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Recognition and fast separation of Paclobutrazol pesticides *via* molecular imprinted silica nanoparticles

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Abstract

In this study, molecular imprinted silica nanoparticles were prepared via surface imprinting method for specific recognition with high affinity and separation of paclobutrazol in honey samples. To prepare molecular imprinted silica nanoparticles, paclobutrazol was used as template molecule, 2-hydroxyethyl methacrylate as monomer, 1,2-ethyleneglycoldimethacrylate as crooslinking agent. Furthermore, molecular imprinted silica nanoparticles were synthesized by single electron transfer living radical polymerization. The prepared nanoparticles can be easily separated and collected by ultracentrifuge with 14000 rpm and exhibited highly selectivity to template molecules. All rebinding studies showed that the molecular imprinted silica nanoparticles had excellent recognition towards paclobutrazol. The recoveries of paclobutrazol in the spiked honey samples changed from 98.7% to 99.9% with the relative deviation from 8.62% to 4.92% According to all results, molecular imprinted silica nanoparticles can be good alternative for selective recognition and efficient separation of pesticides in real samples.

1. INTRODUCTION

Pesticides, contain high bioactive and stable organic compounds, which are thoroughly applied for disease control. Triazoles are new generation pesticides of applied to grain crops, vegetables and fruits [1-3]. These compounds can be gathered in different steps of ecosystem and harm to food chains because of their lipophilic nature [4]. The pesticide residues can be transferred to other agricultural products via food chain. Agricultural products may have pesticide residuals. Considering, there is rare concern about triazole pesticides residual in bee products, there is a demand to develop effective and sensitive methods for the separation of triazole pesticides in honey. Paclobutrazol (PBZ), which is a triazole pesticide, responsible for regulate plant growth inhibit gibberellic acid biosynthesis and increase in abscisic acid and cytokinin hormones [5].

Molecular imprinting is an effective and simple technique for synthesizing of shape memory recognition materials by the polymerization of appropriate functional monomers with target molecules [6]. The imprinted cavities form inside or surface of the polymer after the extraction of template molecule from the polymeric structure. The resulting molecular imprinted polymers are (MIP) specific to the template molecules with high affinity. Silica nanoparticles (SNPs) can be used as core for MIP due to the unique features of silica nanoparticles such as high surface area volume ratio [7]. MIP SNPs have been successfully used in selective separation and recognition, environmental analysis, disease diagnose, biosensing and drug delivery etc. [8]. In recent years, different molecular imprinted silica nanostructures have been investigated for various template molecules such as proteins, antibodies, toxins, hormones and etc. In one example, He et al. synthesized colloidal silica nanoparticles and they modified with 3-methacryloxypropyltrimethoxysilane. These nanoparticles were covered with poly(methacrylic acid-co-acrylamide-(dimethylamino)ethyl methacrylate) via free radical polymerization in the presence of lysozyme protein. The resulting lysozyme imprinted silica nanoparticles were exhibited good mass transfer. Moreover, these nanoparticles were reached adsorption equilibrium in 5 min due to superthin polymer coating [9].

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In 2011, A novel molecular imprinted silica nanoparticles for the specific recognition of porcine serum albumin combining with copper ions were synthesized by Liu et al. In this work, albumin protein was non-covalently imprinted onto the modified silica particles with metal ions. The prepared MIP SNPs were showed rapid mass transport. These MIP SNPs were reached at saturation capacity of 20 mg g^{-1} within 1 min [10].

Ghaemy and coworkers prepared different pesticides (phosalone, diazinon, and chlorpyrifos) imprinted magnetic nanoparticles via surface imprinting method. They used poly(methy methacrylate-co-maleic anhyride) as the functional polymer croslinked with triethylenetetramine. The magnetic molecular imprinted polymers were showed excellent binding affinity toward the template molecules. Adsorption capacities of the prepared nanostructures did not decrease after four adsorption-desorption recycles [11]. Many successful studies have been published about molecular imprinted nanostructures but there has not been reported a work about paclobutrazole imprinted silica nanoparticles, yet.

