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Application of magnetic molecularly imprinted polymers for extraction of imidacloprid from eggplant and honey

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ABSTRACT

A magnetic molecularly imprinted polymer (MMIP) adsorbent for imidacloprid was prepared using non-covalent approach with functionalized nano Fe_3O_4 particles (magnetic cores), imidacloprid (template), acrylic acid (functional monomer), ethylene glycol dimethacrylate (cross linker) and azobisisobutyronitrile (initiator) and used for selective separation of imidacloprid from honey and vegetable samples. The polymers were characterized using FT-IR spectroscopy, SEM and TEM images. For analysis of imidacloprid LC-MS/MS equipment was used. Adsorption kinetics was best explained by pseudo-second-order kinetic model. Adsorption data fitted well into linearized Freundlich equation ($R^2 > 0.98$). Scatchard plot analysis indicates the presence of two classes of binding sites in the MMIPs with the C_{max} of 1889.6 μg g^{-1} and 65448.9 μg g^{-1}, respectively. MMIPs demonstrated much higher affinity for imidacloprid over structurally similar analogues acetamiprid ($\alpha = 23.59$) and thiamethoxam ($\alpha = 17.15$). About 87.1 ± 5.0% and 90.6 ± 5.6% of the added imidacloprid was recovered from MMIPs in case of fortified eggplant and honey samples, respectively.

1. Introduction

Agrochemicals intervention in agriculture has played a crucial role in achieving the food sustainability for the ever increasing population. Over the years, many different classes of pesticides with desirable characteristics especially safety to the environment have been introduced in the market. Imidacloprid (IMD) is one such molecule belonging to neonicitinoid group with systemic activity. It is insect neurotoxin and widely used in agriculture throughout the world (Gervais et al., 2010). In India, imidacloprid is registered for use on cotton, paddy, vegetables, pulses, millets etc. Number of monitoring studies conducted in India and abroad has shown the presence of undesired residues of imidacloprid in fruits, vegetables and cereals (Kapoor et al., 2013; Daragmeh, Shraim, Abulhaj, Sansour, & Ng, 2007). Recently neonicitinoid insecticides including imidacloprid have been blamed for the Colony Collapse Disorder (CCD) observed in honey bee in Europe and North America (Blanchard et al., 2008; Higes et al., 2009; Smith et al., 2013). It has also been reported that sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies (Peng & Yang, 2016).

Developing analytical tools for the detection of trace levels of pesticides in complex matrices is a challenging task and invariably requires one or more sample cleanup steps. In last few years methods like solid phase extraction (SPE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are gaining importance in sample preparation. Even with all these advancements, there is always a growing demand for high throughput methods especially for analysis involving highly heterogeneous and complex matrices. Recently molecularly imprinted polymers (MIPs) with specific binding sites for particular analyte has received tremendous attention of the researchers worldwide for selective and sensitive detection of analyte in complex matrices. Molecularly imprinted polymers (MIPs) are synthetic receptors possessing unique cavities designed for a target molecule. They are produced by a templating process by co-polymerization of functional monomer and cross-linker. The MIPs specifically recognize and bind with the target molecules. The affinities of MIPs are comparable to those of natural receptors (Bui & Haupt, 2010). Their most significant advantages like high stability, long life, and easy preparation have led to their extensive applications in chromatographic separation (Ou et al., 2007), chemical sensors (Malitesta et al., 2012), chiral separation (Alvarez-Lorenzo and Concheiro, 2004), SPE (Urraca, Moreno-Bondi, Hall, & Sellergren, 2007), and catalysis (Pasetto, Maddock, & Resmini, 2005). Magnetic molecularly imprinted polymers (MMIPs) have additional advantage of easy separation. The polymer after use can easily be separated conveniently and economically by using a strong magnet and the tedious steps of centrifugation and filtration can be avoided (Hu, Liu, Zhang, & Li, 2009). Use of MMIP makes the method easier, quicker, simpler, and more effective to perform than MIP-SPE with cartridge

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mode. In recent years, use of MIPs in solid phase extraction has increased manifold. Tang, Gao et al. (2016, Tang, Lan et al., 2016)) have reported successful utilisation of MIPs for detection of clenbuterol and ractopamine in pork. The materials showed fast adsorption kinetic, high adsorption capacity and specific recognition ability. Baeza et al. (2016) have reported MIP-liquid chromatography-tandem mass spectrometry based multiresidue analysis of cephalosporin antibiotics in bovine milk. Selective extraction and determination of a mycotoxin citrinin in rice samples based on magnetic molecularly imprinted polymers has been reported by Urraca et al. (2016).

The objective of this study is to synthesize and characterize the magnetic molecularly imprinted polymers selective for imidacloprid. The magnetic properties have been imparted into the MIPs by encapsulating it with Fe_3O_4 magnetite particles. Prepared magnetic molecularly imprinted polymers (MMIPs) and magnetic non-imprinted polymers (MNIPs) were characterized and their adsorption capacity, kinetics, selectivity, regeneration and reusability have been evaluated. Prepared MMIPs have finally been used successfully for the removal of imidacloprid from spiked eggplant and honey samples.

