



Analytical Methods

Determination of organophosphorus in dairy products by graphitic carbon nitride combined molecularly imprinted microspheres with ultra performance liquid chromatography

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ARTICLE INFO

Keywords:

Organophosphorus
Molecularly imprinted microspheres
Graphitic carbon nitride
UPLC
Dairy products

ABSTRACT

Organophosphorus (OPPs) residues in dairy products are a potential threat to human health. To extract trace amounts of OPPs in dairy products, a graphitic carbon nitride (g-C₃N₄) was synthesized and combined with OPPs-based molecularly imprinted microspheres (MIM) to create a composite material (MIM/g-C₃N₄). Then, the MIM/g-C₃N₄ was used to prepare a solid phase extraction (SPE) cartridge to detect the OPPs in dairy products with UPLC method. The specific surface area of MIM/g-C₃N₄ was 172.208 m²/g, good thermal stability under 300°C, and could reuse up to 15 times. The four OPPs had good linear relationship within the range of 1–10000 ng/mL ($r^2 > 0.999$). The limits of detection were 0.7–2.6 ng/mL, and recoveries from blank dairy samples were 86.4 to 95.3 %. In this study, MIM combined with g-C₃N₄ was firstly utilized for the detection of OPPs in dairy products, which indicated it might be an ideal adsorbent for dairy products pretreatment.

1. Introduction

Organophosphorus (OPPs) is a kind of compound used in agriculture to prevent pests and weeds. Because of high control efficiency and short half-life, the OPPs is extensively used (Xie, Zhou, Zhang, Zhao & Zhao, 2021). Unfortunately, excessive use of OPPs cause it to remain in water or soil, and the residues impact non-target organisms negatively through entry into the food chain. OPPs remaining in soil or water is a direct harm to humans. In addition, OPPs can also enter and accumulate in animals and humans through the food chain, indirectly leading to a series of poisonous symptoms (Fu et al., 2022). After entering the body, OPPs can bind to acetylcholinesterase, which can result in a large accumulation of acetylcholine leading to the toxicity and death (Ghorbani et al., 2021).

Code of Federal Regulations has imposed strict restrictions on the residues of OPPs in different types of foods, and the European Union (EU) stipulates that the maximum residue levels (MRL) of OPPs are less than 3 mg/kg in fruits and vegetables (Pang et al., 2020). Furthermore, the restrictions of OPPs on animal derived products are particularly rigorous. This is because the animals such as cows, sheep, and rabbits can obtain OPPs through directly eating plants contaminated by OPPs. Therefore, the dairy products may contain OPPs when dairy cattle are

directly exposed to contaminated environment (Fu et al., 2022). Moreover, some countries have stricter residue limits for milk that the MRL for OPPs is in the range of 0.002–0.05 ppm (Fu et al., 2022). Unfortunately, there are still cases where organophosphates are found in dairy products at excessive level. According to a previous report from the EU, 80 of 1183 milk samples contained chlorpyrifos above the MRL. In addition, in a batch of 45 milk samples from Egypt, 20 milk samples contained OPPs above the MRL set by the European Commission (Yuan, Yang, Yu, Xie, Guo & Yao, 2021). Dairy products are a complex matrix, and the OPPs in them are hydrolyzed extremely slow. Therefore, it is difficult to detect the OPPs in dairy products (Lin et al., 2020).

The current methods for detecting OPPs residues usually include gas chromatography (Kaur, Rani, Malik, Kabir & Furton, 2019; Nasiri, Ahmadzadeh & Amiri, 2021), high-performance liquid chromatography (HPLC) (Arias et al., 2020; Zhao et al., 2021), capillary electrophoresis (Chen et al., 2021) and enzyme linked immunosorbent assay (ELISA) (Wu et al., 2021). Among the mentioned methods above, HPLC is widely used and favored by researchers due to its high sensitivity, good selectivity, large-scale application and other advantages. However, dairy products contain complex matrices and some other non-target compounds, which may lead to the false positive results during the test (Gao et al., 2022). Therefore, the development of a novel sample preparation

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<https://doi.org/10.1016/j.fochx.2022.100424>

Received 28 April 2022; Received in revised form 4 August 2022; Accepted 5 August 2022