Herein, the surface imprinting method was used to prepare the MIP SNPs for recognition and separation of model pesticide paclobutrazol. First of all, the silica nanoparticles were synthesized by Stöber method [12]. Subsequently, the silica nanoparticles were modified with 3-bromopropyl trimethoxysilane. Brom covered SNPs directly coated with polymer layer in the presence of the model pesticide paclobutrazol were polymerized via single electron transfer polymerization (SET-LRP) of 2-hydroxyethyl methacrylate. The morphology of the all nanoparticles were analyzed by transmission electron microscopy (TEM). The model pesticide recognition and separation features of MIP-SNPs were examined by rebinding kinetics, selectivity and reusability tests.

2. EXPERIMENTAL

2.1. Materials

Tetraethyl orthosilicate (TEOS), Ammonium hydroxide solution 28% NH_3, absolute ethanol, 3-bromopropyl trimethoxysilane (BPTS), 2-Hydroxyethly methacrylate (HEMA), 1,2-ethyleneglycoldimethacrylate (EGDMA), Cu(I)Br, N,N,N',N'',N''-Pentamethyldiethylenetriamine (PMDETA), toluene, formic acid, aceton were obtained from Sigma-Aldrich. Paclobutrazol (PB), Triadimefon, Penconazol and Hexaconazol pesticides were also pruchased from Sigma-Aldrich.

2.2. Instrumentation

PBZ rebinding tests was investigated by using a Shimadzu UV-2450 spectrophotometer. Morphology and size of the nanoparticles were detected by TEM (JEOL JEM 1400 TEM).

2.3. Preparation of Paclobutrazol imprinted silica nanoparticles

Silica nanoparticles were synthesized as in the literature [12]. To synthesis of pesticide imprinted silica nanoparticles, pactobutrazole (PBZ) was used as a template molecule, 2-hydroxyethyl methacrylate (HEMA) was used as a functional monomer and 1,2-ethyleneglycoldimethacrylate (EGDMA) was used a crosslinking agent, and brom functionalized SNPs were used as the core. Surface initiated single electron transfer-living radical polymerization (SET-LRP) of HEMA were carried out surface of the brom functionalized SNPs. For this purpose, HEMA (1 mL), EGDMA (0.073 mL,), PBZ (3 mg), Cu(I)Br (0.027 mg), PMDETA (0.039 mL), 25 mL toluene were mixed in the 50 mL schlenk flask under the nitrogen atmosphere for 30 min. Then 50 mg brom functionalized SNPs added the solution and SET-LRP was initiated. After the polymerization, SNPs were precipitated by ultracentrifuge with 14000 rpm and washed with deionized water and acetone. To obtain the pesticide imprinted SNPs, the template molecules were removed with 10 mL acetone with 1 % formic acid. Finally, the nanoparticles were washed with ultra pure water and under vacuum at 50 ^{o}C for 24 h. The non-imprinted silica nanoparticles (NIP SNPs) were also synthesized by using the same way in the absence of PBZ and they were used as the control nanoparticles in rebinding experiments.

2.4. Paclobutrazol rebinding experiments

1.0 mg MIP SNPs or NIP SNPs were immersed into 1.0 mL paclobutrazol solutions of different concentration in water. The solutions were incubated for 1 h at room temperature. The amount of rebinding pesticide on the MIP and NIP SNPs was quantified based on the difference of PBZ concentration before and after rebinding by using UV-vis spectroscopy at 220 nm. The rebinding capacity was calculated using the following formula:

$$Q = \frac{(C_o - C)V}{M}$$

Where Co is the initial paclobutrazol concentration (mg mL^{-1}), C is the paclobutrazol concentration after rebinding, V is the volume of PBZ solution (mL), and M is the weight of the MIP or NIP SNPs (g) [6]

The rebinding kinetics were examined by different rebinding time from 0 to 60 min with the PBZ concentration at 0.6 mg mL^{-1}.