2. Materials and methods

2.1. Reagents and chemicals

Analytical grade imidacloprid (purity 98.3%), acetamiprid (purity 98.1%) and thiamethoxam (purity 98.8%), ethylene glycol dimethacrylate (EGDMA, 98%) and azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich, Germany. Iron oxide (Fe_3O_4, 98.5%, < 30 nm) was purchased from Nanoshel, USA. Polyvinylpyrrolidone (PVP) and oleic acid were purchased from Thomas Baker (Chemicals) Pvt. Ltd, Mumbai, India. HPLC grade acetonitrile, methanol, buffer capsules (of pH 4.0, 7.0 and 9.2) and anhydrous magnesium sulfate (ENSURE®) were procured from Merck Specialities Private Limited, India. PSA used in QuEChERS was purchased from Supelco, USA. High purity water with a resistivity of 18.2 MΩ.cm was obtained from a Milli-Q water system (Millipore, Billerica, MA, USA).

The stock solution of imidacloprid, acetamiprid and thiamethoxam (1000 μg mL^{-1}) were prepared in methanol and stored in refrigerator at 4 °C. The working solutions were prepared daily by diluting with methanol: water (1:1). Control sample of honey was obtained from the Project Coordinator, All India Coordinated Research Project on Honey bees & Pollinators, ICAR-IARI, New Delhi. Control samples of eggplant were obtained from the research farms of ICAR-IARI. Both the samples were stored in refrigerator at 4 °C.

2.2. LC-MS/MS analysis

LC-MS/MS instrumental parameters were optimized for analysis of imidacloprid, acetamiprid and thiamethoxam. Method was optimized using Shimadzu LCMS-8030 instrument equipped with Zorbax Eclipse Plus C-18 column (Agilent) (3 × 100 mm, 3.5 μ) with 23 min run time and gradient mobile phase flowing at 0.2 mL/min. Composition of mobile phase was: Mobile phase A – 80:20 5 mM ammonium formate:MeOH and Mobile phase B – 90:10 MeOH: 5 mM ammonium formate. Mobile phase programming was started from 45% B for 1 min and gradually increased to 100% B in 13 min. Isocratic flow was maintained from 13 to 18 min and then the system was brought back to the initial values at 19 min. MS parameters were: Electron spray ionization (ESI) in positive mode, DL temperature 250 °C, heat block temperature 400 °C, nebulising gas flow 3 L/min, drying gas flow 15 L/min. The individual standards of the pesticides were first scanned to select precursor ion. MRM optimization was then done to select best product ion and to optimize collision energy, Q1 Pre-bias and Q3 Pre-bias. The optimized parameters are presented in the Table 1. Under optimized instrumental conditions, the calibration curves for imidacloprid, acetamiprid and thiamethoxam were found to be linear from 0.1 to 10 μg mL^{-1} with $R^2 > 0.98$.

2.3. Preparation of MMIPs

Imidacloprid (1.0 mmol, 255.7 mg) was dissolved in 10 mL methanol in an RB flask and to it monomer acrylic acid (4.0 mmol, 288.2 mg) was added. This mixture was stirred for 30 min for preparation of the preassembly solution. In a separate two necked flask, Fe_3O_4 (1.0 g) was mixed with 1.0 mL of oleic acid and stirred for 10 min. Then 20 mmol of EGDMA (ethylene glycol dimethacrylate, 3.96 g) and the preassembly solution were added to the mixture of Fe_3O_4 and oleic acid. This mixture was subjected to ultrasonication for 30 min for preparation of the pre-polymerization solution. Polyvinylpyrrolidone (PVP) (0.4 g), used as dispersant, was dissolved in 100 mL of ethanol: water (80:20) and added to the reaction mixture along with 50 mg of AIBN. The mixture was stirred and purged with nitrogen gas to displace oxygen while the temperature was increased to 60 °C. The reaction was allowed to proceed at 60 °C for 24 h. After polymerization, the polymer was separated by filtration. The template molecules from the polymer network were removed by the optimized Method 3 described under Section 2.6.

The magnetic non-imprinted polymers (MNIPs) were prepared and processed similarly except that the template molecule i.e. imidacloprid was not added in the reaction mixture.

2.4. Characterization of the MMIPs and MNIPs

Surface and internal morphology of the prepared MMIPs and MNIPs were determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Functional groups present in the polymers were characterized by Fourier Transform Infrared Spectroscopy (FT-IR). CarlZeiss-Evo-MA-10 scanning electron microscope (SEM) at 20 kV/EHT and 10 Pa was utilized for the surface morphology characterization of the samples. Gold and palladium (2 mm thick) was coated on the samples and photo was taken under high vacuum. Transmission electron microscopy (TEM) was done using the instrument JEOL 100CX-11. The sample suspension (1% in ethanol) was mounted on the carbon grid, then 10 drops of distilled water was used to wash and then it was stained with 2–3 drops of 2% uranyl acetate. After drying, the grid was examined under transmission electron microscope. FT-IR spectra were recorded using Bruker (Alpha) instrument in the spectral range of 400–4000 cm^{-1}. Pressed tablets prepared by mixing sample with KBr (1:100, w/w) were used for recording the spectra.

