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Synthesis and characterization of molecular imprinting polymer for the removal of 2-phenylphenol from spiked blood serum and river water

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Abstract

Background: The 2-phenylphenol is used as an agricultural fungicide. It is generally applied for the post-harvest treatment of fruits and vegetables to protect against microbial damage. It is also used for waxing of citrus fruits and for disinfection of seed boxes. It has been reported that 2-phenyphenol has some toxic effects human beings due to its disposal in the environment. Therefore, preparation of selective materials for the extraction of 2-phenylphenol is important. For this purpose, molecular imprinting polymer (MIP) were prepared by precipitation polymerization using 2-phenylphenol as the template molecule, styrene as the functional monomer, and divinyl benzene as the cross-linker with a non-covalent approach.

Results: The polymers were characterized by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), Fourier transform infra red spectroscopy (FT-IR), and Brunauer–Emmett–Teller (BET). The results obtained from SEM depicted that the shape of polymer particles is spherical with uniform size in micrometers. The BET results also showed better surface area (131.44 $m^2\ g^{-1}$), pore size (7.9587 Å), and pore volume (5.23 cc g^{-1}) of MIP as compared to NIP. The batch adsorption test was conducted to select a most specific polymer in terms of affinity towards the template. A series of parameters such as initial concentration, polymer dosage, effect of pH, and selectivity with structural analog were conducted. The selectivity of MIP towards the 2pp was very appreciable as compared to its structural analog biphenyl with a good adsorption capacity. Moreover, the MIP as an extractant was successfully applied for extraction of 2-phenylphenol from the spiked blood serum (93%) and river water sample (88%).

Conclusion: Molecular imprinting polymer has been successfully synthesized for the selective extraction of 2-phenylphenol from biological and environmental samples. The synthesized material has been applied for the extraction of 2-phenylphenol from blood serum and river water.

Keywords: Molecularly imprinted polymer, Precipitation polymerization, 2-Phenylphenol, Blood serum, River water

Introduction

2-Phenylphenol is a white-colored, flaky crystalline solid used as a biocide in food preservative. 2-Phenylphenol (orthophenylphenol), and its sodium salt sodium o-phenylphenate (SOPP), were evaluated as broad-spectrum fungicides and disinfectants with widespread agricultural, industrial, and domestic usage [1]. 2pp has historically been among the most widely used home and garden pesticides. 2pp is a waxy substance used as a coating agent to protect a variety of crops from storage diseases [1]. 2pp is a typical compound commonly mixed with other phenolic compounds, and the mixtures can be very irritating at sufficiently higher concentrations [2]. Due to the widespread use of especially 2pp and SOPP, the potential for consumer exposure and some "critical" findings the toxicological database is quite extensive and

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complex [3]. Phenolics are one of the oldest known disinfectant classes [4].

The most frequently used methods for the determination of 2-phenylphenol and other fungicides are high-performance liquid chromatography (HPLC) [5], gas chromatography-mass chromatography (GC–MS) [6], and liquid chromatography-tandem mass chromatography (LC–MS-MS) [7], which always involves in traditional sample preconcentration process, such as solid-phase extraction and solid-phase micro-extraction (SPME) [8]. The main drawback related to those typical sorbents of solid-phase extraction (SPE) is the low selectivity or low binding efficiency [9]. A brand new variety of high-efficiency adsorbents, molecularly imprinted polymers (MIPs), having high sample load capability, high selectivity, low price, and simple preparation, are wide applied for preconcentration and high efficient separation of trace analytes in various matrices, like a natural, agricultural, food products, and environmental samples [10]. MIPs are synthetic polymers (artificial receptors) with extremely specific recognition ability for template molecules. Within the commonest preparation method, monomers form a complex with a template through covalent or non-covalent interactions and are then joined by employing a cross-linking agent.

Now a day's concept of molecular imprinting has been widely recognized as the most advanced technology for the preparation of different recognition materials with selective adsorption. Investigation and development of artificial receptors [11] appear to be of particular interest. In comparison to their biological analogs, major advantages of the MIPs, other than possessing antibody-like molecular selectivity, include physical robustness, resistance to high temperatures and pressures, inertness to acids, bases, and organic solvents, as well as low production cost and ease of preparation [12]. The most widely used technique for preparing MIPs is non-covalent imprinting [13]. In this process, the complex of the template and the functional monomer is formed in situ by non-covalent interactions, such as hydrogen bonding, electrostatic forces, van der Waals forces, or hydrophobic interactions. Synthesis of MIP is a relatively straightforward and inexpensive procedure. The MIP is prepared by mixing the functional monomer, template, cross-linker, and initiator in a proper solvent [14]. There are many benefits of this method which includes easy preparation of the template-monomer complex and very easy removal of the templates from the polymers, fast binding of templates to MIPs, and it has very good application to a wide range of target molecules. However, to maximize the formation of the labile complex of template and monomer, the polymerization conditions must be carefully chosen to minimize non-specific binding sites [15].

Results and discussion

Preparation conditions for polymerization of MIP

A series of imprinted and non-imprinted polymers were synthesized. For polymerization of MIP, all the components in the polymerization mixture are important to produce a good imprint on the polymer. The solvent plays a very important role in producing good porosity in the polymer matrix. A good binding interaction such as hydrogen bonding, hydrophobic interactions, etc. between the template (2-phenylphenol) and monomer (styrene) will produce a good imprint on the polymer. Finally, a cross-linking monomer provides a good mechanical strength to the monomer and template complex. The cross-linking monomer is also responsible for producing a good porous structure of the polymer. In this research, styrene was used as a monomer and DVB was used a cross-linker. The combination of both the monomer and cross-linker has produced a good combination and that resulted in the synthesis of an imprinted polymer with good selectivity towards the target template (2-phenylphenol). A suitable molar ratio of 1:4:16 (2pp/styrene/DVB, template/monomer/cross-linker, respectively) in 75 mL of ACN showed a better affinity and selectivity towards 2-phenylphenol and was chosen to study the all parameters including adsorption conditions, selectivity test, and for application in water and blood serum samples.