The selectivity of the MIP SNPs was investigated using 0.6 mg mL^{-1} Paclobutrazol, Triadimefon, Penconazole and Hexaconazole at rebinding time 40 min.

3. RESULTS AND DISCUSSION

3.1. Paclobutrazol imprinted silica nanoparticles

In recent years, molecular imprinted silica nanoparticles (MIP SNPs) have excellent applications such as separation, recognition, synthetic antibody, catalysis, biosensors, and medicine etc [13]. These nanomaterials could specifically recognize and bind template molecules. In this study, silica nanoparticles were used as an excellent cores for the preparation of MIP by using surface molecular imprinted method due to the high-mechanical strength and large surface area. Well-defined MIP SNPs must have high surface-to-volume ratio, site accessibility and low mass transfer resistance and must be design with well-defined and biocompatible monomer because PBZ imprinted silica nanoparticles may be use to clean-up pesticide residues from food samples.

The synthesis procedure of pesticide imprinted SNPs was demonstrated in Scheme 1. Firstly, uniform silica nanoparticles were synthesized by hydrolysis and condensation of TEOS in ethanol, and in presence of ammonia as catalyst [12]. Then, SNPs were functionalized with BPTS to generate free brom groups. BPTS molecules were bound onto the silica nanoparticles via siloxane bonds formed between the silanol groups of SNPs and silane groups of BPTS. Br coated SNPs were added to HEMA:EGDMA:Cu(I)Br:PMDETA:PBZ solution in toluene and the SET-LRP was initiated to form the recognition cavities. Brom modified SNPs were used as initiator in polymerization solution. While silica nanoparticles were covered with PHEMA shells, at the same time some template molecules were embedded in the polymer layer by surface molecular imprinted method. Template molecules were non-covalently imprinted onto the PHEMA coated SNPs. Recognition cavities were formed by removal of PBZ via ultrasonic vibration in acetone with formic acid solution. The control NIP SNPs were prepared by the same procedure in the absence of the template PBZ. The size and shape of MIP and control NIP SNPs were characterized by TEM. TEM images of NIP and MIP SNPs were provided in Figure 1. The TEM images of NIP and MIP SNPs confirmed that the polymer layer was fully coated onto the silica nanoparticles. The surfaces of MIP and NIP SNPs were very smooth even if surface polymerization was taken place [14]. The shape of all nanoparticles was spherical and average diameters of NIP and MIP SNPs were 45 and 46 nm, respectively. The mean diameters of NIP and MIP SNPs are almost equal, it may be due to the cavities of MIP MNPs were not to change the polymer layer thickness.

3.2. Initial Concentration Effect

The rebinding experiments were go through with different pesticide concentrations range from 0.1 to 1 mg mL^{-1}. Adsorption isotherms of MIP and NIP SNPs were plotted (Figure 2). The amount of rebinding pesticides on the surface of the MIP SNPs was increased linearly when the initial concentration of pesticide increased below 0.6 mg mL^{-1}. However, when the initial pesticide concentration was over 0.6 mg mL^{-1},

plateu region began and reached saturation at high PBZ concentration. The saturation value was 39.6 mg PBZ g^{-1} MIP SNPs. Similarly, this value for the NIP SNPs was 5.5 mg PBZ g^{-1} MIP SNPs. The MIP SNPs had higher rebinding capacity for template molecules than NIP SNPs. This result is due to the specific molecular recognition cavities that showed high rebinding affinity for PBZ on the surface of the MIP SNPs. However, the rebinding capacities of NIP SNPs was lower than MIP SNPs because they had no recognition sites for PBZ and the physical adsorption was dominant on their surface.

