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Preparation and Characterization of Hybrid Molecularly Imprinted Polymer Membranes for the Determination of Citrinin in Rice

(Penyediaan dan Pencirian Membran Hibrid Polimer Molekul Teraan untuk Penentuan Sitrinin pada Beras)

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ABSTRACT

A new method for the determination of Citrinin (CIT) in rice samples by hybrid molecularly imprinted polymer (MIP) membrane prior to its quantification by high performance liquid chromatography with fluorescence detection (HPLC-FD) is described for the first time. Conventional extraction methods, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE) produce large volumes of environmentally hazardous waste and the common sorbents used in SPE often suffered from low selectivity. Hybrid MIP membranes offer the advantage of combining the mechanical integrity of the support membrane and the selectivity of the imprinted polymer. These membranes offer large specific surfaces, providing relatively high imprinting sites per unit mass, and fine porous structures, resulting in accessibility of imprinting sites. Thus, MIPs for CIT with 1-naphthol as mimic template were prepared using divinylbenzene as crosslinker and naphthol methacrylate was hybridized into the polyethersulfone scaffold by phase inversion process. The prepared hybrid MIP membrane was characterized using Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Using the resultant hybrid MIP membranes as sample preparation for HPLC-FD of CIT, detection and quantification limits of 0.5 ng g^{-1} and 1.7 ng g^{-1}, respectively, were obtained. The intra-day and inter-day precision expressed in %RSD ranged from 1.9-2.9% and 2.6-5.9%, respectively. The recoveries of CIT in rice spiked at 5, 25 and 100 ng g^{-1} ranged from 89.7-94.2%. Thus, the hybrid MIP membranes can be valuable material for the practical determination of CIT in rice extracts.

Keywords: Citrinin; high performance liquid chromatography; hybrid molecularly imprinted membrane; rice

ABSTRAK

Kaedah baru untuk penentuan sitrinin (CIT) dalam sampel beras oleh membran hibrid polimer molekul teraan sebelum kuantifikasi oleh kromatografi cecair prestasi tinggi dengan pengesanan pendarflour dijelaskan buat kali pertama. Kaedah pengekstrakan konvensional, seperti pengekstrakan cecair-cecair (LLE) dan pengekstrakan fasa pepejal (SPE) menghasilkan sejumlah besar sisa berbahaya alam sekitar dengan penjerap biasa yang digunakan dalam SPE sering mengalami kepilihan yang rendah. Membran MIP hibrid menawarkan kelebihan menggabungkan integriti mekanikal membran sokongan dan pemilihan polimer teraan. Membran ini mempunyai permukaan khusus yang besar, menyediakan tapak teraan yang agak tinggi bagi setiap jisim unit dan struktur berliang yang halus, menyebabkan akses tapak teraan. Polimer molekul teraan (MIP) untuk CIT telah disediakan dengan 1-naftol (NA) sebagai templat tiruan. Seterusnya, membran hibrid polimer molekul teraan telah disediakan dengan memerangkap zarah MIP ke dalam perancah polietersulfon dengan menggunakan teknik fasa penyongsangan. Membran tersebut telah dicirikan dengan menggunakan transformasi Fourier spektroskopi inframerah (FTIR) dan mikroskopi pengimbasan elektron (SEM). Dengan menggunakan hibrid membran sebagai penyediaan sampel bagi analisis HPLC-FD untuk CIT, had pengesanan dan penentuan 0.5 ng g^{-1} dan 1.7 ng g^{-1} telah tercapai. Ketepatan intra-hari dan antara hari yang dinyatakan dalam % RSD masing-masing adalah antara 1.9-2.9% dan 2.6-5.9%. Perolehan semula CIT yang dipaku ke dalam beras pada 5, 25 dan 100 ng g^{-1} adalah 89.7-94.2%. Oleh itu, membran hibrid MIP adalah bahan bernilai bagi penentuan praktikal CIT dalam ekstrak beras.

Kata kunci: Beras; kromatografi cecair prestasi tinggi; membran hibrid polimer molekul teraan; sitrinin

INTRODUCTION

Citrinin (CIT) (Figure 1a) a fungal metabolite produced by several fungal species of *Aspergillus*, *Penicillium* and *Monascus*, is one of the important mycotoxins that has been reported to contaminate rice, wheat, corn, barley and fruit juices (Čulig et al. 2017; Kiebooms et al. 2016; Pleadin et al. 2016). CIT is known to be nephrotoxic, hepatotoxic and carcinogenic to humans and animals. Kidney and liver are the major target organs. Together with ochratoxin A, these mycotoxins are suspected to be linked in human kidney disease, known as the Balken endemic nephropathy (Ali et al. 2016). The International Agency for Cancer Research (IARC) classified CIT in Group 3 despite limited evidence of its carcinogenicity to experimental animals and no evidence found in humans (Föllmann et al. 2014). Therefore, the presence of CIT in rice, a major staple food

for people in Asian countries has become a major concern. Currently, the State Food and Drug Administration of China has issued advisory CIT limit of 50 μg kg^{-1} in red yeast rice-based food while the European Union recently recommended a maximum permissible limit of 2000 μg kg^{-1} in food supplements based on rice fermented with the red yeast *Monascus purpureus* (Commission Regulation (EU) 2014). China has enforced the limit on CIT for functional food products to be less than 50 μg kg^{-1} while in Japan, the advisory CIT concentration limit in red yeast rice was 200 μg kg^{-1} (Urraca et al. 2016).

