

Fabrication of Molecularly Imprinted Electrospun Nanofibers with Mono-amidoxime Functional Ligand for Efficient Decontamination of Toxic Organophosphates

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 Electronic Supplementary Information

Abstract There is an urgent imperative to discover practical and efficacious artificial catalysts for the expeditious decontamination of toxic organophosphates. Herein, we propose a novel molecularly imprinted approach utilizing electrospun fiber scaffolds. Specifically, an amidoxime-based functional polymer (PMAOX) has been synthesized, which contains amidoxime groups that can act as nucleophiles and ligands for the formation of catalytic active sites. This polymer was blended with polyacrylonitrile (PAN), a well-processable material, to prepare a nanofiber mat using electrospinning techniques. Then, the amidoxime side groups on fiber surface were further cross-linked after the molecular coordination of templates to complete the molecularly imprinting process. This approach can not only enhance the content of functional molecularly imprinted polymers without affecting structural stability, but also combines surface MI technology to fully expose and leverage the advantages of active sites. The as prepared molecularly imprinted electrospun nanofibers MIF-Zn-PMAOX/PAN-6/4 catalyzes the degradation of paraoxon with a half-life of 32 min, and MIF-Ag-PMAOX/PAN catalyzes the degradation of parathion with a half-life of 18 min. The maximum catalytic rate evidence rate enhancements nearly 3700-fold of the self-hydrolysis. Thus, the mono-amidoxime based molecularly imprinted fibers demonstrate the versatility and superiority as self-detoxifying for organophosphates.

Keywords Organophosphates hydrolysis; Electrospinning; Molecular imprinting

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INTRODUCTION

Organophosphorus nerve agents (OPs) covering pesticides and chemical weapons, have been involved in agricultural production and military attacks, which present great threat to the health of the general public or the military.^[1] Specifically, nerve agent (e.g., Soman, Sarin and VX) render instantaneously lethal toxicity by irreversibly inhibiting acetylcholinesterase (AChE), an enzyme regulating in the neurotransmitter acetylcholine levels.^[2] A preferred source of coping strategy for nerve agent degradation is phosphotriesterase (PTE), which is capable of hydrolyzing OPs with rapid efficiency.^[3] Unfortunately, the fragile character of PTE in the natural environment severely limits its application in practical field.^[4,5] Inspiring by highly active catalytic performance of PTE towards OPs, the active bridging Zn-OH-Zn sites have been mimicked by researchers for developing artificial catalyst.^[6] For example, Zr-based metal-organic

frameworks (Zr-MOF) with bimetallic Zr-OH-Zr active sites, have demonstrated effective detoxification against toxic chemicals of OPs, and outperformed in exceptional porosity, high surface area and amenability to modular design.^[7–10] However, the actual implementation of Zr-MOF encounters a crucial challenge posed by its non-processable form with powder.^[11]

Molecular imprinted catalysts are known for the precise construction of the active sites, have been widely used as mimics of natural enzymes with enzyme-like catalytic reactivity but higher stability.^[12,13] Zhao *et al.*^[14–17] have utilized a micellar imprinting approach to construct a variety of artificial enzymes by steps of the nano-confinement polymerization with activities close to those of natural enzymes, such as carbonic anhydrase, glucosidase and aspartic proteases, *etc.* Yuan *et al.*^[18] mixed a rigid-type monomer of tetrakis(*p*-bromophenyl)methane with ZnCl₂, 1-vinylimidazole, methacrylic acid and template molecules of 1-(diethoxyphosphorylmethyl)-4-nitrobenzene to give crosslinked molecularly imprinted porous aromatic frameworks (MIPAF) *via* the Heck coupling reaction, the MIPAF materials showed high catalytic activity for paraoxon, excellent stability and recyclability. Based on the inspiration from phosphotriesterase, the struc-

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ture that hydroxide-bridged binuclear zinc active centers ligated with residues of histidine, aspartate, and lysine play a crucial role on catalytic activity.^[19] The ligand-fixed metal sites are responsible for activating of the organophosphorus substrate, and the bridging hydroxide initiate nucleophilic attack to catalyze hydrolytic reaction of OPs.^[20–22] Therefore, a typical structure of functional monomers that a ligand for stabilizing metal sites cooperates with nucleophile was expected to be one promising enzyme-mimic strategy applying to high-efficiency OPs hydrolysis.^[23,24] Tirel et al.^[25] designed a pyridyl zinc complex incorporating a hydrated aldehyde as a nucleophile, and achieved 70-fold greater hydrolysis rate of phosphate diesters than that for simple pyridyl zinc complexes. Recently, our team reported a series of MIPs incorporating pyridine-amidoxime based functional modules those have been shown to provide synergistic promotion to accelerate OPs hydrolysis to minute level, where amidoxime groups act as a potent nucleophile assist in hydrolytic reaction, and simultaneously served as potential ligand participated in metal coordination.^[26,27] This observation suggests that the amidoxime group exhibits dual functionality as a metal-binding ligand and nucleophile, highlighting its potential. Consequently, in this study, we synthesized an amidoxime-based functional monomer (MAOX) with one pendant amidoxime group and one polymerizable vinyl phenyl group for subsequent molecular imprinting of electrospun nanofibers.

Electrospinning is known for scalable procedure that processing polymers into nonwoven porous nanofibers,^[28,29] which has shown excellent potential in various application including catalysis,^[30,31] adsorption,^[32] tissue engineering,^[30] etc. There are three reported combining ways of molecularly imprinted polymers with electrospinning. The first also the commonly used one is the pre-polymerization of molecularly imprinted polymers before spinning, then doping molecularly imprinted polymer nanoparticles into the spinning polymer solution.^[33,34] The limitation of this approach lies in the constrained filling capacity of molecularly imprinted polymer as a nanoparticle filler, which is determined by the spinning conditions. This constraint further influences the catalytic efficiency of the spun material, which relies on the abundance of molecularly imprinted active sites. The second approach involves molecular imprinting during the electrospinning process. As the formation of imprinting sites relies on solvent removal and physical phase separation of spinning fluid polymers, there is no chemical cross-linking occurring in this process. Consequently, the stability of the resulting imprinting fiber materials may be compromised.^[35] And the third preferred strategy is surface molecular imprinting by polymerization of functional monomers on the electrospun fiber surface with the presence of template.^[36,37] Although the surface imprinting technology can avoid problems above two methods, additional consideration needs to be given to the bonding stability between molecularly imprinted polymers and matrix materials. Therefore, a novel combination of electrospinning and post-molecular imprinting technology was developed in this study through the crosslinking of functional polymer side chains on the surface of electrospun fibers after electrospinning. This approach ensures stable binding of catalytic active

functional components to the matrix while maximizing the efficiency of formed molecular imprinting active sites. In our specific work, homopolymer PMAOX polymerized from MAOX monomers were blended with commercial polyacrylonitrile (PAN) to obtain the blend fiber material *via* electrospinning. Then, the imprinting process is carried out upon fiber surface which incorporates template-coordinating assemblies and crosslinking of the side chain. The post-imprinting approach is more advantageous for rectifying the imprinted cavities in close proximity to the catalytic active center, thereby enhancing substrate diffusion and binding kinetics, which are crucial factors contributing to the development of MIP nanofibers with accelerated catalytic degradation of OPs.

EXPERIMENTAL

Chemicals

N-methylethylenediamine (95%) was purchased from Alfa Aesar. Ethyl trifluoroacetate and hydroxylamine was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Polyacrylonitrile ($M_w=1.5\times10^5$) was purchased from Huachuang Plastic Chemical Co., Ltd., (Suzhou, China). 4-Vinylbenzyl chloride (98%) was purchased from Heowns Reagent Co., Ltd., (Tianjin, China). Acrylonitrile (95%) was purchased from Tianjin Bohua Reagent Co., Ltd., (Tianjin, China). Azobisisobutyronitrile was recrystallized in anhydrous methanol. $Zn(NO_3)_2\cdot6H_2O$, $AgNO_3$, $Cu(NO_3)_2$ and $Co(NO_3)_2$ were purchased from Tianjin Fuchen chemical Reagents Factory (Tianjin, China). Diethyl-4-nitrophenyl phosphate (DENP), and parathion was acquired from Yifang Science & Technology Co., Ltd., (Tianjin, China). All other reagents were analytical reagents and used as received without further purification.

Synthesis of MAOX

As illustrated in Scheme 1, the synthesis of monomer MAOX was divided into 5 steps and the specific steps are described in the electronic supplementary information (ESI).