The Scatchard isotherm model widely used to describe the rebinding feautures of the MIP SNPs, which can be expressed as follows [7]:

$$\frac{Q}{C_e} = \frac{Q_{max}}{K_d} - \frac{Q}{K_d}$$

where Q (mg g^{-1}) is the amount of rebinding PB to the MIP SNPs at equilibrium, Qmax (mg g^{-1}) is the apparent maximum rebinding capacity. Ce is the free concentration of PB at equilibrium (mg mL^{-1}), and K_d is the dissociation constant related to the the affinity of recognition sites (mg mL^{-1}). The values of K_d and Q_{max} can be calculated from the slope and intercept of the linear plot of Q/C_e versus Q. The K_d and Q_{max} values were obtained for NIP SNPs 0.421 mg mL^{-1} and 11.02 mg g^{-1}, while K_d and Q_{max} values of MIP SNPs 0.103 mg mL^{-1} and 49.76 mg g^{-1}. The K_d values of MIP and NIP SNPs indicated that rebinding efficiency of MIP SNPs was higher than NIP SNPs owing to the specific molecular recognition cavities. According to these results, MIP SNPs showed excellent accessibility to target molecules.

3.3. Rebinding Time Effect

The rebinding time effect of the pesticide on the MIP and NIP SNPs experiments were carried out with different incubation time from 0 to 60 min at 0.6 mg mL^{-1} pesticide concentration. Kinetic curves were showed in Figure 3. During the first 25 min, the rebinding rates of the MIP and NIP SNPs increased fastly and over the 40 min the kinetic curve became relatively flat and rebinding capacities reached equilibrium. Generally, surface molecularly imprinted polymers reach adsorption equilibrium in 30-120 min [15]. MIP SNPs reached the saturation within 40 min. PBZ molecules easily reached the binding sites at the surface of the MIP SNPs for easy diffusion of template molecules into the recognition cavities. The rebinding could be attributed to the geometric and functional affinity between the PBZ and cavities on the surface of the MIP SNPs [16]. However, the control NIP SNPs showed high resistance for specific recognition since they had no recognition sites for PBZ. As a result the binding process onto the NIP SNPs may be physical adsorption.

3.4. Rebinding Selectivity of MIP SNPs

The rebinding selectivity of MIP SNPs were determined by using target pesticide paclobutrazol and other competitor pesticides (Triadimefon, Penconazol and Hexaconazol) under the same conditions. The imprinting factor (α) and the selectivity factor (β) were calculated following equations [17]:

$$\alpha = \frac{Q_{MIP}}{Q_{NIP}}$$

$$\beta = \frac{\alpha_{TEMP}}{\alpha_{NONTEMP}}$$

Where Q_{MIP} and Q_{NIP} (mg g^{-1}) are the rebinding amount of PBZ for MIP and control NIP SNPs. α_{TEMP} is imprinting factor of template pesticide and $\alpha_{NONTEMP}$ is the imprinting factor of non-template pesticide. The selectivity test results were demonstrated in Figure 4 and Table 1.

As shown in Table 1, the calculated imprinting factors of template molecule (Paclobutrazol) and other pesticides (Triadimefon, Penconazol and Hexaconazol) were about 7.31, 3.53, 3.18 and 3.09, respectively. These results could be attributed that the PBZ binding sites of the MIP SNPs were recognize the template molecules in shape, size and functionality. As a result, MIP SNPs indicated the specific selectivity towards the template molecule due to the specific recognition sites for PBZ.

3.5. Regeneration of MIP SNPs

To test the regeneration of the MIP SNPs, 20 regeneration cycles were carried out with paclobutrazol. The acetone:formic acid (9.0:1.0, v/v) mixture was used as an eluent. After the MIP SNPs was treated 0.6 mg mL^{-1} pesticide solution for 40 min, the MIP SNPs were washed with eluent under ultrasonic vibration for 20 min and SNPs were precipitated by ultrasantrifuge with 14000 rpm. The results were demonstrated in Figure 5. After the 14 cycles of regeneration, the rebinding capacity of MIP SNPs decreased about 9.0 % in PBZ solution. These results indicated good retention of the activity of the MIP SNPs during the 14 regeneration cycles.

