50 mM Na-acetate buffer (pH 4.0). This was carried out to study the influence of buffer and pH on the affinity of the MIPs for abacavir. The same eluting solvent (0.25 M HCl in 50% of methanol) was used for the recovery of abacavir hemi-sulphate in all experiments.

2.5. Polymers binding capacity

The binding capacity of MIPs in SPE was assessed in a series of breakthrough experiments [18]. A solution of abacavir hemi-sulphate (0.75 g l^{-1}) (a concentration typical of that found in an industrial process) was prepared in different solvents: water, 50 mM Na-acetate buffer (pH 4.0) and 50 mM Na-acetate buffer (pH 4.0 with the addition of 10 or 20% of IPA). This was carried out to study the influence of both the presence of buffer and of organic solvent on the polymers binding capacity. Sequential aliquots of these solutions were passed through the cartridges and the amount of abacavir left in the filtrates was quantified by using a spectrophotometer at 254 nm. Breakthrough curves were then obtained and the binding capacity values were extrapolated from the 50% point of the breakthrough curve.

The binding capacity of the blank polymer, BP3, was only tested for 0.75 g l^{-1} of abacavir hemi-sulphate under optimal conditions (50 mM Na-acetate buffer pH 4.0).

The effect of the abacavir hemi-sulphate concentration on the binding capacity of MIP3 and BP3 was also studied. In this case the SPE experiments were performed by sequentially loading aliquots of abacavir hemi-sulphate solutions with different concentrations (0.1–15 g l^{-1}), prepared in 50 mM Na-acetate buffer (pH 4.0). As previously described the filtrates were collected and the amount of abacavir hemi-sulphate was evaluated by using a spectrophotometer at 254 nm. The binding capacity values were again extrapolated from the 50% point of the breakthrough curve.

3. Results and discussion

3.1. Computer modelling

The molecular modelling based on the LEAPFROG™ algorithm was used to identify the best candidates for the molecular imprinting of abacavir. The interaction energies obtained by docking the template and the monomer structures, minimised both in vacuum and in water are reported in Table 2. In the case of abacavir minimised in vacuum, the complexes of acrylamide, bisacrylamide and itaconic acid with the template are shown in Fig. 1A, C and E, respectively. The complexes of abacavir minimised in water with the three functional monomers are similarly reported in Fig. 1B, D and F. Almost identical results were obtained in the two cases (water and vacuum), except that the binding energies and/or the interaction positions of the monomers with abacavir were slightly different. We anticipate that there is a good chance that these three complexes exist in the real pre-polymerisation mixture. As can be seen from Fig. 1, acrylamide and bisacrylamide have different points of interaction with abacavir, whereas itaconic acid is interacting in exactly the same position with abacavir as acrylamide. This suggests that these two monomers cannot be used together, since they will most likely compete for the same binding sites on abacavir, namely the NH_2 group and one of the nitrogen atoms in the six-membered heterocyclic ring. Therefore, three different polymers were synthesised, two containing only acrylamide (MIP2) or itaconic acid (MIP3) based on a template–functional monomer molar ratio of 1:4 and the third polymer (MIP1) containing both acrylamide and bisacrylamide in a molar ratio 1:2:2.

3.2. SPE experiments

The affinity of the MIPs for 10 mg l^{-1} of abacavir hemi-sulphate prepared in different solvents was tested, as explained in Section 2. Fig. 2 shows the binding and recovery results expressed in %, obtained under different conditions. All three MIPs gave binding greater than 90% in almost all of the tested conditions. The respective recoveries, calculated taking into account the total amount of analyte, were always greater than 80%. With regard to the influence of the pH, a small improvement in binding capacity was observed at pH 4.0. At this pH abacavir binding exceeded 97% and this amount of drug was also

Table 2
Result of LEAPFROG™ algorithm: tabulated binding energies of complexes between the monomers and abacavir minimised both in vacuum and in water

