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Preparation and characterization of novel molecularly imprinted polymers based on thiourea receptors for nitrocompounds recognition

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ABSTRACT

Molecularly imprinted polymers (MIPs) for the recognition of nitro derivatives are prepared from three different (thio)urea-bearing functional monomers. The binding capability of the polymers is characterized by a batch binding experiment. The imprinting factors and affinity constants (K) of the imprinted polymers exhibit the same tendency as the binding constants (K_a) of the functional monomers to the target substance in solution. Not only nitrofurantoin is efficiently bound by these MIPs but also a broad spectrum of other nitro compounds is bound with at the intermediate level, addressing that these (thio)urea-based monomers can be utilized to prepare a family of MIPs for various nitro compounds, which can be applied as recognition elements in separation and analytical application.

1. Introduction

The non-covalent molecularly imprinted polymers (MIPs) introduced by Mosbach [1] have been developed based on selective functional receptors for a broad range of target analytes [2–6]. In particular, the design and synthesis of receptors for the binding of carboxylic acids and carboxylates has been mentioned in the literature [7–9] because of their abundance in biological systems. However, only a few hydrogen-binding receptors have been developed for the specific recognition of other isosteric anions such as phosphates, sulfonates, and only one for the nitro groups [10]. Receptors for the nitro group can be deduced from binders for carboxylic acid or carboxylate because the nitro group has electronic structural similarity and characteristics of molecular interaction [10,11]. Aryl(thio)urea derivatives forming bifurcate hydrogen donor–acceptor interactions are used as receptors for these oxoanions both in polar and in non-polar solvents [12,13]. Previously, we reported the synthesis and characterization of the different (thio)urea-based receptors with electron-deficient aryl moieties, which can be utilized for the recognition of nitro group such as nitrofurantoin (NFT) [14]. The receptors showed efficient binding of the nitro group containing substances induced by their electronic properties. Therefore, these receptors can be exploited as molecular recognition element in MIPs.

NFT is an antibiotic drug, which has been used as a food additive in animal farms. Although the European Union has prohibited it since 1997 because of its mutagenic and carcinogenic properties, it is still illegally used in many Asian countries [15] and allowed for ophthalmic use in animals for food production [16]. LC–MS–MS is a standard method for detection of nitrofurans including NFT. However, some disadvantages of the method such as long analysis time and high cost for the detection of nitrofurans. Furthermore, the time needed for sample preparation and the low selectivity of ordinary solid-phase extraction methods are also major issues. Because MIPs can be highly selective materials for targets, they have been considered as alternative sorbents for the molecular recognition and extraction of nitro compounds.

Non-covalent MIPs for nitro compounds such as 4-nitrophenol were prepared as solid-phase extraction (SPE) materials [17,18], used as selective sorbents in liquid chromatography [19] or coated on electrodes as sensor elements [20] using commercial functional monomers. Gas-phase sensor for the detection of 2,4,6-trinitrotoluene (TNT) used acrylamide-based MIPs [21–23]. Edmiston's group [24,25] used a covalently bound TNT template in combination with waveguide spectrometry for the detection of TNT in the gas phase. The detection of TNT and 2,4-dinitrotoluene (DNT) in aqueous solution using a fluorescence-labeled imprinted polymer prepared by a non-covalent approach was also reported [26]. Other examples of MIPs for other nitro compounds have been described. For example, a MIP was used as a SPE sor-

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bent for the determination of nitroimidazole [27], prepared in the form of nanogels for 4-nitro-4′-acetamino-diphenylcarbonate recognition [28] and synthesized on the electrode surface by electropolymerization technique for the detection of nitrobenzene [29]. A phosphate imprinted polymer prepared from other thiourea derivatives showed also a low binding of nitrobenzene [30]. Up to now there has been no reports on MIPs, which were prepared from simple (commercial) monomers, that can selectively bind not only NFT but also other nitro compounds.