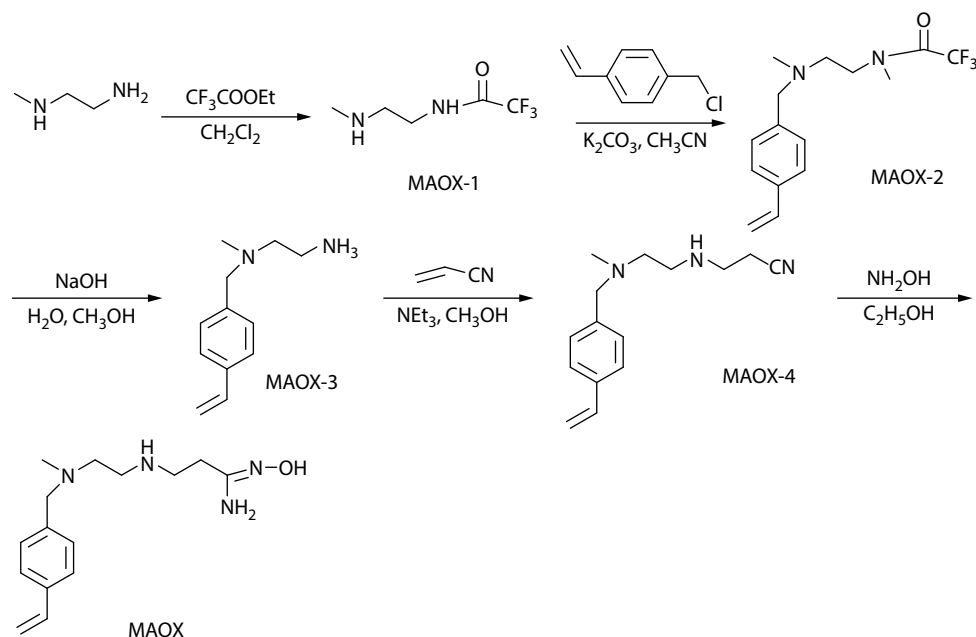
Preparation of Metal-MAOX Assemblies and PMAOX

As a typical run, MAOX (50 mg, 0.18 mmol), $Zn(NO_3)_2\cdot6H_2O$ (54 mg, 0.18 mmol) were dissolved in 10 mL of CH_3OH . After stirring for 12 h at room temperature, the mixture was evaporated under reduced pressure to obtain Zn-MAOX as viscous light-yellow liquid. The sample M-MAOX coordinated by other metals is consistent with the above method.

A mixture of MAOX (0.8 g, 2.9 mmol) and *N,N*-dimethylformamide (3.4 mL) was deaerated by argon purge for 30 min. 2,2-Azobisisobutyronitrile (AIBN, 40 mg) was added to the reaction solution, the reaction was then carried out at 80 °C for 12 h under argon. The PMAOX was obtained by precipitation in a large amount of tetrahydrofuran. The obtained polymer was dried under vacuum to yield 0.67 g (~84%) of PMAOX.

Preparation of PMAOX/PAN Electrospinning Nanofibers

Three different ratios of PMAOX/PAN electrospun nanofibers were prepared in this work. A certain concentration of PMAOX/PAN (4/6, W/W, 15 wt%; 5/5, W/W, 15 wt%; 6/4, W/W, 20 wt%) spinning solution was prepared in *N,N*-dimethylformamide (DMF). Before the co-electrospinning process, the prepared polymer solution was stirred 30 min to prepare homoge-



Scheme 1 Synthetic route to MAOX.

neous solution. The blending polymer solution was then transferred into a 1 mL syringe equipped with a metallic needle of 0.4 mm inner diameter. The applied voltage was at 14 kV and the tip-to-collector distance was kept at 28 cm. The speed of the collecting disc is kept at 74.5 r/min.

Preparation of MIF-Metal-PMAOX/PAN Nanofibers

Diethyl(4-nitrobenzyl) phosphonate (D4NP) as a structural analogue of organophosphorus nerve agents, was chosen as better template molecule than the target substrate itself in this work, owing to its hydrolytic stability. For the preparation of a molecularly imprinted Zn-PMAOX/PAN nanofiber mat, the PMAOX/PAN fiber mat (4/6: 12.5mg, 5/5: 10 mg, 6/4: 8.3 mg) was put into 4 mL of tetrahydrofuran containing 11 μ L of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ aqueous solution (500 mg/mL) and 5 mg of template diethyl(4-nitrobenzyl)phosphonate (D4NP), and the mixture was shaken for 12 h at room temperature. Subsequently, 20 mg of epichlorohydrin was added into the above solution for crosslinking at 60 $^{\circ}\text{C}$ for 4 h, the solution was refluxed for another 4 h at 60 $^{\circ}\text{C}$. Afterwards, the sample was washed for several times with methanol to extract the unreacted crosslinker. The obtained materials was treated with 2 mL of 2,2-bipyridyl/ CH_3OH solution (100 mmol/L) and 2 mL of $\text{NaOH}/\text{H}_2\text{O}$ solution (100 mmol/L) to remove template. Then They were washed with methanol and water three times and treated with 11 μ L of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ aqueous solution (500 mg/mL) for 12 h. Finally, MIF-Zn-PMAOX/PAN catalyst was obtained after drying for 3 h under reduced pressure. Non-imprinted fiber mat was prepared in the absence of template D4NP following the same procedure as described above.

Characterization

^1H -NMR spectra were recorded on a Unity Inova 400 MHz spectrometer with tetramethylsilane as the internal standard. Attenuated total reflectance Fourier transformation infrared (ATR-FTIR, Bio-Rad, FTS6000, America) spectroscopy was utilized to detect the chemical structure of the materials. The morphology of the fibers obtained from co-electrospinning was observed us-

ing an JSM-7500 scanning electron microscope (SEM) after platinum sputtering onto the fibers.

Decontamination of Organophosphates

The kinetics of organophosphates degradation was assessed by UV-2550 spectrophotometer. The reaction medium contains the following substances: 4 mL of Tris-HCl buffer (20 mmol/L, pH=7–10), 900 μ L of CH_3OH , 5 mg functional materials and 100 μ L of organophosphate substrate (10 mg/mL DENP). Except for the experiment exploring the effect of pH on catalytic performance, all other hydrolysis performance tests were conducted under the condition of pH=9. Reaction progress was monitored at $\lambda=400$ nm. During the kinetic testing, we take out 50 μ L of the reaction liquid and dilute with 350 μ L of Tris-HCl, and then put it into the UV-2550 to detect the formation of *p*-nitrophenol. The reaction half-life ($t_{1/2}$) and the pseudo first rate constant (k_{obs}) were derived as in Eqs. (1) and (2), where C_0 and C_t represent the initial substrate concentration and the current concentration, respectively. Fitting the first three points to acquire the k_{obs} .

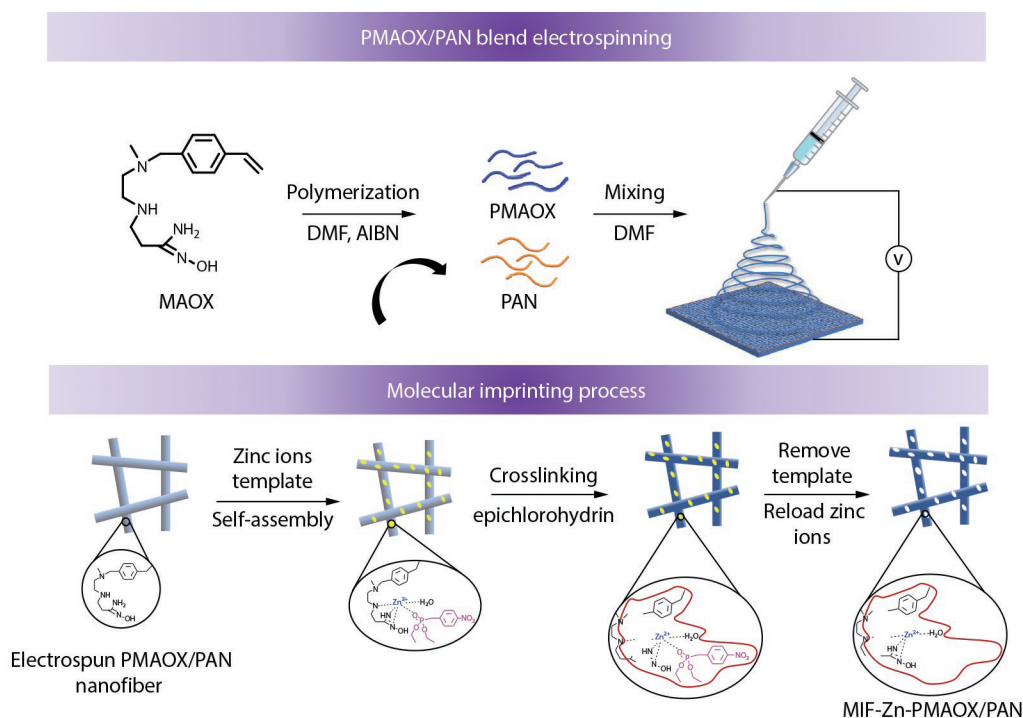
$$\ln\left(\frac{C_t}{C_0}\right) = -k_{\text{obs}}t \quad (1)$$