Monomers	Binding energy (kcal mol^{-1}) in vacuum	Binding energy (kcal mol^{-1}) in water
Acrylamide	−33.40	−35.38
Bisacrylamide	−29.25	−31.38
Itaconic acid	−23.99	−27.81
Urocanic acid	−23.50	−23.57
Methacrylic acid	−17.00	−18.39

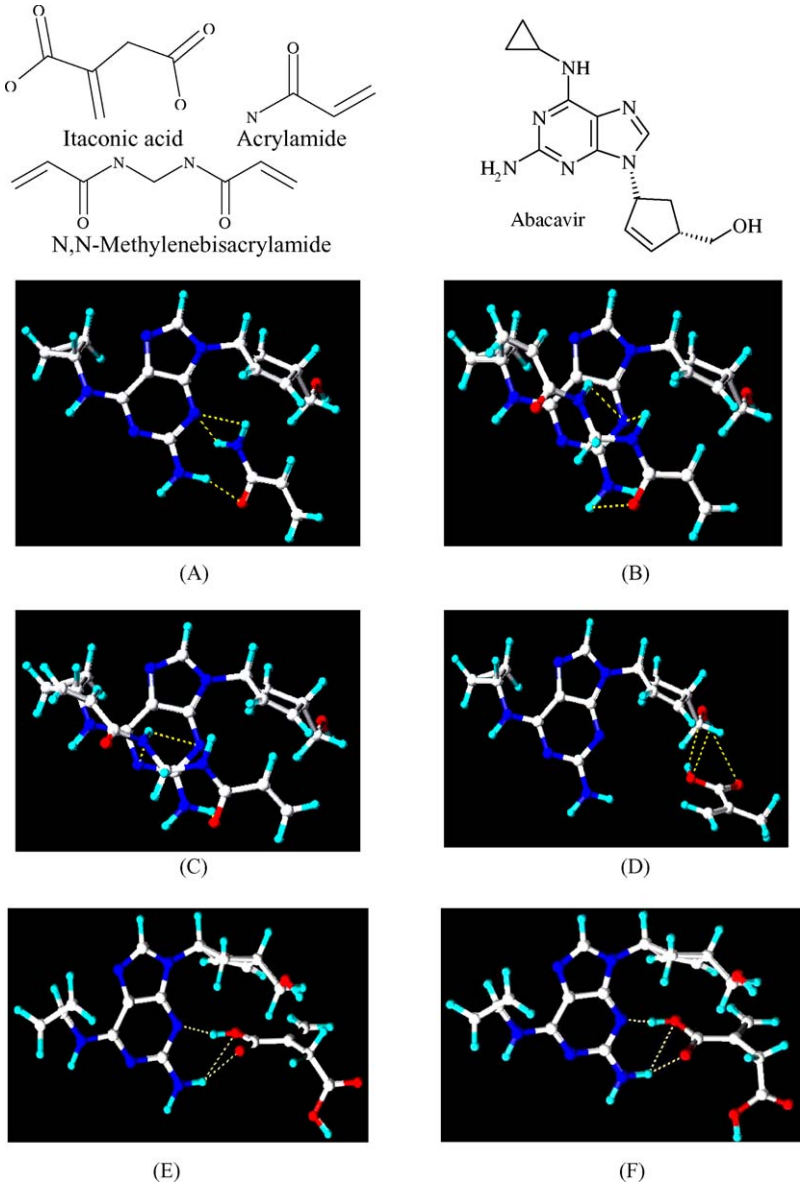

Fig. 1. LEAPFROG^{TM} results: the interactions between abacavir and the three best monomers (minimised both in vacuum and in water). (A) Abacavir–acrylamide in vacuum; (B) abacavir–acrylamide in water; (C) abacavir–bisacrylamide in vacuum; (D) abacavir–bisacrylamide in water; (E) abacavir–itaconic acid in vacuum; (F) abacavir–itaconic acid in water.