Recently, we synthesized MIPs, which recognize the imide (–CO–NH–CO–) moiety of NFT [31]. The aim of this work is to address the nitrofunctionality of NFT. The interaction relies on the formation of hydrogen bonds between the nitro moiety of the analyte and (thio)urea-based moiety of the functional monomers in the MIP. The bulky polymers were prepared by non-covalent imprinting approach and then the binding efficiency of the polymers was characterized in batch binding study. All binder solutions were analyzed using UV–Vis spectrophotometry. We present MIPs with high affinity for the nitro group of NFT and assess their selectivity by studying the binding of various nitro derivatives.

2. Experimental

2.1. Chemicals and reagents

1-Hydroxycyclohexyl phenyl ketone (Irgacure 127) and pentaerythritol triacrylate (PETRA) were purchased from Sigma–Aldrich, Germany. All reagents used in this work are of analytical grade. All solvents purchased from either Sigma–Aldrich or Roth are of HPLC grade.

2.2. The synthesis of the analog template and functional monomers

The analog template, carboxyphenyl aminohydantoin (CPAH), and the functional monomers, 1-(4-vinylphenyl)-3-(3,5-bis(trifluoromethyl)phenyl thiourea (VTU), and 1-(4-vinylphenyl)-3-(pentafluorophenyl) thiourea (PTU) were synthesized and described in our previous report [14,31]. 1-(4-Vinylphenyl)-3-(3,5-bis(trifluoro-methyl)phenyl urea (VFU) was synthesized earlier by Sellergren's group [32].

2.3. Preparation of MIPs

The mixture of 0.5 mmol of CPAH and 0.5 mmol of the functional monomer (VFU, VTU, or PTU) was added to 6 mL of DMSO:acetonitrile (67:33) in 10 mL glass vials and then incubated for 4 h at 25 °C to allow the self-assembly. Next, 6 mmol of crosslinker (PETRA) and 0.5 wt.% of photoinitiator (Irgacure 127) were added. After purging the mixture with argon for 5 min, it was preincubated at 4 °C for 2 h before polymerization was initiated by 6 h exposure under ultraviolet light at 366 nm. The non-imprinted control polymer was prepared with the same procedure but without the template. The molar ratios of template:monomer:crosslinker were 1:1:12 following the previous literature [33]. The bulky polymers were ground, sieved, and then washed with hot methanol in a soxhlet apparatus overnight for template removal. A UV–Vis spectrophotometer at a wavelength of 300 nm was used for monitoring the template removal.

2.4. Batch rebinding studies

The binding kinetics of the imprinted polymers for both NFT and CPAH were investigated as follows. 10 mg of the imprinted polymer particles was mixed with 5 mL of 0.1 mM CPAH or NFT solution in acetonitrile + 2% DMSO. The DMSO addition was necessary because CPAH and NFT are not soluble in pure acetonitrile. The mixture was incubated at 25 °C under continuous stirring. Sample solutions were taken after 0.5 h, 1.5 h, 3 h, 6 h, 9 h, and 12 h, respectively, then transferred to a centrifuge tube, and centrifuged for 10 min at 10,000 rpm. The supernatant was analyzed by UV–Vis spectrophotometry at a wavelength of 300 or 370 nm for unbound CPAH or NFT, respectively. The concentration of CPAH or NFT in the supernatant was calculated using a standard solution calibration curve. The amount of the analyte bound (B, μmol/g) to the imprinted polymer was calculated by subtracting the concentration of the unbound target molecule from the initial concentration [34,35].

2.5. Adsorption isotherm

The binding capacity of the imprinted polymers with varying concentrations of the analyte was also evaluated from batch binding studies. The conditions were the same as in our previous study [31]. For example, acetonitrile with 2% DMSO was used as the solvent and to ensure that equilibrium was reached, the incubation time for the target binding to all polymers was 24 h at 25 °C under continuous stirring. 10 mg imprinted polymer was equilibrated with varied initial concentration (0.001–2.5 mM) of the analyte (5 mL) in each tube. After 24 h incubation time, the supernatant was analyzed by UV–Vis spectrophotometry at the maximum wavelength of each binder. The amount of the analyte bound was determined using an individual calibration curve for each analyte.