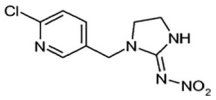
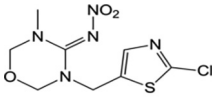
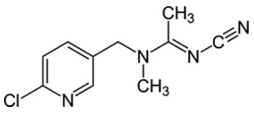
2.5. Binding experiment

Kinetic experiment was conducted in a tube containing 3 mL of 50 μg mL^{-1} imidacloprid solution (prepared in 1:1, MeOH: water) and 20 mg of MMIP. Tubes were kept on shaker. Three tubes were taken out at different time (0, 5, 10, 20, 30 60, 90, 120 180 and 240 min) and processed. Supernatant was diluted and analysed for imidacloprid residues to determine binding efficiency of MMIP as a function of time.

For binding isotherm study, 20 mg of MMIPs or MNIPs were weighed in glass tubes and to it 3 mL of varying concentration of imidacloprid solution (1, 5, 10, 20, 50 and 100 μg mL^{-1}) prepared in 1:1 methanol: water was added. All sets of MMIPs and MNIPs were shaken simultaneously for 2 h on a horizontal shaker. After shaking, the tubes were centrifuged and the supernatant was analysed by LC-MS/MS.

To investigate the relative selectivity of the prepared MMIPs and MNIPs, sorption experiment was conducted with 3 mL solution containing 50 μg mL^{-1} each of imidacloprid, acetamiprid and thiamethoxam. Contents were shaken with 20 mg of MMIPs or MNIPs. After 2 h, the tubes were centrifuged and the residues of imidacloprid, acetamiprid and thiamethoxam were quantified in the supernatant by LC-

Table 1
LC-MS/MS parameters for quantification of imidacloprid, acetamiprid and thiamethoxam.

Pesticide	Chemical structure	RT (Min)	Precursor (*m/z*)	Product (*m/z*)	Q1 Pre-bias	Collision energy	Q3 Pre-bias
Imidacloprid		3.22	256.0	209.10	−13	−15	−16
Thiamethoxam		2.79	292.0	211.1	−14	−14	−17
Acetamiprid		3.58	222.9	126.1	−24	−20	−27

MS/MS.

Buffer solutions of pH 4.0, 7.0 and 9.2 were prepared by dissolving the content of the respective pH capsules in 100 mL Milli Q water. Measured quantity of the imidacloprid stock solution was added to the different buffer solutions to get 50 μg mL^{-1} concentrations. The fortified buffer solutions (3 mL) were stirred with 20 mg of MMIPs or MNIPs on horizontal shaker. After 2 h the samples were centrifuged and supernatant analysed for imidacloprid residues.

In all the above experiments, the amount of imidacloprid bound on to the polymers was calculated by subtracting the amount present in the supernatant from the initial amount added to the mixture. All the treatments were replicated thrice.

2.6. Elution optimization

For removal of template molecule from the polymeric matrix three methods were tried:

Method 1: The template molecules from the polymer network were removed by Soxhlet extraction with methanol/acetic acid (8:2, v/v) for 12 h. The extract was concentrated and reconstituted in methanol and analysed by LC-MS/MS to determine the extraction percentage. To remove residual acetic acid, the polymer particles were washed with pure methanol, dried under vacuum at 50 °C, and stored at ambient temperature.

Method 2: The polymers were dispersed in 5 mL acetone: acetic acid (8:2, v/v) and sonicated for 1 min. Supernatant was collected in a tube and the matrix was once again dipped in 5 mL of washing solvent and sonicated for 1 min. Process of washing was repeated one more time. All the three washings were combined, concentrated, reconstituted and analysed by LC-MS to determine the amount of imidacloprid extracted from the polymer. To remove residual acetic acid, the MMIP particles were washed with pure methanol, dried under vacuum at 50 °C, and stored at ambient temperature.

Method 3: This method was similar to Method 2 except that the solvent system used for extraction was methanol: acetic acid (8:2, v/v).

Based on the highest extraction percentage and the ease, Method 3 was selected for further studies.

2.7. Regeneration/reuse of MMIPs

To the 3 mL solution of imidacloprid 50 μg mL^{-1} (prepared in 1:1, MeOH-Water), 20 mg of MMIPs were added. After 2 h of shaking, the contents were centrifuged and the MMIPs recovered. The recovered MMIPs were regenerated by washing as per Method 3 (described above) and reused in the next adsorption cycle.

2.8. Application of MMIPs for separation of imidacloprid from fortified honey and eggplant samples

Homogenised eggplant sample (10 g) was fortified at 5 μg g^{-1} level using imidacloprid solution and then mixed with 10 mL of acetonitrile, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl. Tubes were vortexed for two minute and then centrifuged at 3500 rpm for 5 min. The supernatant (2 mL) was drawn, mixed with 50 mg of MMIP and shaken for fifteen minutes at room temperature. Subsequently, the MMIPs with absorbed imidacloprid were separated from the solution using external magnetic field. In case of honey, 2 g sample was fortified at 5 μg g^{-1} level with imidacloprid solution. The sample was diluted to 10 mL with distilled water and then mixed with 50 mg of MMIP. After 15 min of shaking, the MMIPs were separated from the solution using external magnetic field. The solution (vegetable/honey) in the tube is analysed by LC-MS/MS to determine unbound imidacloprid. The separated MMIPs from vegetable/honey solution were washed with three 5 mL portions of methanol-acetic acid (8:2, v/v) with intermittent ultra sonication for 1 min. The combined eluents were concentrated and then reconstituted in 2 mL methanol for further analysis by LC-MS/MS.