$$t_{1/2} = \ln(2)/k_{\text{obs}} \quad (2)$$

RESULTS AND DISCUSSION

Preparation of MIF-Zn-PMAOX/PAN

The procedure for the synthesis of molecularly imprinted fibers MIF-Zn-PMAOX/PAN is illustrated in Scheme 2. Prior to the electrospinning, the self-designed amidoxime based functional monomers MAOX were initiated to polymerize into homopolymers of PMAOX, and then, PMAOX and PAN were mixed together with different feeding ratio to acquire spinning solutions for the fabrication of nanofibers. A class of functional PMAOX/PAN blend fibers with mass ratios of 4/6, 5/5 and 6/4 were acquired via electrospinning. Those fibers were employed as post-modifying scaffold followed with molecularly imprint-



Scheme 2 Schematic illustrating of the preparation process of MIF-Zn-PMAOX/PAN electrospun nanofiber.

ing process upon its surface to afford MIF-Zn-PMAOX/PAN. At first, the chemical structure of PMAOX, PAN and PMAOX/PAN blend fiber materials were determined by FTIR spectra. As Fig. 1(a) shows, the broad bands at 3306 cm^{-1} could be found in spectra of original functional polymer of PMAOX and blended polymer fibers of PMAOX/PAN, which represents the stretching vibration band of amino and hydroxyl groups on the amidoxime group. In addition, the stretching vibration bands at 880 and 1650 cm^{-1} corresponding to the N—O bond and imine bond (—C=N) of amidoxime group, respectively. With the blending of PMAOX with PAN, the characteristic absorption band of cyano group in PAN appears at 2243 cm^{-1} , indicating the successful preparation of the blend fiber material. Moreover, the stretching vibration absorption bands of amino and hydroxyl groups on the amido group and the stretching vibration absorption band of C=N are more significant along with the in-

crease of the proportion of PMAOX in PMAOX/PAN blend fiber.

Based on as-prepared electrospun fiber of PMAOX/PAN, specific binding sites of OPs substrates constructed via molecularly imprinting with template molecules (D4NP). In specific, the assemblies of imprinting module, including metal ions, templates and ligand, were mixed with crosslinker of epichlorohydrin, and followed with coupling reaction between epichlorohydrin and amine group of amidoxime upon fibrous surface of PMAOX/PAN. Different from traditional molecular imprinting polymerization of directly cross-linking polymer chains to form molecularly imprinted networks, MIF-Zn-PMAOX/PAN forms an interwoven network structure through the crosslinking between adjacent polymer side chains pendant on the electrospun fibers, and to realize the fixation of templates. This method is most likely to maximize the effective exposure of the constructed reactive imprinting

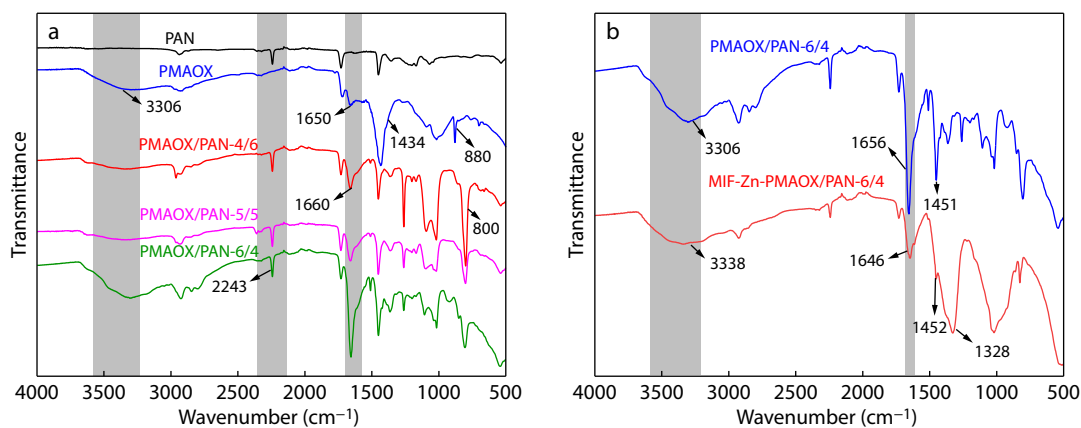


Fig. 1 FTIR spectra of (a) PMAOX and blended electrospun fibers of PMAOX/PAN; (b) Electrospun fibers of PMAOX/PAN-6/4 and molecularly imprinted fibers of MIP-Zn-PMAOX/PAN-6/4.

sites. As shown in Fig. 1(b), comparing with the PMAOX/PAN-6/4 fiber, molecular imprinted fibers of MIF-Zn-PMAOX/PAN-6/4 appears a new absorption band at 1328 cm^{-1} , which is due to the C—N bond formed by epoxy-amino reaction. This suggests the successfully crosslinking of blend nanofibers. Meanwhile, the FTIR spectra provide insights into the structural changes occurring before and after metal coordination. In MIF-Zn-PMAOX/PAN-6/4, the characteristic bands of the imine bond band in the amidoxime group exhibits a red shift from 1656 cm^{-1} to 1646 cm^{-1} as compared with that in PMAOX/PAN-6/4, indicating coordination of the imino group with zinc ions in MIF-Zn-PMAOX/PAN-6/4. By summarizing the above analyses, the appearance of these characteristic bands confirmed the successful preparation of MIF-Zn-PMAOX/PAN-6/4 blend fiber.

The morphologies of electrospun nanofibers changed with the concomitant procedure involving coordinate self-assembly and molecular imprinting. As shown in Fig. 2, the scanning electron microscope (SEM) of the blend fiber material PMAOX/PAN-6/4 shows typical fibrous morphology, smooth surface and uniform fiber diameter. After the imprinting post-treatment, the homologous surface get increase in roughness for fibrous samples of MIF-Zn-PMAOX/PAN-6/4 and the nonimprinted NIF-Zn-PMAOX/PAN-6/4. Particularly, MIF-Zn-PMAOX/PAN-6/4 exhibit notably enhanced rough surface as compared to NIF-Zn-PMAOX/PAN-6/4, which mainly caused by template-eluted process with the formation of imprinted cavities on fiber surface. Besides, the energy-dispersive X-ray analysis of MIF-Zn-PMAOX/PAN-6/4 fiber shows the existence of zinc, indicating the coordinately bound between zinc ions and the functional ligand in PMAOX.

Hydrolytic Degradation of Organophosphate Catalyzed by Molecularly Imprinted Fibrous Catalysts

Catalytic activity of MAOX/PMAOX

Firstly, the catalytic activity of MAOX functional monomers for the hydrolysis of OPs was investigated separately. Paraaxon was

selected as target substrate, and the hydrolysis process catalyzed by metal complex Zn-MAOX under different pH conditions (pH=7, 8, 9, 10) was monitored by measuring the hydrolysate *p*-nitrophenol. The hydrolysis reaction kinetics curves and apparent reaction rate constant and half-life of Zn-MAOX catalytic degradation of are displayed in Fig. 3 and Table 1. As shown in Fig. 3(a), obviously, with the increase of pH value, catalytic degradation performance of Zn-MAOX for paraoxon improved with concomitant increasing pH values within the pH range of 7–10, and the relevant reaction rate constant k_{obs} increases from $1.0 \times 10^{-3}\text{ min}^{-1}$ to $20.8 \times 10^{-3}\text{ min}^{-1}$. This indicates that the more basic exogenous medium condition is conducive to accelerate the catalytic hydrolysis process by rapid deprotonation that can assist in the nucleophilic attack step of hydrolysis. Moreover, the higher alkalinity of reaction environment will offer more hydroxyl ligands participating in the regeneration of nucleophilic reagents, which increases the efficiency of catalytic cycle.