2.6. Crossreactivity study

To evaluate the selectivity of the imprinted polymers, the structurally related compound nitrofurazone (NFZ), furazolidone (FZD), 5-nitroanthranilic acid (NAA), 4-nitrobenzyl bromide (NBB), *p*-nitrophenol (NP), 2-nitrofuran (NF), and the non-related compound *p*-aminophenol (AP), and 5,5-diphenylhydantoin (DPH), Fig. 4, were used instead of NFT at a concentration of 0.1 mM in the batch-rebinding experiment. The concentration of non-bound compounds was measured by UV–Vis spectrometry at 300 nm (CPAH), 370 nm (NFT), 370 nm (NFZ), 370 nm (FZD), 350 nm (NAA), 275 nm (NBB), 405 nm (NP), 300 nm (NF), 310 nm (AP), and 240 nm (DPH), using as individual calibration curves for each compound.

3. Results and discussion

3.1. Equilibrium binding characteristics and adsorption isotherm

Three polymer formulations (MIP-VFU, MIP-VTU, and MIP-PTU) were prepared from the functional monomers VFU, VTU, and PTU (Fig. 1), respectively, by the non-covalent imprinting approach with photoinitiated polymerization using CPAH as the template in DMSO:acetonitrile (67:33) as the porogen. The non-imprinted control polymers NIP-VFU, NIP-VTU, and NIP-PTU were prepared using the same procedure but without the template. Acetonitrile seemed a natural choice for the solvent to conduct the binding study, because it is commonly used for sample preparation and as the mobile phase in the chromatography for nitrofurans detection [36–39]. However, the experiments with 2% DMSO were carried out because of the absolute minimum required to dissolve 1 mM of CPAH and NFT. At this low volume fraction of DMSO did not interfere with the UV–Vis spectrophotometry measurement as previously reported [31]. Therefore, the batch binding protocol for MIPs applied in this work can be directly used with established sample preparation techniques.

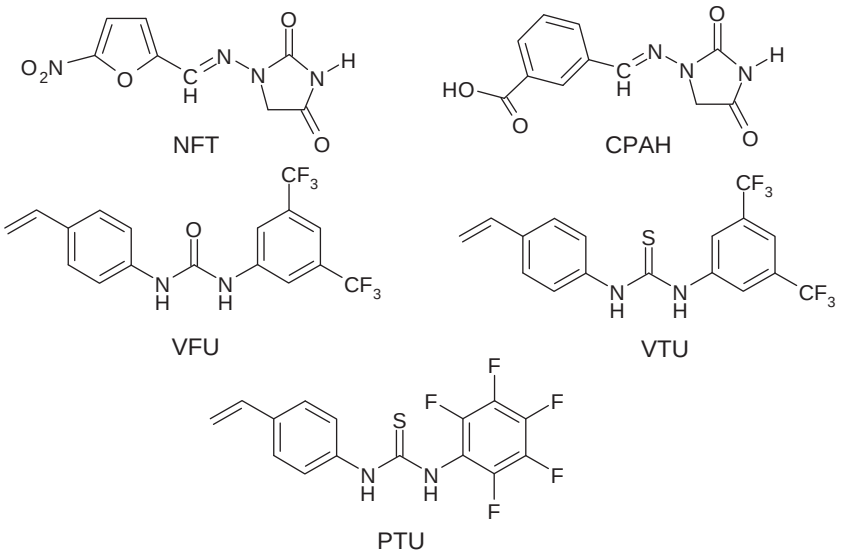

Fig. 1. Chemical structures of NFT, an analog template CPAH and functional monomers VFU, VTU and PTU.

