BMC Chemistry

RESEARCH Open Access

Development of a metal-organic framework-based nanosensor for determination of cyclosporine in plasma samples

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Abstract

According to the narrow therapeutic range and multiple adverse effects of cyclosporine and the need for its therapeutic drug monitoring (TDM), in this study, an efficient zeolitic imidazolate framework-8 metal-organic framework (ZIF-8 MOF) based nanoprobe was designed for simple, rapid and high sensitive its quantification in plasma samples. After the successful synthesis of the ZIF-8 MOF, under the optimum condition, the fluorescence emission of ZIF-8 MOF, measured at an excitation wavelength of 370 nm and an emission wavelength of 417 nm, was enhanced with increasing cyclosporine concentration, due to the specific interactions between cyclosporine and the nanoprobe, including hydrogen bonding and hydrophobic effects. The nanoprobe showed a linear correlation between the analytical response and cyclosporine concentration in the concentration range of 0.01–1.0 μg mL^{-1}, with a detection limit of 0.003 μg mL^{-1}. Acceptable precision was achieved, evidenced by intra-day and inter-day relative standard deviations of 0.4% and 0.5%, respectively. Recovery between 97.1% and 102.1% in plasma samples indicated the method's reliability in practical applications.

Keywords Nanoprobe, Metal-organic framework, Cyclosporine, Plasma

Introduction

Cyclosporine, originating from the fungus *Tolypocladium inflatum*, has an essential role in developing modern immunopharmacology [1]. Medical uses of cyclosporine are commonly in psoriasis, Crohn's disease, and organ transplants [2]. Its therapeutic dose in plasma samples is reported to be 0.1–0.4 μg.mL^{-1} [3]. The compound is neutral and has a molecular weight of 1203 Daltons. It is abundant in hydrophobic amino acids, insoluble in water and saturated carbohydrates, highly soluble in various organic solvents, and very challenging to crystallize [4]. Cyclosporine usage in transplantation medicine has been demonstrated to induce various harmful cellular side effects. One of the most severe side effects of the drug is nephrotoxicity, limiting its employment as an immunosuppressant in clinical applications. In addition to nephrotoxicity, other adverse effects could include hepatotoxicity, neurotoxicity, and myocardial toxicity [5, 6]. The narrow therapeutic ranges and high variability of cyclosporine in blood levels make therapeutic drug monitoring essential for maintaining patients within the required therapeutic concentrations [7]. This is crucial for optimizing clinical outcomes and reducing the risk of

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toxicity or rejection following organ transplantations due to interindividual and intraindividual variability [8]. Some analytical methods have been reported for the cyclosporine analysis, for example, capillary electrophoresis [9], reverse phase-high performance liquid chromatography with a UV detector (RP-HPLC) [13–15], electrochemical [16], and spectroscopy [17] techniques. Many of these techniques require lengthy processes, extensive sample preparation, and costly reagents. Among the different methods, optical-based methods are fast and user-friendly, making them ideal for point-of-care applications. To improve the selectivity and sensitivity of optical techniques, it is essential to create reliable platforms [18] by using advanced materials [19, 20]. Metal-organic frameworks (MOFs) are highly porous crystalline structures created by connecting inorganic metal nodes or clusters with organic ligands through strong coordination bonds [21, 22]. Due to their desirable properties, including ordered crystalline structures, adjustable pore sizes, large surface areas, chemical versatility, and thermal stability, MOFs have been extensively utilized in sensor and biosensor systems [23]. A representative MOF material is the zeolitic imidazolate framework (ZIF). Its structure is primarily derived from the connection of transition metal cations (such as Zn and Co) that are tetrahedrally coordinated, using imidazolate groups as the linking units. These frameworks are known for their unique properties, such as electron transfer capabilities, extensive functionalities, unimodal micropores, and strong chemical and thermal stability [24, 25]. ZIF-8, a pioneer member of the ZIF class, has been widely used owing to its significant pore volume and surface area (> 1600 m^2 g^{-1}) and facile synthesis [26, 27]. ZIF-8 MOF exhibits a large intrinsic second-order nonlinear optical response due to the noncentrosymmetric octupolar symmetry and the presence of electron-rich aromatic rings of the imidazole linkers, highlighting its significance in biomedical research [28]. The aim of this study was to validate a sensing platform for cyclosporine analysis in plasma samples using ZIF-8 MOF. A published method was used to synthesize ZIF-8 MOF to attain the desired structural stability and morphology. Cyclosporine was chosen as the target analyte because of its extensive therapeutic applications. The detection mechanism of the ZIF-8 MOF based nanoprobe was driven by specific interactions between cyclosporine and the nanoprobe. By restricting nonradiative relaxation pathways through hydrogen bonding and hydrophobic interactions, the fluorescence of ZIF-8 MOF is effectively amplified. The validated probe was then successfully applied for a straightforward and economical analysis of cyclosporine in the plasma of patients under cyclosporine treatment. A schematic illustration describing the whole procedure was shown in Scheme 1. The platform provides a novel approach, allowing for the development of simple MOF-based nanoprobes suitable for clinical applications.