Then, the impact of the specific composition of catalytic active module on the catalytic performance was further explored, that is, the substrate-binding metal sites. Specifically, the hydrolysis of OPs catalyzed by metal complexes using various coordinated metal ions were executed. For typical OPs of paraoxon and parathion, different type of Lewis acid metal ions activates the P=O/S bond by combining the oxygen atom or sulfur atom of P=O/S bond in different reactive levels.^[27] Therefore, a series of functional polymer with different coordinated metal ions including zinc nitrate, copper nitrate, silver nitrate, cobalt nitrate, were prepared here to investigate the impact of active metal sites on the hydrolysis of OPs (Figs. 3b and 3c). As shown in Fig. 3(b), for paraoxon, the hydrolytic rates catalyzed by different metal-PMAOX materials show the tendency: Zn-PMAOX > Ag-PMAOX > Cu-PMAOX > Co-PMAOX. The maximum catalytic rate constant of Zn-PMAOX is $7.9 \times 10^{-3}\text{ min}^{-1}$ ($t_{1/2}=88\text{ min}$). In the catalytic degradation of parathion, distinct activity trends are observed among vari-

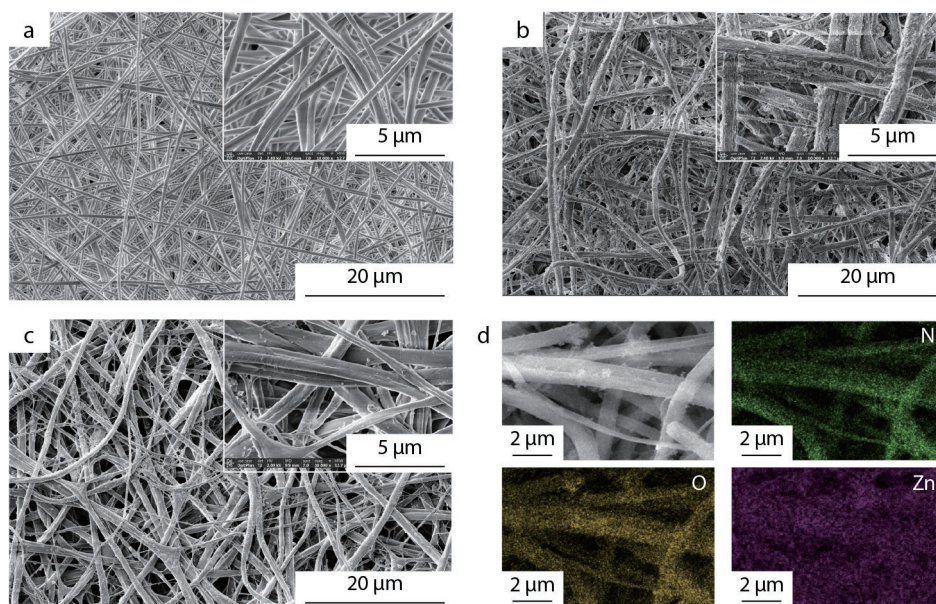


Fig. 2 The morphology of electrospun fibers of (a) PMAOX/PAN-6/4, (b) MIF-Zn-PMAOX/PAN-6/4 and (c) NIF-Zn-PMAOX/PAN-6/4; (d) Energy-dispersive X-ray analysis of MIF-Zn-PMAOX/PAN-6/4.

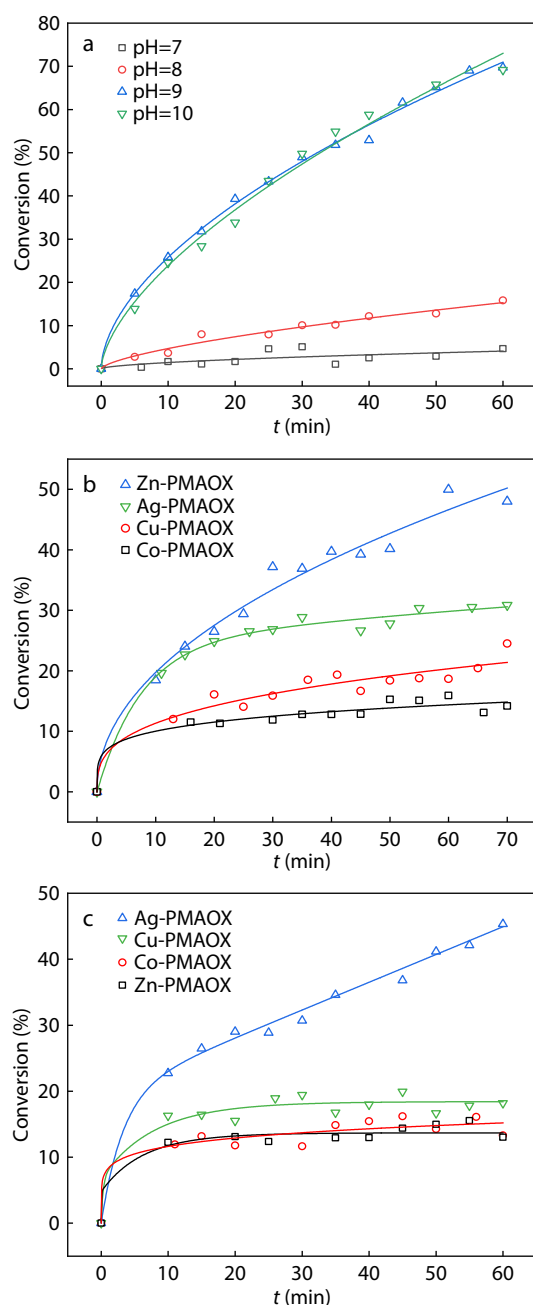


Fig. 3 Under the constant temperature water bath at 30 °C, (a) the kinetic curve of the catalytic degradation of paraoxon by Zn-MAOX in Tris-HCl buffer solutions with different pH values; In Tris-HCl buffer solution with pH=9, the hydrolysis curve of (b) paraoxon and (c) parathion catalyzed by M-PMAOX with different metal coordinations. The initial concentration of parathion is 0.727 mmol/L.

Table 1 Catalytic performance of paraoxon by Zn MAOX at different pH values.

pH	k_{obs} (min ⁻¹)	$t_{1/2}$ (min)	Substrate
7	1.0×10^{-3}	716	Paraoxon
8	2.5×10^{-3}	282	Paraoxon
9	18.5×10^{-3}	37	Paraoxon
10	20.8×10^{-3}	33	Paraoxon

ous metal-PMAOX complexes, the sequence is Ag-PMAOX > Cu-PMAOX > Co-PMAOX > Zn-PMAOX (Fig. 3c). The reactivity disparities among these materials are predominantly attributed to the intrinsic properties of the transition metal present at the active sites. The binding reactivity of metal towards organic ligands as well as organophosphates determine the stability of active sites and the activation of the phosphorus center. According to Pearson's hard/soft acid/base (HSAB) principle, the divalent metal cations exhibit a relatively higher Lewis acidity compared to Ag ions, resulting in stronger binding with oxygen atoms in DENP.^[38,39] Conversely, sulfur in parathion demonstrates a softer character when compared to oxygen.^[38] Thus, the superior catalytic performances were observed for Zn-PMAOX and Ag-PMAOX complexes toward DENP and parathion hydrolysis, respectively. Moreover, amidoxime, featuring an amino and imine nitrogen as the primary coordination atom, represents a class of soft Lewis base ligands that facilitate the formation of stable and efficient active sites between Ag and the functional polymer PMAOX through enhanced coordination. This is also the underlying factor contributing to the excellent catalytic performance exhibited by Ag-PMAOX material in both of DENP and parathion hydrolysis.

Catalytic activity of MIF-M-PMAOX/PAN

Molecular imprinting is a powerful technique to form a substrate-selective active center with enzyme-like reactivity through constructing template-complementary imprinted pockets in terms of molecular size, structural shape and position of catalytic groups. Hence, we have compared the catalytic performance differences between molecularly imprinted and non-molecularly imprinted functional fiber catalysts, as well as reactivities of MIF-Metal-PMAOX/PAN-6/4 toward different organophosphorus substrates. As shown in Fig. 4(a), the hydrolysis performance of MIF-Zn-PMAOX/PAN-4/6 for paraoxon under the buffer condition of pH=9 with apparent reaction rate constant k_{obs} of $4.9 \times 10^{-3} \text{ min}^{-1}$ ($t_{1/2}=143 \text{ min}$, Table 2), which is obvious better than that of nonimprinted NIF-Zn-PMAOX/PAN-4/6 with k_{obs} of $4.1 \times 10^{-3} \text{ min}^{-1}$ ($t_{1/2}=171 \text{ min}$, Table 2). This shows that the formation of imprinting sites has a significant effect in promoting the catalytic degradation of organophosphorus agents. The interchain cross-linking of PMAOX using epichlorohydrin then fixes the spatial structure of active site in the catalytic pocket, which is conducive to achieving higher reactivity for degradation of OPs owing to the rapid and selective substrate binding through template-imprinted cavities shaped as substrate-matching structure. Moreover, the porosity and high specific surface area of the electrospun fiber matrix facilitate rapid mass transfer rates and the creation of densely packed accessible active sites. These remarkable properties of the MIF-Zn-PMAOX/PAN further enhance its catalytic activity for OPs degradation.