FT-IR spectroscopy

The FT-IR spectroscopy of the imprinted polymers was done to identify the functional groups present in the obtained MIP particle. The FT-IR analysis was conducted to ensure the interaction among 2PP, Styrene, and DVB. The IR spectra (Fig. 1 and Table 1) of MIPs with the template are little different from NIP because of the existence of 2-phenylphenol as a template in the polymer matrix of MIPs. A strong broad peak at ~ 3500–3200 cm^{-1} attributed to the vibration mode of O–H stretch was observed in the IR spectra of the MIPs with the template (2-phenylphenol) while no such spectra were observed in NIP due to the absence of template molecule (2-phenylphenol). This gives a clear indication of the interaction between monomer and template. Besides that, a sharp band at ~ 1702–1631 cm^{-1} indicated the presence of C=C of alkene in the spectrum, of MIPs with the template. A strong sharp peak at ~ − 3453 cm^{-1} observed in the MIPs with template indicated the presence of OH in the polymer matrix, while the disappearance of these peaks in the MIPs after the process of washing proved that the template, 2-phenylphenol, was completely removed from the polymer matrix. There are also strong peaks located at 2925.78 cm^{-1}, 2922.56 cm^{-1}, 2922.89 cm^{-1}, and 2922.14 cm^{-1} wavelength of MIP1, MIP2, MIP3,

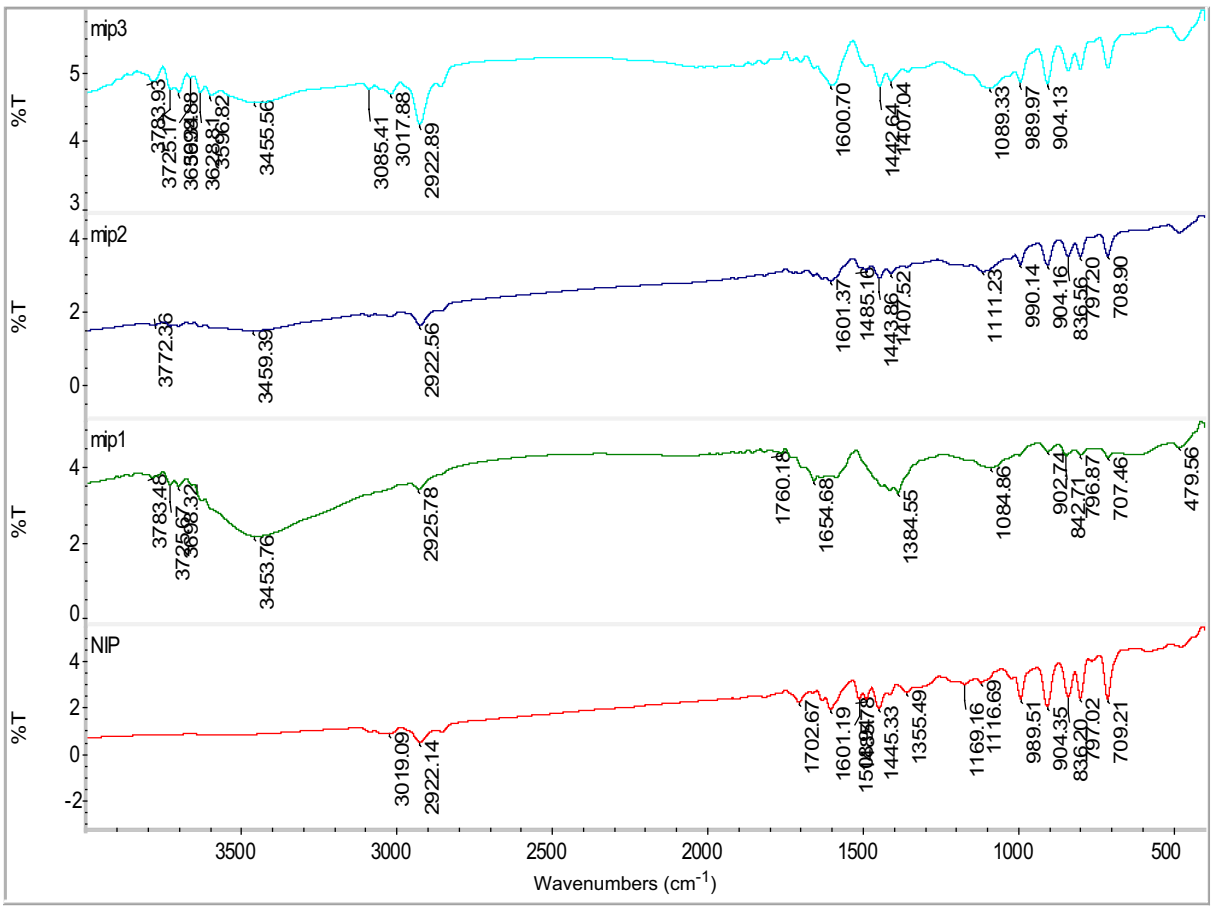

Fig. 1 FT-IR spectra of MIPs and NIP

Table 1 Wave numbers with mode of vibration of MIPs and NIP

Mode of vibration	Wavenumber (cm^{-1})
O–H stretching	3500–3200
C–H stretching	2925.78–2922.14
=C–H bending	900–850
C=O stretch	1702–1631
C=C stretch (in ring)	1180–1047
C–H bending	836–707

