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A Triphenylamine-Functionalized Silica Nanoparticle for Fluorescent Detection of Fe²⁺/Fe³⁺ in Red Wine

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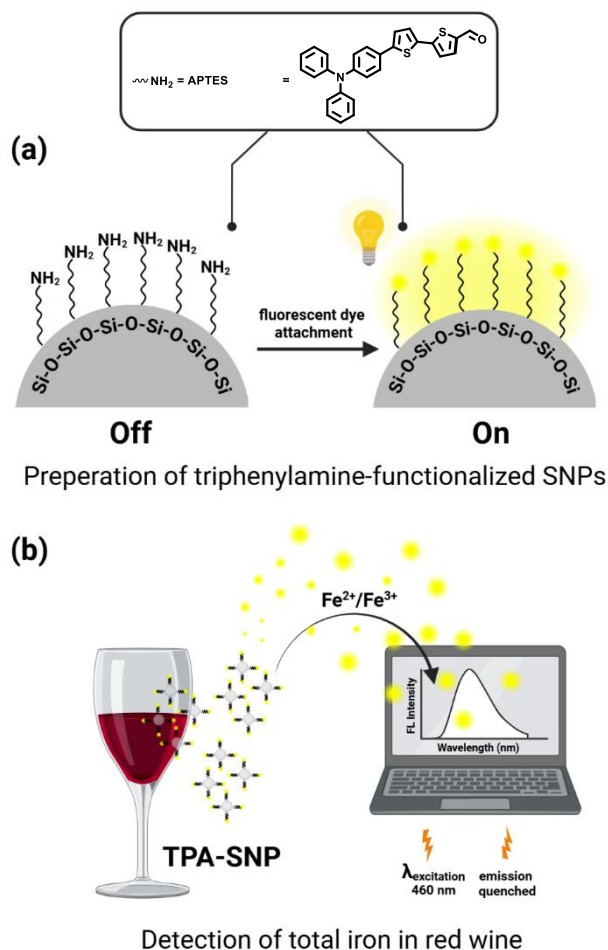
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Abstract

Iron plays a crucial role in numerous physiological processes; however, both iron deficiency and iron overload can lead to severe health disorders, including anaemia, organ dysfunction, neurodegenerative diseases, and cancer. Therefore, the development of rapid, sensitive, and reliable sensing platforms for iron ion detection has attracted considerable attention in recent years. In this study, a novel triphenylamine-functionalized silica nanoparticle probe (**TPA-SNP**) was developed for the selective and sensitive detection of Fe²⁺ and Fe³⁺ ions. The sensing platform combines the strong fluorescence properties and electron-donating characteristics of triphenylamine with the enhanced stability provided by silica nanoparticles. In addition, the incorporation of thiophene units contributes to efficient π -conjugation and charge transfer, improving the optical performance of the system. The synthesized **TPA-SNP** probe exhibited a rapid fluorescence “turn-off” response toward Fe²⁺/Fe³⁺ ions with high selectivity and sensitivity over a broad pH range. Finally, the efficient determination of total iron in red wine samples demonstrates the practical applicability of the method without the need for specialized equipment.

Keywords: silica nanoparticles, triphenylamine, iron detection, fluorescent nanoprobe

Graphical Abstract



1. Introduction

Iron is an essential trace element in the human body and vital for various physiological functions [1]. While adequate iron levels are necessary for maintaining health, both deficiency and excess can pose significant health risks. Iron overload (hyperferremia) may lead to many health problems, including osteoporosis, several cancer types, organ malfunction, hemochromatosis, and neurodegenerative diseases such as Alzheimer's and Parkinson's[2–4]. Conversely, iron deficiency can result in anaemia and several cellular dysfunctions[5]. This leads to a greater emphasis on developing user-friendly, highly sensitive, and rapid sensing platforms for the detection of iron in both biological and environmental systems.

Unlike traditional methods such as atomic absorption spectrometry (AAS) [6], inductively coupled plasma mass spectrometry (ICP-MS) [7], and electrochemical techniques [8], optical molecular probes—specifically chromogenic and fluorogenic types—have gained significant

attention for iron ions analysis [9–13]. These techniques offer a more versatile approach due to their inexpensively, rapid response, and potential for on-site detection.

Among the various fluorophores, triphenylamine (TPA) demonstrates significant fluorescence ability due to its remarkable electron-donating capacity and serves as an efficient fluorescent probe owing to its chemical stability and elevated fluorescence quantum yield [14]. In addition, thiophene-based systems are widely employed due to their notable π conjugation which facilitates charge transfer across the molecule, and their ease of derivatization, enabling a variety of applications, including organic light-emitting diodes (OLEDs), electrochromic devices, solar cells, capacitors, and fluorescence-based sensing systems[15]. This pairing often increases fluorescence quantum yield, gives large Stokes shifts, and allows emission color tuning by changing the thiophene length or substitution pattern [16]. In reported TPA–thiophene fluorophores, solution and solid-state emission can both be strong, which is valuable because many fluorophores weaken when aggregated.