The adsorption kinetic and isotherms of the MIP- and NIP-VFU from batch binding studies were evaluated as shown in Fig. 2. The adsorption rate of NFT to MIP- and NIP-VFU (Fig. 2a) shows two stages, a quick stage at the beginning and a slow stage before the equilibrium. The binding curves were fitted by an exponential function showing that the equilibrium was reached after ca. 3 h. Moreover, the binding capacity of MIP for NFT could be determined from this curve and the amount of NFT bound to MIP-VFU was three times higher than for the control polymer NIP-VFU. Fig. 2b demonstrates that at all concentrations, the MIP-VFU binds the target molecules CPAH and NFT considerably stronger than 5,5-diphenylhydantoin (DPH). DPH contains a hydantoin ring, i.e., its molecular shape is similar to NFT but no group is isosteric to the carboxylic acid of CPAH or the nitro group of NFT. The negligible binding capacity of the MIP for DPH shows the dominating influence of the VFU for the binding while the nonspecific interaction with the polymer backbone is obviously weak. In analogy to the MIP-VFU the MIPs containing the functional monomer VTU and PTU, respectively, showed high binding for the target analytes but low binding to DPH. With all imprinted polymers, an apparent saturation of the binding curve was found at ca. 1 mM CPAH or NFT. This effect was obviously caused by the limited solubility of the binders but not by the actual saturation of the MIPs. For MIP-VFU, the saturation values for CPAH and NFT are 5.95% and 5.31% of the theoretical maximum binding capacity, respectively.

The imprinting factors (Table 1) of the MIPs were determined at the concentration of 1 mM by the ratio of the amount bound by the MIP to the amount bound by the respective NIP. Because the NIP contains the same amount of the functional monomer but no target substance during the polymerization, the imprinting factor reflects the influence of the cavities formed after the removal of the target molecule in the imprinted polymer. With MIP-VFU, the imprinting factor was 3.39 and 2.61 at 1 mM for CPAH and NFT, respectively. These values of both compounds were slightly higher when compared with the imprinting factor of MIP-VTU and MIP-PTU, which decrease in the order MIP-VFU > MIP-VTU > MIP-PTU. In a previous report [14], we presented the binding constant (K_a), for the interaction between CPAH and NFT with the three different functional monomers. The strength and the efficiency for the binding of CPAH or NFT in the batch binding experiments in the polar solvent system (acetonitrile + 2% DMSO) by the MIPs containing VFU, VTU, and