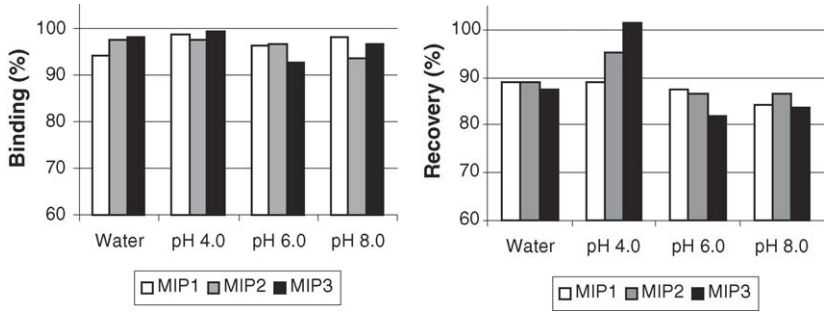

Fig. 2. MIP binding (%) and recovery (%) for 10 mg l^{-1} of abacavir hemi-sulphate prepared in different solutions: water, 50 mM Na-phosphate buffer (pH 6.0 and 8.0) and 50 mM Na-acetate buffer (pH 4.0).

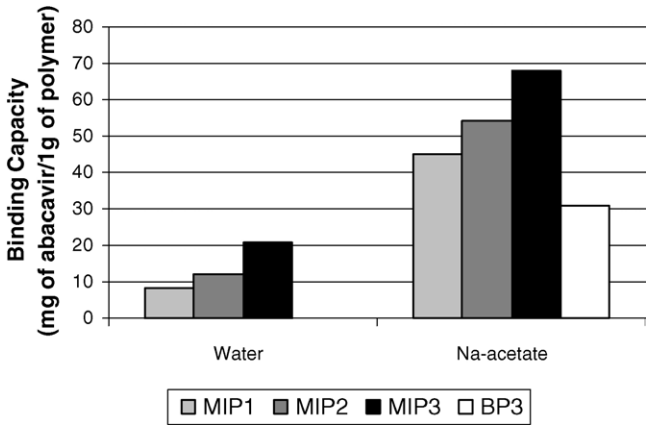

Fig. 3. Binding capacities of MIPs and BP3 for 0.75 g l^{-1} abacavir hemi-sulphate prepared in water and 50 mM Na-acetate buffer pH 4.0.

recovered quantitatively. Therefore on the basis of this result, 50 mM Na-acetate buffer (pH 4.0) was chosen as solvent for the following binding capacity experiments, both in the presence and absence of organic solvents.