In order to explore the optimal blending ratio between PMAOX and PAN, we ensure that the content of functional component PMAOX is constant during the catalytic degradation performance test, that is, the equivalent PMAOX mass is fixed at 5 mg, so as to explore the efficiency of PMAOX in molecular imprinted blends nanofibers with different proportions. As shown in Fig. 4(b), when PMAOX and PAN are mixed in different proportions, the catalytic performance of the

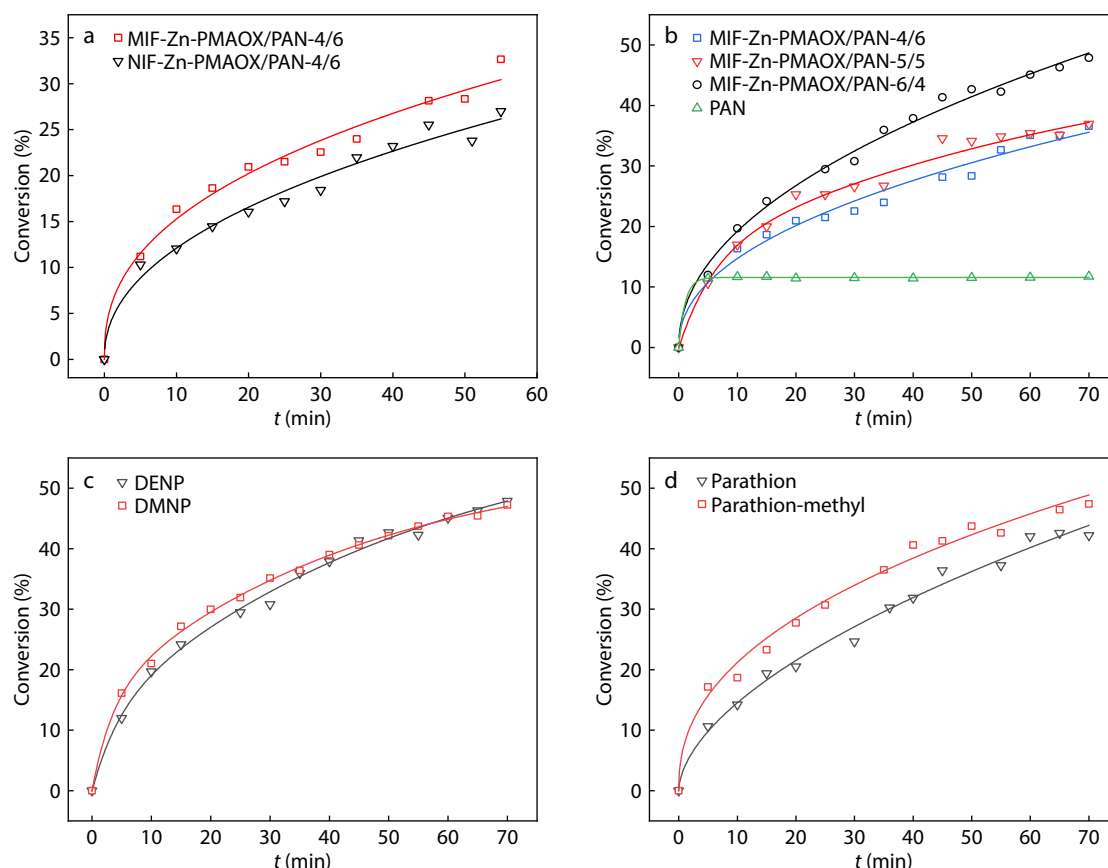


Fig. 4 The hydrolysis kinetics curve of (a) MIF-Zn-PMAOX/PAN-4/6 and NIF-Zn-PMAOX/PAN-4/6; (b) Different proportions of MIF-Zn-PMAOX/PAN-m/n for paraoxon; (c) MIF-Zn-PMAOX/PAN-6/4 for the hydrolysis kinetics curve of paraoxon and methyl paraoxon; (d) MIF-Ag-PMAOX/PAN-6/4 for parathion and methyl parathion. The initial concentration of paraoxon/parathion is 0.727 mmol/L, and methyl paraoxon/methyl parathion is 0.809 mmol/L and measurements executed in Tris-HCl (pH=9) buffer solution.

Table 2 Catalytic degradation performance of different fiber materials on different organophosphorus substrates.

Catalysts	pH	Substrate	k_{obs} (min^{-1})	$t_{1/2}$ (min)
MIF-Zn-PMAOX/PAN-4/6	9	Paraoxon	4.9×10^{-3}	143
NIF-Zn-PMAOX/PAN-4/6	9	Paraoxon	4.1×10^{-3}	171
MIF-Zn-PMAOX/PAN-5/5	9	Paraoxon	5.0×10^{-3}	138
MIF-Zn-PMAOX/PAN-6/4	9	Paraoxon	7.7×10^{-3}	90
MIF-Zn-PMAOX/PAN-6/4	9	Methyl paraoxon	6.7×10^{-3}	103
MIF-Ag-PMAOX/PAN-6/4	9	Parathion	7.1×10^{-3}	98
MIF-Ag-PMAOX/PAN-6/4	9	Methyl parathion	8.1×10^{-3}	86
Self-hydrolysis	9	Paraoxon	3.0×10^{-6} [26]	—
Self-hydrolysis	9	Parathion	1.9×10^{-6} [27]	—

formed molecular imprinted nanofibers improved with the increase of the content of effective component PMAOX. As compared to MIF-Zn-PMAOX/PAN-4/6, MIF-Zn-PMAOX/PAN-5/5 with higher PMAOX proportion, catalytic reaction rate constants of k_{obs} from $4.9 \times 10^{-3} \text{ min}^{-1}$ of MIF-Zn-PMAOX/PAN-4/6 ($t_{1/2}=143 \text{ min}$) increased to $5.0 \times 10^{-3} \text{ min}^{-1}$ ($t_{1/2}=138 \text{ min}$). Notably, when the blending ratio of PMAOX is increased to MIF-Zn-PMAOX/PAN-6/4, k_{obs} is $7.7 \times 10^{-3} \text{ min}^{-1}$ ($t_{1/2}=90 \text{ min}$), the performance is improved more significantly. This indicates that in the MIF-Zn PMAOX/PAN blend nanofibers with the higher blending ratio of PMAOX/PAN, is able to achieve higher catalytic efficiency resulting from the higher the effective component content exposed on the surface. When the

proportion of PMAOX is low, the reactive component of PMAOX is most likely to be embedded by PAN with relatively large component content and lead to a lower catalytic hydrolysis rate. However, if the proportion of PMAOX components is further increased, it will have a negative impact on the spinning performance and be difficult to shape. Thus, MIF-Zn-PMAOX/PAN-6/4 indicates a preferred PMAOX/PAN ratio that balances catalytic performance with material processability.

As shown in Fig. 4(c), we also measured the catalytic degradation performance of the molecularly imprinted nanofibers for paraoxon and methyl paraoxon. As shown in Fig. 4(d), silver coordinated MIF-Ag-PMAOX/PAN-6/4 also has good catalytic performance for a variety of sulfur-containing phos-

phate esters, showing a wide range of substrate tolerance of the material. Therefore, the molecularly imprinted electrospinning-fiber catalysts showed broad-spectrum substrate tolerance and excellent catalytic performance for both oxophosphate and thiophosphate. Among them, the catalytic rate of MIF-Zn-PMAOX/PAN-6/4 for the hydrolysis of paraoxon is 2600 times of its self-hydrolysis rate, and the hydrolytic degradation of parathion catalyzed by MIF-Ag-PMAOX/PAN-6/4 is 3700 times of its self-hydrolysis rate, which may provide a referential guidance for the preparation of protective materials against OPs. Additionally, we revealed decent reusability of the MIF-Zn-PMAOX/PAN-6/4 for catalytic hydrolysis of paraoxon after the three-cycle reuse experiment. As Fig. 5(a) displays, catalytic activity of MIF-Zn-PMAOX/PAN-6/4 basically maintaining complete initial activity in second cycle, but a performance decline begins to occur in the third cycle. MIF-Zn-PMAOX/PAN-6/4 can maintain over 70% catalytic activity. Regarding the decline in catalytic activity, our hypothesis posits that it stems from the inhibition of hydrolysis reaction caused by the entrapment of hydrolysis product *p*-nitrophenol

within the imprinting pores of the molecularly imprinted polymer. The robust adsorption between *p*-nitrophenol and functional polymer PMAOX could be readily achieved under the hydrogen bonding and π - π stacking.^[40] Furthermore, the adsorption capacity of MIF-Zn-PMAOX/PAN-6/4 for *p*-nitrophenol was investigated through UV-visible absorbance spectroscopy. As shown in Fig. S2 (in ESI), the obvious adsorption of *p*-nitrophenol can be observed from the evolution of the UV-Vis absorption spectra before and after addition MIF-Zn-PMAOX/PAN-6/4 for 60 min. In order to assess the binding strength of *p*-nitrophenol, the sample containing solution after adsorption was subjected to ultrasonic treatment for 10 min. Subsequently, the concentration of *p*-nitrophenol was re-evaluated. It was observed that a partial desorption and diffusion of *p*-nitrophenol occurred into the solvent, however, there still remained a partial adsorption onto MIF-Zn-PMAOX/PAN-6/4. The retention of some products inside the material ultimately leads to a decrease in catalytic activity by the inevitable effect of product inhibition. In addition to reusability, the thermal stability of polymer catalytic materi-