Available online 9 August 2022

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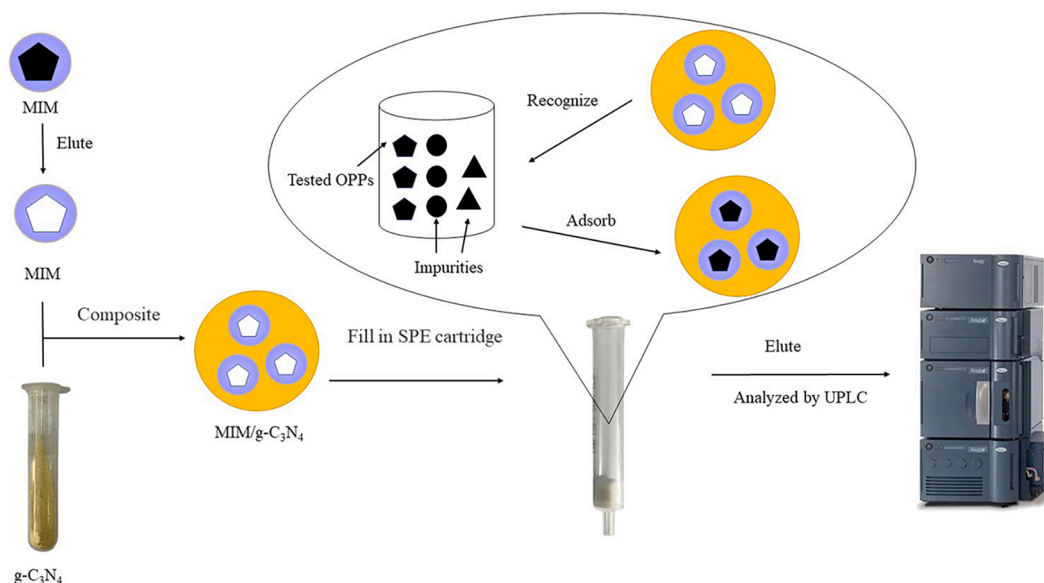


Fig. 1. The principle of MIM/g-C₃N₄ detection method based on UPLC.

method is essential for preconcentration and extraction of trace residues of OPPs in dairy products prior to detection.

At present, two commonly used sample extraction and purification methods are liquid–liquid extraction and solid-phase extraction (SPE) (Lin et al., 2020). Among the available methods, the SPE method is favoured by researchers due to its simple operation, good reproducibility, short extraction time, and high concentration capacity (Hashemi & Rezania, 2019). On this basis, some materials such as C18, C8 and PSA were widely used as extraction fillers in the SPE step. (Khan, Arain & Soyak, 2020). As a carbon nitride material, graphitic carbon nitride (g-C₃N₄) has two-dimensional structure, consisting of triazine ring units linked by an amine group. It possesses unique properties including excellent catalytic activity, chemical stability and easy surface modification (Hashemi & Rezania, 2019). These physicochemical properties make g-C₃N₄ potential as a novel sample pretreatment material. However, the fillers mentioned above such as C18 and g-C₃N₄ belong to nonspecific adsorption materials that the competitive adsorption may happen during the sample pretreatment progress, which affects the measurement accuracy. Furthermore, the developed extraction methods based on these fillers cannot be reused. (Wang et al., 2022). So, it is worthwhile to develop a filler with a specific recognition ability to eliminate the interference of impurities in the pretreatment process.

Molecular imprinting technology has been a rapidly developing analytical technique in recent years. It simulates the specific binding of antigens and antibodies in organisms, prepares the polymers that match with a specific target compound in spatial configuration. The prepared polymers are called molecular imprinting polymers (MIP) or molecularly imprinted microsphere (MIM) (He et al., 2021; Liu, Lian, Li, Majid & Wang, 2021). With excellent recognition ability, MIM or MIP can directly adsorb target analytes and minimize background interference. Therefore, MIM or MIP can be applied to the pretreatment and quantitative analysis of trace residues in biological samples with complex matrix.

At present, there have been some reports of combining MIP and g-C₃N₄ for developing novel analytical methods. Researchers have used MIP, g-C₃N₄, or other materials in combination for the applications in photochemistry, electrochemistry and detection of some hazardous metal ions (Liu et al., 2020; Pelin Böke, Karaman, Medetalibeyoglu, Karaman, Atar & Lütfi Yola, 2020; Xie, Yang & Li, 2020). In addition, according to some recent reports, g-C₃N₄ binding to MIP was used for the detection or processing of aflatoxin and tetracycline (Mao et al., 2021; Sun et al., 2019). However, to our best knowledge, there are no

reports using MIM/g-C₃N₄ for the detection of OPPs in dairy products.

In this study, for the combinative utilization of the specific recognition ability of MIM and the adsorption ability of g-C₃N₄, a composite material (MIM/g-C₃N₄) was synthesized through the direct polymerization of MIM over the surface of g-C₃N₄. And then, with the application of MIM/g-C₃N₄ as filler, a SPE cartridge was prepared for the detection of OPPs in dairy products. The synthesized MIM/g-C₃N₄ was characterized using some instruments and techniques to determine its structure and the functional group. Some parameters that could affect the SPE performance such as loading solvent, elution solvent, pH and salt concentration were optimized. In addition, the regeneration experiments were also performed to evaluate the stability and reusability of MIM/g-C₃N₄. Finally, the proposed SPE method was successfully applied with UPLC to selectively recognize and detect OPPs in dairy samples (Fig. 1). This study highlighted the potential of MIM/g-C₃N₄ for OPPs residue analysis in dairy products.

Materials and methods

2.1. Chemicals and reagents

Coumaphos, chlorpyrifos, diazinon, parathion, ribavirin, amantadine, sulfisomidine and oxytetracycline were obtained from Chemical Metrology & Analytical Science Division, National Institute of Metrology (Beijing, China). Divinylbenzene was supplied from Aladdin Industrial Corporation (Shanghai, China). Ammonium acetate, methacrylic acid, melamine, and 2,2-azobis (isobutyronitrile) (AIBN) and were provided by Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China).