Xu et al. (2006) has reviewed the qualitative and quantitative analytical methods for CIT. Several methods for the determination of CIT in food, including colorimetric, immunochemical and chromatographic techniques have been reported (Yirga et al. 2017). Colorimetric techniques based on the natural fluorescence of CIT are disadvantaged due to the low recoveries and the lack of sensitivity. Immunochemical techniques such as enzyme linked immunosorbent assays and electrochemical immunosensor have been developed as rapid screening method for the presence of mycotoxins. These methods, although are highly specific as they are based on antibody-antigen interactions, but the cross-reactivity with structurally related compounds is common. To date, high performance liquid chromatography (HPLC) with fluorescence detection remains the most commonly used analytical method.

Sample preparation constitutes a very important step in chemical analysis. Its function is mainly to remove potentially interfering components present in the sample and as well as to preconcentrate the analytes in order to achieve the desired sensitivity. The most widely used method for the extraction and clean-up of CIT are liquid-liquid extraction which involved chloroform as the extracting solvent. Alternatively, solid-phase extraction (SPE) that involved immunoaffinity columns (IACs) or aminopropyl SPE can be used (Hartl & Stenzel 2007; Marley et al. 2016). These columns, however, are rather expensive, designed for single use and with the exception of IACs, lack selectivity. To increase selectivity, molecularly imprinted polymer (MIPs) has been introduced.

MIPs involve the formation of template-monomer complex with either covalent or non-covalent interactions, followed by copolymerization in the presence of a suitable cross-linker. After removing the template, imprinted cavities of specific size and shape were left inside the polymer network, exhibiting sites with molecular recognition properties for the target molecule. It is considered as an interesting alternative for clean-up, which contrary to IACs, do not suffer from storage limitations and stability problems when in contact with organic solvents. The highly cross-linked and three-dimensional network of MIPs is stable, robust and resistant to a wide range of pH, solvents and temperature, exhibiting advantages of high selectivity, stability, reusability, easy and low cost of preparation (Martín-Esteban & Sellergren 2012).

The high selectivity of MIPs has lent themselves as sorbents in SPE (MISPE). MISPE allows simultaneous and pre concentration of target analytes and clean-up extracts, removing undesirable sample matrix components. MISPEs have been used for the determination of CIT in rice samples (Guo et al. 2010) and maize (Appell et al. 2015). The previous reported methods prepared MIP using the bulk polymerization, where the resulting polymer needs to be ground to obtain regular sized particles before loaded into the SPE cartridge. The process of crushing, grinding and sieving to obtain the appropriate particle sizes however, is tedious and time-consuming and often produces particles that are irregular in size and shape. Of more concern is the destruction of interaction sites during the grinding step. Since only a portion of the original polymer is used, this method uses high amount of the template. Template bleeding is also considered as one of the main drawbacks of MISPE. The difficulty in removing the entire template molecule, even after extensive washing is often encountered. The leakage of these trace amounts of the template remaining in the MIP is an obstacle in the accurate and precise assay of the target analyte (Sarafraz-Yazdi & Razavi 2015; Vasapollo et al. 2011).

To overcome the disadvantages of traditional bulk polymerization, several alternative polymerization methods have been proposed. Precipitation polymerization represents a more practical approach as regular beads can be formed. This technique is easy, faster than the bulk polymerization method and provides regular beads in good yields (Tamayo et al. 2007). However, in one-step precipitate polymerization method, the binding sites are inside the network, the mass transfer of target molecules is very slow thus limits the effective binding sites of the target analytes (Yi et al. 2013). Core-shell MIPs particles prepared by multi-step precipitation polymerization was developed to address this issue (Son et al. 2011).

Investigations of hybrid MIP membranes have attracted significant interest in order to enhance the sensitivity and selectivity of MIPs. The preparation of hybrid MIP membranes using phase inversion technique demonstrated excellent membrane properties for binding and separation of template and their derivatives (Faizal et al. 2009; Takeda & Kobayashi 2006; Yoshikawa et al. 2016). In this study, core-shell MIP particles were synthesized using 1-naphthol (NA) (Figure 1(b)) as the mimic template with two-step precipitation polymerization method and the hybrid MIP membranes were prepared using phase inversion technique for the extraction of CIT in rice samples. To the best of our knowledge, this work is the first to report on the development of hybrid MIP membrane for the extraction of CIT.