In order to compare the results of MMIPs with the conventional method, the fortified vegetable samples were also processed as per QuEChERS technique. Homogenised eggplant sample (10 g) fortified with imidacloprid at 5 μg g^{-1} level and mixed with 10 mL acetonitrile, 4 g of anhydrous $MgSO_4$ and 1 g of NaCl. Tubes were first vortexed for two minutes and then centrifuged at 3500 rpm for 5 min. 2 mL supernatant was withdrawn in eppendorf tube and mixed with 50 mg PSA (Agilent) and 150 mg anhydrous $MgSO_4$. Eppendorf tube was vortexed for 2 min and then centrifuged. Supernatant was filtered through 0.45 μ syringe filter and analysed by LC-MS/MS. All the experiments were conducted in triplicate and the mean value has been reported in results.

3. Results and discussion

3.1. Characterization of the MMIPs and MNIPs

Characterization of the MMIPs and MNIPs was done by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FT-IR). The SEM images of MMIPs and MNIPs (Fig. 1A), show that the MMIPs possess spherical structures with rough surfaces. The loose and porous structures with many cavities may be responsible for the selective binding of the template molecules as compared to MNIPs. The TEM images of MMIPs and MNIPs (Fig. 1B) revealed that the three-dimensional structure of MMIPs appeared more porous, irregular and looser, presumably due to the presence of the imprint in comparison with that of MNIPs (Miao et al., 2015). Both MMIPs and MNIPs gave almost identical spectra because of the same chemical composition and functional groups

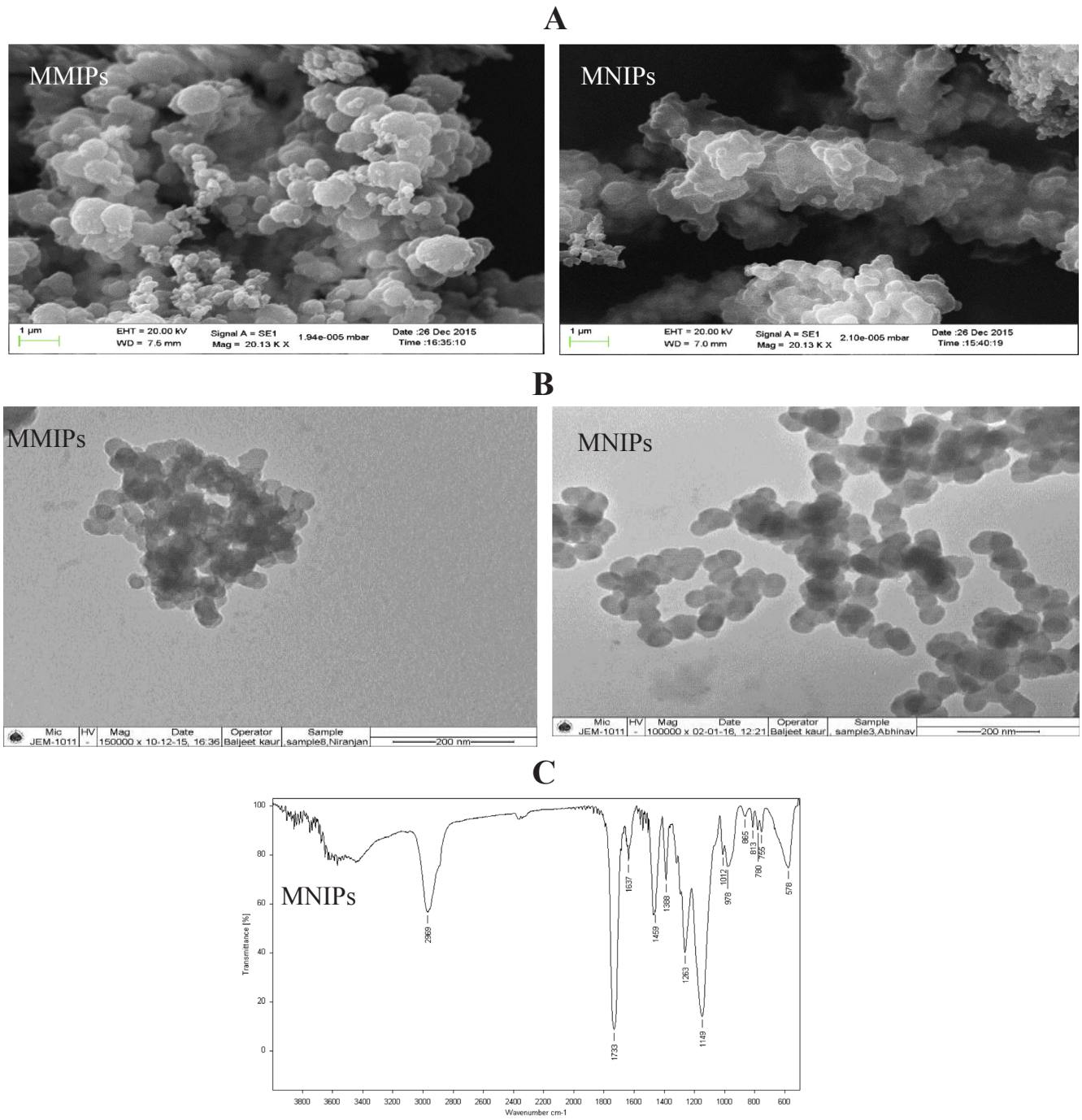


Fig. 1. SEM (A), TEM (B) and FTIR (C) spectra of MMIPs/MNIPs.

present in them. FT-IR spectra of MMIPs (Fig. 1C) revealed characteristic peaks at 1733 cm^{-1} for carbonyl stretching and at 1149 cm^{-1} for C—O stretching. This suggests the incorporation of cross-linker EDGMA in the polymeric matrix. Broad peak at around 3600–3400 cm^{-1} may be assigned to —OH stretching vibration in acrylic acid. Peaks at 2969 cm^{-1} may be assigned to C—H stretching and at 1459 cm^{-1} to C—H bending vibrations. A characteristic band of Fe-O appeared at 574 cm^{-1} indicating the inclusion of Fe_3O_4 in the matrix.