2.2. Synthesis of g-C₃N₄

The synthesis of g-C₃N₄ was performed based on the previous study with some modifications (Ma et al., 2015). Briefly, melamine weighted at 12 g was heated to 550 °C in a quartz tube furnace for 4 h. The obtained carbonized product was mixed with HNO₃ (65 wt%, 35 mL) for 24 h, and the mixture was then placed in absolute ethanol for 8 h of sonication. Finally, the mixture was centrifuged at 6583 g and the precipitate was corrected. After washed by ultrapure water, the precipitate was dried until the weight was constant, and then the g-C₃N₄ was obtained.

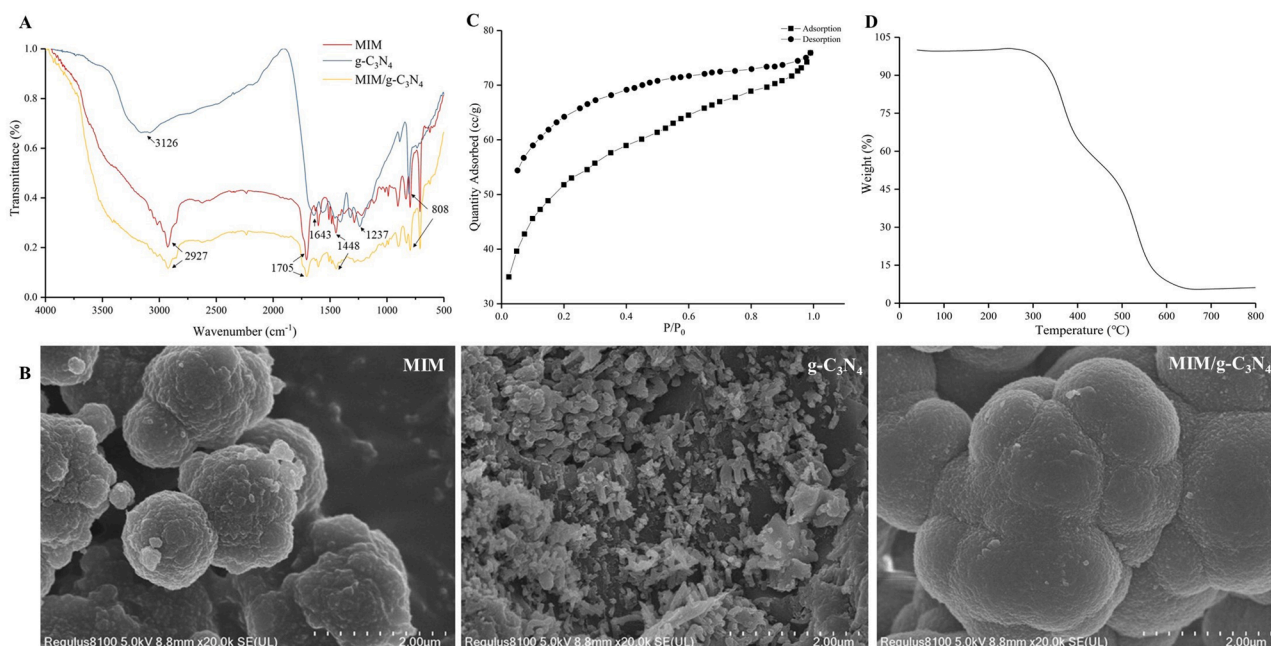


Fig. 2. Characterization of the materials. (A) FT-IR of MIM, g-C₃N₄ and MIM/g-C₃N₄; (B) SEM images of MIM, g-C₃N₄ and MIM/g-C₃N₄; (C) N₂ sorption–desorption isotherm of MIM/g-C₃N₄; (D) The thermogravimetric of MIM/g-C₃N₄.

2.3. Synthesis of MIM/g-C₃N₄

The synthesis of MIM is based on a previous report (Jia, He, Cui, Liu & Wang, 2019). Briefly, 6.4 mL acetonitrile, 0.1 mmol template OPPs, 2.1 mL toluene, 20 mg AIBN and 2 mmol divinylbenzene were put into a flask. After sonication for 5 min, the mixture was stirred and blew under nitrogen for 15 min. Under the sealed condition, the flask was stirred in water bath at 60 °C for 24 h.

The sediments were prepared by centrifugation of the collected suspension (8000 rpm, 30 min). For removing the template molecules, methanol/acetic acid (95:5, v/v) was used to extract the sediments in a Soxhlet apparatus for 72 h, then the methanol/acetic acid was replaced by pure methanol, and the extraction process was continued for 24 h. Finally, the sediments, after washed with methanol, were dried to constant weight at 110 °C to obtain MIM. Without the template molecule, the non-imprinted microsphere (NIM) was synthesized by the same method. The MIM/g-C₃N₄ was synthesized by directly polymerizing the MIM on the g-C₃N₄ surface (Wang et al., 2022). The MIM/g-C₃N₄ and NIM/g-C₃N₄ were produced by adding 30 mg g-C₃N₄ to the MIM and NIM synthesis process.