Experimental Details

Chemicals and materials

CIT (≥ 98%) and sodium hydrogen carbonate was purchased from Sigma (St. Louis, MO, USA). Methanol and sodium hydroxide (NaOH) were purchased from Wako Pure

Chemical Industries (Osaka, Japan). NA and triethylamine and methacryloyl chloride were purchased from Tokyo Kasei Industry (Tokyo, Japan) and were used without further purification. Magnesium sulphate was purchased from Kanto Chemical (Tokyo, Japan). Tetrahydrofuran, chloroform, divinylbenzene (DVB), N-methyl-2-pyrrolidone (NMP), acetone, azobisisobutyronitrile, HPLC grade acetonitrile, methacrylic acid, acetic acid, ethanol and dimethyl sulfoxide were purchased from Nacalai Tesque (Kyoto, Japan). The DVB was purified three times using silica gel column to remove inhibitor prior to use. Polyethersulfone (PES) was purchased from BASF (Ludwigshafen, Germany).

PREPARATION OF NA MONOMER (NAM)

NAM was synthesized following the procedure described earlier (Faizal et al. 2008). Briefly, to a 200 mL flask, NA (20 mmol) was added in tetrahydrofuran solution (50 mL) and the mixture was slowly stirred and cooled to 0°C. Triethylamine (40 mmol) and methacryloyl chloride (40 mmol) were added slowly to the solution. After stirring for 24 h at room temperature, the triethylamine salt was removed by filtration and the tetrahydrofuran solution was concentrated by evaporation. Chloroform (60 mL) was added and the solution was washed with water (8 × 50 mL) in order to remove the salts. The chloroform layer was dried with magnesium sulphate and the solution was further concentrated and then evacuated under vacuum.

PREPARATION OF CORE-SHELL MIP PARTICLES

DVB (50 mmol) and azobisisobutyronitrile (1 mmol) were dissolved in 100 mL of acetone:water (122:28, v/v) and poured into a 300 mL three neck, round bottom flask. The flask was kept in an oil-bath and the stirring speed was kept at 300 rpm. After purging the nitrogen gas for 30 min, the temperature of the mixture was increased to 65°C under continuous introduction of nitrogen gas. After 2 h, NAM (10 mmol, prepared in section 2.2) which has been dissolved in 50 mL acetone:water (122:28, v/v) was slowly dripped into the mixture. The polymerization was carried out for 24 h. After the polymerization was completed, the white precipitate formed was filtered and washed with acetone:water mixture thrice before being dried in a vacuum oven. The dried polymer (P(NAM-co-DVB)) was hydrolyzed with 1 M of sodium hydroxide solution at 60°C for 12 h, filtered, washed with water until neutral and dried in vacuum oven again (Figure 1(c)). A non-imprinted polymer (NIP) was prepared using the same method of the copolymers and the terpolymers when no NAM was added to the polymerization mixture.

PREPARATION OF HYBRID MIP MEMBRANE

To prepare hybrid MIP membranes, the resultant core-shell MIP particles were mixed with 20% PES in NMP solution (2 g of PES in 8 g of NMP). The contents of the powders in the polymer solution were 10 wt. % to the scaffold polymer in the solution; 1 g of MIP into 20% PES in NMP solution. The mixture was stirred at 50°C for 12 h. The obtained viscous solution was casted on the surface of a glass plate (150 mm × 200 mm) at 50°C. The thickness of the membrane was controlled at 100 μm using a polyester-film spacer (Lumirror, Japan). The casted solution was coagulated immediately in water at 25°C and kept for 12 h to solidify the membrane. The obtained membrane was then washed with excess of water to remove the NMP solution.

CHARACTERIZATION OF CORE–SHELL MIP PARTICLES AND HYBRID MIP MEMBRANE

The core-shell MIP particles were characterized using scanning electronic microscope (SEM) (JSM-5310LVB; JEOL, Japan) and confirmed by laser diffraction particle size analyzer (SALD7000; Shimadzu Corp., Japan). Fourier transform infrared (FT-IR) spectra were recorded for both MIP particles and hybrid MIP membrane using