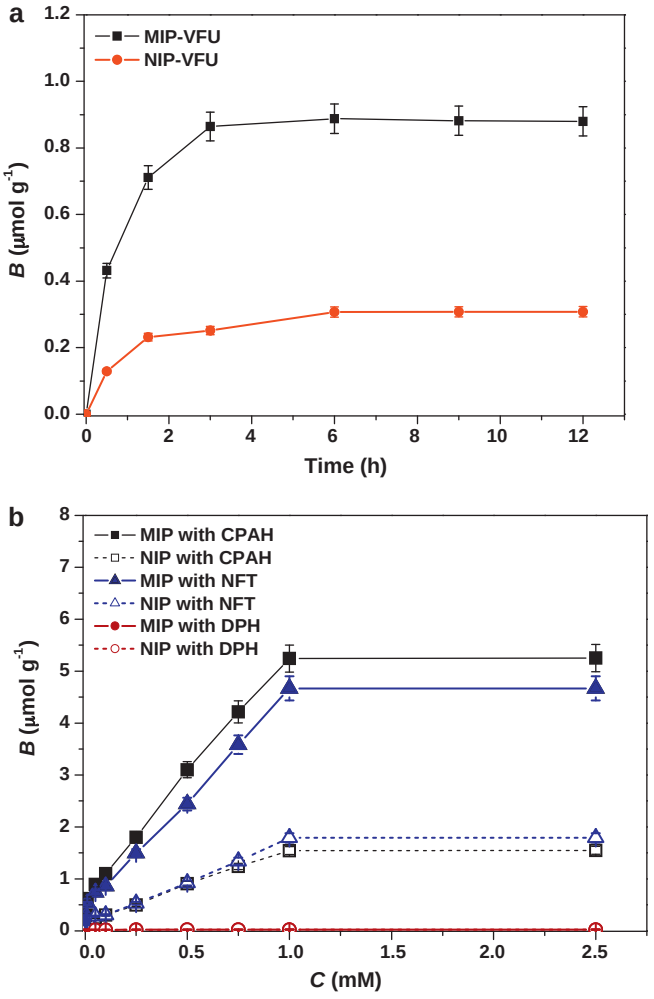

Fig. 2. Adsorption kinetics (a) of MIP- and NIP-VFU for NFT. *Conditions*: 10 mg of polymer with 5 mL of 0.1 mM NFT solution in acetonitrile + 2% DMSO, 25 °C; Adsorption isotherms (b) of CPAH, NFT, and DPH to the polymers MIP- and NIP-VFU. *Conditions*: 10 mg of polymer with 5 mL of 0–2.5 mM binder solution in acetonitrile + 2% DMSO, 24 h, 25 °C.

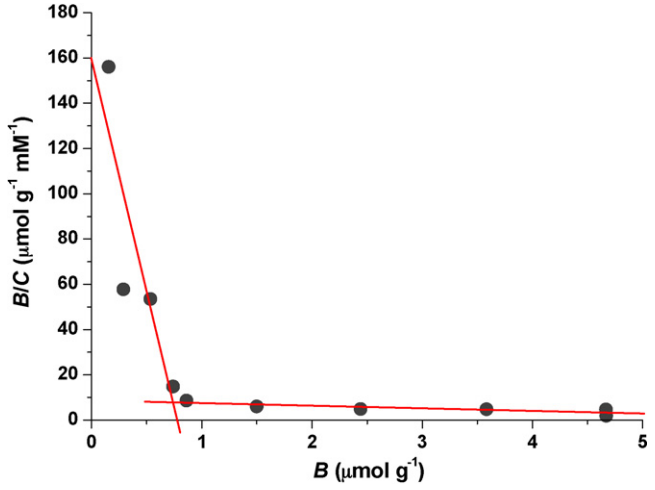

Fig. 3. Scatchard plot of the adsorption isotherm of NFT to the MIP-VFU at equilibrium.

PTU in this research, respectively, follows the tendency of these K_a values (see Table 2).

To determine the affinity and binding sites of the imprinted polymer expressed by affinity constants (K) and apparent maximum number (B_{max}) of the imprinted polymer, we evaluated the data in the form of a Scatchard plot (Fig. 3). The Scatchard analysis is a graphical representation of the linearized form of the Langmuir equation (Eq. (1)). It is obtained by replotting the adsorption isotherm for the analyte bound (B) as the X axis versus the ratio of the analyte bound to the concentration of free analyte (B/C) as the Y axis. The slope of the graph is equal to $-K$ and the X axis intercept is equal to B_{max} with an acceptable fit (R^2 = 0.97–0.99) [40,41]. In this work, the same K and B_{max} were also obtained using program LMMpro version 1.06.

$$\frac{B}{C} = KB_{max} - KB \quad (1)$$

The Scatchard plot of the batch-binding experiments shows that there are two classes of binding sites in the imprinted polymer MIP-VFU. The affinity constants (K) and the apparent maximum number (B_{max}) are given in Table 2. The results indicate that at a lower concentration of CPAH and NFT, the occupied binding sites possess a higher affinity. On the other hand, at a higher concentration the analyte could bind to cavities of the polymers with higher density, but with a lower affinity [42]. The affinity constants (K) calculated from the batch-binding of the MIPs decrease in the order MIP-VFU > MIP-VTU > MIP-PTU, thus showing the same tendency of the binding constants (K_a) determined for the interaction between

Table 1
Binding capability of imprinted polymers at saturation concentration (1 mM) from batch binding studies.