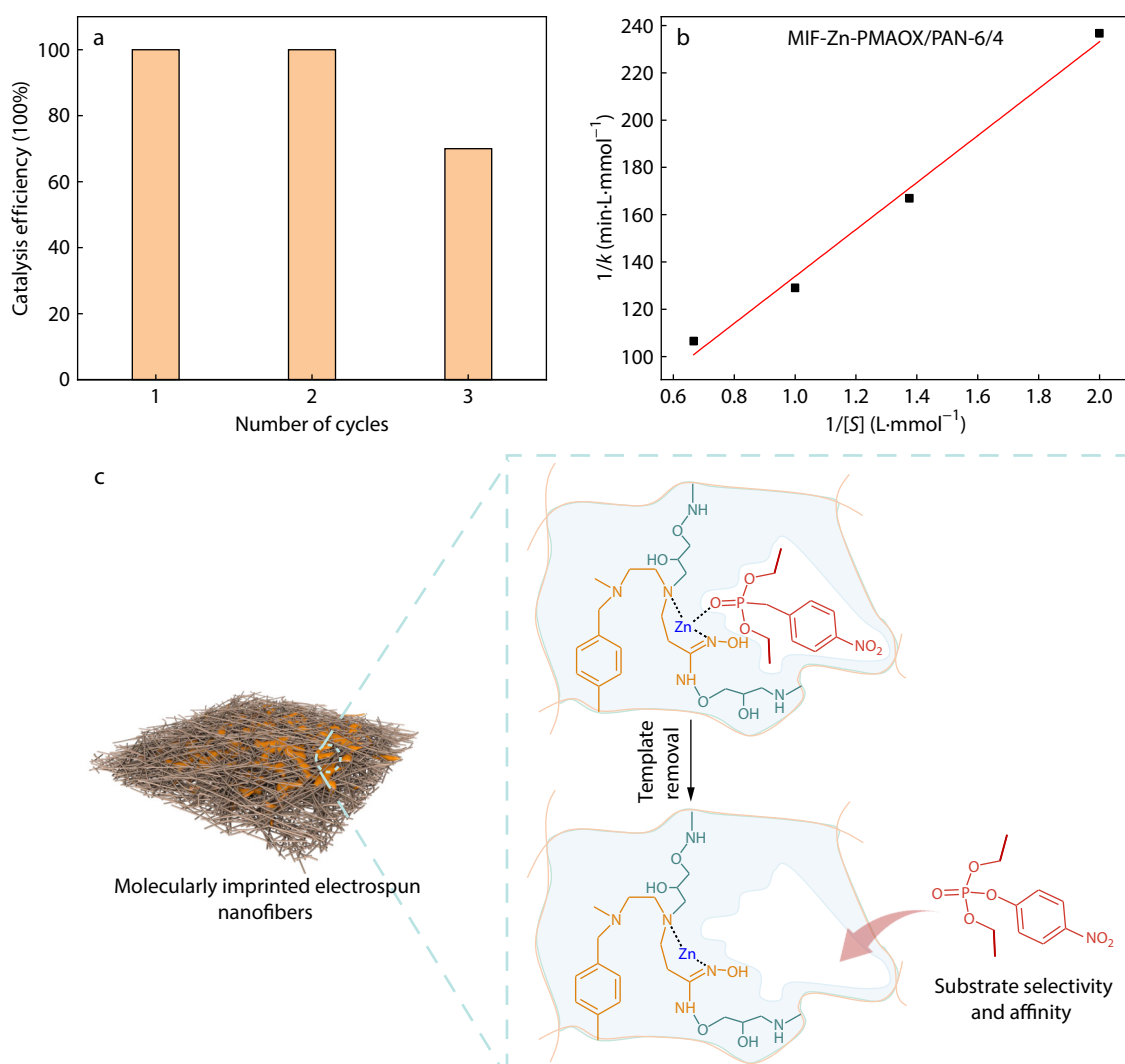


Fig. 5 (a) Reusability of MIF-Zn-PMAOX/PAN-4/6; (b) Lineweaver-Burk plot of hydrolysis kinetics data for paraoxon catalyzed by MIF-Zn-PMAOX/PAN-4/6; (c) A schematic representation of DENP selective binding by molecularly imprinted nanofibers.

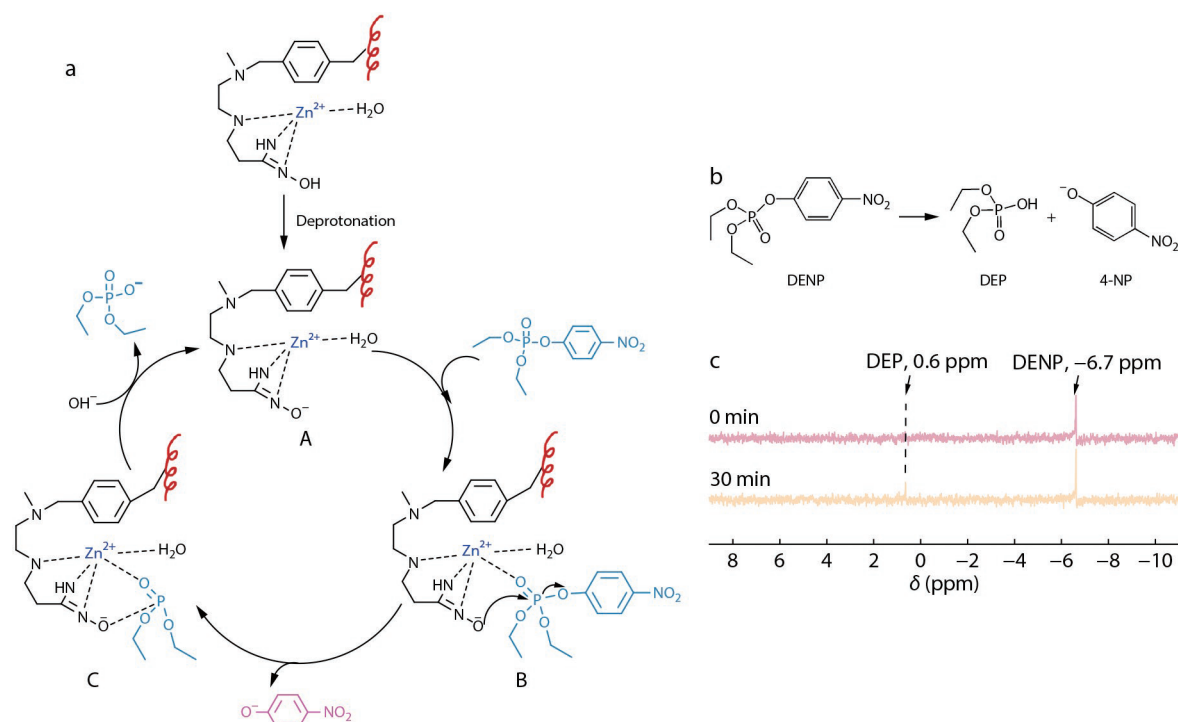


Fig. 6 (a) Possible hydrolytic reaction mechanism of paraoxon catalyzed by MIF-Zn-PMAOX/PAN; (b) Hydrolysis reaction of DENP; (c) ³¹P-NMR spectra of catalytic hydrolysis of paraoxon.

als must be considered to realize the actual detoxification application. We selected MIF-Zn PMAOX/PAN-4/6 as the representative catalyst and measured its thermal weight loss performance during the heating procedure. Fig. S1 (in ESI) shows that the integrity of the MIF-Zn PMAOX/PAN-4/6 catalyst can be well maintained below 150 °C and the thermal weight loss rate does not exceed 10% At 200 °C. Therein, a small amount of weight loss may come from incomplete drying of water and solvents. This result meets the actual usage requirements of the catalyst material.