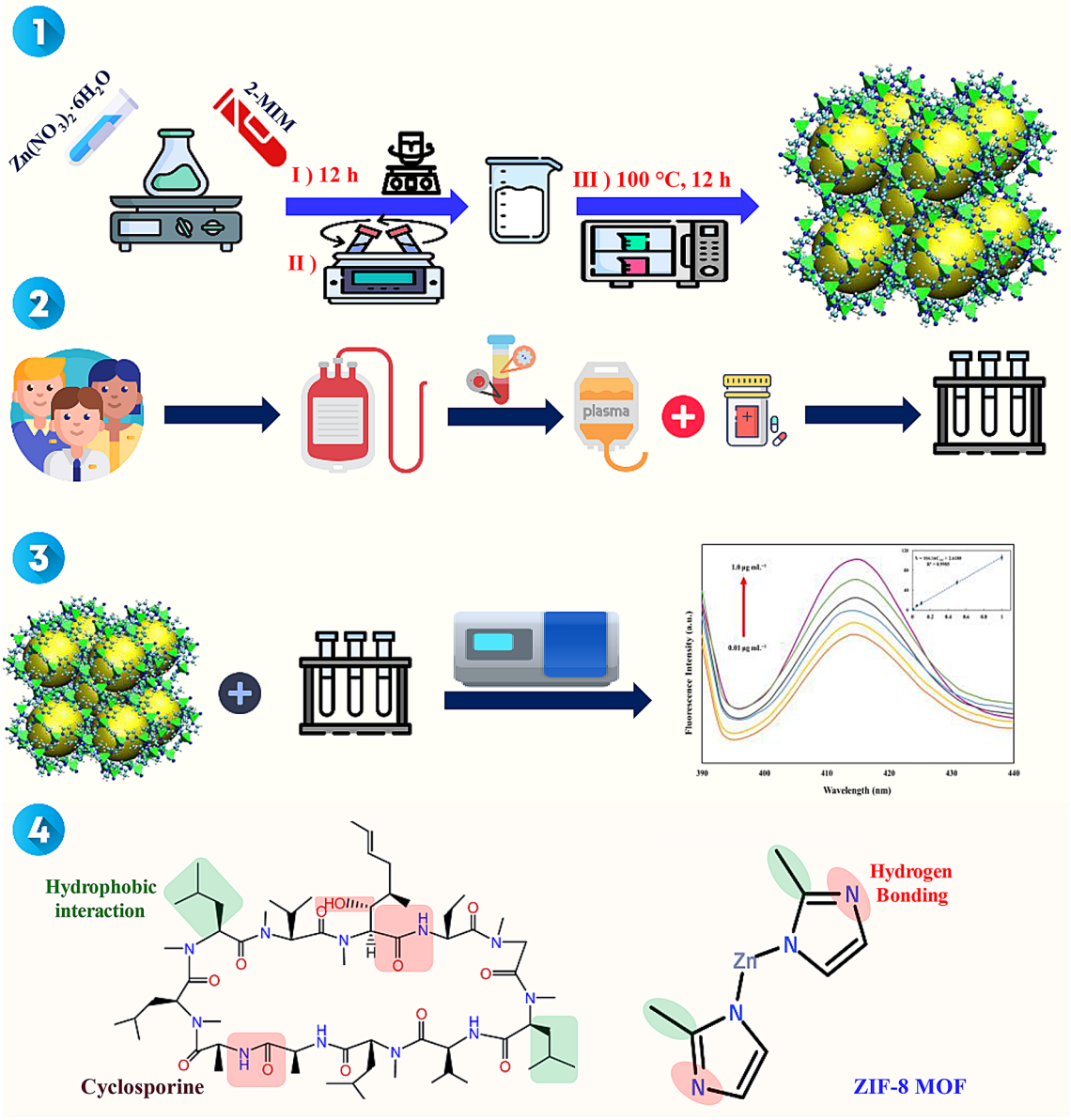
Experimental section

Reagents and solutions

Zinc nitrate ($Zn(NO_3)_2{\cdot}6H_2O$, 98.0%), 2-methylimidazole (≥ 99.0%), and methanol (≥ 99.9%) for the preparation of ZIF-8 (Zn) were purchased from Merck (Darmstadt, Germany). For preparing the buffer, Sodium dihydrogen phosphate (NaH_2PO_4, 99.9%) was purchased from Merck (Darmstadt, Germany). The buffer pH was set in different levels using sodium hydroxide (NaOH, ≥ 99.0%) and hydrochloric acid (HCl, 37.0%), that were supplied from Merck (Darmstadt, Germany). Cyclosporine was obtained from Zahravi Pharmaceutical Company (Tabriz, Iran), and ethanol (≥ 99.9%) was purchased from Merck (Darmstadt, Germany) for the preparation of a stock standard solution of cyclosporine (1000 μg mL^{-1}). Ultrapure deionized water for the dilution process was sourced from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). All reagents were kept at 4 °C before use.

Instruments and characterization

The fluorescence spectra were recorded using an FP-750 spectrofluorometer (Jasco, Japan) equipped with a xenon lamp. A 1 mL standard quartz cell was used for the fluorescence measurements, with excitation and emission bandwidths set at 10 nm and 20 nm, respectively. The synthesis of ZIF-8 (Zn) was conducted in a Biotage oven (Initiator 8 EXP, 2450 MHz frequency). The particle size distribution of the prepared nanoprobe was examined, and their shape and size were analyzed using a CM120 transmission electron microscope (TEM) (Philips, The Netherlands). Powder X-ray diffraction (XRD) patterns were analyzed with a Siemens diffractometer (Tongda Co., China) utilizing filtered Cu-Kα radiation at 35 kV across a 2θ range of 4° to 70°. To verify the chemical bonding of ZIF-8 MOF, Fourier transforms infrared (FT-IR) spectroscopy was accomplished on AVATAR FT-IR (Thermo Fischer Scientific, USA) over 4000–400 cm^{-1} as spectral width. A vega 3 scanning electron microscope (SEM) (Tescan, Czech Republic) equipped with Tescan-vega 2 EDAX software (XMU, Czech Republic) for Energy Dispersive Spectroscopy (EDS) measurement implementing an accelerating voltage to 20 kV was used for the analysis of ZIF-8 MOF atomic composition. The Brunauer-Emmett-Teller (BET) analysis was conducted using the BELSORP-mini II instrument (MicrotracBEL, Japan) to measure the surface area and pore size distribution of ZIF-8 MOF through the volumetric gas adsorption technique. UV-Vis absorption spectra of the prepared solutions were recorded on a spectrophotometer model UV-1800 using a micro quartz cell (Shimadzu, Japan). Dynamic light scattering (DLS) technique



Scheme 1 General layout for synthesizing ZIF-8 MOF, cyclosporine determination, and the possible interaction between the ZIF-8 MOF and cyclosporine

(Microtrac, USA) was employed for the zeta potential measurement of ZIF-8 MOF. pH adjustments were made using a model 744 digital pH meter (Metrohm, Switzerland).