and NIP polymers, respectively. These peaks correspond to the C-H stretching from alkane functional group. In addition, a sharp band from 1180 to 1047 cm − 1 represents the C=C (alkene) of styrene in the polymer matrix. Some other absorption peaks were observed in the polymer structure including the C=C stretch of aromatic compounds ranging from 1450 to 1600 cm^{-1} indicate the existence of benzene ring that revealed the presence of the polymer cross-linker (DVB) in the polymer matrix. The strong peaks between ~ 900 and 850 cm^{-1} attributed to the bending mode of =C–H were observed and also peaks between 836 and 707 cm^{-1} are due to the aromatic C–H bending vibrations because of DVB.

Scanning electron microscope (SEM)

The morphological characterization (shape and size) of imprinted polymers was studied by using SEM. The SEM image (Fig. 2) depicts uniform shape and size of polymer particles were achieved. All the polymer particles were spherical in shape and the size is in the micro range [NIP (0.68 µm) and MIP (1.22 µm)]. This is due to the advantage of precipitation polymerization method [16–18] and the favorable uniform size distribution between the interaction of monomer and template. The uniform shape and size of polymer particles may be due to the solvent (acetonitrile). The acetonitrile as a solvent has been considered as the best solvent for the production of uniform shape and size of polymer

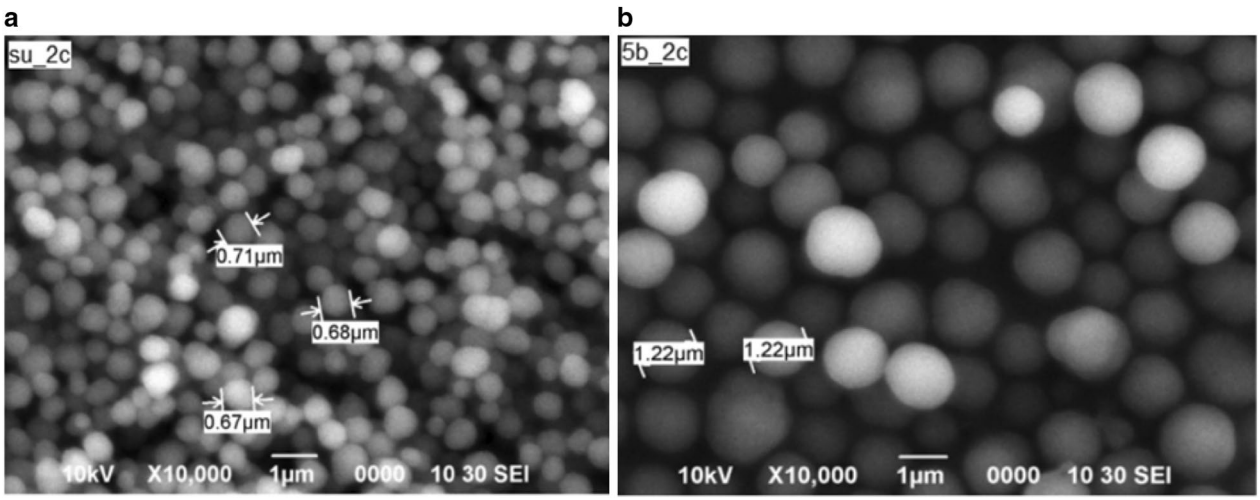

Fig. 2 SEM image for **a** NIP and **b** MIP

particles. Another factor responsible may be due to the presence of divinyl benzene as a cross-linker in the polymer matrix. The cross-linker (DVB) basically provides the mechanical strength to the polymer and enhances the stability of recognition sites in a polymer matrix [19].

Energy dispersive X-ray (EDX)

A quantitative energy dispersive X-ray (EDS) analysis was performed to observe the amount of main chemical constituents such as carbon (C) and oxygen (O) present in the imprinted polymer. It was (Fig. 3) observed that the significant amount of carbon (91.39%) and oxygen (8.21%) were present in the sample. This indicated the polymer back bone is mainly composed of carbon with substituent elements present in the chain.

Brunauer–Emmett–Teller (BET) of MIP and NIP

The specific surface areas, pore diameters, and pore volumes of MIP and NIP are shown in Table 2. The results reported that all the parameters such as specific surface area, average pore diameter, and pore volume of MIP were greater than that of NIP. This indicated that the presence of template during the synthesis of MIP has created a cavity and confined the shrinkage of the pores effectively in the process of the polymerization and hence the synthesized MIP had a greater pore size and pore volume than NIP. This also indicated that the formation of a complementary spatial structure for the selective recognition of the template by the MIP. This also provides information about the presence of template during the synthesis could be the possible way for the selective extraction of analytes from various samples.

Batch binding analysis

The batch binding of all the MIPs (MIP1, MIP2, MIP3) and NIP were carried out in order to select the MIP with maximum efficiency. The binding efficiency of MIP was calculated by using batch binding experiments. Figure 4 shows that MIP3 have the highest efficiency as compared to other MIPs and NIP because MIP3 contains a larger molar ratio of functional monomers as compared to other MIPs which might have produced more specific interaction (binding) sites hence the binding efficiency also increases. The MIP3 attained highest binding efficiency at 90 min it means the adsorption process was very fast. Further increase in contact time did not produce any significant change in adoption efficiency and it remains almost the same. In the case of NIP, efficiency is the lowest due to the absence of complementary binding sites. The low efficiency of other MIPS may be due to scattered binding sites or low-affinity binding sites which leads to low selectivity of MIP.