Despite their advantages, the potential limitations of small molecule-based fluorescent probes under varying environmental conditions should be taken into consideration. Changes in pH, ionic strength, or the presence of reactive species may influence fluorescence response and sensing performance. For instance, many organic dyes have been reported to undergo photobleaching or fluorescence quenching in complex biological and environmental matrices, which can compromise signal accuracy and long-term stability [17, 18].

To overcome these limitations, silica nanoparticles (SNPs) provide a chemically stable matrix that shields and encapsulates fluorophores from the external environment [19–21]. The silica matrix acts as a physical barrier, preventing direct exposure of the fluorescent molecule to the surroundings and thereby maintaining its optical performance even under difficult conditions. This enhanced stability is particularly vital for applications involving biological tissues or environmental samples with varying pH levels and oxidative stress. Owing to these advantages, silica nanoparticle-based fluorescent probes have been extensively investigated for sensing applications [22–27]. Numerous studies have reported SNP-supported sensing platforms for the selective detection of Fe^{2+} and Fe^{3+} ions in aqueous and environmental samples, highlighting the benefits of nanoparticle immobilization in terms of signal stability and analytical performance [28–33]. A comparison of representative silica nanoparticle-based iron sensing systems reported in the literature is presented in Table SI.

Although extensively investigated for sensing applications, the combination of triphenylamine-based fluorophores with silica nanoparticles has been rarely explored. As far as we know, there is no report in the literature about the covalent immobilization of triphenylamine-based probe onto the silica nanoparticle platform for iron ion sensing. The development of such organic–inorganic hybrid systems is of great interest because they combine the excellent optical properties of organic fluorophores with the structural stability, high surface area, and tunable functionality of silica materials. Herein, we report a novel triphenylamine-functionalized silica nanoprobe (**TPA–SNP**) as a hybrid sensing platform for fluorescence detection of total iron (Fe^{2+} and Fe^{3+}). The experimental findings demonstrated that **TPA–SNP** probe displays a rapid "turn-off" response, enhanced selectivity, and sensitivity. The dual responsiveness toward Fe^{2+} and Fe^{3+} represents an advantage for total iron determination, simplifying the analytical procedure compared with methods requiring separate detection of each oxidation state. The practical applicability of the **TPA–SNP** was further demonstrated through the determination of total iron in red wine samples, highlighting its potential for real-sample analysis without the need for specialized equipment.

2. Materials and Methods

2.1. Chemicals

(3-Aminopropyl)triethoxysilane (APTES, $\geq 99\%$), tetraethyl orthosilicate (TEOS, $\geq 98\%$), ammonia solution (NH_4OH , 28%), 4-(Diphenylamino)phenylboronic acid ($\geq 95.0\%$), N-bromosuccinimide ($\geq 99.0\%$), bis(triphenylphosphine)palladium(II) dichloride ($\geq 98.0\%$), 2-bromothiophene ($\geq 98.0\%$), and 2-bromothiophene ($\geq 95.0\%$), 5-formylthiophene-2-boronic acid ($\geq 95.0\%$) potassium phosphate buffered saline (0.01M pH 7.2–7.6), o-phthalaldehyde ($\geq 97\%$), salicylaldehyde ($\geq 98\%$), ethylenediamine ($\geq 99\%$), 2-Mercaptoethanol ($\geq 99\%$), magnesium sulfate anhydrous (MgSO_4 , $\geq 99\%$), ethanol ($\geq 99.8\%$), N,N-Dimethylformamide (DMF, $\geq 99\%$), dichloromethane (CH_2Cl_2 , $\geq 99\%$) toluene ($\geq 99\%$), n-hexane (HPLC grade, $\geq 99.0\%$) ethyl acetate (EtOAc , HPLC grade, $\geq 99.8\%$), and tetrahydrofuran (HPLC grade, THF, $\geq 99\%$) and all water-soluble sulfate, nitrate, or chloride metal salts ($\geq 95\%$) were purchased from Sigma-Aldrich.