ZIF-8 (Zn) synthesis

A solution of 1.0 g of $Zn(NO_3)_2 \cdot 6H_2O$ in 40:10 mL of methanol and water was quickly mixed with a solution containing 3.0 g of 2-methylimidazole in 50 mL of methanol, resulting in a white suspension of ZIF-8. This suspension was stirred at room temperature for 12 h. Pure ZIF-8 nanoparticles were then achieved through repeated centrifugation and washing with water and methanol. The collected nanoparticles were subsequently dried in a vacuum at 100 °C overnight in a drying oven [29].

Samples Preparation

In order to optimize and validate the proposed nanoprobe, plasma samples were obtained from the Blood Transfusion Organization of Eastern Azerbaijan, located in Tabriz, Iran. To assess the probe's performance in a real-world setting, samples were collected from hospitalized patients who were being treated with cyclosporine. Donors provided signed consent approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1403.062). For sample preparation before analysis, 1000 µL of the real plasma sample or a

blank plasma sample spiked with different concentrations (0.01–1.0 μg mL^{-1}) of stock standard solution of cyclosporine (1000 μg mL^{-1} in ethanol) was mixed with 1000 μL of acetonitrile to precipitate the proteins. Following vortex mixing, the mixture was centrifuged at 5000 rpm for 5 min. The clear supernatant was then separated for the cyclosporine analysis.

General procedure

Fluorescence detection was performed in a 2 mL vial using a batch process. Under optimized conditions, 10 μL of 0.1 mol L^{-1} phosphate buffer (PBS, pH 9.0) was added to a microtube containing 400 μL of the prepared plasma from the previous section containing different concentrations of cyclosporine (0.01–1.0 μg mL^{-1}). Then, 100 μL of ZIF-8 MOF (0.48 mg.mL^{-1} in water) was incorporated. The total volume in the vial was then adjusted to 0.6 mL with deionized water. After incubating the sample for 2 min, the fluorescence intensity of the nanoprobe was measured at approximately $\lambda_{max} = 417$ nm following excitation at 370 nm. In this setup, ΔF (F–F_0) served as the analytical response, where F and F_0 show the sensor's response in the presence and absence of cyclosporine, respectively. All experiments were conducted at room temperature.

Results and discussions

Characterization of the ZIF-8 MOF

Morphology of the ZIF-8 MOF was studied by TEM analysis, as presented in Fig. 1A. As can be seen, the synthesized ZIF-8 nanoparticles present a rhombic dodecahedral morphology and possess particle sizes ~ 100–150 nm. The crystallinity and phase purity of ZIF-8 MOF were examined using XRD analysis, as shown in Fig. 1B. The diffraction peaks observed at 7.4°, 10.5°, 12.8°, 14.7°, 16.5°, 18.1°, 24.6°, and 26.7° related to the (011), (002), (022), (013), (222), (233), and (134) crystal planes in the structure of ZIF-8 MOF. This pattern is consistent with those reported in the literature [30]. To get further evidence of the successful synthesis of ZIF-8 MOF and confirm the bonds formed between ZIF-8 MOF and cyclosporine, FTIR spectroscopy was performed (Fig. 1C). The absorption band appeared at 3136 cm^{-1} was assigned to the respective C–H stretching vibrational modes of the aromatic imidazole ring. The band observed at 2946 cm^{-1} was associated with the characteristic band of the aliphatic C–H stretching of the methyl group presented in the linker. The absorption band attributed to the stretching of the imidazole ring was observed at 1419 cm^{-1}. The absorption bands appeared at 900–1350 cm^{-1} were corresponded to the in-plane bending of the imidazole ring and the peaks below 800 cm^{-1} were appertained as the out-of-plane bending of the imidazole ring. The absorption band appeared at 420 cm^{-1} was the characteristic of the Zn-N stretching mode of ZIF-8 MOF [31]. Figure 1D and showed the EDS analysis of ZIF-8 MOF. As can be seen, zinc and carbon atoms are present in ZIF-8 MOF. The presence of nitrogen atoms in the structure of ZIF-8 MOF is from the 2-methylimidazole linker [32]. In order to investigate and quantify the pore size and surface area of the ZIF-8 MOF, the BET analysis, incorporating nitrogen adsorption/desorption isotherms, was employed. As Fig. 1E showed, the ZIF-8 MOF exhibited an excellent BET surface area (1792 $m^2\ g^{-1}$) and pore volume (1.0 $cm^3\ g^{-1}$). To explore the surface charge of the ZIF-8 MOF, surface zeta-potential was measured by DLS technique, which provide a negative value of -0.3 mv.

Herein, the stability study was also conducted. As you can see in Fig. 2, the intensity of the synthesized probe remained stable for about a month, after which it gradually decreased.

Detection mechanism

The performance of the synthesized nanoprobe was investigated following the verification of the preparation process. The interaction between the nanoprobe and cyclosporine was characterized using spectral analysis. The fluorescence intensity of the ZIF-8 (Zn) MOF increased upon the addition of cyclosporine. This enhancement is attributed to interactions between cyclosporine and the ZIF-8 framework, which is composed of zinc ions (Zn^{2+}) and 2-methylimidazole linkers. However, the enhancement in fluorescence signal could be attributed to the following modifications: (i) Hydrogen bonding: Interactions between the hydroxyl (-OH) and amide (-NH) groups in cyclosporine and the nitrogen atoms of the imidazole rings in 2-methylimidazole could also promote a more organized alignment within the ZIF-8 structure. This alignment could enhance fluorescence emission efficiency because it creates a more optimal molecular arrangement for energy transfer. (ii) Hydrophobic interactions: The non-polar regions of cyclosporine could interact with the methyl groups in 2-methylimidazole, promoting closer packing within the ZIF-8 framework. This could create an environment that enhances the fluorescence properties of the probe by restricting non-radiative relaxation pathways, thus increasing the intensity of the signal. The porous structure of ZIF-8 facilitates the diffusion of cyclosporine into the framework, promoting these interactions throughout the material, not just on the surface. This structural feature enhances the system's sensitivity and specificity, allowing for an amplified fluorescence signal in response to cyclosporine. The intensity of the fluorescence signal increases as the cyclosporine concentration rises, making this a highly sensitive tool for detecting cyclosporine in various samples.