The calculated static adsorption Q for MIP and NIP was found to be 99.52% and 46.50%, respectively. The imprinting factor α between MIP and NIP was 2.14. The specific adsorption ratio for MIP and NIP was 53.28% which is good enough for MIP. This indicated that the selected MIP can be used for the extraction of 2pp from biological and environmental samples.

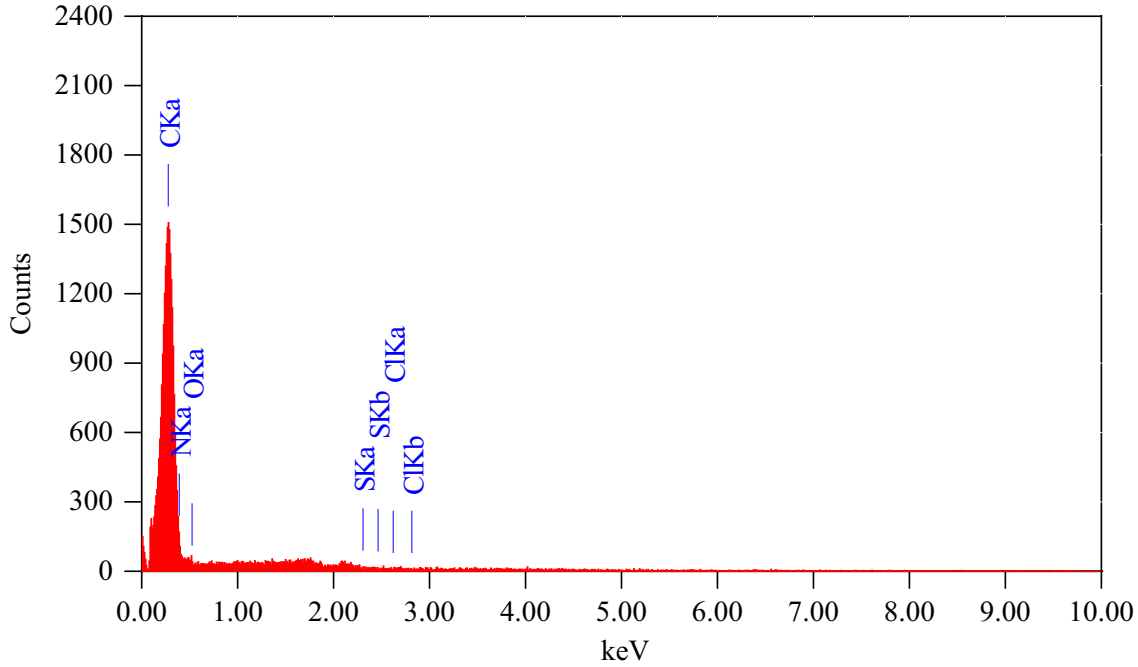

Fig. 3 EDX of molecular imprinted polymer

Table 2 BET of MIP and NIP

Properties	Magnitude MIP	Magnitude NIP
Surface area ($m^2\ g^{-1}$)	131.44	19.931
Average pore radius (Å)	7.9587	3.9679
Total pore volume (cc g^{-1})	5.23	3.954

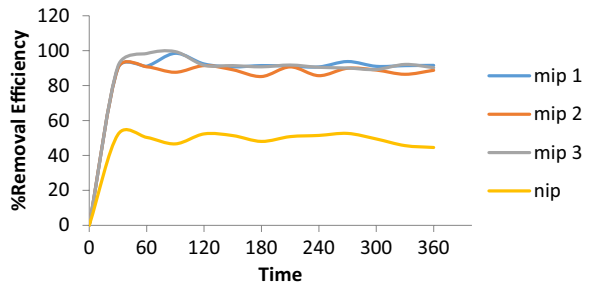

Fig. 4 Effect of contact time on adsorption efficiency

Effect of initial concentration

An adsorption isotherm is an important tool for the theoretical evaluation and interpretation of the binding capacity of MIP. In this adsorption study, the experiment was carried out at different concentration of 2pp (10,15, 20, 25, 30, 35, and 40 ppm). Figure 5 shows that the binding capacity for MIP significantly increased with the increase in concentration (10 ppm to 25 ppm) of the template and then decreased gradually with further increase in concentration (30 ppm to 40 ppm). The maximum binding capacity was achieved at 25 ppm and this may be due to the availability of an optimum number of binding sites in MIP. Further increase in template concentration may have caused overcrowding around the MIP and also the availability of limited binding sites. This is the main reason that MIPs demonstrate low affinity and low selectivity at high template concentrations. Conversely, MIPs show high affinity and high selectivity at low template concentrations where the MIP's binding characteristics are dominated by high-affinity sites.