3.6. Practical Application of MIP SNPs

To verify the applicability of the proposed method, honey samples were analyzed. The recovery test were conducted with spiking honey samples. As shown in Table 2, the recoveries from 98.7% to 99.9% with the relative deviation from 8.62% to 4.92% were obtained for Paclobutrazol. The results indicated that the MIP SNPs was applicable for the recognition and separation with high affinity and selectivity of PBZ in different honey samples.

4. CONCLUSION

In this study, it was developed efficient and selective method for specifically recognize and separate paclobutrazol in honey samples. It was chosen the 2-hydroxyethyl methacrylate coated silica nanoparticles as the solid support for rebinding the template molecule, paclobutrazol. This support provide a good platform for pesticide imprinting. The pesticide imprinted silica nanoparticles have high surface area, a uniform well-defined spherical structure and high affinity to template molecules. Moreover, these nanoparticles possess fast and selective recognition with high affinity of PBZ from aqueous solutions. After MIP SNPs were reused and regenerated 14 times, the fourteenth rebinding capacity was excellent. According to all results, molecular imprinted silica nanoparticles have potential applications in the selective recognition and separation of paclobutrazol in real samples. As a result, these MIP SNPs can be use as support for recognition with high affinity of PBZ. In addition, PBZ imprinted silica nanoparticles may be used to clean-up pesticide residues from food samples.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors

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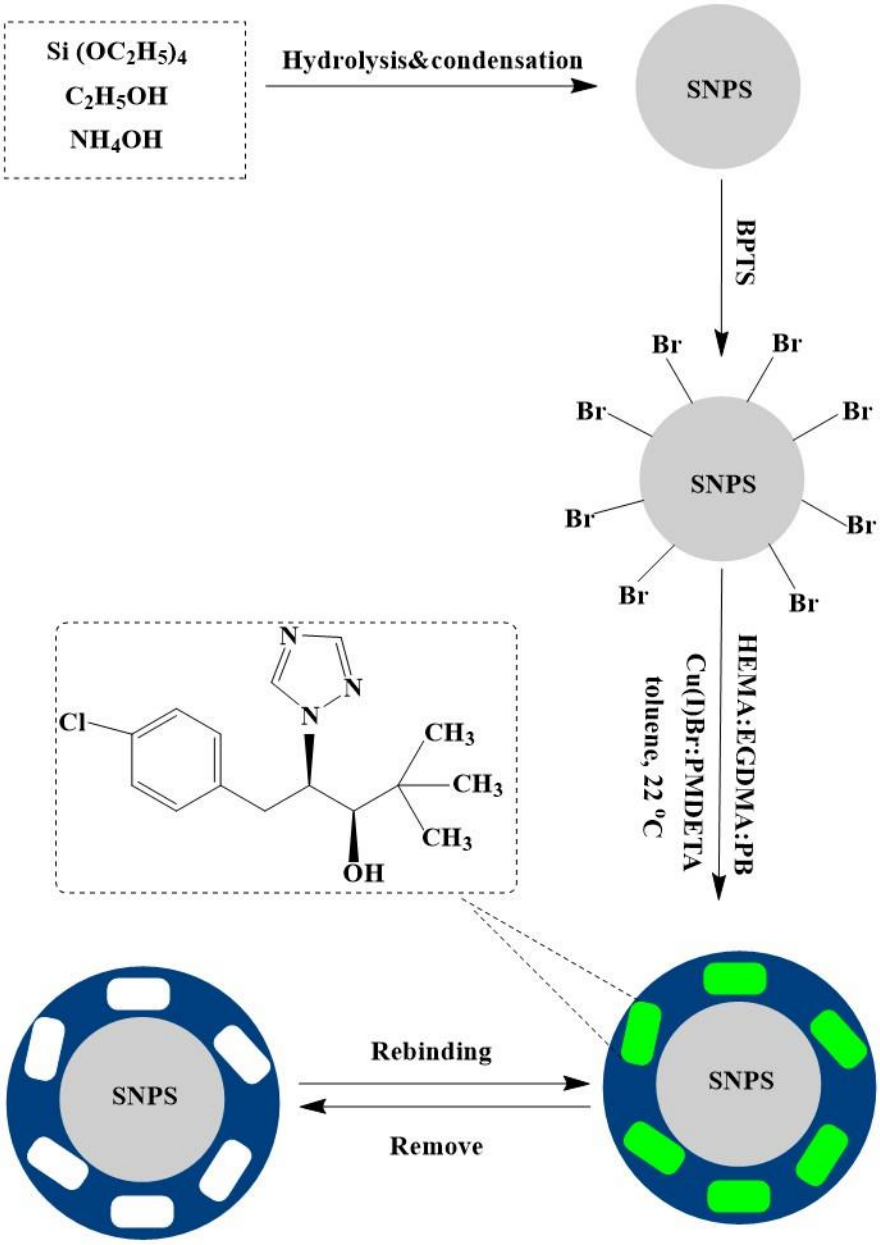
APPENDIX: FIGURES and TABLES