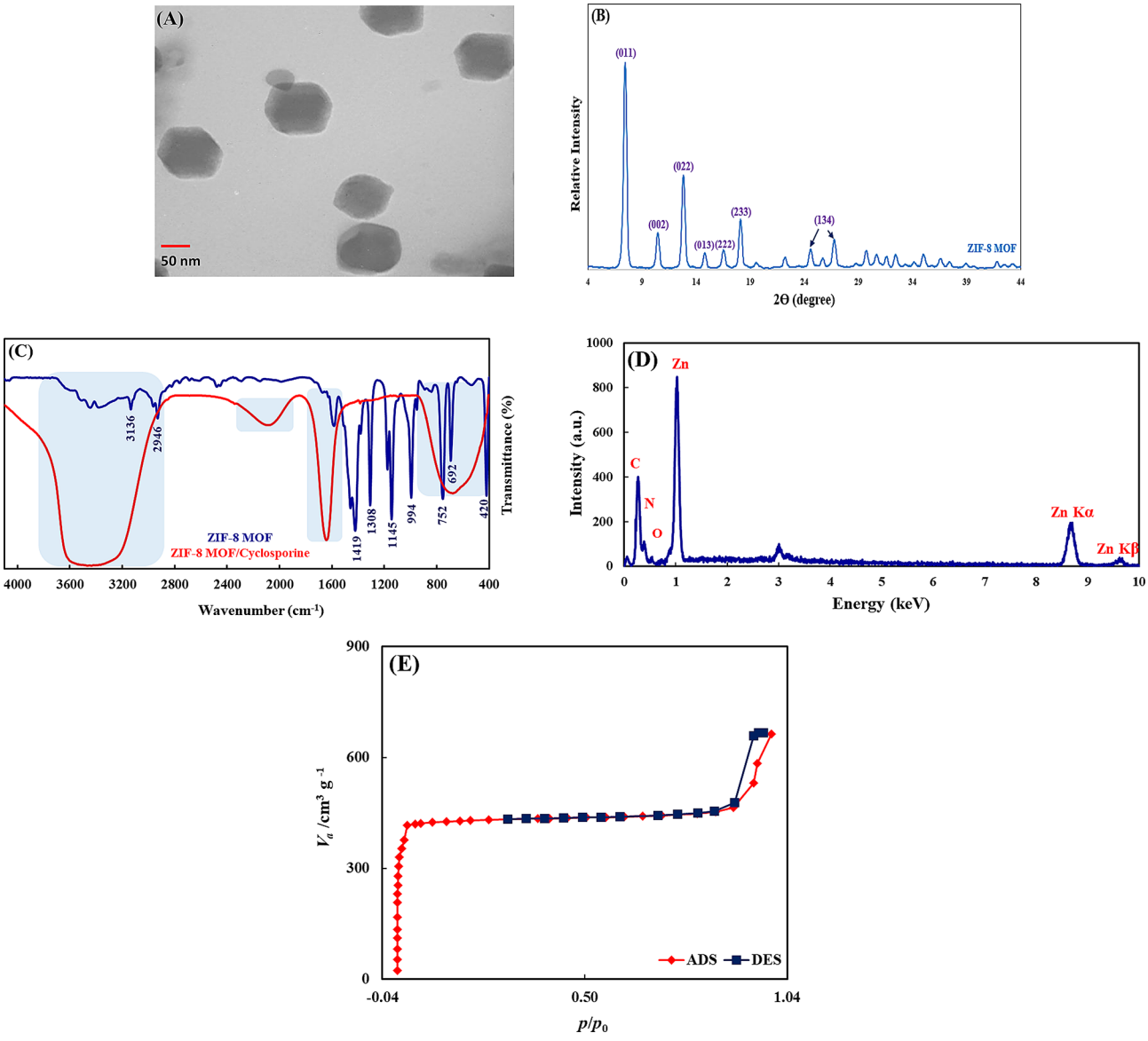


Fig. 1 (**A**) TEM image and (**B**) XRD pattern (**C**) FT-IR spectra of ZIF-8 MOF and ZIF-8 MOF/Cyclosporine, (**D**) EDS analysis, and (**E**) N_2 adsorption isotherm of synthesized ZIF-8 MOF

FT-IR and UV-Vis spectroscopy analyses were carried out to verify the mechanism. FT-IR (Fig. 1C) analysis confirms the interaction between ZIF-8 MOF and cyclosporine through hydrogen bonding and hydrophobic interactions. The peaks at 3200–3400 cm^{-1} in ZIF-8 MOF, assigned to N-H and O-H stretching, shift and broaden upon cyclosporine incorporation, suggesting hydrogen bond formation between cyclosporine's carbonyl and amide groups with ZIF-8's imidazole units. Additionally, the characteristic aliphatic C-H stretching peaks of ZIF-8 MOF at range of 2800–3200 cm^{-1} are significantly reduced after cyclosporine loading, indicating hydrophobic interactions. The amide-related band at 1642 cm^{-1} remains visible, while the peak at 1583 cm^{-1} disappears, reflecting structural changes due to drug incorporation. These spectral changes provide strong evidence of an interaction between the cyclosporine and ZIF-8 MOF structure. Furthermore, the absorption spectra of the ZIF-8 MOF probe in the absence and presence of cyclosporine (Fig. 3) provide more evidence for the interaction between the probe and cyclosporine. The partial shift and enhancement in the peak position and intensity of the probe's absorption spectrum suggests an interaction between the cyclosporine and ZIF-8 MOF probe.