Effect of dosage of MIP

In this study different amount of polymer was used to evaluate the binding capacity. It is clear from Fig. 6 that the binding capacity has decreased with the increased amount of polymer dosage. This may be due to the aggregation of polymer particles. This aggregation may have reduced the number of available binding sites due to the self-association of polymer particles. Because of the less availability of binding sites have decreased the

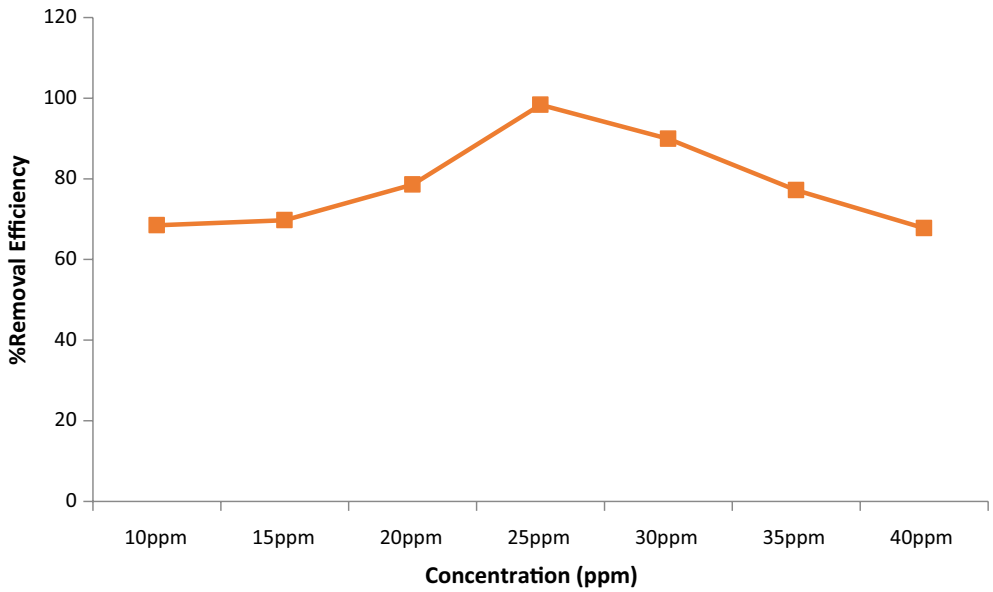

Fig. 5 Effect of concentration on the binding efficiency of MIP

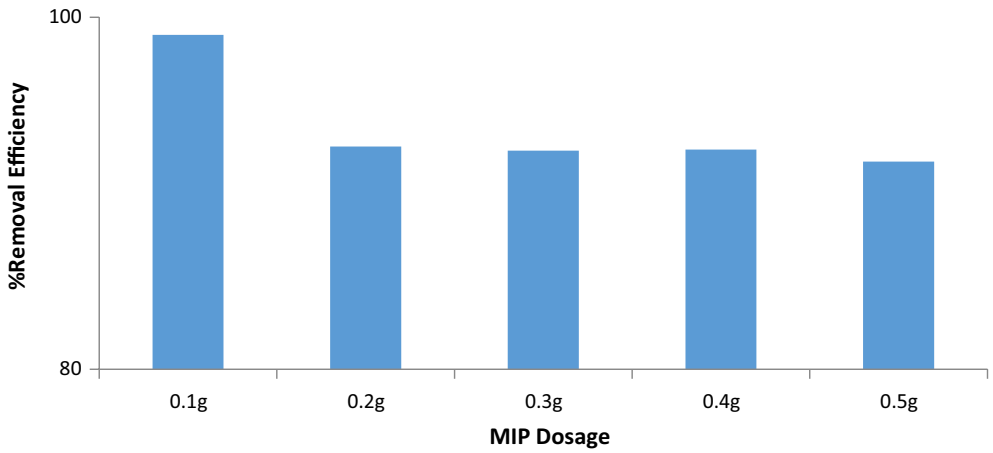

Fig. 6 Effect of polymer dosage on binding efficiency

binding capacity with an increased amount of dosage of the polymer.

Effect of pH

The effect of pH on the binding capacity of the polymer was tested at different pH. The pH of the template solution was changed from acidic basic conditions. The importance of this study explained the change in functionality of the template molecule could also change the binding efficiency of the polymer. From this study, it was observed that both in acidic and basic conditions binding efficiency was low as compared to the neutral medium. The results presented in Fig. 7 also indicated that with the increase in both acidic and basic nature of template solution the binding efficiency also decreased. This can be concluded from the results that with the change in the functionality of the template the recognition at the binding site of polymer towards the template also decreased.

Selectivity of the MIP

The adsorption selectivity of the MIP towards 2-phenylphenol and its structural analog, biphenyl as competitive substrates were used to study the complementary interactions between polymer and template. The MIP had shown relatively high adsorption capacity towards

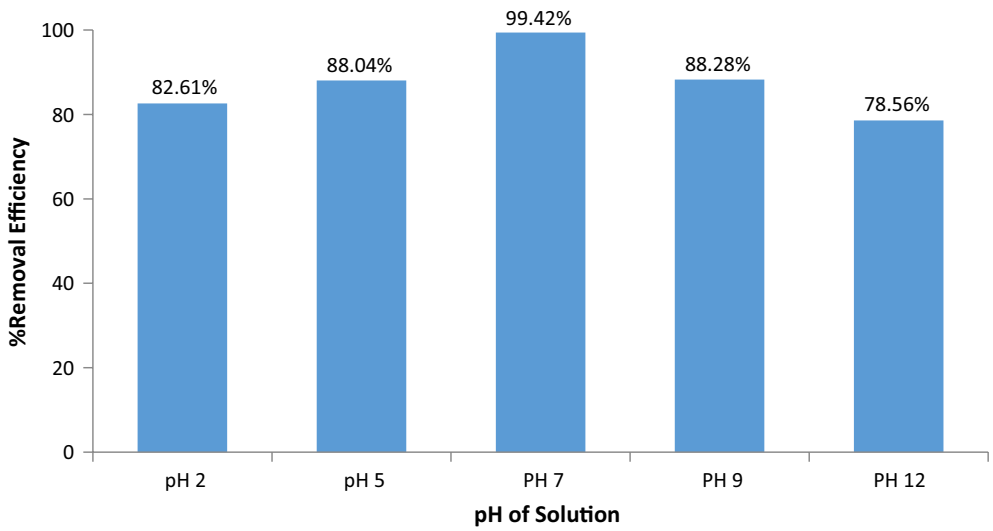

Fig. 7 Effect of pH on the binding capacity

Table 3 Selectivity of MIP

Template	KD (MIP) (mL g^{-1})	KD (NIP) (mL g^{-1})	*k*sel	*k*
2-Phenylphenol	90	16.8	2.11	3.1
Biphenyl	42.56	24.54	0.68	