Polymer	CPAH			NFT		
	Bound amount (B, μmol/g of polymer)	Imprinting factor	Efficiency of binding (%)	Bound amount (B, μmol/g of polymer)	Imprinting factor	Efficiency of binding (%)
MIP-VFU	5.240 ± 0.262	3.39	5.95 ± 0.30	4.670 ± 0.233	2.61	5.31 ± 0.30
NIP-VFU	1.545 ± 0.077			1.790 ± 0.089		
MIP-VTU	4.290 ± 0.214	2.94	4.29 ± 0.21	3.775 ± 0.189	2.28	3.78 ± 0.30
NIP-VTU	1.460 ± 0.073			1.655 ± 0.083		
MIP-PTU	4.115 ± 0.206	2.88	4.20 ± 0.21	3.600 ± 0.180	2.25	3.67 ± 0.30
NIP-PTU	1.430 ± 0.072			1.600 ± 0.080		

Table 2
Binding affinity of the imprinted polymers obtained by Scatchard analysis.

Polymer	CPAH					NFT				
	Binding constant (K_a, M^{-1})[a]	K_1 (M^{-1})[b]	B_{max1} (μmol)[b]	K_2 (M^{-1})[c]	B_{max2} (μmol)[c]	Binding constant (K_a, M^{-1})[a]	K_1 (M^{-1})[b]	B_{max1} (μmol)[b]	K_2 (M^{-1})[c]	B_{max2} (μmol)[c]
MIP-VFU	1470 ± 89	129.16	1.03	2.08	6.46	1370 ± 55	121.29	0.92	1.69	5.98
MIP-VTU	470 ± 44	79.94	0.90	1.76	5.48	450 ± 33	78.79	0.71	1.35	5.11
MIP-PTU	430 ± 36	71.36	0.80	1.72	5.29	280 ± 18	68.50	0.70	1.42	4.81

[a] Binding constant was determined in solution between the functional monomers (VFU, VTU, or PTU) with the template CPAH or target NFT by our previous report [14].
[b] K and B_{max} evaluated from linear line (R^2 = 0.97–0.99) using Langmuir analysis are the affinity constant and the apparent maximum number, respectively. K_1 and B_{max1} are the parameters for the higher affinity binding sites.
[c] K_2 and B_{max2} are the parameters for the lower affinity binding sites.

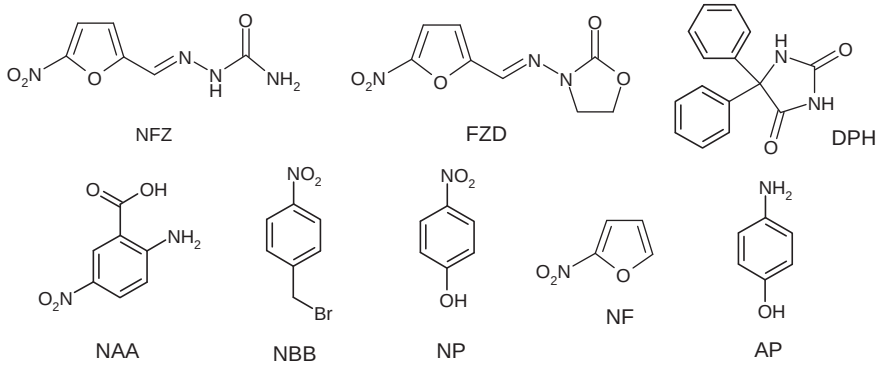

Fig. 4. Chemical structures of nitro compounds (NFZ, FZD, NAA, NBB, NP, and NF) and structually non-related compounds (AP and DPH) using in crossreactivity studies.