2.2. Instruments

^1H and ^{13}C NMR spectra were recorded on a Varian VNMRJ 600 nuclear magnetic resonance spectrometer. UV–Vis absorption analyses were performed using a Shimadzu 1900i spectrophotometer, while fluorescence emission studies were carried out with a Shimadzu

fluorescence spectrophotometer. All optical measurements were conducted in Hellma quartz microcuvettes. Transmission electron microscopy (TEM) images were acquired using a JEOL JEM 2100 HRTEM operated at an accelerating voltage of 200 kV. Surface modification of the silica nanoparticles was characterized by Fourier transform infrared (FTIR) spectroscopy employing a Shimadzu IRTracer-100 instrument. FTIR spectra were collected over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 128 accumulated scans. The synthesis of the TPA-Al compound was performed under controlled conditions using an Anton Paar Microwave 400 synthesis reactor. pH values were measured with a Mettler Toledo Seven Compact pH meter fitted with an InLab Expert Pro-ISM electrode. Centrifugation procedures were conducted using a Sigma 3K15 centrifuge. Dynamic light scattering (DLS) analyses were carried out on an Anton Paar Litesizer DLS 101 instrument. All experiments and measurements were performed in triplicate.

2.3. Synthesis of Silica Nanoparticles (SNPs)

Silica nanoparticles (SNPs) were prepared according to the Stöber method. Briefly, ammonium hydroxide (2 mL), ethanol (30 mL), and deionized water (0.5 mL) were introduced into a round-bottom flask and stirred vigorously at 70°C for 15 min. Following homogenization of the reaction medium, 3.32 mL of TEOS was added dropwise to the mixture. The resulting solution was then refluxed at 70°C for 3 h and allowed to cool to room temperature. The obtained nanoparticles were collected by centrifugation at 14,000 rpm for 30 min. Subsequently, the SNPs were washed at least three times with ethanol to remove residual reagents and dried under vacuum to obtain a white nanoparticle powder. The synthesized SNPs were further characterized by TEM and FTIR analyses.

2.4. Synthesis of T-T-AL

A solution of 2-bromothiophene (200 mg, 1.23 mmol) and $\text{PdCl}_2(\text{PPh}_3)_2$ (86 mg, 0.123 mmol) in tetrahydrofuran (THF) (3 mL) was prepared. An aqueous solution (3 mL) containing 5-formylthiophene-2-boronic acid (192 mg, 1.23 mmol) and K_2CO_3 (828 mg, 6.0 mmol) was added to this mixture. The reaction mixture was stirred under a nitrogen atmosphere and heated at 90°C for 20 min under microwave irradiation. After completion of the reaction, it was quenched by the addition of water (10 mL) and extracted with dichloromethane (DCM) (3×30 mL). The combined organic layers were dried over anhydrous MgSO_4 , filtered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel using DCM/hexane (80/20) as the eluent to afford T-T-AL (165

mg, 69% yield). ^1H NMR (600 MHz, CDCl_3) δ 9.85 (s, 1H), 7.66 (d, $J = 4.0$ Hz, 1H), 7.37 – 7.33 (m, 2H), 7.24 (d, $J = 3.8$ Hz, 1H), 7.06 (dd, $J = 5.0, 3.7$ Hz, 1H). ^{13}C NMR (150 MHz, CDCl_3) δ 185.3, 149.8, 144.3, 140.1, 138.7, 131.0, 129.8, 128.8, 126.9.

2.5. Synthesis of Br-T-T-AL

T-T-AL (165 mg, 0.85 mmol) was dissolved in DMF (5 mL) and the solution was cooled in an ice bath. N-Bromosuccinimide (NBS) (182.6 mg, 1.02 mmol) was added portionwise under dark conditions, and the reaction mixture was stirred at the same temperature for 4 h. The reaction mixture was extracted using NaHCO_3 solution, and the solvent was removed under reduced pressure. The crude product was precipitated with methanol to give a yellow solid in 86 % yield (200 mg). ^1H NMR (700 MHz, CDCl_3) δ 9.80 (s, 1H), 7.59 (d, $J = 3.9$ Hz, 1H), 7.11 (d, $J = 3.9$ Hz, 1H), 7.03 (d, $J = 3.8$ Hz, 1H), 6.97 (d, $J = 3.9$ Hz, 1H). ^{13}C NMR (176 MHz, CDCl_3) δ 182.6, 145.8, 142.0, 137.4, 137.3, 131.2, 126.2, 124.4, 114.2.