2.4. Evaluation of adsorption capacity of MIM

In this study, four OPPs including coumaphos, chlorpyrifos, diazinon and parathion and four other compounds (ribavirin, amantadine, sulfisomidine and oxytetracycline) were used to determine the adsorption capacities of the MIM. In this study, MIMs (coumaphos MIM, chlorpyrifos MIM, diazinon MIM, 30 mg, respectively) were put into a cartridge and washed by methanol (1 mL) and water (1 mL), then the standard solutions (10 µg/mL, 1 mL) were filtered through the cartridge by using a vacuum pump. The filtrate was collected and the concentration of the analyte was calculated from the standard curve. The standard curves of each drug were plotted by UPLC and calculated the adsorption capacity. The adsorption amounts Q (µg/mg) were calculated as follows: $Q = (C_0 - C_e) V/m$. In this equation, C_0 (µg/mL) represents the initial concentration of the OPPs solution, C_e (µg/mL) indicates the final concentration, and m represents the weight of adsorption material added.

2.5. Development of MIM/C₃N₄-SPE method

The MIM/g-C₃N₄-SPE cartridge was prepared as follows: briefly, 30 mg MIM/g-C₃N₄ was put into an empty cartridge (80 × 5.6 mm, the inner diameter) and washed with methanol (1 mL) and water (1 mL). Then, the vacuum pump was used to filter the standard solution (1 mL/min). Ultrapure water was used to clean the cartridge, and the remaining analyte was eluted. The filtrate was dried with nitrogen at 40 °C. Then, the residue was reconstituted by 1 mL methanol and filtered through 0.22 µm for UPLC analysis. In order to evaluate the reusability, the used cartridge was rinsed with methanol and water at 3 mL, respectively.

2.6. Sample preparation of dairy products

Milk, yogurt and milk powder were purchased randomly from local markets, which were analyzed by the developed method. The milk and yogurt samples processing methods were carried out according to the previous reports (Ghorbani et al., 2021; Pan et al., 2022). Briefly, 0.3 g MgSO₄, 0.1 g CH₃COONa and 1.5 mL of acetonitrile were added to 5 mL milk to precipitate protein. After centrifugation of the mixture (6583 g, 10 min), the precipitate was discarded and the supernatant was taken. The supernatant was evaporated to 5 mL at 40 °C, and diluted with ultrapure water to 10 mL. Equivalent of 10, 100 ng/mL of the OPPs was added to the resulting liquid. The supernatant was dried with nitrogen at 40 °C, and the resulting dried product was dissolved by methanol/water (1:9, v/v, 1 mL), filtered (0.22 µm) and analyzed by UPLC. For milk powder, 10 g milk powder was dissolved in ultrapure water and then fully homogenized at 37 °C, then the homogenate was processed as the milk sample (Alahlah, El Maoudi, Bouchriti, Triqui & Bougtab, 2020). In this study, standard curves of the OPPs in matrix-matched solution and mobile phase solution were prepared, and the matrix effect was calculated according to the previous study (Zhou et al., 2022).

2.7. UPLC condition for OPPs analysis

Analytes were chromatographically determined on an ACQUITY H-CLASS UPLC system (Waters Technology, Milford, USA). A HSS C18 column (2.1 × 100 mm, 1.8 µm) maintained at 40 °C was used to separate the four OPPs. The mobile phases were made up of solvent A

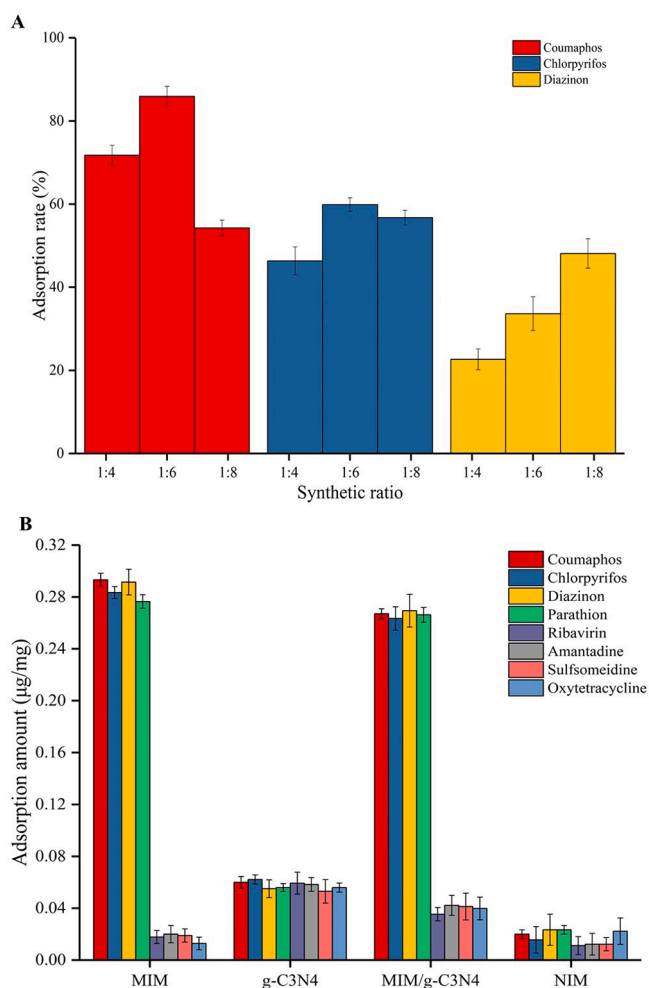


Fig. 3. Evaluation of the adsorption capacity. (A) Adsorption rates of MIM with different ratios of template molecules; (B) Adsorption amounts of the four materials.