3.3. MIPs binding capacity

For industrial applications, the use of adsorbents possessing a high binding capacity would be highly desirable. Separations and purification could be performed on small amounts of adsorbent with consequent savings in solvent, energy, costs and waste minimisation. Therefore, an evaluation of the binding capacity of our MIPs was fundamental to whether they possess the properties required by the industry. The MIP binding capacities were initially evaluated both in the optimal buffer composition, 50 mM Na-acetate buffer (pH 4.0) and in water (Fig. 3). As was explained in Section 2, breakthrough curves were determined for all the MIPs and the binding capacity values were extrapolated from the 50% point of the breakthrough curves. This is exemplified in Fig. 4, which shows the binding capacity of a cartridge containing 100 mg of MIP3 loaded with 0.75 g l^{-1} of abacavir hemi-sulphate in 50 mM Na-acetate buffer (pH 4.0). In this case the 50% point of the breakthrough curve corresponded to the loading of 9.1 ml of 0.75 g l^{-1} of drug into the cartridge. This gives a binding capacity of 68 mg of drug/g of polymer, which corresponds to 6.8% by weight. This was also the best overall binding capacity obtained by any of the three MIPs studied in either solvents (see Fig. 3). This value is particularly high if we consider that the theoretical maximum binding capacity is 5% corresponding to the percentage of template (by weight) used in the polymerisation mixture (see Table 1). Therefore, in order to study both the specificity and the nature of the interaction of MIP3 with abacavir, the binding capacity of its corresponding blank polymer (BP3) was also evaluated in the optimised loading solvent, 50 mM Na-acetate buffer (pH 4.0). The binding capacity of BP3 was three times lower than that of the corresponding MIP (see Fig. 3). This difference in performances did indeed demonstrate that MIP3 exhibited specific binding for the template, but it could also demonstrate that the high capacity of this MIP is due to a combination of both specific and non-specific adsorption. In order to obtain more information about the nature of the interaction between MIP3 and abacavir hemi-sulphate, the influence of the drug concentration on the binding capacity of MIP3 and BP3 was also studied. As explained in Section 2 the SPE experiments were performed with different concentrations of abacavir dissolved in 50 mM Na-acetate buffer (pH 4.0). The breakthrough curves were obtained as before and the values extrapolated from the 50% point of the curves. The results of these experiments are reported in Fig. 5. This figure shows that the binding capacities of both MIP3 and BP3 are directly proportional to the concentration of the target analyte: the higher the abacavir hemi-sulphate concentration, the higher the observed binding capacity of the two polymers. We believe that the reason for these unusually high binding capacities is cooperative binding between abacavir bound to the polymers and further abacavir from solution, resulting in stacks or clusters at the polymer surface. Cooperative binding is not new to MIPs,

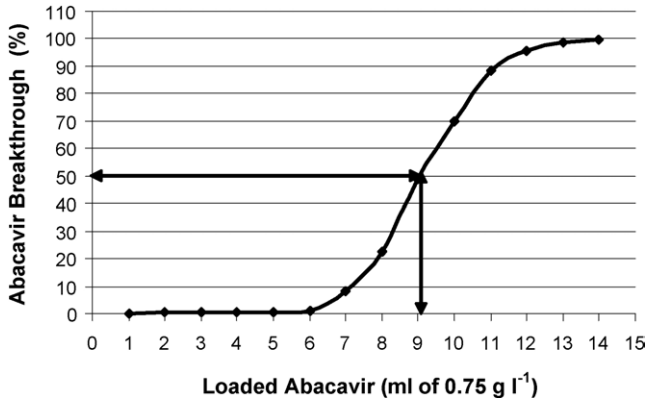

Fig. 4. Breakthrough curve for 0.75 g l^{-1} of abacavir hemi-sulphate in 50 mM Na-acetate buffer (pH 4.0) adsorbed by MIP3 (100 mg of polymer packed in a SPE cartridge). The binding capacity value is extrapolated from the 50% point of the breakthrough curve.

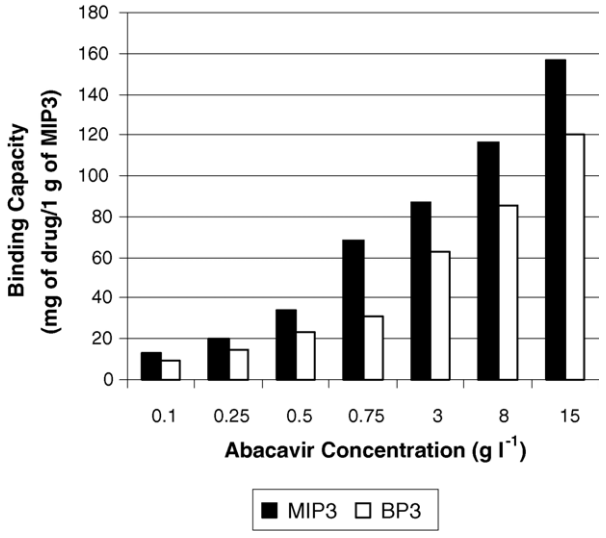

Fig. 5. Influence of abacavir hemi-sulphate concentration on the binding capacity of MIP3 and BP3. The abacavir hemi-sulphate solutions were prepared in 50 mM Na-acetate buffer (pH 4.0).