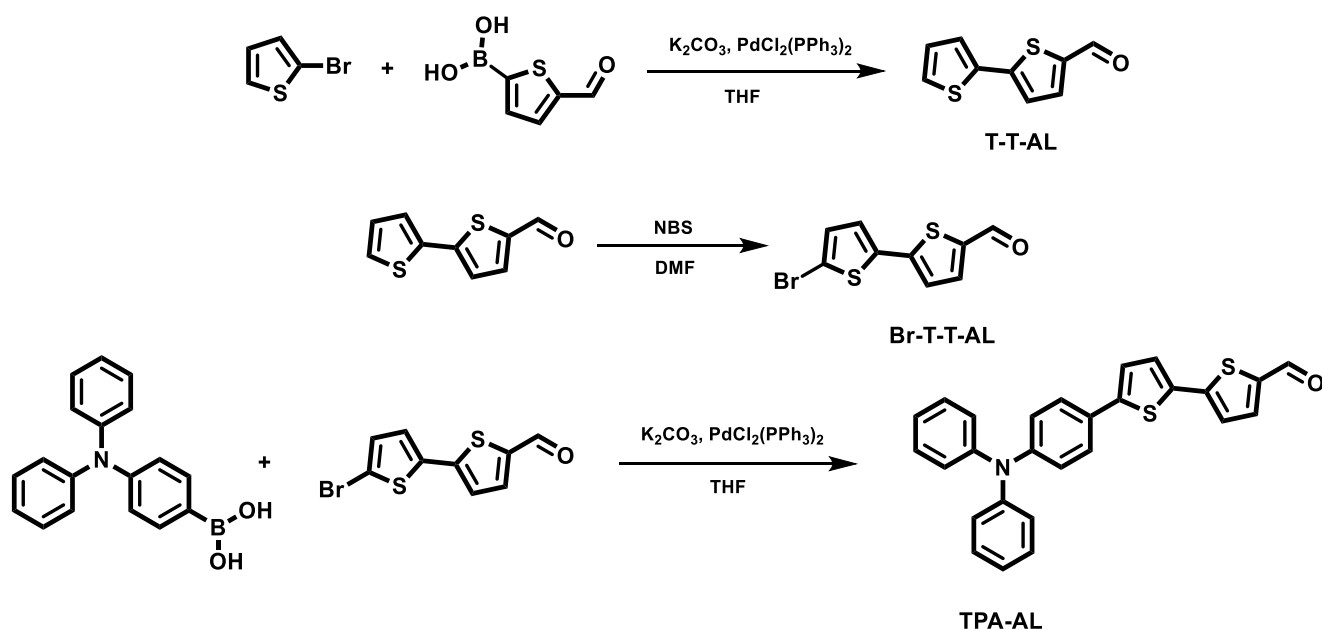
2.6. Synthesis of TPA-AL

A solution of Br-T-T-AL (200 mg, 0.73 mmol) and $\text{PdCl}_2(\text{PPh}_3)_2$ (51 mg, 0.07 mmol) in tetrahydrofuran (THF) (2 mL) was prepared. An aqueous solution (2 mL) containing 4-(diphenylamino)phenylboronic acid (200 mg, 0.73 mmol) and K_2CO_3 (600 mg, 4.38 mmol) was added to this mixture. The reaction mixture was stirred under a nitrogen atmosphere and heated at 90 °C for 20 min under microwave irradiation. After completion, the reaction was quenched with water (30 mL) and extracted with dichloromethane (DCM) (3×30 mL). The combined organic phases were dried over anhydrous MgSO_4 , filtered, and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using DCM/hexane (80/20) as the eluent to afford **TPA-AL** as a product (200 mg, 66 % yield). ^1H NMR (600 MHz, CDCl_3) δ 9.85 (s, 1H), 7.66 (d, $J = 4.0$ Hz, 1H), 7.47 – 7.43 (m, 2H), 7.31 (d, $J = 3.8$ Hz, 1H), 7.28 (dd, $J = 8.6, 7.4$ Hz, 4H), 7.23 (d, $J = 3.9$ Hz, 1H), 7.17 (d, $J = 3.8$ Hz, 1H), 7.14 – 7.11 (m, 4H), 7.08 – 7.04 (m, 4H). ^{13}C NMR (150 MHz, CDCl_3) δ 185.1, 150.7, 150.1, 149.9, 148.9, 143.9, 140.2, 136.7, 132.1, 129.9, 129.7, 129.3, 127.5, 126.4, 126.1, 125.8, 125.8. HRMS (ESI): m/z : Calcd. for $(\text{C}_{27}\text{H}_{19}\text{NOS}_2) [\text{M}]^+$: 437.0908 found, 437.0908.