(10 mM ammonium acetate in water) and B (10 mM ammonium acetate in methanol). The pH value of the two mobile phases was 5.0 with the adjustment of acetic acid. The flow rate of the mobile phase was 0.25 mL/min under the gradient elution progress in Table S1. The injection volume was set to 10 µL, and the OPPs were detected at 254 nm and 275 nm.

2.8. Analysis and processing of experimental data

In this study, each measurement was repeated three times, and the data were expressed as mean \pm standard deviation (SD). The limits of quantitation (LOQ) and limits of detection (LOD) were determined at signal-to-noise ratios of 10 and 3, respectively. The intraday recoveries were calculated when 6 samples were measured on the same day, and the interday recoveries were calculated when one sample was measured for 6 consecutive days. The coefficient of variation (CV) was calculated with SD/average.

3. Results and discussion

3.1. Characterization of the composite material

The results of Fourier transform infrared spectra were shown in Fig. 2A. It was found that there were distinctive peaks at 3126 cm^{-1} , 1643 cm^{-1} , 1237 cm^{-1} and 808 cm^{-1} in the spectrum of g-C₃N₄, which

were corresponding to the stretching vibration of N—H bond, C—N bond, C=N bond and out-of-plane bending of triazine. The peak of MIM/g-C₃N₄ contained the peak of MIM (C—H bond 2927 cm^{-1} , C=O bond 1705 cm^{-1} , C—O bond 1448 cm^{-1}) and g-C₃N₄ (triazine ring 808 cm^{-1}). These results indicated that MIM was successfully bound to g-C₃N₄.

The results of scanning electron microscope were showed in Fig. 2B. It was manifest that pure MIM was a sphere with a uniform shape, and the g-C₃N₄ was an irregular flake. Notably, the MIM was evenly distributed over the surface of g-C₃N₄. These results indicated that the synthesis process was feasible and the MIM/g-C₃N₄ were obtained.

Measurement of the N₂ adsorption-desorption isotherms of the MIM/g-C₃N₄ was performed at 77.3 K. MIM/g-C₃N₄ was a specific adsorption material, its ability to identify OPPs was based on imprinted three-dimensional (3D) cavities. Through the calculation of isotherms in Fig. 2C, the Brunauer-Emmett-Teller surface area and Barret-Joyner-Halenda total pore volume were 172.208 m^2/g and 0.113 cc/g , which indicated that the MIM/g-C₃N₄ had good adsorption capacity.

Under the environment of flowing air, the thermogravimetric data of MIM/g-C₃N₄ were obtained from 40 to 800 °C (10 °C/min). The results in Fig. 2D showed that the MIM/g-C₃N₄ started to lose weight massively at 300 °C, which suggested that the MIM/g-C₃N₄ began to decompose at 300 °C. In addition, the material had a very slight increase in mass at 250–300 °C. The mass gain is because when the temperature rises, the density of nitrogen becomes smaller, resulting in a smaller buoyancy, and the mass of the sample is higher. In this study, all operations of dairy production analysis were performed at room temperature, so the thermal stability of MIM/g-C₃N₄ could fulfill the request of the subsequent experiments.

3.2. The adsorption capacity of MIM, NIM g-C₃N₄ and MIM/g-C₃N₄

The 3D cavities formed by MIM is determined by the template molecule. Therefore, the choice of template molecule is particularly critical. In this study, three template molecules (coumaphos, chlorpyrifos and diazinon) were selected for the synthesis of MIM, and three kinds of MIM of each template molecule were synthesized by changing the ratio of template molecules to functional monomers, and then their respective adsorption capacities were compared to select the optimal scheme. It could be seen from Fig. 3A that, when the ratio of coumaphos to functional monomers was 1:6, the adsorption capacity was obviously higher than those of the other MIMs. So, the MIM with a 1:6 ratio of coumaphos to functional monomers was selected to synthesize of MIM/g-C₃N₄.

As shown in Fig. S1, the core structure of the four OPPs was thiophosphate ester, which was almost the same in the 3D conformations of the molecules. Their tetrahedral structures were centered on phosphorus atoms, bond angles between 102° and 115° (Table S2), and the side chains were connected to oxygen atoms in the core structure. Compared with the other 3 OPPs, coumaphos has two 6-membered rings, which have large steric hindrance. The other 3 OPPs could enter the 3D cavities imprinted with coumaphos. Compared with coumaphos, both chlorpyrifos and diazinon have only one six-membered ring, and the steric hindrance is smaller, so the formed 3D cavities are smaller. It is more difficult for other OPPs molecules to enter, so the adsorption capacity of MIM synthesized with chlorpyrifos and diazinon was weaker than coumaphos.