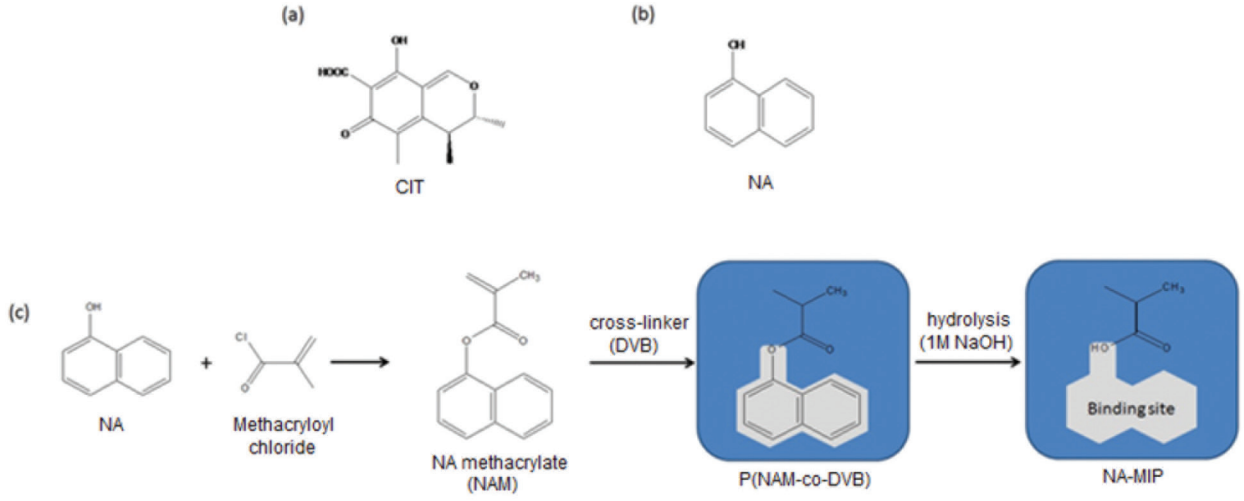

FIGURE 1. Chemical structure of (a) CIT, (b) NA and (c) formation of binding site using NA as mimic template

IR Prestige-21 FT-IR 8400s; Shimadzu Corp., Japan with KBr pellet technique (KBr: sample ratio 200: 1). For the membrane cross section, the membranes were placed in liquid nitrogen and were broken before pasting on a sample holder. After the gold coating, the SEM images were captured with the accelerating voltage set at 15 kV at magnification of 500 times and 7,500 times, respectively.

STANDARD CIT SOLUTIONS

Stock solution of CIT (5000 μg mL^{-1}) was prepared by dissolving the solid standard in methanol and stored at -18°C and protected from light. Standard solutions were prepared from appropriate dilutions of the stock solution with methanol:water (80:20, v/v).

BATCH BINDING OF CIT TO MIP AND NIP PARTICLES

An aliquot (40 mL) of the sample solution was added to a sample vial (50 mL) and 0.05 g of MIP or NIP was dispersed. The initial solution concentration was 50 μM of mixture (CIT and NA) and 20-100 μM of CIT. The solution was shaken at 30°C for 24 h. The binding amount [S] (μmol/g) was calculated using:

$$[S] = (Co-Ct)\ V/W \quad (1)$$

where Co and Ct represent the molar concentrations of the compounds that were measured before and after the binding process (24 h), respectively. V represents the solution volume and W is the mass of the imprinted polymer.

EXTRACTION OF CIT WITH HYBRID MIP MEMBRANE

For hybrid MIP membrane operation, 50 mL volume ultrafiltration cell (UF-8050; Amicon Inc.) was used. 25 mL of the aqueous solution (pH adjusted to 4.0 using 1 M HCl) was introduced into the cell and the solution was permeated through the membrane under atmospheric pressure. After the permeation, the membrane was removed, dried with lint-free tissue and placed in a 10 mL vial. 10 mL methanol:acetic acid (98:2, v/v) was added and the analytes were desorbed by ultrasonication for 10 min. After desorption, the membrane was removed from the desorption vial and the extract was injected directly into the HPLC for analysis.

REAL SAMPLE

Blank rice sample (10 g) was weighed into a conical flask (250 mL) and was fortified with CTN working solution to achieve different concentration levels and were left in the fume cupboard overnight. 100 mL of 1% sodium hydrogen carbonate solution was then added. The suspension was shaken at 200 rpm for 30 min and passed through a Whatman No. 4 paper filter. The pH for 20 mL of the filtrate was adjusted to 4.0 by using 1.0 M HCl before performing the extraction procedure (Refer to Standard CIT solutions).

HPLC CONDITIONS

A HPLC system (CCPS; Tosoh Corp., Tokyo, Japan) equipped with fluorescence detector (RF-10AXL; Shimadzu Corp., Japan) was used. The separation was performed on Poroshell 120 EC-C18 analytical column (100 mm × 4.6 mm × 2.7 μm) (Agilent Technologies, Wilmington, DE, USA) operated at 30°C. The mobile phase consisted of acetonitrile, water, and acetic acid (33:66:1, v/v) with the flow rate of 1.0 mL min^{-1}. CIT exhibits natural fluorescence and the detector wavelengths were set at excitation and emission wavelength of 333 nm and 460 nm, respectively.

RESULTS AND DISCUSSION

Mimic templates were usually selected when synthesizing mycotoxin MIPs due to its high toxicity and high cost. They interact with the functional monomer in a similar manner as CIT and is an inexpensive and safer alternative for imprinting polymers (Appell et al. 2015). In this study, NA which is similar to CIT, was selected as the mimic template (Figure 1). The MIP particles were formed by the copolymerization of NA with DVB as crosslinker. In this method, NAM addition was delayed for 2 h after the polymerization of DVB started in order to create the imprinted polymer shell on the surface of DVB seeds. Hybrid MIP membrane were then prepared by embedding the core-shell MIP particles into the polyethersulfone scaffold using phase inversion technique. The effect of extraction and desorption conditions using the hybrid MIP membranes for the determination of CIT in rice were studied.