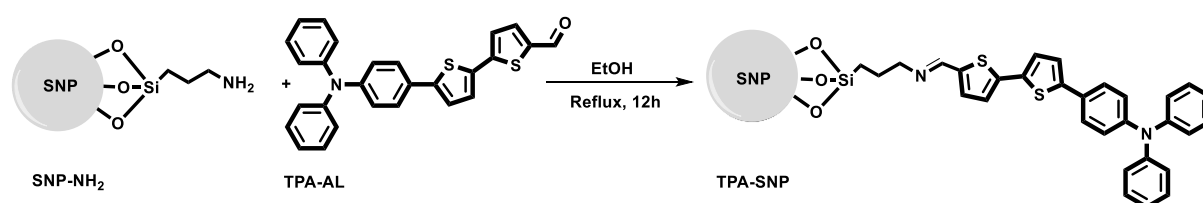
2.7. Preparation of TPA-SNP

SNP-NH₂ (50 mg) was suspended in a solution of **TPA-AL** (50 mg) dissolved in hot ethanol (5 mL) by ultrasonication for 15 min. The resulting mixture was then stirred under reflux at 100

°C for 12 h. After completion of the reaction, the mixture was allowed to cool to room temperature. The product was collected by centrifugation (10,000 rpm) and washed with ethanol at least three times until the supernatant became colorless in order to remove the unreacted TPA–AL. Finally, the obtained yellow solid TPA–SNP was dried in a vacuum oven.



Scheme 1. Synthetic route for the preparation of TPA–AL.



Scheme 2. Synthetic route for TPA–SNP.

2.8. Amine quantification of SNP–NH₂

The surface amine content of the aminated silica nanoparticles (SNP–NH₂) was determined in our previous study using both fluorescence spectroscopy and UV–Vis spectroscopy methods. Briefly, the fluorescence-based assay relied on the reaction between primary amines and o-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol, while the UV–Vis method involved Schiff base formation between salicylaldehyde and ethylenediamine as a model amine compound. Detailed experimental procedures and calibration methods have been reported our previous work [33].

Based on these analyses, the amine density on the surface of SNP-NH₂ was determined to be $117.0 \pm 10.2 \mu\text{mol g}^{-1}$ using the fluorescence method and $113.9 \pm 5.1 \mu\text{mol g}^{-1}$ using the UV-Vis method. These values were used to estimate the available surface amine groups for subsequent functionalization in this work.

2.9. Fluorescence detection of Fe²⁺ and Fe³⁺

A stock solution of the probe (TPA-SNP) was prepared at a concentration of 1 mg/mL in DMF:DMSO mixture. Solutions of metal ion salts at a concentration of 20 mM were prepared, including Ag⁺, K⁺, Na⁺, Li⁺, Fe²⁺, Ba²⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺, Fe³⁺, Al³⁺, Ce³⁺, and Cr³⁺ using triple-distilled deionised water (UPW). During the measurements, metal ion solutions were introduced to the probe solution (750 μL) using a micropipette. The TPA-SNP concentration for UV-vis and fluorescence emission measurements was maintained at 25 ppm in a 1:1 THF:PBS (10 mM pH 7.4) (v/v) solution. Regarding the “turn-off” measurements, emission spectra were collected from 450 to 700 nm following excitation at 380 nm immediately after each addition (with both excitation and emission slit widths set to 10 nm / 10 nm). Each measurement was conducted a minimum of three times. For the fluorescence analysis of total iron in wine, two bottles of different brand red wine samples were purchased from a local supermarket in Istanbul, Türkiye. The samples were diluted 10-fold with deionized water, and diluted wine samples was used for each analysis.

3. Results and discussions

3.1. Synthesis and characterization of SNPs

The chemical identification of the TPA-AL molecule was confirmed using ¹H NMR, ¹³C NMR (Supporting Information, SI), and HRMS analyses. TPA-AL modified silica nanoparticles (TPA-SNP) and silica nanoparticles (SNPs) were examined using transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential, Fourier-transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA).

TEM analysis (Figure 1) revealed that both SNPs and TPA-SNP exhibited well-dispersed, uniform and spherical morphologies. The average particle diameter of the bare SNPs was determined to be $110 \pm 9 \text{ nm}$, whereas a slight increase to $114. \pm 10 \text{ nm}$ was observed after functionalization with TPA unit. According to the DLS analysis, the average hydrodynamic diameters of the obtained SNPs and SNP-NH₂ were $120.01 \pm 1.4 \text{ nm}$ and $135.20 \pm 3.10 \text{ nm}$, respectively, with low polydispersity index (PDI) values of 0.13 ± 0.01 and 0.12 ± 0.02 (Table

1, Figure S6). The mean hydrodynamic diameter of the **TPA–SNP** was measured as 182.01 ± 2.26 nm, with a PDI value of 0.18 ± 0.04 . The larger particle sizes obtained from DLS compared to TEM can be attributed to the hydrodynamic nature of DLS measurements, which include the solvation layer and surface-bound organic groups. However, TEM technique provides the intrinsic size of the crystals.

Table 1. TEM and DLS characterization of SNPs, SNP–NH₂, and TPA–SNP.