3.2. Binding studies

The binding properties of MMIPs and MNIPs have been estimated by conducting the isothermal absorption experiment in the concentration range 1–100 µg mL^{-1}. The amount of pesticide adsorbed at equilibrium, C_s (µg adsorbate/g adsorbent), was calculated using the following mass balance equation:

$$C_s = (C_o - C_e).\ V/m$$

where C_o and C_e (µg mL^{-1}) are the initial and equilibrium liquid-phase concentrations of the pesticide respectively, V is the pesticide solution volume (mL) and m is mass of the polymer (mg). Binding isotherms for imidacloprid obtained by plotting initial concentration of imidacloprid against adsorbed concentration (C_s) showed that the amount of imidacloprid bound to the polymers (both MMIPs and MNIPs) at equilibrium increased with the increasing initial concentration of imidacloprid (Fig. 2A). Furthermore, significantly high binding of imidacloprid on MMIPs as compared to MNIPs revealed good selectivity of MMIPs for the template molecule imidacloprid.

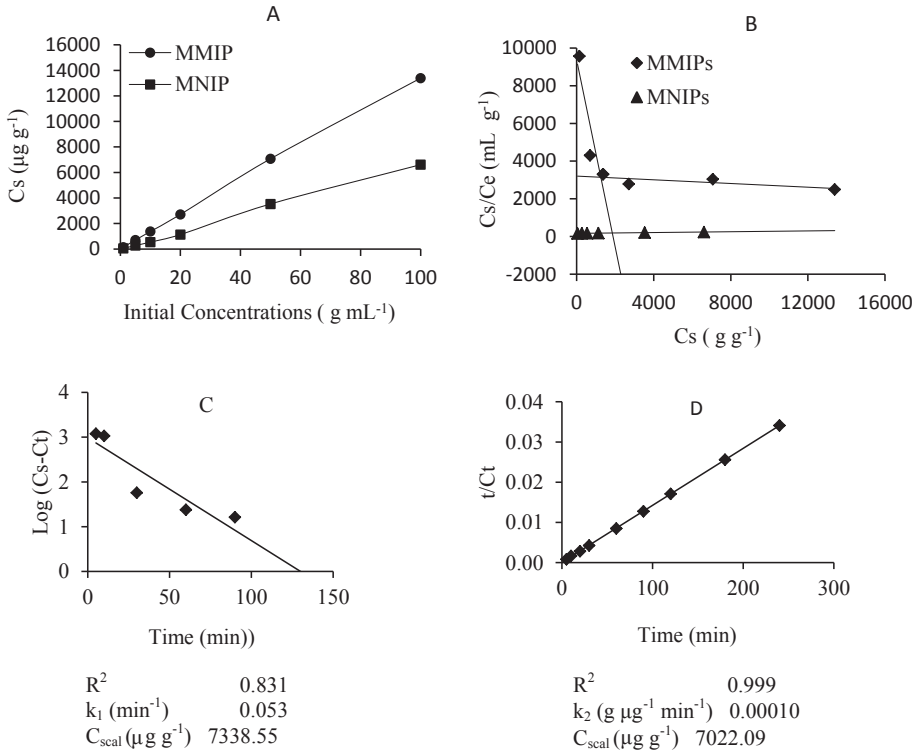


Fig. 2. Binding isotherms (A), Scatchard plot analysis (B), Pseudo first order kinetics (C) and pseudo second order kinetics (D) of binding of imidacloprid onto MMIPs and MNIPs.

Scatchard plot analysis was conducted to assess the binding site heterogeneity of the prepared polymers. Sorption data was fitted into following Scatchard equation:

$$C_s/C_e = (C_{max} - C_s)/K_{dc}$$

Where, C_s (µg g^{-1}) is the amount of pesticide bound to the polymers at equilibrium; C_e (µg mL^{-1}) is the equilibrium concentration of pesticide in solution; K_{dc} (µg mL^{-1}) is the dissociation constant of the binding sites and C_{max} (µg g^1) is the apparent maximum binding amount. The values of K_{dc} and the C_{max} can be calculated from the slope and intercept of the linear plot of Cs/Ce versus Cs (Ma & Chen 2014).