After ensuring the template molecule, the adsorption capacity of the four adsorbents was measured. It could be seen from Fig. 3B that the adsorption capacity of MIM for OPPs was obviously higher than the results of other compounds. The g-C₃N₄ could adsorb eleven compounds, but the adsorption effect was not obvious. This is due to the physical properties of g-C₃N₄ itself, which means that g-C₃N₄ is a non-specific adsorbent. After synthesizing MIM and g-C₃N₄ into a composite material, it was found that MIM/g-C₃N₄ had a high adsorption performance and the ability to specifically recognize the OPPs (Fig. 3B). In

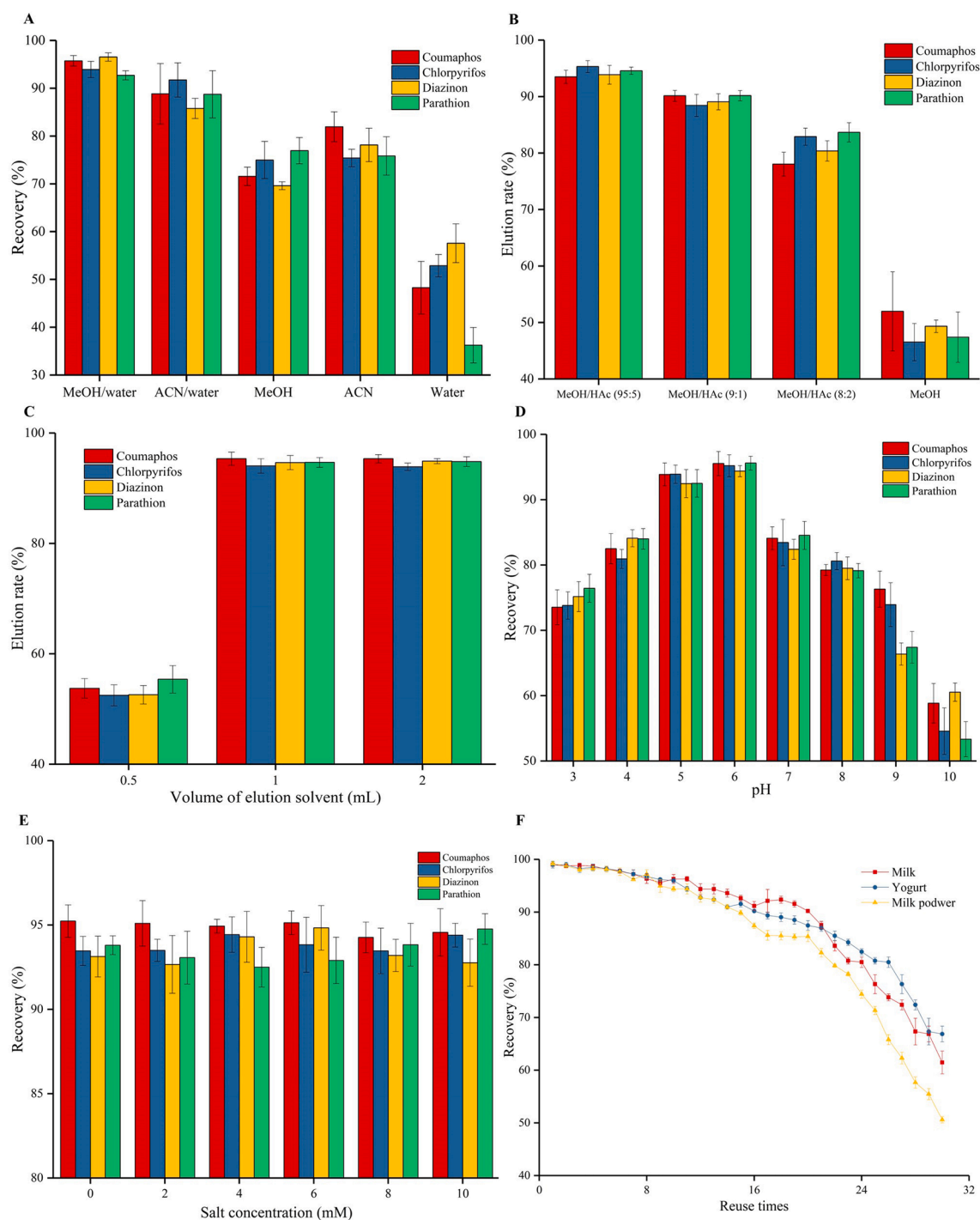


Fig. 4. Optimization of the parameters in SPE method. (A) Loading solvent; (B) Different elution solutions; (C) Volume of elution solution; (D) Recoveries at different pH; (E) Recoveries at different salt concentrations; (F) Reuse times.

addition, since NIM did not add template molecules during the synthesis process, there were no imprinted cavities on its surface, so it could not adsorb these compounds. What's more, since the 3D cavity of MIM/g-C₃N₄ did not match the molecular structure of the other four compounds, MIM/g-C₃N₄ could not adsorb the other four compounds. This indicated that the MIM/g-C₃N₄ had good anti-interference ability, and it could specifically recognize the four OPPs.

3.3. Parameter optimization of SPE method

In this study, an SPE method was developed based on MIM/g-C₃N₄

composites, and the parameters affecting the purification effect were optimized.