Sample	TEM Size (nm)	DLS Size (nm)	PDI
SNPs	110.2 ± 9.1	120.01 ± 1.40	0.13 ± 0.01
SNP–NH ₂	—	135.20 ± 3.10	0.12 ± 0.02
TPA–SNP	114.3 ± 10.3	182.01 ± 2.26	0.18 ± 0.04

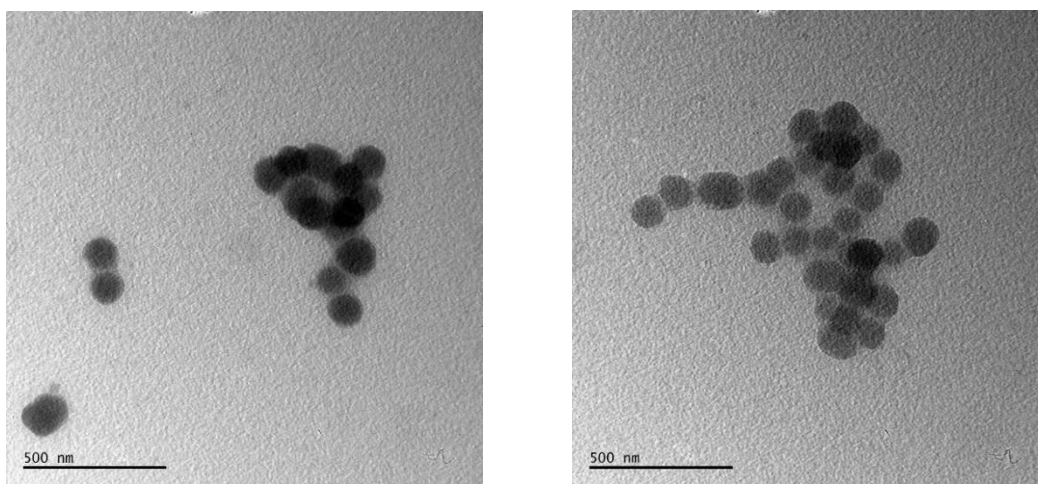


Figure 1. TEM images of (a) SNPs and (b) TPA–SNP at 200 kV. Scale bar: 500 nm.

The FTIR spectroscopy was performed to confirm the effective functionalization of silica nanoparticles within the 400–4000 cm^{−1} range. As shown in Figure 2, the spectrum of bare SNPs exhibited asymmetric stretching band related to siloxane (Si–O–Si) at 1058 cm^{−1}, stretching vibrations of the silanol (Si–OH) group at 799 cm^{−1}, and bending vibrations attributed to siloxane at 436 cm^{−1}. The broad peak at around 3400 cm^{−1} was ascribed to the O–H stretching vibration of Si–OH groups. Following modification with APTES, a noticeable decrease in the intensity of the –OH stretching vibration bands, indicating the consumption of surface hydroxyl groups during silanization. The emergence of additional bands at 2932 cm^{−1} and 2881 cm^{−1}, corresponding to C–H stretching of methylene (–CH₂–) groups. The bands observed at 3075 cm^{−1}, 1558 cm^{−1}, and 1489 cm^{−1}, can be ascribed to the N–H stretching and bending vibrations of the amine groups, further supporting the formation of SNP–NH₂. After conjugation of TPA–

AL, a new peak at 1634 cm^{-1} was appeared corresponding to the C=N stretching vibration of the imine bond, confirming the successful covalent attachment of the TPA moiety onto the nanoparticle surface.