CHARACTERIZATION OF CORE-SHELL MIP PARTICLES

Figure 2 portrays the relative size distribution of resultant spheres in acetone/water medium. With the increase of the polymerization time from 0.5 to 2 h, the size distribution of the P(DVB) was changed from 0.2-0.5 mm to 200-800 mm. After the copolymerization of P(NAM) was then carried out at 2 h in the P(DVB), the P(NAM-co-DVB) had an average size distribution in 100-400 mm. The SEM picture of the P(NAM-co-DVB) was inserted in Figure 2. This presented that the particle size was in the range of 0.2-0.5 mm, meaning that the light scattering results were observed in aggregated distribution of the particles in water medium.

The resultant copolymer particles were analyzed by FT-IR. In Figure 3, it was clear that the stretching band of C=O group on NAM at 1749 cm^{-1} and the DVB band at 700 cm^{-1} can be found in the copolymer. After the hydrolysis process, the OH band intensity at 3500 cm^{-1} increased significantly, indicating the formation of COOH group in the copolymer.

BATCH BINDING EXPERIMENT

Batch binding experiments were performed to observe the selective recognition of the resultant MIP particles towards the template (NA) and CIT. These systems would expect

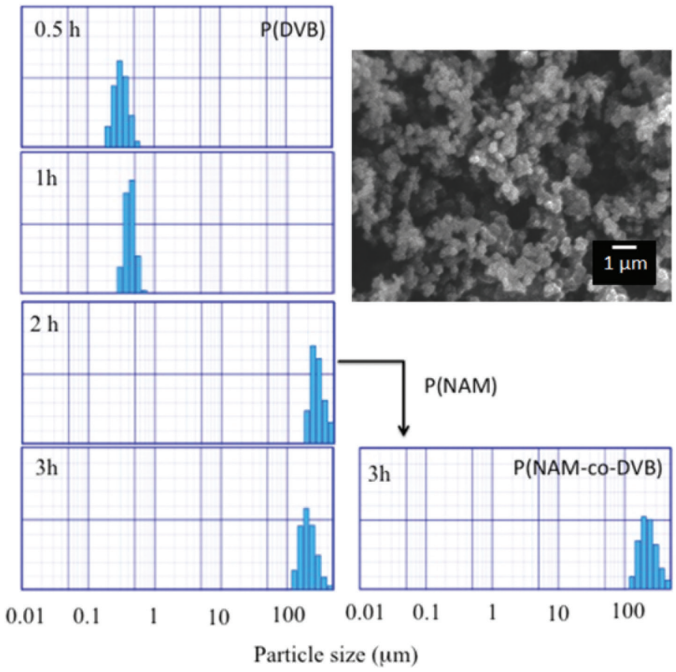

FIGURE 2. The relative size distribution of resultant spheres in acetone/water medium with the polymerization time from 0.5 to 2 h with the corresponding SEM micrograph

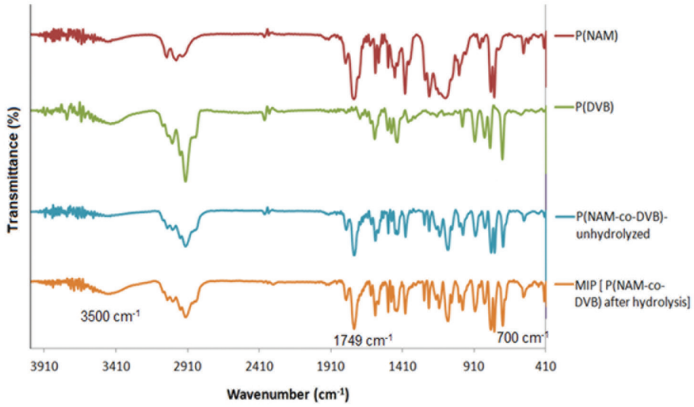

FIGURE 3. FTIR spectra for MIP particles prepared using NAM

as mimic imprinting to CIT. The MIP showed selective adsorption of NA, and CIT compared to the NIP, indicating that the mimic templates was successfully selected (Figure 4(a)). No significant binding of CIT and NA was observed on the NIPs. The selectivity for different concentrations of CIT towards the particles is shown in Figure 4(b).

CHARACTERIZATION OF HYBRID MIP MEMBRANE

The core-shell MIP particles were used for the preparation of hybrid MIP membrane. The scaffold PES was dissolved in NMP solution. PES and MIP particles are water insoluble. Therefore, after placing into the water bath, the PES with MIP particles will solidify to form membranes by phase inversion. Through the membrane formation, the scaffold polymer solution containing MIP will invert into the solid porous membrane (Takeda et al. 2007).

Figure 5 presents the SEM images of the MIP, cross section of the resultant hybrid MIP membrane and the PES membrane. As shown in the figure, the membranes contained sponge-like pores, showing typical membranes prepared by the phase inversion method. The resultant membrane thickness was about 175 µm for the PES membrane with pore diameter from 12.5-37.5 µm. The membrane thickness of hybrid MIP membrane (Figure 5(b)) was about 90 µm. The presence of particles with diameter in the range of 0.2-0.5 µm in the membrane wall was observed. This suggested that the hybridization was successful as the MIP particles were embedded in the pores of the PES scaffold as shown in Figure 4(d).