Optimization procedure

To ensure a method's best performance, it's critical to optimize experimental conditions during probe design.

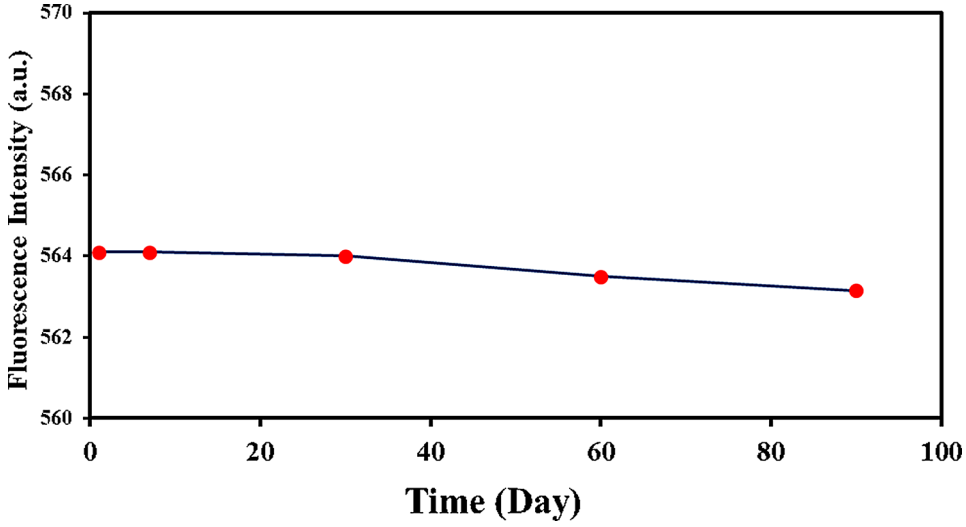


Fig. 2 Stability study of the ZIF-8 MOF based probe intensity

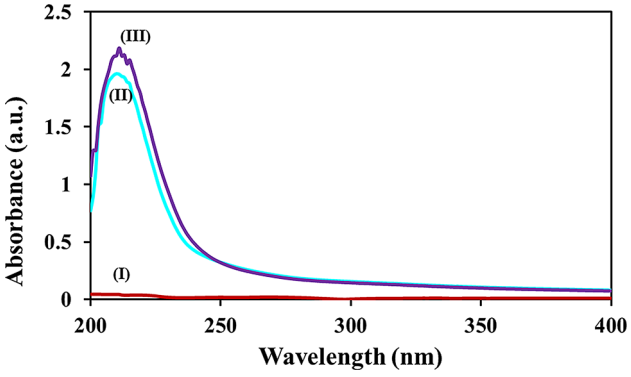


Fig. 3 UV–vis absorption spectra of cyclosporine (I), ZIF-8 MOF (II), and ZIF-8 MOF after the addition cyclosporine (III)

During the initial steps of the study, three critical factors—pH, ZIF-8 MOF concentration, and incubation time—that significantly affected the analytical response were identified. A target cyclosporine concentration of 1.0 μg mL^{-1} was selected for the optimization process. Among these variables, pH plays a key role in controlling and fine-tuning the reaction environment for optimal probe sensitivity. The impact of pH on the system's response was evaluated using PBS (0.1 mol L^{-1}) over a pH range of 4.0 to 11.0. As the pH increased, an acceptable enhancement in the nanoprobe's response was detected, reaching its maximum response (ΔF) at pH 9.0. Beyond this point, the response declined at higher pH levels, as illustrated in Fig. 4A. The maximum fluorescence response was reached at pH 9.0, likely due to ZIF-8 being more negatively charged at higher pH [33] enhancing its interaction with positively charged cyclosporine with pk_a of 13.2. The reduction in fluorescence response at pH values exceeding 9.0 is attributable to a combination of factors, including the compromised structural integrity of the ZIF-8 MOF under strongly alkaline conditions and the fluorescence quenching effect of elevated hydroxide ion concentrations. This may occur through the promotion of non-radiative decay pathways or through interactions with the fluorophore's excited electronic states. This quenching effect would result in a lower observed fluorescence intensity, even if the binding interaction between ZIF-8 and cyclosporine remains unchanged. The optimal amount of ZIF-8 MOF was determined by adjusting the volume used during the design process and assessing its effect on the system's performance. The results showed that the nanoprobe achieved its highest fluorescence response with 100 μL of ZIF-8 MOF. Consequently, 100 μL was selected as the optimal volume, as illustrated in Fig. 4B. The decrease in fluorescence beyond this volume can be related to the self-quenching effect of the probe at elevated concentrations. The duration required for the reaction between cyclosporine molecules and ZIF-8 MOF to achieve equilibrium is called the incubation time. To assess this, a time-course experiment was conducted where ZIF-8 MOF was added to a solution of cyclosporine in PBS (pH 9). As the incubation time increased from 1 to 10 min, the nanoprobe's response rose until 2 min before gradually decreasing over time (Fig. 4C). Based on these observations, the fluorescence response was highest at 2 min. The optimized conditions for the reaction were established as follows: pH 9.0, ZIF-8 MOF concentration of 100 μL, and incubation time of 2-minutes.