the 2-phenylphenol as compared to its competitive template (Table 3). Moreover,2-phenylphenol had the highest selectivity than that of the structural analog, demonstrating that 2-phenylphenol more specifically imprinted on the MIP. The higher the similarity in chemical structure, the stronger the selective adsorption. It is also obvious from the results that the adsorption capacity of MIP is higher than NIP. This also indicated that the specific binding sites are available on MIP that led to the higher binding efficiency as compared to NIP. The imprinting sites formed in the MIP have the capability to distinguish target molecules through their size, shape, and functional group distribution. However, NIP only adsorbs 2pp and biphenyl on the surface due to the non-existence of the imprinting sites in the polymer network.

Extraction of 2pp from blood plasma and River water

The extensive use of 2-phenylphenol in medicine and as antifungal, antimicrobials has generated this idea to use MIP in the extraction of 2-phenylphenol from blood serum and river water. Therefore, 2-phenylphenol was spiked in blood serum and river water and then extracted with the most selective polymer MIP (Table 4). This will lead us a way forward to expand the application of these imprinted polymer particles. The extraction efficiency of MIP3 (93%) from the spiked plasma sample was higher as compared to NIP(50%). In this way, these polymer particles can act as promising sorbents for the extraction of 2-phenylphenol from biological samples as well as in natural products. In a similar way, the extraction efficiency of MIP (88%) from the spiked water sample was higher as compared to NIP (46%). In this way, these polymer particles can act as promising sorbents for the extraction of 2-phenylphenol from a water sample.

Conclusion

MIP has been successfully used as sorbents for selective removal of 2pp. MIP for 2pp was synthesized using precipitation polymerization method. Precipitation

Table 4 Extraction efficiency of MIP 3 and NIP in different samples containing 2-phenylphenol

Samples	Amount of MEL added (μg mL^{-1})	MIP 3			NIP		
		Amount of MEL found (μg mL^{-1})	Recovery (%)	RSD (%)	Amount of MEL found (μg mL^{-1})	Recovery (%)	RSD (%)
Blood serum	25	23.25	93	0.23	12.5	50	0.54
River water	25	22	88	0.26	11.5	46	0.77

polymerization is able to produce uniform size and shape of MIP microspheres. In this research uniform shape and size of imprinted polymer particle for 2-phenyl phenol were produced. The MIP showed high selectivity and binding capacity towards 2-phenylphenol against the structural analog biphenyl. Batch binding assay of template molecules and analog with similar functional groups shows that the adsorption recognition mechanism of the MIP, suggesting that its high selective ability mainly depended on the binding sites from functional groups. The MIP was also successfully applied in spiked human blood serum and water analysis, achieving high recoveries up to 93% and 88%, respectively. This means that the MIP was able to effectively extract 2-phenylphenol from complicated blood serum and water samples. Thus, the MIP prepared was proven to be a promising material for solid-phase extraction and the best extractant of fungicide 2-phenyl phenol from human blood serum and river water.

Materials and methods

Materials and reagents

The material and reagents are obtained as follows: 2-phenylphenol (Merck Chemicals), styrene, divinylbenzene, DVB (Merck Chemicals), acetonitrile (Mallinckrodt Chemicals), azo-bis-isobutyronitrile, AIBN (R&M Chemicals), methanol (R&M Chemicals), acetic acid (J.T. Chemicals), (HmbGChemicals), and acetone(HmbGChemicals).

Instrumentation

FT-IR spectra of the MIP and the NIP were obtained by FT-IR analysis. KBr disks of the MIP and the NIP were respectively prepared, and their spectra were recorded at 4000–400 cm^{-1} on an FT-IR (Model ThermoScientificNicoletiS10). Electron micrographs were taken for evaluation of the morphology with a scanning electron microscope (SEM) (Model JEOL JSM-6390LA). Sample powder was attached to the sample holder, dried, and sputtered with gold in an Ion Sputtering Coater. Morphologies of the MIP and the NIP were observed under SEM scanning at the voltage of 20 kV. The surface area and pore size were determined at 77 K by N2 adsorption and desorption isotherms using multipoint Brunauer–Emmett–Teller (BET) method. The high-performance liquid chromatography (HPLC) (Model Shimadzu LC-20) was performed by using C18 Column and Uv-detector. The other equipment used in this research were bath sonicator (Model Branson 2510), centrifuge (Model Hettich EBA20), shaker (Model N-Biotek 101MT), and water bath (Model Memmert W350T).