FT-IR spectra of the PES scaffold, the MIP spheres and the hybrid MIP membranes were obtained to confirm the presence of the MIP in the hybrid MIP membrane (Figure 5(e)). It was found that in the hybrid MIP membrane spectra,

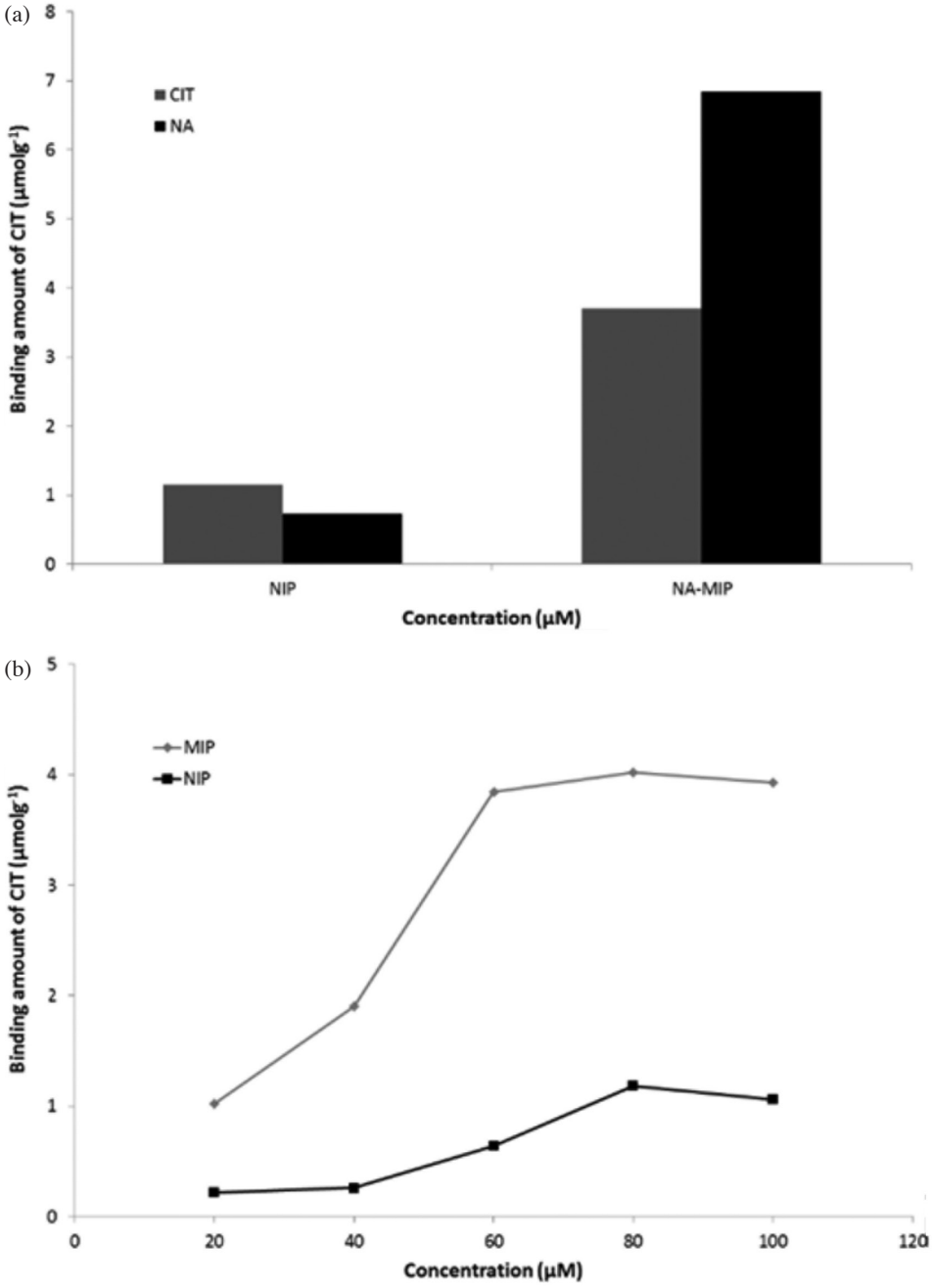

FIGURE 4. Batch binding experiment using MIPs and NIP with (a) mixture solution of CIT and NA and (b) with different concentrations of CIT

the stretching band of C=O group near 1749 cm^{-1} which represented MIP spheres, and the asymmetric stretching bands of $>S(=O)_2$ group near 1325 and 1299 cm^{-1} due to the PES scaffold were found. This indicates that the MIP spheres were hybridized successfully in the PES scaffold membrane.

OPTIMIZATION OF CIT EXTRACTION ABILITY OF HYBRID MIP MEMBRANES

Several extraction conditions were investigated to evaluate the different factors that affect the extraction ability of CIT on hybrid MIP membranes. Optimization was carried out by triplicate analysis with 100 ng mL^{-1} CIT.

Effect of MIP content The influence of MIP content in the hybrid MIP membrane for the extraction of CIT was investigated. The loading contents of the MIP particles in the hybrid MIP membrane were varied from 0-10 wt. % in the 20 wt. % of PES. It was found that the PES membrane without the MIP particles showed binding ability of 30 ng mL^{-1}. As expected, when the loading content of the MIP particles increased, the binding ability of the membranes increased. The maximum binding ability was achieved when 10 wt. % of MIP was loaded.