Interference study

The specificity and selectivity of the developed fluorescent probe were evaluated to assess potential interference from drugs co-administered with cyclosporine and some possible ions. To test selectivity, probe responses

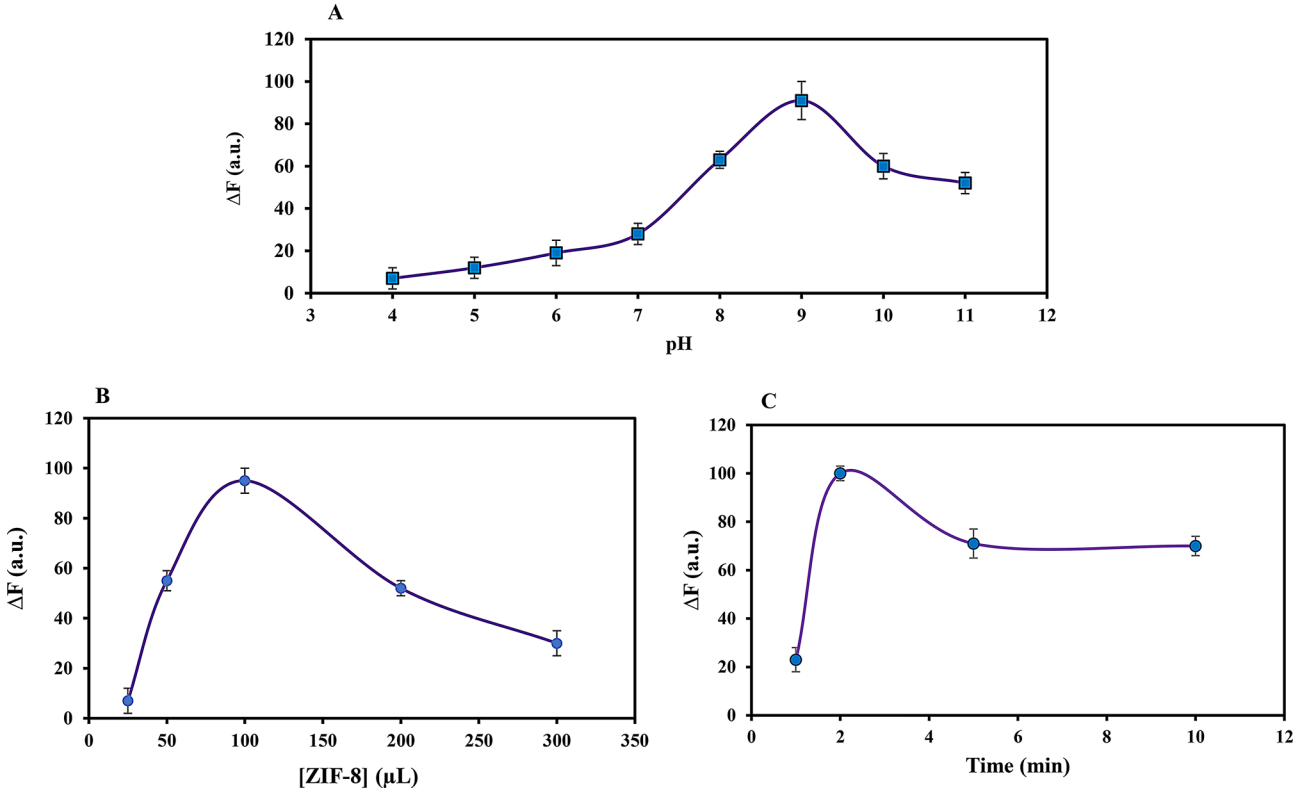


Fig. 4 Impact of (**A**) pH, (**B**) amount of ZIF-8 MOF, and (**C**) incubation time on the response of the system

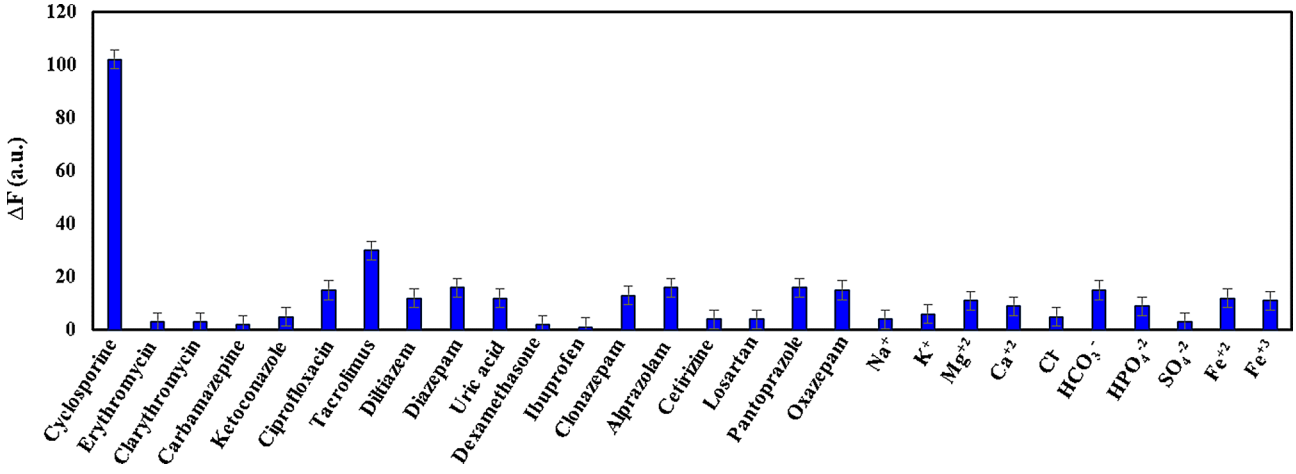


Fig. 5 Method selectivity study in optimal conditions (Condition: pH 9.0, ZIF-8 MOF concentration of 100 µL, and incubation time of 2-minutes) in the presence of some possible and our lab-available interfering pharmaceuticals and ions in plasma with concentrations of 1 µg mL^{-1}

were measured in plasma samples against various our lab-available interfering substances. The response was tested with cyclosporine (1.0 µg mL^{-1}) and each interfering substance at a concentration of 1.0 µg mL^{-1}. It should be noted that although the therapeutic concentration of most investigated drugs in plasma is below 3.0 µg mL^{-1}, interference tests were also conducted at this concentrations (three times higher than cyclosporine concentration 1.0 µg mL^{-1}), yielding similar results. This indicates that the examined interferents did not affect the response to probe toward cyclosporine. As illustrated in Fig. 5, the presence of these substances had a non-significant impact on the probe's response. Therefore, the method appears well-suited for tracing cyclosporine in plasma samples, even in patients taking these medications. Notably, the lack of functionalization in ZIF-8 (Zn) MOF with complexing or biorecognition elements may restrict its ability to selectively detect cyclosporine over similar

immunosuppressants. These pharmaceuticals were not accessible to us for examination of their effects. However, the study acknowledges the need for further exploration, including the functionalization or coupling with the separation/preparation techniques to enhance selectivity for distinguishing structurally similar compounds.