Table 5 Template-monomer-crosslinker ratios for synthesis of MIPs and NIP

Polymer	Template (mmol)	Monomer (mmol)	Cross-linker (mmol)
MIP1	1	2	16
MIP2	1	3	16
MIP3	1	4	16
NIP		4	16

Synthesis of molecularly imprinted polymer (MIP) and NIP

The MIPs for 2-phenylphenol were prepared by non-covalent precipitation polymerization approach with a variable molar ratio of the functional monomer as given in Table 5 for the synthesis of different MIPs and NIP. Firstly, the template was added to a conical flask containing 75 mL of acetonitrile followed by the addition of monomer (styrene), cross-linker (DVB), and an initiator (AIBN) into the reaction flask. After that, the mixture was sonicated for 15–20 min and purged with nitrogen gas in an ice water bath for 15 min. The conical flask was sealed under nitrogen protection to prevent oxidation (oxygen inhabitation). The reaction flask was immersed in a water bath at 60 °C for the first 8 h and later the temperature was raised and set at 85 °C for 4 h in order to complete the polymerization process. The resulted polymer particles were extracted out by using the centrifugation at 6000 rpm for 20 min. NIP was also prepared in the same way but without the addition of the template molecule.

Extraction of 2-phenylphenol from Molecularly Imprinted Polymer (MIP)

The template 2-phenylphenol was removed by washing the MIPs successively in the mixture of methanol and acetic acid (8:2, v/v) until the template was not detected by HPLC at 270 nm. At last the polymer particles were washed with acetone 2–3 times in order to remove the acetic acid from the polymer matrix. The MIPs were finally dried at 60 °C for 6 h in a regular oven.

Batch binding assay

The adsorption test was conducted in order to evaluate the binding capacity of MIPs and NIP [13]. A series of 100 mL conical flasks containing 100 mg(0.1 g) of the MIPs (MIP1, MIP2, and MIP3) and NIP beads were added with a 10 mL of a 25 ppm solution of 2-phenylphenol. The conical flasks were shaken on the shaker at 250 rpm at room temperature and the samples were collected at different time intervals (0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 min). The collected samples were centrifuged at 6000 rpm for 30 min in order to remove any suspended particles and supernatant was

used for further analysis. The concentration of 2-phenylphenol after adsorption was recorded by using reversed-phase high-performance liquid chromatography (RP-HPLC). The HPLC was conducted by using the C18 column (Length 250 × width 4 mm, diameter 5 µm) with the mobile phase consisting of acetonitrile, ultra-pure water, and acetic acid in the ratio of 60:39.5:0.5, v/v/v, respectively. The flow rate was set at 0.6 mL min^{-1} with UV detection at 270 nm with a run time of 10 min and the injection volume was set at 20 µL.

The binding capacity of MIPs and NIP of 2-phenylphenol was calculated by using the following equation:

$$\text{Binding capacity } Q\ (\%) = [C_i - C_f/C_i] * 100 \quad (1)$$

where C_i is the initial 2-phenylphenol concentration in the solution and C_f is the final 2-phenylphenol concentration in the solution.

The imprinting factor was calculated by Eq. (2):

$$\alpha = \text{QMIP}/\text{QNIP} \quad (2)$$

where, α = imprinting factor, QMIP = the adsorption capacity of the MIP (µmol g^{-1}), and QNIP is the adsorption capacity of the NIP (µmol g^{-1}).

The specificity adsorption ratio was calculated by Eq. (3):

(3)

Effect of initial concentration

The adsorption isotherms were studied using MIP (100 mg) in 2pp solution (10 mL) at different concentration (10, 15, 20, 25, 30, 35, and 40 ppm). A series of conical flasks were used for this study and were agitated on a shaker at a time interval of 90 min. In this study, both contact time and polymer dosage were kept constant. The amount of template adsorbed on the polymer was monitored by HPLC and evaluated by Eq. 1.

Effect of polymer (MIP) dosage

MIP dosage was studied using 25 ppm of 2pp (10 mL) for the different amount (100, 200, 300,400, and 500 mg) of MIP at a constant contact time, initial concentration and pH. A series of conical flasks were used for this study and were agitated on a shaker at a time interval of 90 min. The amount of template adsorbed on the polymer was monitored by HPLC and evaluated by Eq. 1.

Effect of pH

For pH study, the pH of the template solution was adjusted to 2, 5, 7, 9, and 12 with hydrochloric acid (HCl) and sodium hydroxide (NaOH), keeping the other parameters (contact time, concentration, and polymer dosage) constant. A series of conical flasks were used for this study and were agitated on a shaker at a time interval of 90 min. The amount of template adsorbed on the polymer was monitored by HPLC and evaluated by Eq. 1.

Selectivity of the MIP

Firstly, the MIP and NIP (100 mg) containing in two different conical flasks were added with 20 mL of a mixed substrate solution (containing 15 ppm 2-phenylphenol and 15 ppm biphenyl solutions). The flasks were shaken at room temperature for 90 min (time for maximum absorbance of 2pp-MIP). The resulting solution was centrifuged for 20 min and filtered to remove suspended MIP particles and then the concentration was determined HPLC at 270 nm.

The selectivity of the MIP was calculated by the specific factor β,

$$\beta = Q(\text{biphenyl})/Q(\text{2-phenylphenol}) \quad (4)$$

where

$$Q(\text{biphenyl}) = (\text{QMIP} - \text{QNIP})\text{biphenyl}$$

And

$$Q(\text{2 - phenylphenol}) = (\text{QMIP} - \text{QNIP})\text{2-phenylphenol.}$$

Specific adsorption ratio (%)

$$= [(\text{QMIP} - \text{QNIP})/\text{QMIP}] \times 100\%.$$

Extraction of 2-phenylphenol from blood serum and river water

About 10 mL of drug—free fresh human blood was collected and then the whole blood was allowed to clot by leaving it undisturbed for 30 min at room temperature. After that, the clotted blood was centrifuged at 5000 rpm for 15 min. The supernatant collected is the serum. After that, the blood serum was diluted with ultra-pure water in the ratio of 1:10, respectively. Next, 5 mL of diluted blood serum was spiked with 5 mL of 25 µg mL^{-1} 2-phenylphenol. About 100 mg of selected MIP and NIP were added into conical flasks containing 10 mL spiked blood serum. The flask containing the whole mixture was agitated on a shaker for 90 min. The supernatant solution was collected after filtering the whole mixture. The extraction efficiency was evaluated by using Eq. 1.