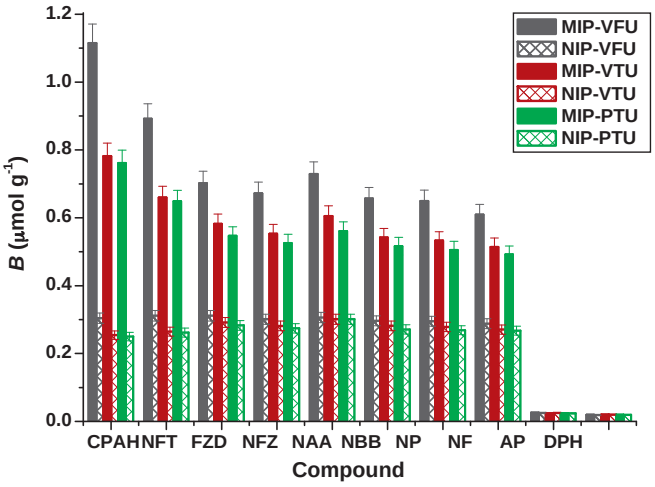

Fig. 5. Crossreactivity of three different imprinted polymers. 10 mg of imprinted polymer with 5 mL of 0.1 mM binder in acetonitrile + 2% DMSO, 24 h, 25 °C.

the functional monomer VFU > VTU > PTU and the binders (CPAH or NFT) in the solution by the titration method [14].

3.2. Crossreactivity

To investigate the selectivity of the imprinted polymers, the interaction of the MIPs with the following binders, which selected from four groups, (Fig. 4) was characterized: (i) compounds structurally related to NFT and containing a nitrogroup (NFZ and FZD), (ii) compound similar to NFT but without an isosteric ligand (DPH), (iii) nitrocompounds structurally dissimilar to NFT (NAA, NBB, NP and NF), and (iv) the structurally nonrelated compound (AP). Fig. 5 compares the binding at the concentration of 0.1 mM, i.e., when binding is dominated by the high-affinity binding sites, in acetonitrile + 2% DMSO. The data show that the crossreactivity based on the imprinting effect (MIP/NIP amount bound from Fig. 5) of all three polymers might be categorized into three classes with respect to the structural analogy to the template: (i) high binding for the template CPAH and NFT, (ii) intermediate crossreactivity for substances bearing a nitrogroup: NAA, NFZ, FZD, NBB, NP, and NF, (iii) low binding for AP and DPH, which have no isosteric group of the target.

The binding of all nitro groups containing substances to the non-imprinted polymer was almost equal, indicating the interaction with the accessible groups of the functional monomer. Because of the different extent of binding to the MIP, the imprinting factor for structurally similar nitrocompounds is higher (2.5–3.0) when compared with dissimilar nitro substances. DPH and AP bind with almost identically very low intensity to both the MIP and the NIP, which may reflect the weak interaction with the polymer backbone. The imprinting factor IF ~ 2 for NP from batch-binding experiments in this work is higher than previously reported by other group [18] with IF ~ 1 using 4-vinylpyridine as a functional monomer of the MIP. These findings are in accordance with other reports [31,43] that the functional group of the polymer is of key importance for the recognition process and binding capacity.

4. Conclusions

The (thio)urea-based MIPs bind the target CPAH in highest amount followed by NFT, which resembles CPAH in shape and size and possesses the isoelectronic NO_2 group. Other compounds such as NFZ, FZD, NAA, NBB, NP, and NF carrying an NO_2 group are bound via hydrogen bonds to the functional monomer. Determined by the lower degree of similarity of shape and size to the target, the binding strength is at an intermediate level. The dominating effect of hydrogen bonding by the functional monomer is exemplified by the very low binding of DPH and AP both missing the nitro group. DPH bearing an imide group in the furan ring similar to CPAH and NFT is devoid of an isosteric group for binding, which causes the negligible binding. Although the structure of AP is close to NP, the MIPs selectively bind to the nitro group of NP stronger than AP. Therefore, these (thio)urea-based MIPs can be further used as sorbents not only for nitrofurantoin recognition and extraction but also a broad spectrum of nitro compounds. With the aim to further increase the sensitivity and selectivity, we are presently preparing MIPs addressing two different functional groups of the target, i.e., a diaminopyridine derivative for the imide functionality and (thio)urea derivatives for the nitro group.

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