Scatchard plot for MMIPs consisted of two linear parts with different slopes which signify the presence of heterogeneous binding sites (Fig. 2B). The linear regression equation for the left part of the curve (concentration range 1–10 µg mL^{-1}) was Cs/Ce = -4.9657x + 9401. The K_{dc} and C_{max} were calculated to be 0.201 µg mL^{-1} and 1889.6 µg g^{-1} of dry polymer, respectively. The linear regression equation for the right part of the curve (concentration range 10–100 µg mL^{-1}) was Cs/Ce = −0.0049x + 3207.0 with K_{dc} and C_{max} values of 20.4 µg mL^{-1} and 65448.9 µg g^{-1} of dry polymer, respectively. The Scatchard plot of MNIPs was a single straight line which indicates the presence of homogenous binding sites. The linear regression equation of the line curve was Cs/Ce = 0.0112x + 164.79 with K_{dc} and C_{max} values of 89.3 µg mL^{-1} and 14715.7 µg g^{-1}, respectively.

3.3. Kinetic adsorption experiment

Fig. 2 shows the kinetics of imidacloprid sorption onto MMIPs. Kinetic data was subjected to pseudo first order and pseudo second order equations represented as $\ln (C_s - C_t) = \ln C_{scal} - k_1 t$ and $t/C_t = 1/k_2 C_{scal}^2 + t/C_{scal}$, respectively. C_s and C_t are the amount of imidacloprid adsorbed at equilibrium and at time t respectively. k_1 and k_2 are the equilibrium rate constants for the pseudo first order and pseudo second order sorption model and C_{scal} is the theoretical adsorption capacity of the respective model. The values of these constants can be calculated from the intercept and slope of the linear plot of $\ln (C_s - C_t)$ versus t for pseudo first order model and t/C_t versus t for pseudo second order model. As shown in Fig. 2C & D, the experimental data of imidacloprid adsorption on MMIPs fitted better into pseudo second order model in terms of higher R^2 value of 0.999 and closer values of experimental (7071.14 µg g^{-1}) and theoretical (7022.09 µg g^{-1}) absorption capacities.

3.4. Optimization of extraction conditions

3.4.1. MMIPs amount

To determine minimum amount of MMIPs required for optimum adsorption, the sorption experiment was conducted by dispersing different amounts of MMIPs ranging from 10 to 100 mg in the imidacloprid solution. The results revealed that 20 mg of MMIPs were sufficient to adsorb 94.5% of the imidacloprid from the solution (Fig. 3A). Further increase in the sorbent amount did not affect the adsorption significantly.

3.4.2. Extraction time

Extraction time required for optimum extraction was determined by conducting the sorption studies from 0 to 120 min. The results indicated that the imidacloprid sorption increased from 78.4% to 93.7% with the increase in extraction time from 5 min to 20 min (Fig. 3B). After 20 min no significant change in adsorption was noticed. The MMIPs showed very fast adsorption because of the presence of specific imprinted sites on the surface.

3.4.3. Elution condition

In order to elute bound imidacloprid from MMIPs, three methods viz. Method-1: Soxhlet extraction with methanol: acetic acid (8:2), Method-2: dipping and sonication with acetone: acetic acid (8:2) and Method-3: dipping and sonication with methanol: acetic acid (8:2) was tried. In the last two methods, extraction step was repeated three times using 5 mL solvent each time and one minute of intermittent sonication. Out of the three methods, Method 3 gave the highest recovery with the mean removal of about 92.2% followed by Method 1with the mean

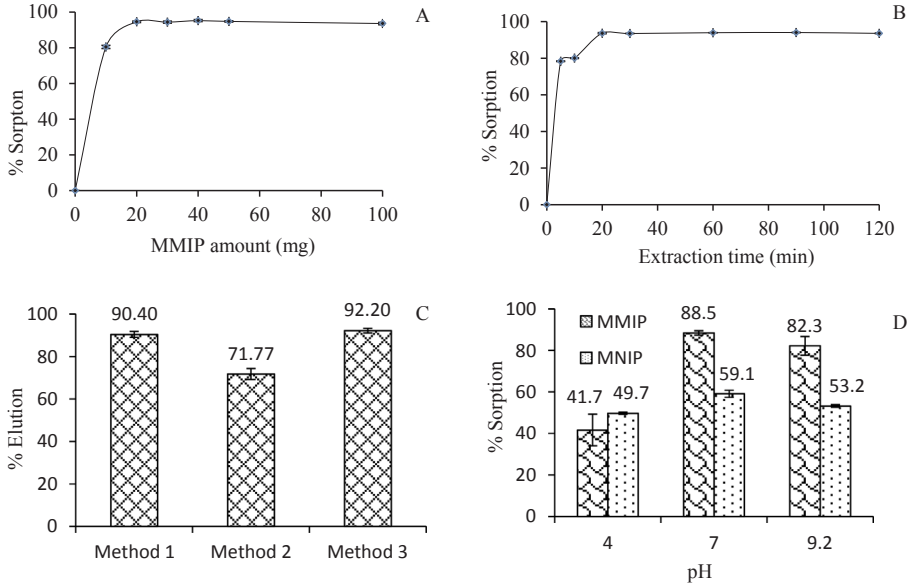
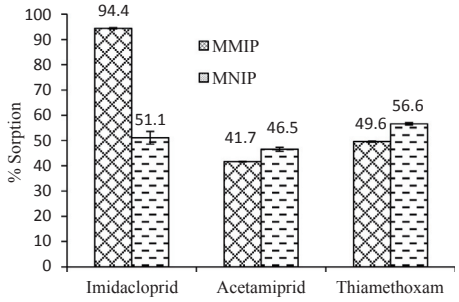


Fig. 3. Effect of sorbent amount (A), extraction time (B), elution condition (C) and solution pH (D) on sorption of imidacloprid.