3.3.1. Loading solvent

Loading solvent provides the appropriate environment for the analyte and affects the recovery. During the experiment, four tested OPPs were diluted with different solvents to 1 µg/mL and loaded onto the MIM/g-C₃N₄ SPE cartridge, and the recoveries were determined, respectively. The results in Fig. 4A showed that, when methanol/water (1:9, v/v) was used as the loading solvent, the recovery of OPPs was the highest. So, it was chosen for the subsequent experiments.

Table 1
Recoveries of the four OPPs from standards fortified blank dairy samples (n = 6).

Samples	OPPs	Added (ng/mL)	Intraday recovery		Interday recovery	
			Recovery (%)	CV (%)	Recovery (%)	CV (%)
Milk	Coumaphos	10	92.8	7.5	91.1	6.6
		100	90.6	6.4	92.2	8.3
	Chlorpyrifos	10	88.9	8.7	87.9	5.9
		100	93.0	6.5	95.4	6.7
	Diazinon	10	86.4	5.8	86.1	7.1
		100	88.6	6.9	86.3	3.0
Parathion	10	90.7	5.8	90.1	8.2	
	100	92.5	9.0	87.7	6.5	
Yogurt	Coumaphos	10	88.1	5.4	89.9	5.1
		100	92.6	7.4	93.5	7.1
	Chlorpyrifos	10	87.5	3.1	89.4	6.6
		100	93.1	8.1	90.3	4.5
	Diazinon	10	89.6	4.7	92.7	8.0
		100	94.6	5.0	91.1	4.2
Parathion	10	87.1	6.3	89.6	5.5	
	100	91.3	5.3	91.6	8.9	
Milk powder	Coumaphos	10	95.3	3.5	94.4	3.9
		100	86.6	6.4	90.8	5.2
	Chlorpyrifos	10	94.5	6.5	91.1	5.6
		100	86.6	9.8	88.4	3.6
	Diazinon	10	93.1	6.0	87.5	4.4
		100	94.2	5.5	93.4	9.5
Parathion	10	89.1	7.8	92.0	7.1	
	100	89.0	7.6	95.0	5.6	

3.3.2. Elution solvent

In this study, four OPPs (1 µg) were diluted with loading solvent and loaded onto MIM/g-C₃N₄ SPE cartridges. After the adsorption process was completed, the cartridges were eluted with different elution solutions. The results in Fig. 4B showed that methanol/acetic acid (95:5, v/v) had an optimal elution effect, so it was chosen as the elution solvent. During the elution process, acetic acid could break the chemical bond between MIM and OPPs, and the OPPs could easily be eluted, so methanol/acetic acid often performed well as an elution solvent. After that, the volume of elution solvent was optimized. The results in Fig. 4C indicated that 1 mL of elution solvent could elute almost all of the adsorbed OPPs (elution rate > 90 %), so the volume of elution solvent was set at 1 mL for the subsequent experiment.

3.3.3. pH

During the extraction process, the pH of the solution can affect the physicochemical properties of OPPs, thereby affecting the purification effect. In this study, the recoveries under pH from 3.0 to 10 were measured to evaluate the optimal pH. It could be seen from Fig. 4D that the sample recovery rates at the pH of 5.0 and 6.0 were better than other recoveries. In this study, the four selected and tested OPPs all contain phosphorothioate structures, which are easily converted into corresponding phosphoric acid and alcohol in acidic (pH less than 4) and alkaline (pH > 8) solutions (Moinfar, Khodayari, Abdulrahman, Aghaei, Sohrabnezhad & Jamil, 2022). Therefore, the solution pH was adjusted to 6.0 in subsequent experiments.

3.3.4. Salt concentration

According to a previous report, adding salt to the sample may have two opposing effects. On the one hand, adding salt to the sample can lead to analyte precipitation due to the salting-out effect, which helps the MIM/g-C₃N₄ to adsorb the analyte. On the other hand, the addition of salt causes the solution to become viscous, which is not conducive to the adsorption of OPPs (Gao et al., 2021). During the experiment, the ionic strength was examined by adding 0–10 mM NaCl to the tested samples. The results in Fig. 4E indicated that the ionic strength made no difference on the adsorption effect. Although the addition of salt made the solution viscous, the adsorption capacity was not affected due to the large pore size of the MIM/g-C₃N₄. Based on these results, the salt was

not used in the SPE process.

In summary, the optimal parameters of the extraction process were: methanol/water (1:9, v/v) was used as loading solvent, methanol/acetic acid (95:5, v/v, 1 mL) was used as elution solvent, solution pH was adjusted to 6, and no salt was added.

3.4. Method performance

In order to evaluate the method performance, four OPPs were diluted with blank milk, yogurt and milk powder extracts, respectively, and determined according to the optimized conditions mentioned above. The results in Table S3 indicated that the LODs of the four OPPs were ranged from 0.2 to 0.8 ng/mL and the LOQs were 0.7–2.6 ng/mL, and the four OPPs had a good linearity from 1 to 10000 ng/mL ($r^2 > 0.999$).