Effect of pH CIT is a weak acid, with pKa values of 3.55 for the acidic group and 4.8 for the basic groups. Therefore,

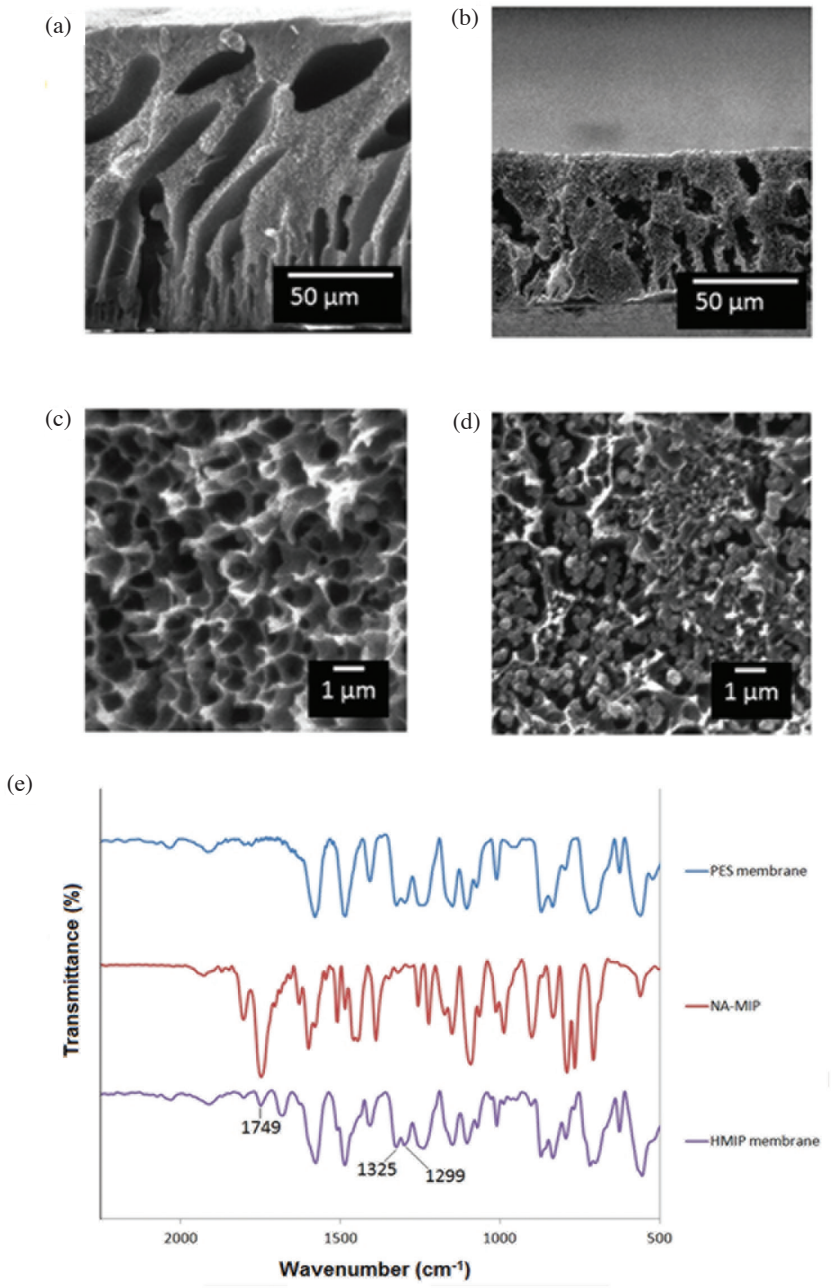

FIGURE 5. (a) SEM microgram of PES membrane, (b) SEM microgram of hybrid MIP membrane at magnification of 500 times, (c) SEM microgram of PES membrane and (d) SEM microgram of hybrid MIP membrane at magnification of 7,500 times, and (e) FT-IR spectra of PES, MIP and hybrid MIP membranes

the pH of the sample solution should be adjusted to be acidic to promote its extraction, as under neutral and alkaline conditions it is present predominantly in the dissociated form, which cannot be extracted. The pH of the sample solutions was varied from 1-5 by the addition of hydrochloric acid (1.0 M). It was found that the optimum pH for CIT extraction was pH4.0. The influence of sample volume (10-30 mL) was also investigated. When more than 25 mL of sample was fed, no additional enhancement of peak area was observed. Thus, 25 mL of sample was used in all experiments.

Effect of desorption solvent and time After binding, CIT was desorbed in 10 mL of organic solvents *via* ultrasonication. Various organic solvents (e.g. methanol, acetonitrile, methanol:acetic acid (98:2 v/v) and acetonitrile:acetic acid (98:2 v/v)) were tested. Methanol:acetic acid (98:2 v/v) was found to be the best desorption solvent as the highest peak areas were obtained, followed by acetonitrile.