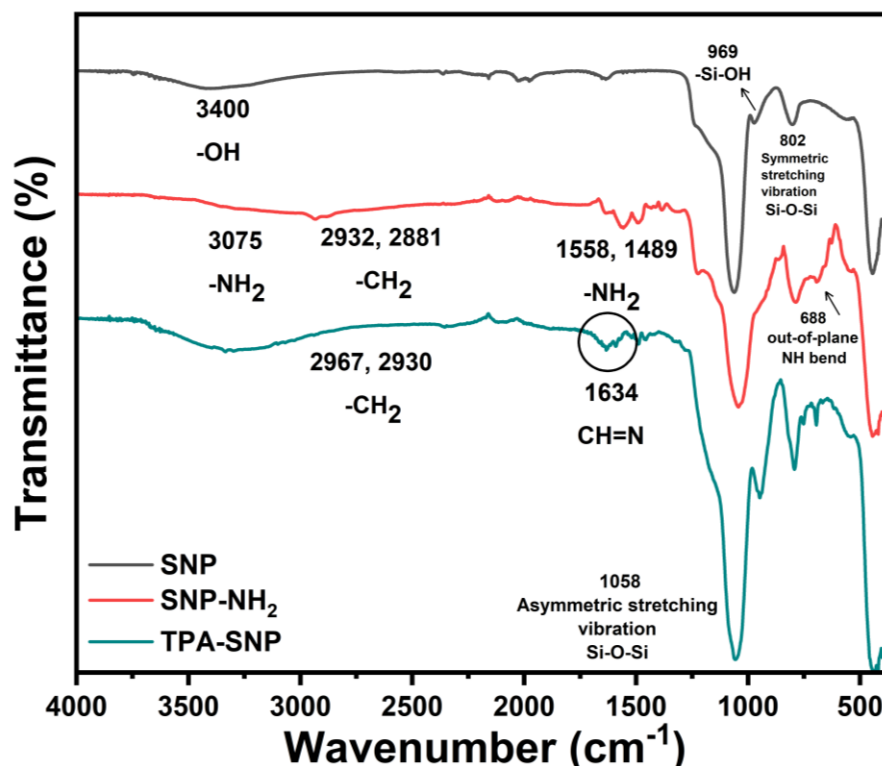


Figure 2. FTIR spectra of SNPs, SNP-NH₂ and TPA-SNP.

The surface amine density of SNP-NH₂ nanoparticles was quantified using the OPA fluorescence assay and UV-Vis Schiff base method following our previously reported protocol [33]. The amine contents were determined as $117.0 \pm 10.2\text{ }\mu\text{mol g}^{-1}$ and $113.9 \pm 5.1\text{ }\mu\text{mol g}^{-1}$, respectively.

TGA analysis of SNP-NH₂ and **TPA-SNP** was performed in the temperature range of 25–950°C at a heating rate of 10 °C/min (Figure S1). The initial weight loss was observed below 200°C which was due to the thermal evaporation of physically adsorbed water from the nanoparticle surface. The difference in weight loss between **TPA-SNP** and SNP-NH₂ is approximately 8.2 % within the temperature range of 200 to 400°C; corresponding to the thermal decomposition of organic moieties attached to the silica surfaces. The findings clearly demonstrate that the thermal stability and decomposition of organic compounds on the surface of SNPs existed at extreme temperature ranges.

3.2. Sensing studies

The fluorescence sensing behavior of **TPA–SNP** were examined comprehensively in THF/PBS (1:1, v/v) medium, both in the absence and presence of Fe^{2+} and Fe^{3+} ions. This solvent system provides an optimal environment for preserving the colloidal stability of **TPA–SNP** while facilitating the sensitive fluorescence measurements. As shown in Figure 3a **TPA–SNP** exhibited pronounced yellow fluorescence emission at 525 nm upon excitation at 380 nm ($\phi F = 0.38$). Upon the introduction of $\text{Fe}^{2+/3+}$ ions, the fluorescence intensity dramatically diminished, accompanied by a significant decrease in quantum yield ($\phi F = 0.03$).

The UV-Vis absorption spectrum of **TPA–SNP** displayed dual absorption peaks at 308 and 405 nm. Notable alterations were detected in the spectrum following the introduction of $\text{Fe}^{2+/3+}$ ions: the short-wavelength band transitioned from 308 to 302 nm, indicating a hypsochromic shift, whereas the long-wavelength band moved from 405 to 426 nm, demonstrating a bathochromic shift and an enhancement in intensity (hyperchromic effect). A significant color change was observed from colorless to pale yellow (Figure 3b).