Adsorbent	Molecule	K_d (mL/g)	α	β
MMIPs	Imidacloprid	5057.98		
	Acetamiprid	214.43	23.59	19.62
	Thiamethoxam	294.95	17.15	21.41
MNIPs	Imidacloprid	313.30		
	Acetamiprid	260.62	1.20	
	Thiamethoxam	391.06	0.80	

Fig. 4. Binding selectivity and selectivity parameters of MMIPs for imidacloprid.

removal of 90.4%. Method 2 was least efficient with elution of only 71.7% of bound imidacloprid (Fig. 3C). Based on the results Method 3 was selected for further studies.

3.5. Effect of pH on adsorption capacity of MMIPs

Effect of solution pH on the sorption efficiency of MMIPs was studied by conducting the sorption experiment at different pH viz 4.0, 7.0 and 9.2. The result shown in Fig. 3D revealed that highest sorption by MMIPs was obtained at neutral pH = 7 (88.5%) followed by alkaline pH = 9.2 (82.3%). In acidic condition (pH = 4), the sorption was lowest probably due to the formation of salt by the slightly basic imidacloprid molecule. Soekamto et al. (2017) have also observed low sorption of β-sitosterol on molecularly imprinted polymers at low pH. They have mentioned that at low pH, compound containing certain functional groups get protonated and this protonation affect the interaction of compound with the active site of the MIP. The obtained data revealed that the sorption of imidacloprid by the MMIPs was strongly influenced by the solution pH. As expected, no significant change in sorption characteristic of MNIPs towards imidacloprid was observed at different pH.

3.6. Reusability of MMIPs

The binding and rebinding studies were conducted to determine if the regenerated MMIPs obtained after elution of bound imidacloprid can be reused for removal of imidacloprid from aqueous matrix. Results revealed that the MMIPs can be regenerated by washing the bound imidacloprid as per Method 3 and can be reused without appreciable loss of their efficiency for at least three adsorption–desorption cycles. The removal efficiency of regenerated MMIPs was found to be 91.1, 89.6 and 88.7% for the three successive cycles.

3.7. Selectivity studies

Adsorption selectivity of MMIPs for imidacloprid was compared to two other structurally similar neonicotinoid molecules namely acetamiprid and thiamethoxam. MMIPs exhibited much higher binding affinity for the template molecule imidacloprid than for the structural analogues probably due to the presence of template selective molecular recognition sites in the MMIPs. There was not much difference in the adsorption capacity of MNIPs towards template molecule and the structural analogues (Fig. 4).

The static distribution coefficient (K_d), separation factor (α) and relative separation factor (β) were used to determine the selectivity of MMIPs (Ma & Chen 2014).

$$K_d = C_s/C_e; \alpha = K_{d1}/K_{d2}; \beta = \alpha 1/\alpha 2$$

where C_s and C_e are the adsorbed and unadsorbed concentrations, respectively, and K_d defines the adsorption capacity of the polymer. Separation factor 'α' measures the selectivity of polymer and is dependent on the distribution coefficient of template (K_{d1}) and the analogue (K_{d2}). High value of α-factor signifies greater selectivity (Tan, Wangrangsimakul, Bai, & Tong, 2007). Relative separation factor (β) is calculated as the ratio of separation factors of MMIPs (α1) and MNIPs (α2). The results of selectivity experiment presented in Fig. 4 revealed that adsorption capacity of MMIPs for imidacloprid was much higher (K_d 5057.98) than that for acetamiprid (K_d 214.4) and thiamethoxam (K_d 294.95). Separation factor values of 23.59 and 17.15 obtained for imidacloprid/acetamiprid and imidacloprid/thiamethoxam combination indicate that the MMIPs are selective for imidacloprid. In case of

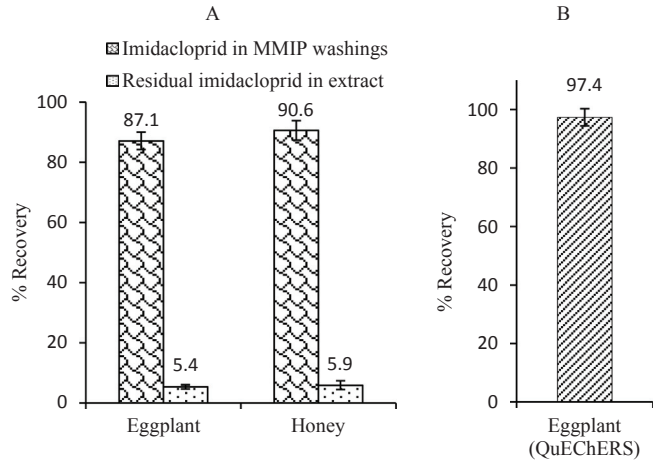


Fig. 5. Recovery (%) of imidacloprid (A) from eggplant and honey sample using MMIPs and (B) from eggplant using QuEChERS.

MNIPs, the adsorption capacities for imidacloprid and its structural analogues were almost the same and the separation factors were close to 1, indicating lack of selective imprints in the prepared MNIPs. The value of β was 19.62 and 21.41 for acetamiprid and thiamethoxam, respectively. High values of β further indicate good selectivity and strong separation capacity exhibited by the MMIPs in comparison to MNIPs.