To evaluate the reuse performance of the material, three matrix-matched standard solutions were loaded onto three cartridges, respectively, and then reused 30 times. The results in Fig. 4F showed that MIM/g-C₃N₄ had a good reusability. After 15 times of use, the OPPs recoveries of the three matrix-matched solutions began to be lower than 90 %. In order to ensure the accuracy of the measurement and the rigor of the experiment, the reusable times were set as 15 times. These results showed that MIM/g-C₃N₄ was a recyclable and durable adsorbent. The reduction in recovery rate might be due to the following two reasons: first, the matrix-matched solution contained impurities, which occupied adsorption cavities and were difficult to elute, so the accumulated impurities reduced the adsorption capacity of the material. Second, the elution solvent could not completely elute the adsorbed analytes, and these accumulated analytes occupied the cavities of the MIM/g-C₃N₄, which resulted in the reduce of the cartridge absorption.

The chromatograms of the four matrix-matched OPPs solutions were shown in Fig. S2. It can be seen that there were no impurity peaks around the target analyte, this indicated that the cartridge had a good specific purification effect. Then the four tested OPPs were added into the blank milk, yogurt and milk powder samples for analysis, respectively. The results of the dairy products were summarized in Table 1, in which the intraday recoveries were 86.4 to 95.3 % and interday recoveries were 86.1 to 95.4 %. When the matrix effect is between –20 % and 20 %, it indicates the sample preparation method is feasible and has a weak matrix effect. In present study, the results of matrix effect were in

Table 2
Details of some SPE methods for OPPs.

Material	EM	Analytes	DM	LOD (ng/g or ng/mL)	Application	Reuse time	Ref.
Ionic liquid	CPE	5 OPPs	HPLC	2.0–4.3	coarse cereals	No	(Zhang et al., 2022)
PVA/MGO	MSPE	5 OPPs	GC-MS	20–80 pg/mL	juice and water	No	(Nasiri, Ahmadzadeh & Amiri, 2021)
MHPA	QuEChERS	11 OPPs	GC-MS	0.74–8.16	juice	No	(Wang et al., 2019)
Monoclonal antibody	Coating hapten	Diazinon	ELISA	0.58	vegetables	No	(Wu et al., 2021)
MIP	SPME	5 OPPs	HPLC	0.02–2.00	vegetables, water and barley	No	(Huang, Ma, Feng, & Wei, 2019)
MIM/g-C ₃ N ₄	SPE	4 OPPs	UPLC	0.2–0.8	milk, yogurt and milk powder	Yes (15 times)	This study

EM: extraction method; DM: detection method; PVA/MGO: magnetic graphene oxide coated with polyvinyl alcohol; CPE: cloud point extraction; MSPE: magnetic solid-phase extraction; SPME: solid-phase microextraction;

the range of –11.3 % to 9.58 % (Table S3), which suggested there was no obvious matrix effect. In summarize, this method has a good performance with satisfactory sensitivity and accuracy.

3.5. Real sample detection

In this study, some samples from the local market were analyzed using MIM/g-C₃N₄ cartridge. From the results, all the samples were negative. Nevertheless, the threat of OPPs to human health cannot be underestimated, especially for the elderly and children.

3.6. Comparison with other methods

With the application of SPE method using MIM/g-C₃N₄, this study firstly detected the OPPs in dairy products. In order to compare with related studies, the detection methods of OPPs including MIP, HPLC and SPE methods were summarized in Table 2. Compared with the materials reported in other studies, the SPE method based on MIM/g-C₃N₄ can be reused for 15 times, does not require complicated operation process, and costs less. Due to the physical properties such as strong adsorption and easy modification, g-C₃N₄ has great potential in sample pretreatment. With the combination of g-C₃N₄ to MIM, the synthesized MIM/g-C₃N₄ can be well applied to detect designated compounds with specific recognition ability. Moreover, the composite material of MIM/g-C₃N₄ is simple to synthesize and can be reused many times. Considering the application in OPPs detection, MIM/g-C₃N₄ has a good potential in the purification process of dairy products.

4. Conclusion

In present study, a MIM/g-C₃N₄ composite material was prepared, and a novel SPE cartridge was developed using the MIM/g-C₃N₄ for the purification and detection of OPPs in dairy products. The MIM/g-C₃N₄ has the advantages of specific recognition ability, good extraction effect and reusability. The prepared SPE cartridge was combined with the UPLC method to detect OPP in dairy products, and the results were satisfactory. Therefore, MIM/g-C₃N₄ may become a potential excellent material in the pretreatment of dairy products for OPPs residue testing.

CRedit authorship contribution statement

Yinchuan Pan: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Xu Liu:** Formal analysis, Investigation. **Jing Liu:** Data curation, Formal analysis. **Jianping Wang:** Conceptualization, Project administration, Resources. **Juxiang Liu:** Supervision, Visualization. **Yanxia Gao:** Investigation, Methodology, Resources. **Ning Ma:** Conceptualization, Funding acquisition, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was financially supported by the Precision Animal Husbandry Discipline Group Construction Project of Hebei Agricultural University (No. 1090064; Baoding, China) and earmarked fund for CARS (No. CARS-36, Beijing, China). The authors have not stated any conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100424>.

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