In order to explore the affinity between MIF-Zn-PMAOX/PAN-6/4 and OPs, we prepared four kinds of paraoxon solutions with different initial concentrations (0.5, 0.7, 1, 1.5 mmol/L) for catalytic degradation experiments, and used the reaction rate of $1/k$ to plot $1/[S]$ to obtain Lin Bayes diagram to get the value of Michaelis constant, K_m . K_m is a characteristic physical quantity of the enzyme, and its value reflects the affinity between the enzyme and the substrate molecule.^[41] Herein, the calculated value of K_m is able to clarify enzyme-mimetic properties of the molecularly imprinted functional fibers catalyst and its affinity with organophosphorus substrate. As shown in Figs. 5(b) and 5(c), the K_m for MIF-Zn-PMAOX/PAN-6/4 is 2.87 mmol/L, which indicates that molecular imprinting process successfully render the synthetic MIF-Zn-PMAOX/PAN-6/4 catalyst has good affinity to the organophosphorus substrate.

We tentatively raised the catalytic mechanism of for the hydrolysis of paraoxon in Fig. 6(a) and further verify its reliability by ³¹P-NMR. Due to the porous morphology of electrospun fiber materials, in combination with molecular imprinting technology, pores that match the substrate are retained near the active site, facilitating rapid diffusion of the substrate into

close proximity with the active site and thereby accelerating the recognition process between the active center and the substrate. In a slightly alkaline catalytic medium, the amidoxime group first deprotonates to form a more nucleophilic oxime anion intermediate A. Then, the oxygen atom in the P=O bond of the substrate coordinates with the zinc ion, and the substrate is fixed near the active center to form intermediate B. The more nucleophilic oxime anion launches a nucleophilic attack on the phosphorus atom in the P=O bond, causing the leaving group *p*-nitrophenol to leave to form intermediate C. Finally, the deprotonated water molecules attack the intermediate C and dephosphorylation occurs, making the catalyst return to the initial state A and enter the next catalytic hydrolysis cycle. The hydrolytic degradation process of DENP catalyzed by MIF-Zn-PMAOX/PAN was further investigated by ³¹P-NMR spectra. As displayed in Fig. 6(c), the solely substrate DENP peak (−6.7 ppm) was observed in ³¹P-NMR spectra at 0 min. By tracking the peak variation after a period of time, the results indicate an obvious substrate degradation with 38% conversion that DENP break down into hydrolyzed product diethyl phosphate (0.61 ppm). This result is consistent with the final hydrolysate in the hydrolysis reaction mechanism proposed above.

CONCLUSIONS

In summary, we developed a novel post molecular imprinting method to prepare molecularly imprinted electrospun nanofiber for OPs decontamination. The electrospinning technique was employed to produce a nano-fiber mat, specifically utilizing a blend of the functional amidoxime-based polymer PMAOX and the easily processable PAN. Subsequently, through

a coupling reaction between epichlorohydrin and amine groups, adjacent polymer side chains containing amidoxime groups on the electrospun PMAOX/PAN fibers were further crosslinked with epichlorohydrin to immobilize molecularly imprinted polymer networks centered around template molecules. This approach finally give rise to a type of functionalized reactive fibrous catalyst MIF-Zn-PMAOX/PAN with good processability and catalytic ability toward organophosphate toxicants. Significantly, our molecularly imprinted electrospun nanofiber capitalizes on its inherent design flexibility to achieve efficient catalytic degradation of two types of organophosphorus toxicants, namely paraoxon and parathion, through the modulation of different coordinated metals for the formation of imprinted active sites. The apparent reaction rate constant (k_{obs}) of MIF-Zn-PMAOX/PAN-6/4 catalytic degradation of paraoxon is $7.7 \times 10^{-3} \text{ min}^{-1}$, $t_{1/2}=90 \text{ min}$, that is 2600 times faster than self-hydrolysis. Additionally, the k_{obs} of MIF-Ag-PMAOX/PAN-6/4 catalytic degradation of parathion is $7.1 \times 10^{-3} \text{ min}^{-1}$, $t_{1/2}=98 \text{ min}$, 3700 times of the self-hydrolysis. Summarily, the performance of molecularly imprinted electrospun nanofibers imply its potential value in preparing high-performance protective materials against OPs.

Conflict of Interests

The authors declare no interest conflict.



Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-024-3075-y>.

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REFERENCES

- Iqbal, A.; Malik, S.; Nurulain, S. M.; Musilek, K.; Kuca, K.; Kalasz, H.; Fatmi, M. Q. Reactivation potency of two novel oximes (K456 and K733) against paraoxon-inhibited acetyl and butyrylcholinesterase: *in silico* and *in vitro* models. *Chemico-Biological Interactions* **2019**, *310*, 108735.
- Zhang, L.; Murata, H.; Amitai, G.; Smith, P. N.; Matyjaszewski, K.; Russell, A. J. Catalytic detoxification of organophosphorus nerve agents by butyrylcholinesterase-polymer-oxime bioscavengers. *Biomacromolecules* **2020**, *21*, 3867–3877.
- Su, S.; Jung, D.; Gong, X.; Idrees, K. B.; Hanna, S. L.; Islamoglu, T.; Gianneschi, N. C.; Farha, O. K. Aggregation-suppressed porous processable hexa-zirconium/polymer composites for detoxification of a nerve agent simulant. *Chem. Mater.* **2022**, *34*, 4983–4991.
- Chen, Y.; Luo, Z.; Lu, X. Construction of novel enzyme-graphene oxide catalytic interface with improved enzymatic performance and its assembly mechanism. *ACS Appl. Mater. Interfaces* **2019**, *11*, 11349–11359.
- Hoskin, F. C.; Roush, A. H. Hydrolysis of nerve gas by squid-type diisopropyl phosphorofluoridate hydrolyzing enzyme on agarose resin. *Science* **1982**, *215*, 1255–7.
- Fan, F.; Zheng, Y.; Zhang, Y.; Zheng, H.; Zhong, J.; Cao, Z. A comprehensive understanding of enzymatic degradation of the G-type nerve agent by phosphotriesterase: revised role of water molecules and rate-limiting product release. *ACS Catal.* **2019**, *9*, 7038–7051.
- Luo, H.-B.; Castro, A. J.; Wasson, M. C.; Flores, W.; Farha, O. K.; Liu, Y. Rapid, biomimetic degradation of a nerve agent simulant by incorporating imidazole bases into a metal-organic framework. *ACS Catal.* **2021**, *11*, 1424–1429.
- Jiang, P.; Wang, L.; Li, J.; Liu, W.; Chen, Z.; Guo, T. Facile *in-situ* strategy for incorporating amphoteric dopamine into metal-organic framework with optimized degradation capacity of nerve agents simulant. *Chem. Eng. J.* **2022**, *448*, 137702.
- Moon, S. Y.; Liu, Y.; Hupp, J. T.; Farha, O. K. Instantaneous hydrolysis of nerve-agent simulants with a six-connected zirconium-based metal-organic framework. *Angew. Chem. Int. Ed.* **2015**, *54*, 6795–6799.
- de Koning, M. C.; van Grol, M.; Breijaert, T. Degradation of paraoxon and the chemical Warfare Agents VX, Tabun, and Soman by the metal-organic frameworks UiO-66-NH₂, MOF-808, NU-1000, and PCN-777. *Inorg. Chem.* **2017**, *56*, 11804–11809.
- Ma, K.; Cheung, Y. H.; Kirlikovali, K. O.; Xie, H.; Idrees, K. B.; Wang, X.; Islamoglu, T.; Xin, J. H.; Farha, O. K. Fibrous Zr-MOF nanozyme aerogels with macro-nanoporous structure for enhanced catalytic hydrolysis of organophosphate toxins. *Adv. Mater.* **2023**, *n/a*, 2300951.
- Mo, W.; Zhu, Z.; Kong, F.; Li, X.; Chen, Y.; Liu, H.; Cheng, Z.; Ma, H.; Li, B. Controllable synthesis of conjugated microporous polymer films for ultrasensitive detection of chemical warfare agents. *Nat. Commun.* **2022**, *13*, 5189.
- Wang, L.; Jiang, P.; Liu, W.; Li, J.; Chen, Z.; Guo, T. Molecularly imprinted self-buffering double network hydrogel containing bi-amidoxime functional groups for the rapid hydrolysis of organophosphates. *J. Hazard. Mater.* **2023**, *444*, 130332.
- Arifuzzaman, M. D.; Zhao, Y. Artificial zinc enzymes with fine-tuned active sites for highly selective hydrolysis of activated esters. *ACS Catal.* **2018**, *8*, 8154–8161.
- Arifuzzaman, M. D.; Bose, I.; Bahrami, F.; Zhao, Y. Imprinted polymeric nanoparticles as artificial enzymes for ester hydrolysis at room temperature and pH 7. *Chem. Catal.* **2022**, *2*, 2049–2065.
- Li, X.; Zangiabadi, M.; Zhao, Y. Molecularly imprinted synthetic glucosidase for the hydrolysis of cellulose in aqueous and nonaqueous solutions. *J. Am. Chem. Soc.* **2021**, *143*, 5172–5181.
- Zangiabadi, M.; Zhao, Y. Selective binding of complex glycans and glycoproteins in water by molecularly imprinted nanoparticles. *Nano Lett.* **2020**, *20*, 5106–5110.
- Yuan, Y.; Yang, Y.; Faheem, M.; Zou, X.; Ma, X.; Wang, Z.; Meng, Q.; Wang, L.; Zhao, S.; Zhu, G. Molecularly imprinted porous aromatic frameworks serving as porous artificial enzymes. *Adv. Mater.* **2018**, *30*, 1800069.
- Tsai, P. C.; Fan, Y.; Kim, J.; Yang, L.; Almo, S. C.; Gao, Y. Q.; Raushel, F. M. Structural determinants for the stereoselective hydrolysis of chiral substrates by phosphotriesterase. *Biochemistry* **2010**, *49*, 7988–7997.
- Prokop, Z.; Oplustil, F.; DeFrank, J.; Damborsky, J. Enzymes fight chemical weapons. *Biotechnol. J.* **2006**, *1*, 1370–80.
- Kirlikovali, K. O.; Chen, Z.; Islamoglu, T.; Hupp, J. T.; Farha, O. K. Zirconium-based metal-organic frameworks for the catalytic hydrolysis of organophosphorus nerve agents. *ACS Appl. Mater. Interfaces* **2020**, *12*, 14702–14720.
- Bigley, A. N.; Raushel, F. M. Catalytic mechanisms for