Scheme 1. *Synthetic route of PB imprinted MIP SNPs by surface imprinting method.*

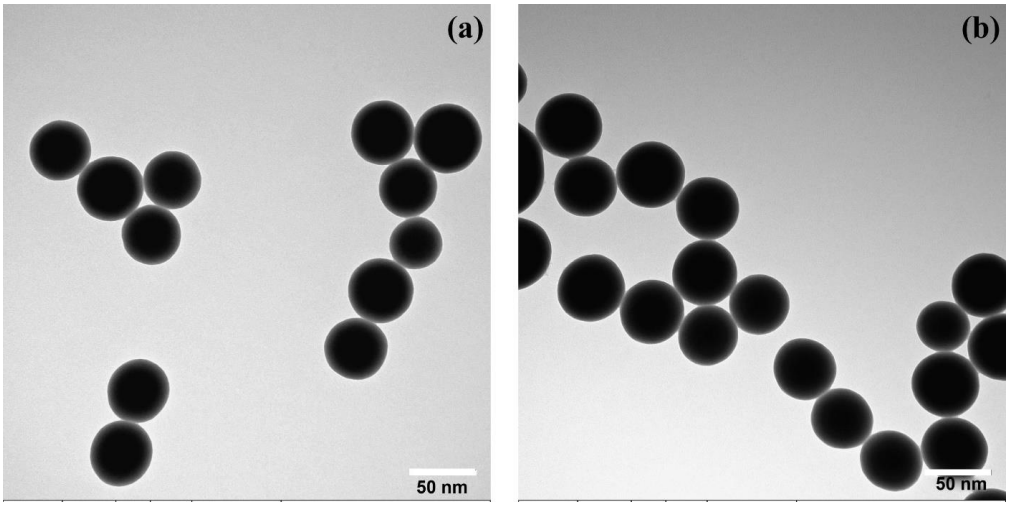


Figure 1. *TEM images of NIP SNPs (a), and MIP SNPs (b).*

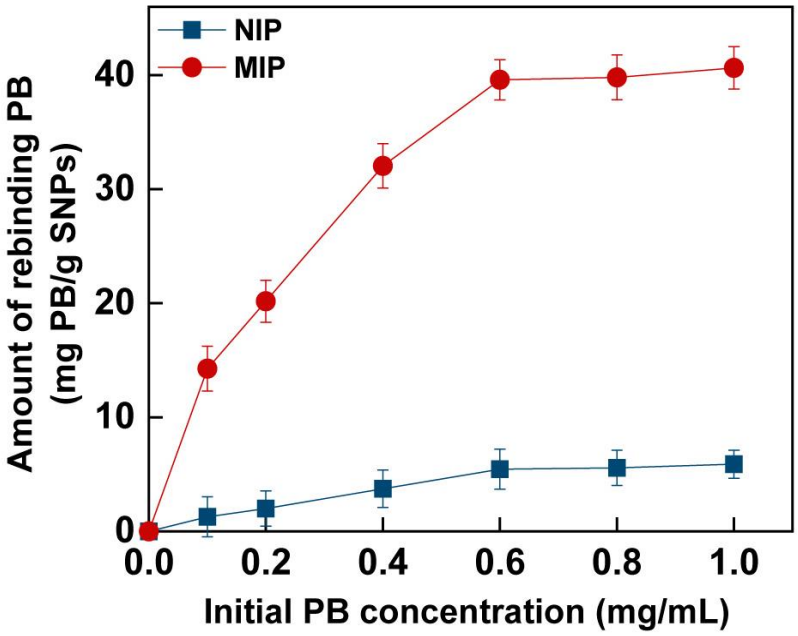