Analytical figures of merit

The optimal conditions for the partial validation of the method were determined following the guidelines provided by the Food and Drug Administration (FDA). Initially, the method's concentration-dependent behavior was investigated under the above-mentioned optimal conditions. Figure 6 displays the fluorescence spectra of ZIF-8 MOF, which shows a peak at 417 nm when excited at 370 nm. The intensity of this peak raised with higher concentrations of cyclosporine. The results indicated a linear correlation between fluorescence intensity and cyclosporine concentration within the range of 0.01 to 1.0 µg mL^{-1}, as illustrated in the inset of Fig. 6. The regression equation was given by $\Delta F\ (F - F_0) = 104.2\ C_{cyc} + 2.62$ ($R^2 = 0.999$). In this equation, C_{cyc} shows the concentration of cyclosporine, F_0 was the fluorescence intensity without cyclosporine, and F was the fluorescence intensity at 417 nm in the presence of cyclosporine. The calculated limits of detection (LOD), determined using the formula $3S_b/m$ (where S_b is the standard deviation of the blank and m is the calibration slope), and the limit of quantification (LOQ) based on $10S_b/m$, were found to be 0.003 µg mL^{-1} and 0.01 µg mL^{-1}, respectively. The precision of the proposed method was investigated by performing repeat tests on the same day and on different days throughout the study. The relative standard deviations (*RSDs*%) for five cyclosporine measurements at 1.0 µg mL^{-1} were 0.4% for intra-day and 0.5% for inter-day measurements. A comparative analysis of the validated method against other reported method for cyclosporine analysis in the literature is presented in Table 1. As shown, the current study demonstrated comparable sensitivity to other methods. Although the optical methods cannot compete with the chromatographic methods such as HPLC and capillary electrophoresis. However, these methods are more time-consuming and need an additional sample preparation step before sample injection. In contrast to chromatographic techniques, optical methods can be made portable and used for point-of-care analysis. Furthermore, poor selectivity and reproducibility/repeatability limited the applications of electrochemical methods in clinical applications. Recent research efforts have been directed toward the development of novel probes with tailored properties to address specific application requirements. In this study, we report the development of a ZIF-8 based fluorescence

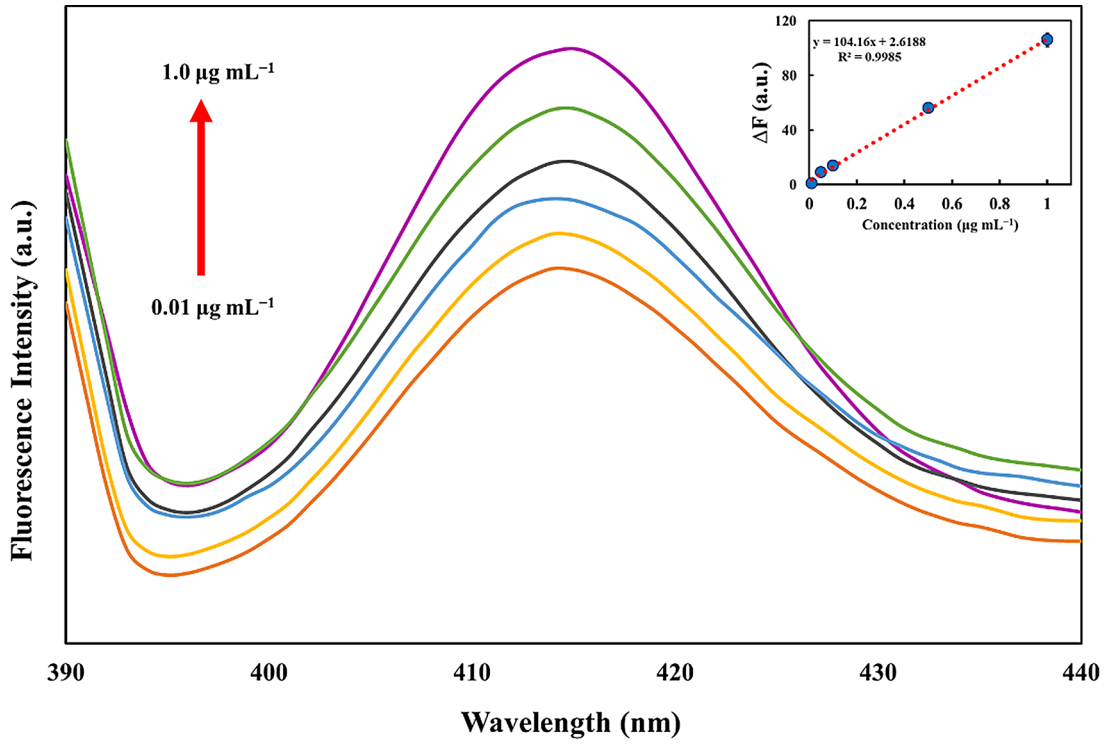


Fig. 6 Fluorescence intensity of ZIF-8 MOF in the absence and presence of cyclosporine in the range of 0.01–1.0 µg mL^{-1}. Inset: Calibration curve obtained for cyclosporine in plasma samples. Condition: pH 9.0, ZIF-8 MOF concentration of 100 µL, and incubation time of 2-minutes, an excitation wavelength of 370 nm and an emission wavelength of 417 nm

Table 1 Comparison of analytical features of the reported method with other methods reported in the literature