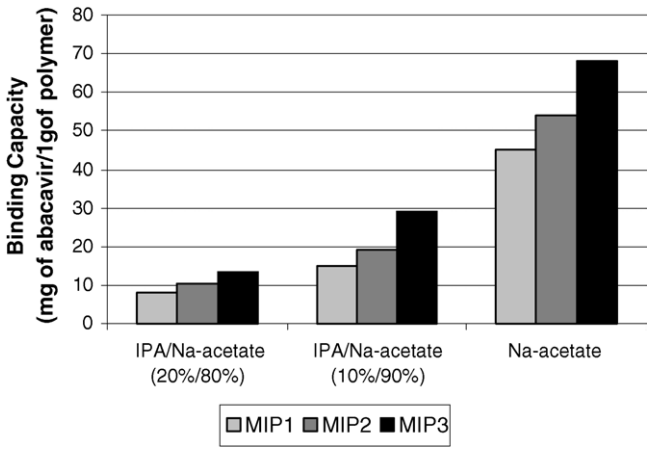

Fig. 6. Influence of an organic solvent on the binding capacities of MIPs for 0.75 g l^{-1} of abacavir hemi-sulphate prepared in 50 mM Na-acetate buffer (pH 4.0) with 10 and 20% of IPA. The binding capacities of MIPs in the absence of organic solvent are also reported.

in fact it was already observed by Nicholls and co-workers during the detection of (−)-nicotine by a methacrylic acid–ethylene dimethacrylate based MIP [19].

In the final part of our study the influence of a small percentages of an organic solvent, e.g. isopropanol (IPA), on the binding capacity was investigated. For these experiments, solutions of 0.75 g l^{-1} of abacavir hemi-sulphate were prepared in 50 mM Na-acetate buffer (pH 4.0) with the addition of 10 and 20% of IPA. The SPE experiments were performed as before. The results of this investigation are presented in Fig. 6, together with the best result obtained in the absence of organic solvent (i.e. in aqueous buffer alone). Fig. 6 shows that the binding capacity is greatly affected by the presence of IPA. The binding capacity of MIP3 in the presence of 20% of IPA decreased from 6.8 to 1.4% (14 mg of abacavir/g of polymer). The poor performance of MIPs in mixed solvents has been discussed previously. Sellergren and co-workers have shown that MIPs have high binding in two cases—in water, where significant contribution to binding originates from hydrophobic interactions, and in organic solvents, where the binding relies almost entirely on electrostatic interactions [20]. In mixed solvents or in solvents with intermediate polarity both hydrophobic and electrostatic interactions are too weak to support strong binding and this would account for the loss of binding capacity in our system. Therefore, it is possible to conclude that the synthesised MIPs might not be suitable for those industrial applications where mixed solvents are used.

4. Conclusions

In order to develop materials suitable for industrial applications, MIPs with high binding capacity for abacavir hemi-sulphate were developed by computational design, resulting in the selection of functional monomers possessing very high affinity for the template. Three MIPs based on the three “best” monomers used alone or in combination were synthesised and tested in SPE experiments. The MIP based on itaconic acid (MIP3) possessed a very high binding capacity, up to 15.7% in 50 mM Na-acetate buffer (pH 4.0). Furthermore, the binding capacity of the polymer was proportional to the concentration of abacavir. The higher the concentration of abacavir hemi-sulphate in the loading solution the higher was the binding capacity of the polymer. The affinity of MIP3 for abacavir was greatly reduced in the presence of organics solvents. However, when the percentage of the organic solvent was lower than 20%, MIP3 was still capable of binding abacavir, but with a lower capacity. These characteristics make this MIP a very good candidate for scaled-up SPE experiments and therefore potentially suitable for many industrial applications.

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