Figure 2. *Initial concentration effect of rebinding PB on the surface of NIP and MIP SNPs.*

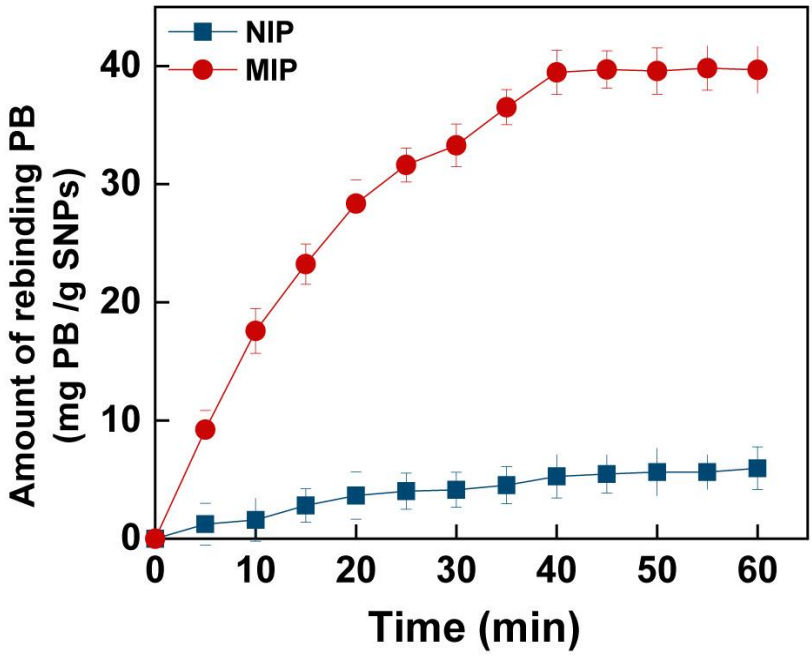


Figure 3. *Rebinding time effect of rebinding PB on the surface of NIP and MIP SNPs.*