The desorption (ultrasonication) time was investigated between 3-15 min with 10 mL of methanol:acetic acid (98:2 v/v). Longer desorption time gave higher peak area. However, there is no increase in peak area after 10 min. Thus, 10 min was selected.

Adopted extraction conditions The adopted conditions were: 10 wt. % of MIP embedded in 20 wt. % PES; pH4.0; sample volume, 25 mL; desorption solvent, methanol:acetic acid (98:2 v/v); desorption time, 10 min.

METHOD VALIDATION

Under the optimized binding conditions, matrix matched calibrations were done by spiking known amount of CIT into rice sample that were originally free from CIT. This approach enables the assessment of possible matrix effects to be evaluated. Linear range for rice was 2.5-250 ng g^{-1}. For each level, three replicate extractions were performed. The regression equations and correlation of determination were y = 1908.2x + 3298.5 and r^2 = 0.9984, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the equations:

$$LOD = 3.3s_a/b,$$

$$LOQ = 10s_a/b$$

where s_a is the standard deviation of the intercept and b is the slope of the regression line obtained from the calibration graph. The LOD and LOQ were 0.5 and 1.7 ng g^{-1}, respectively, suggesting that the method possess sufficient sensitivity for the analysis of citrinin in rice.

Recovery studies were carried out by spiking CIT to the non-contaminated rice and at different concentrations, i.e. 5, 25 and 100 ng g^{-1}. Three replicate samples were studied at each concentration. Good recoveries were obtained for all samples, ranging from 89.7-94.2%. Figure 6 shows the chromatogram of the extracts from the blank and the spiked rice samples.

Intra-day precision (repeatability) was estimated at three concentration levels of CIT that were spiked to the samples. Inter-day precision (reproducibility) was performed by spiking to the matrix with three concentration levels of CIT and all samples were analyzed on three different days. Intra-day and inter-day precisions for peak areas, expressed as the percentage relative standard deviation (RSD), were 1.9-3.8% and 2.6-5.9%, respectively, indicating the good precision of the developed method.

Table 1 shows the important analytical characteristics of the present method when were compared with the previous reported methods. It is clear that analytical performance of the present method is comparable to those provided by other MIP-based or more conventional methods. On the other hand, the hybrid MIP membranes have the advantages of high capacity to bind the target molecule in the matrix. This is due to large surface area, faster transport of substrate molecule and faster equilibrium of binding cavities. In addition, with these, low energy consumption, compactness, and ease of scaling up would be advantage.

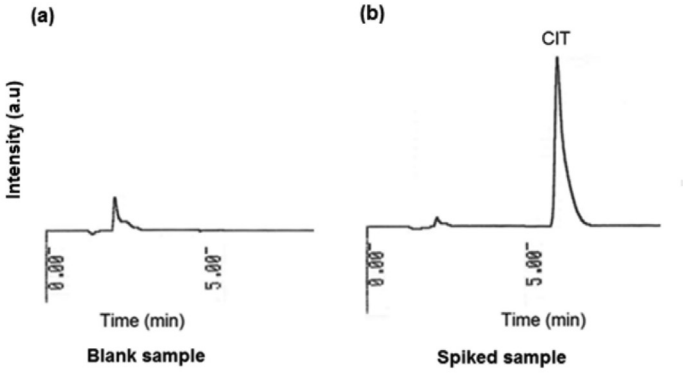

FIGURE 6. HPLC chromatogram of the extracts from (a) blank and (b) rice sample spiked with 5 ng g^{-1} of CIT

TABLE 1. Comparison of the developed method with the previous study for the determination of CIT using MIP

Linear range	LOD	LOQ	Recovery (%)	Reference
2.5-250 ng g^{-1}	0.5 ng g^{-1}	1.7 ng g^{-1}	89.7 - 94.2	Current work
1-15 μg kg^{-1}	0.6-0.9 μg kg^{-1}	1.7-3.3 μg kg^{-1}	77-92%	Hartl & Stenzel (2007)
Wheat: 10-200 μg/kg, red yeast rice :100-3000 μg/kg	3 μg kg^{-1}	10 μg kg^{-1}	80 to 110%	Marley et al. (2016)
1.5-100 μg kg^{-1}	0.5 μg kg^{-1}	-	86.7 - 97.7	Guo et al. (2010)
10-3000 ng g^{-1}	0.01 μg g^{-1}	0.03 μg g^{-1}	82.3-91.5	Appell et al. (2015)
5–200 μg L^{-1}	0.7 μg kg^{-1}	**2.3** μg kg^{-1}	94.4 - 98.2	Urraca et al. (2016)

SPE; Solid phase extraction
MISPE; Molecularly imprinted solid phase extraction

CONCLUSION

Core-shell MIP targeted for CIT using NA as mimic template were prepared using two-step precipitation method. Hybrid MIP membrane were then prepared by embedding the core-shell MIP particles into the polyethersulfone scaffold using phase inversion technique and was used in the determination of CIT in rice for the first time. High recoveries (89.7-94.2%) and satisfactory precision (1.9-5.9%) obtained suggest that the method can be a viable option for the analysis of CIT in food matrices.

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