Method	Sample	LOD (μg mL^{-1})	Linear range (μg mL^{-1})	Reference
RP-HPLC[a]	Capsule dosage form	0.180	4.0–24.0	[12]
LC-MS/MS[b]	Blood	5.600	15.4–4400.0	[13]
Spectrofluorometry	Plasma	0.007	0.01–10	[17]
Capillary electrophoresis	Blood	1.080	3.0-30.1	[34]
Time-resolved fluoroimmunoassay	Blood	0.010	0.01-1.0	[35]
Potentiometry	solution	0.940	2.0–8.0	[36]
ZIF-8 based fluorescence method	Plasma	0.003	0.01-1.0	This work

[a] Reverse phase-high performance liquid chromatography

[b] Liquid chromatography-tandem mass spectrometry

Table 2 Cyclosporine analysis in real plasma samples by validated probe in optimal conditions (Condition: pH 9.0, ZIF-8 MOF concentration of 100 ML, and incubation time of 2-minutes)

No.	Gender	Added (μg mL^{-1})	Found (μg mL^{-1})[a]	Recovery (%)[b]
1	Female	-	0.092 ± 0.008	-
		0.1	0.194 ± 0.010	102.1
		0.5	0.582 ± 0.009	97.9
2	Male	-	0.088 ± 0.005	-
		0.1	0.188 ± 0.009	100.8
		0.5	0.578 ± 0.009	98.1
3	Male	-	0.093 ± 0.004	-
		0.1	0.195 ± 0.011	101.7
		0.5	0.591 ± 0.012	99.4
4	Male	-	0.094 ± 0.007	-
		0.1	0.197 ± 0.008	102.7
		0.5	0.588 ± 0.007	98.6
5	Male	-	0.091 ± 0.004	-
		0.1	0.192 ± 0.008	101.7
		0.5	0.576 ± 0.010	97.1

[a] average of three replication ($n = 3$) + standard deviation

[b] Recovery (%) = [cyclosporine concentration in samples (after spiking– before spiking)/Added] × 100

method for the facile detection of cyclosporine in plasma samples.

Real samples analysis

To evaluate the analytical practicality and potential of the validated ZIF-8 MOF method for cyclosporine determination, five plasma samples obtained from patients treated with cyclosporine were analyzed. The results of these real sample analyses were summarized in Table 2. The accuracy of the method was evaluated through recovery tests, where cyclosporine was spiked at concentrations of 0.1 and 0.5 μg mL^{-1}. The recovery percentages ranged from 97.1 to 102.1%, indicating high accuracy and demonstrating that the method was free from matrix effects, making it suitable for cyclosporine quantification in complex biological samples such as plasma samples. Furthermore, sample 1 was examined using HPLC-UV, a standard analytical technique. The concentrations of cyclosporine in patient sample 1 was determined to be 0.0916 ± 0.006 μg.mL^{-1}. A paired t-test (2-tailed) was conducted to compare these results with those obtained from the validated method (0.092 ± 0.008 μg.mL^{-1}), confirming no significant difference between the two sets of results at a 95% confidence level. These outcomes indicate that the proposed method shows strong promise for accurately measuring cyclosporine in the biological samples analyzed.

Conclusion

In this study, we synthesized ZIF-8 MOFs as a fluorescence nanoprobe for detecting cyclosporine in plasma samples. The findings indicated a progressive increase in fluorescence intensity with increasing cyclosporine concentrations. This increase is attributed to specific hydrogen bonding and hydrophobic interactions between the drug and the ZIF-8 framework. These interactions improved the fluorescence performance, resulting in a strong linear correlation between fluorescence intensity and cyclosporine concentration (0.01–1.0 μg mL^{-1}), and a low LOD of 0.003 μg mL^{-1}. The nanoprobe demonstrated excellent precision, with RSDs of 0.4% for intra-day measurements and 0.5% for inter-day measurements, indicating the reproducibility and reliability of the method. Additionally, recovery between 97.1% and 102.1% in plasma samples showcased the method's accuracy, emphasizing its potential for real-world clinical diagnostics. These results highlight the capability of the ZIF-8 MOF nanoprobe to detect cyclosporine with high sensitivity, precision, and accuracy. Compared to traditional methods like chromatography techniques, the ZIF-8 MOF based optical method offers several advantages, including faster response times, more straightforward sample preparation, user-friendly operations, and cost-effectiveness. However, the study also acknowledges the need for further exploration, including the functionalization of ZIF-8 MOF or coupling with the separation/preparation techniques to enhance selectivity distinguishing structurally similar compounds, further validation in highly complex biological matrices and the integration of the nanoprobe into point-of-care diagnostic devices.

Acknowledgements

This work was supported by the Research Affairs of Tabriz University of Medical Sciences (Tabriz, Iran) under the grant number 73050.

Author contributions

RM: Investigation, writing– original draft, ZK: Design of the work, writing– original draft, ZG: Validation, AG: Validation, ER: Analysis, Conceptualization, Writing—review & editing. AJ; Supervision, Writing—review & editing. All authors read and approved the final manuscript.

Funding
This work was supported by the Research Affairs of Tabriz University of Medical Sciences (Tabriz, Iran) under the grant number 73050.

Data availability
Data supporting this study are included within the article.

Declarations

Ethics approval and consent to participate
All methods were performed in accordance with the relevant guidelines and regulations by in the declaration of Helsinki (ethics approval and consent to participate). The aims and methods of the study were explained for all participants, and necessary assurance was given to them for the anonymity and confidentiality of their information. Informed consent (with explaining the goals and methods of the project) was obtained from participants. The interviewers were reading text of informed consent form verbatim for illiterate participants and obtained informed consent of them. The participants had the right to withdraw during the study or at any other time. All sample donors or participants filled out and signed the informed consent of project with ethical code IR.TBZMED.REC.1403.062 confirmed by the ethics committee at Tabriz University of Medical Sciences verified.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 November 2024 / Accepted: 13 March 2025
Published online: 27 March 2025

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