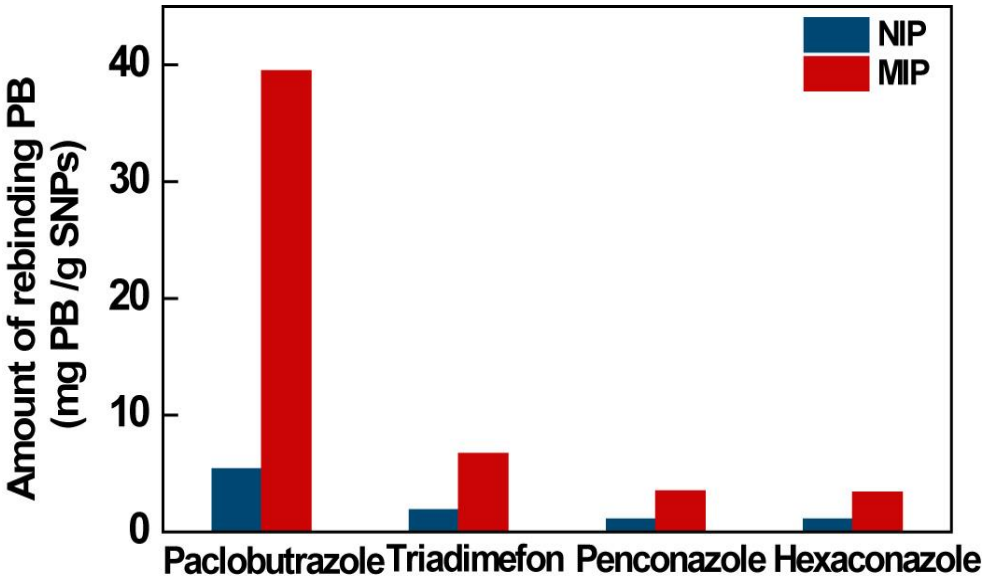


Figure 4. *Selectivity of PB compared with other pesticides on the surface of NIP and MIP SNPs.*

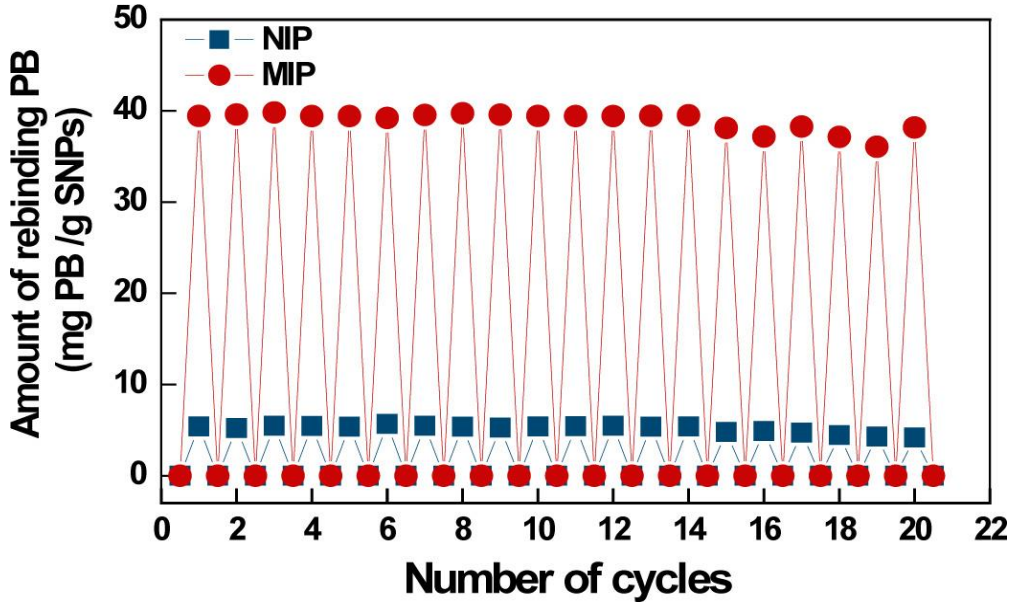


Figure 5. *Regeneration experiments for the NIP and MIP SNPs.*

Table 1. *The adsorption capacities, imprinting factors (α) and selectivity factors (β) of PB, Triadimefon, Penconazole and Hexaconazole for MIP SNPs*

Toxin	Q_{MIP} (mg g^{-1})	Q_{NIP} (mg g^{-1})	α	β
Paclobutrazol	39.5	5.4	7.31	-
Triadimefon	6.7	1.9	3.53	2.07
Penconazole	3.5	1.1	3.18	2.30
Hexaconazole	3.4	1.1	3.09	2.37

Table 2. *Recovery of PB in honey samples after extracted from MIP SNPs (n = 5).*

Added PB (µg/mL)	Found PB (µg /mL)[a]	Recovery (%)[b]	R.S.D. (%)[c]
4.75	4.69 ± 0.28	98.7	5.97
9.50	9.43 ± 0.61	99.3	6.47
19.0	18.8 ± 1.62	98.9	8.62
38.0	37.83 ± 1.87	99.6	4.92

[a] Determination by molecularly imprinted method.
[b]Recovery = measured spiked sample concentration /initial spiked sample concentration×100%.
[c] Relative standard deviation, R.S.D. = (standard deviation/mean) ×100%.