3.8. Applicability of the Method

Potential of prepared MMIPs for removing imidacloprid from fortified honey and eggplant samples was investigated in laboratory. Imidacloprid residues in the MMIPs washings and the unbound residual imidacloprid in leftover extract were quantified using LC-MS/MS. Calculation of recovery percent revealed that 87.1 ± 5.0% and 90.6 ± 5.6% of the added imidacloprid was recovered from the MMIPs washings of vegetable and honey, respectively. Around 5.4 ± 1.2% and 5.9 ± 2.5% of residual unbound imidacloprid was also detected in the leftover vegetable and honey extract, respectively (Fig. 5A). Recovery of imidacloprid from fortified eggplant was also conducted using conventional QuEChERS method. Results of the study revealed the recovery of 97.4 ± 5.2% of the added imidacloprid (Fig. 5B). Results obtained in the proposed method using MMIPs are in good agreement with the most widely used QuEChERS method.

The analytical results obtained in our study were also compared with the earlier reported results for analysis of imidacloprid in different matrices (Fernandez-Alba, Valverde, Agüera, Contreras, & Chiron, 1996; Bonmatin et al., 2003; Garcia-Chao et al., 2010; Paradis, Berail, Bonmatin, & Belzunces, 2014; Jovanov et al., 2014; Brahim, Ammar, Abdelhedi, & Samet, 2016). The results presented in Table 2 indicate that the sensitivity, recovery and precision of our method is similar to the earlier reported methods. In addition, the selectivity exhibited by the imprinted polymers due to the presence of selective recognition sites makes the separation of imidacloprid from the matrix easier and faster. Prepared MMIPs have the potential for use as solid phase adsorbent for selectively removing the imidacloprid from complex matrices.

4. Conclusions

Imidacloprid selective magnetic molecularly imprinted polymers were synthesized by precipitation polymerization and characterized by FTIR, SEM and TEM techniques. Prepared MMIPs showed high adsorption capacity and good selectivity for imidacloprid. Scatchard analysis revealed the presence of two types of binding sites in MMIPs. Kinetic data fitted well to the pseudo-second order equation. Selective

Table 2
Comparison of our method with the literature reported methods for analysis of imidacloprid.

Sample	Sample preparation	Detection	LOD	Recovery (%)	Precision (RSD, %)	Reference
Soil	Extraction by mixing with 100 mL of methanol/NH_4OH 0.05% (3/1) for 1 min and clean up on a glass frit containing 2 g of wet Celite	LC/APCI-MS/MS	0.1 μg/kg	85	2.9	Bonmatin et al. (2003)
Pollen	Extraction with 20 mL of EtOH/H_2O (75/25)	HPLC-DAD	0.3 μg/kg	78–85	4.7	Fernandez-Alba et al. (1996)
Plant	Extraction by grinding with 100 mL of methanol/H_2SO_4 0.04% (4/1) for 1 min.	HPLC	0.1 μg/kg	78.3–81.9	4.7	
Pepper, tomato and cucumber	Extraction by blending with 100 mL of acetone and clean up using a 0.1-g C_{18} disposable cartridge	HPLC	0.01 mg/kg	95	4.7	
Honey	Extraction with 3 mL of a solution of water: methanol	LC–MS–MS	0.83–4.83 ng/g	104.5	11.3	Garcia-Chao et al. (2010)
Pollen	QuEChERs method	HPLC-MS/MS	0.2 ng/g	127.3	18.6	Paradis et al. (2014)
Honey	Dispersive liquid–liquid microextraction (DLLME)	LC-MS/MS	0.5–1.5 μg L^{-1}	69.2–113.4	3.21–10.20	Jovanov et al. (2014)
Honey	QuEChERS sample preparation	LC-MS/MS	1.0–2.5 μg L^{-1}	71.8–94.9	4.19–12.81	
Apple, cabbage, cucumber, lettuce	Elution from MIPH using acetic acid/methanol (10:90, v/v)	Molecular imprinted photonic crystal hydrogel sensor	Not mentioned	87.6–97.8	< 10	Wang et al. (2013)
Plum juice	SWV (square-wave voltammetric method)	Electrochemically pretreated boron-doped diamond (BDD) electrode	8.6 μmol/L	96.6	1.02	Brahim et al. (2016)
Tomato, cabbage, chilli, lettuce	Extraction with acetonitrile	Electrochemical sensor based on Pt-In nanoparticles and a Bromophenol blue doped molecularly imprinted film	1.2×10^{-11} mol/L	93.6–105.5	< 3	Li et al. (2016)
Rice	DSPE extraction with DLLME clean up	MIP/GN modified glassy carbon electrode (GCE)	0.10 μM	83.8	3.79	Zhang et al. (2017)
Eggplant and Honey	20 mg MMIP, extraction with 5 mL methanol: acetic acid (8:2, v/v) followed by ultra sonication for 1 min	LC-MS/MS	0.05 μg/g	94.98 (eggplant) 94.15 (Honey)	0.66 (eggplant) 2.06 (Honey)	Our study

sorption of imidacloprid from honey and eggplant sample and subsequent quantification of residues using LC-MS/MS was successfully attempted. The synthesized magnetic polymers, with added advantage of easy separation, possess a great potential as SPE sorbent for rapid, cost-effective, and efficient separation of targeted compounds from complex biological and environmental matrices.

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