- phosphotriesterases. *Biochim. Biophys. Acta* **2013**, 1834, 443–53.
- 23 Yang, M.-Y.; Iranzo, O.; Richard, J. P.; Morrow, J. R. Solvent deuterium isotope effects on phosphodiester cleavage catalyzed by an extraordinarily active Zn(II) complex. *J. Am. Chem. Soc.* **2005**, 127, 1064–1065.
 - 24 Shu, J.; Yue, J.; Qiu, X.; Liu, X.; Ren, W.; Li, Q.; Li, Y.; Xu, B.; Zhang, K.; Jiang, W. Binuclear metal complexes with a novel hexadentate imidazole derivative for the cleavage of phosphate diesters and biomolecules: distinguishable mechanisms. *Inorg. Chem. Front.* **2021**, 8, 2684–2696.
 - 25 Tírel, E. Y.; Bellamy, Z.; Adams, H.; Lebrun, V.; Duarte, F.; Williams, N. H. Catalytic zinc complexes for phosphate diester hydrolysis. *Angew. Chem. Int. Ed.* **2014**, 53, 8246–8250.
 - 26 Pan, J.; Liu, S.; Jia, H.; Yang, J.; Qin, M.; Zhou, T.; Chen, Z.; Jia, X.; Guo, T. Rapid hydrolysis of nerve agent simulants by molecularly imprinted porous crosslinked polymer incorporating mononuclear zinc(II)-picolinamine-amidoxime module. *J. Catal.* **2019**, 380, 83–90.
 - 27 Zheng, S.; Pan, J.; Wang, J.; Liu, S.; Zhou, T.; Wang, L.; Jia, H.; Chen, Z.; Peng, Q.; Guo, T. Ag(I) pyridine-amidoxime complex as the catalysis activity domain for the rapid hydrolysis of organothiophosphate-based nerve agents: mechanistic evaluation and application. *ACS Appl. Mater. Interfaces* **2021**, 13, 34428–34437.
 - 28 Gibson, P.; Schreuder-Gibson, H.; Rivin, D. Transport properties of porous membranes based on electrospun nanofibers. *Colloids and Surfaces A: Physicochem. Engin. Aspects* **2001**, 187–188, 469–481.
 - 29 Chen, L.; Bromberg, L.; Lee, J. A.; Zhang, H.; Schreuder-Gibson, H.; Gibson, P.; Walker, J.; Hammond, P. T.; Hatton, T. A.; Rutledge, G. C. Multifunctional electrospun fabrics via layer-by-layer electrostatic assembly for chemical and biological protection. *Chem. Mater.* **2010**, 22, 1429–1436.
 - 30 Wu, K.; Zhang, X.; Yang, W.; Liu, X.; Jiao, Y.; Zhou, C. Influence of layer-by-layer assembled electrospun poly(L-lactic acid) nanofiber mats on the bioactivity of endothelial cells. *Appl. Surf. Sci.* **2016**, 390, 838–846.
 - 31 McCarthy, D. L.; Liu, J.; Dwyer, D. B.; Troiano, J. L.; Boyer, S. M.; DeCoste, J. B.; Bernier, W. E.; Jones, J. W. E. Electrospun metal-organic framework polymer composites for the catalytic degradation of methyl paraoxon. *New J. Chem.* **2017**, 41, 8748–8753.
 - 32 Lu, A. X.; McEntee, M.; Browe, M. A.; Hall, M. G.; DeCoste, J. B.; Peterson, G. W. MOFabric: electrospun nanofiber mats from PVDF/UiO-66-NH₂ for chemical protection and decontamination. *ACS Appl. Mater. Interfaces* **2017**, 9, 13632–13636.
 - 33 Zhang, L.; Guo, Y.; Chi, W. H.; Shi, H. G.; Ren, H. Q.; Guo, T. Y. Electrospun nanofibers containing *p*-nitrophenol imprinted nanoparticles for the hydrolysis of paraoxon. *Chinese J. Polym. Sci.* **2014**, 32, 1469–1478.
 - 34 Moraes Segundo, J. d. D. P. d.; Oneide Silva de Moraes, M.; Brito, W. R.; d'Ávila, M. A. Incorporation of molecularly imprinted polymer nanoparticles in electrospun polycaprolactone fibers. *Mater. Lett.* **2020**, 275, 128088.
 - 35 Perez-Puyana, V.; Wieringa, P.; Guerrero, A.; Romero, A.; Moroni, L. (Macro)Molecular Imprinting of Proteins on PCL Electrospun Scaffolds. *ACS Appl. Mater. Interfaces* **2021**, 13, 29293–29302.
 - 36 Wu, K.; Yang, W.; Jiao, Y.; Zhou, C. A surface molecularly imprinted electrospun polyethersulfone (PES) fiber mat for selective removal of bilirubin. *J. Mater. Chem. B* **2017**, 5, 5763–5773.
 - 37 Ma, X.; Lin, H.; He, Y.; She, Y.; Wang, M.; Abd El-Aty, A. M.; Afifi, N. A.; Han, J.; Zhou, X.; Wang, J.; Zhang, J. Magnetic molecularly imprinted polymers doped with graphene oxide for the selective recognition and extraction of four flavonoids from Rhododendron species. *J. Chromatography A* **2019**, 1598, 39–48.
 - 38 Devic, T.; Serre, C. High valence 3p and transition metal based MOFs. *Chem. Soc. Rev.* **2014**, 43, 6097–6115.
 - 39 Mian, M. R.; Chen, H.; Cao, R.; Kirlikovali, K. O.; Snurr, R. Q.; Islamoglu, T.; Farha, O. K. Insights into catalytic hydrolysis of organophosphonates at M–OH sites of azolate-based metal organic frameworks. *J. Am. Chem. Soc.* **2021**, 143, 9893–9900.
 - 40 Chen, J. Q.; Zheng, Q. Q.; Xiao, S. J.; Zhang, L.; Liang, R. P.; Ouyang, G.; Qiu, J. D. Construction of two-dimensional fluorescent covalent organic framework nanosheets for the detection and removal of nitrophenols. *Anal. Chem.* **2022**, 94, 2517–2526.
 - 41 Soorya, V. C.; Berchmans, S. Flower like Bi structures on Pt surface facilitating effective cholesterol biosensing. *Mater. Sci. Eng. C* **2016**, 64, 183–189.