Firstly, the river water was filtered by using gravitational filtration to remove any suspended particles. Then, the presence of 2pp in the collected river water was observed by using RP-HPLC. Then, 2pp was spiked in river water with a total concentration of 25 µg mL^{-1}. After that, 100 mg of MIP3 and NIP were added into two separate conical flasks containing 10 mL of 25 µg mL^{-1} 2pp solution, respectively. The conical flasks were agitated on a shaker 90 min. After agitation at the appropriate time

intervals, the samples were collected and concentrations of 2pp after adsorption were recorded by using RP-HPLC. The removal efficiencies of MIP and NIP of 2pp in river water were evaluated by the Eq. (1).

Abbreviations
2pp: 2-phenylephenol; SOPP: sodium o-phenylphenate; DVB: divinylbenzene; AIBN: azo-bis-isobutyronitrile; HPLC: high-performance liquid chromatography; SEM: scanning electron microscope; FT-IR: Fourier transform infrared spectroscopy; EDX: energy dispersive X-ray; BET: Brunauer–Emmett–Teller; MIP: molecular imprinting polymer; NIP: non-imprinted polymer.

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Authors' contributions
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References

1. Eastmond DA, Balakrishnan S. Genotoxicity of pesticides. In: Hayes' handbook of pesticide toxicology. 3rd ed. 2010; p. 357–80.
2. Gammon DW, Moore TB, O'Malley MA. A toxicological assessment of sulfur as a pesticide. In: Hayes' handbook of pesticide toxicology. 3rd ed. 2010; p. 1889–1901.
3. Bomhard EM, Brendler-Schwaab SY, Freyberger A, Herbold BA, Leser KH, Richter M. O-phenylphenol and its sodium and potassium salts: a toxicological assessment. Crit Rev Toxicol. 2002;32(6):551–626.
4. Ye L, Mosbach K. Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors. Chem Mater. 2008;20(3):859–68.
5. Zhao RJ, Yuan T, Jiang J, Shi C. Cheng Application of bamboo charcoal as solid-phase extraction adsorbent for the determination of atrazine and simazine in environmental water samples by high-performance liquid chromatography-ultraviolet detector. Talanta. 2008;76:956–9.
6. Geeta S, Padma K. Extraction, gas chromatography–mass spectrometry analysis and screening of fruits of *Terminalia chebula* Retz. for its antimicrobial potential. Pharmacognosy Res. 2013;5(3):162–8.
7. Cristina B, Guillermina F, Jordi M, Yolanda P. Solid-phase microextraction liquid chromatography/tandem mass spectrometry to determine postharvest fungicides in fruits. Anal Chem. 2003;75(14):3606–15.
8. Karen DB, Janusz P. Determination of phenols by solid-phase microextraction and gas chromatographic analysis. Environ Sci Technol. 1993;27(13):2844–8.
9. Xu S, Li J, Chen L. Molecularly imprinted polymers by reversible addition—fragmentation chain transfer precipitation polymerization for preconcentration of atrazine in food matrices. Talanta. 2011;85(1):282–9.
10. Song X, Li J, Wang J, Chen L. Quercetin molecularly imprinted polymers: preparation, recognition characteristics and properties as sorbent for solid-phase extraction. Talanta. 2009;80(2):694–702.
11. Opik A, Menaker A, Reut J, Syritski V. Molecularly imprinted polymers: a new approach to the preparation of functional materials/molekulaarselt jaljendatud polumeerid–uus valjund funktsionaalsete materjalide valmistamiseks. Proc Estonian Acad Sci. 2009;58(1):3–12.
12. Yang HH, Zhou WH, Guo XC, Chen FR, Zhao HQ, Lin LM, Wang XR. Molecularly imprinted polymer as SPE sorbent for selective extraction of melamine in dairy products. Talanta. 2009;80(2):821–5.
13. Yan H, Row KH. Characteristic and synthetic approach of molecularly imprinted polymer. Int J Mol Sci. 2006;7(5):155–78.
14. Komiyama M, Takeuchi T, Mukawa T, Asanuma H. Molecular imprinting from fundamentals to applications. Germany: Weinheim; 2003. p. 9–17.
15. Saifuddin N, Nur YA, Abdullah SF. Microwave enhanced synthesis of chitosan-graft polyacrylamide molecular imprinting polymer for selective removal of 17b-estradiol at trace concentration. Asian J Biochem. 2011;6(1):38–54.
16. Bhawani SA, Sen TS, Ibrahim MN. Synthesis of molecular imprinting polymers for extraction of gallic acid from urine. BMC Chem. 2018;12(1):19.
17. Chow J, Leong A, Bhawani SA. Synthesis and characterization of molecular imprinting polymer microspheres of cinnamic acid: extraction of cinnamic acid from spiked blood plasma. Int J Pol Sci. 2016;1–5.
18. Roland RM, Bhawani SA. Synthesis and characterization of molecular imprinting polymer microspheres of piperine: extraction of piperine from spiked urine. J Anal Methods Chem. 2016;2016.
19. Alexander C, Andersson HS, Andersson LI, Ansell RJ, Kirsch N, Nicholls IA, O'Mahony J, Whitcombe MJ. Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. J Mol Recognit. 2006;19(2):106–80.

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