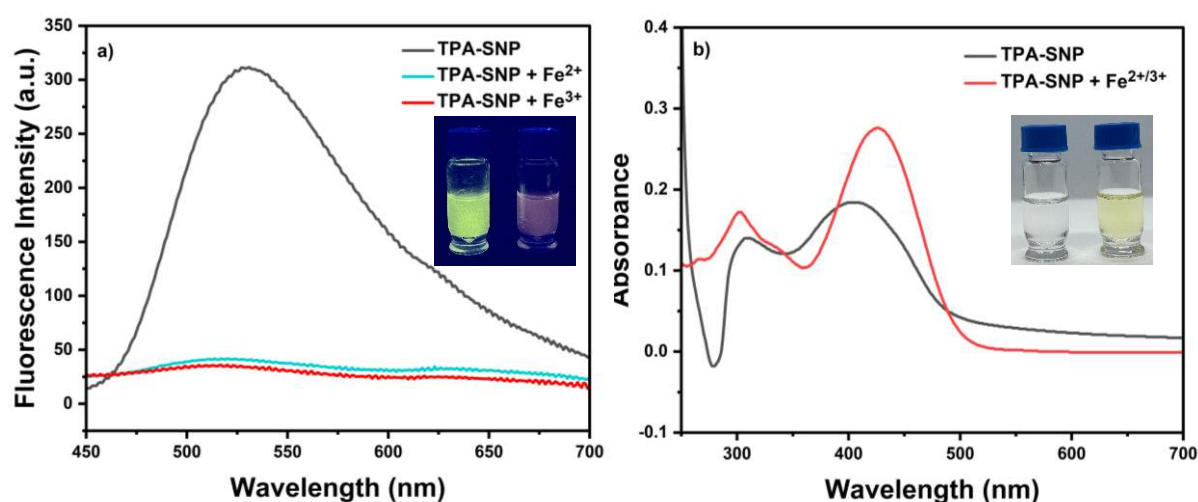


Figure 3. (a) Emission spectra and (b) absorption spectra of **TPA–SNP** at a concentration of 25 ppm in the presence of 100 μM $\text{Fe}^{2+/3+}$.

The interaction between **TPA–SNP** and $\text{Fe}^{2+/3+}$ ions initiated within 30 seconds and achieved complete saturation in approximately 3 minutes, demonstrating a rapid response capability of the sensing platform (Figure S2).

To evaluate the sensing performance, fluorescence titration studies were carried out by increasing the concentration of $\text{Fe}^{2+/3+}$ ions from 0 to 100 μM . As shown in Figure 4 and Figure S3, a significant decrease in fluorescence intensity was recorded with increasing ion concentration. **TPA–SNP** demonstrated a "turn-off" response by forming the starting aldehyde

molecule (TPA–AL). Based on linear regression analysis, the limits of detection (LOD) were calculated to be 1.24 μM for Fe^{2+} and 0.97 μM for Fe^{3+} (Figure S3).

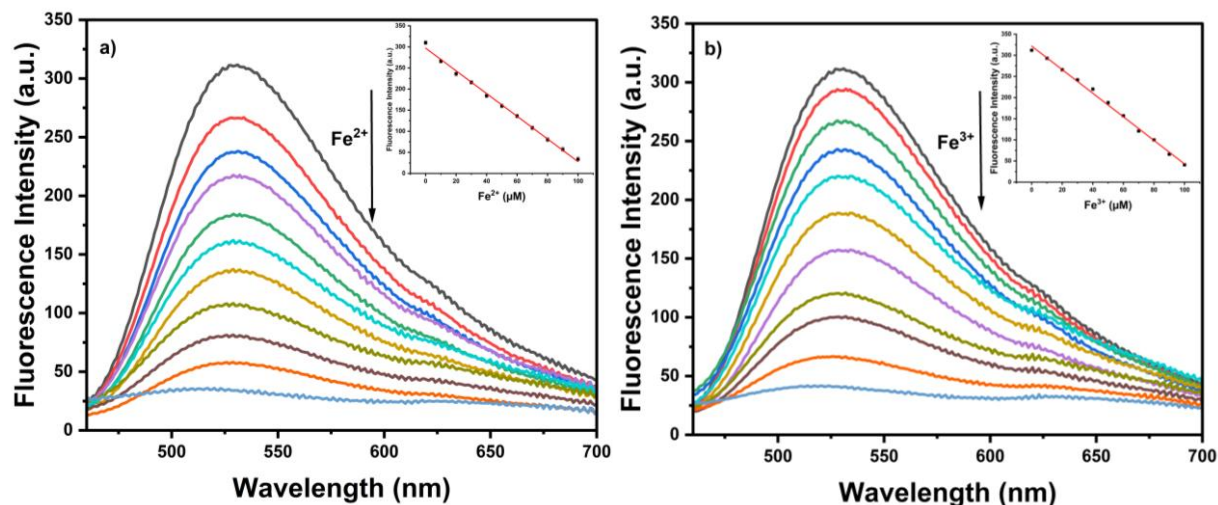


Figure 4. (a) Fluorescence titration spectra TPA–SNP (25 ppm) in the presence of Fe^{2+} (b) Fe^{3+} (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μM) (Inset: Fitting curve spectra of TPA–SNP (25 ppm) with increasing concentration of $\text{Fe}^{2+/3+}$)

The selectivity experiment is essential for the optimal functioning of a detection system. The selectivity of TPA–SNP toward $\text{Fe}^{2+}/\text{Fe}^{3+}$ ions was further evaluated in the presence of various competing metal ions, including Ag^+ , K^+ , Na^+ , Li^+ , Ba^{2+} , Ca^{2+} , Cd^{2+} , Hg^{2+} , Co^{2+} , Cu^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Zn^{2+} , Al^{3+} , Cr^{3+} , Sn^{2+} . The TPA–SNP demonstrated notable spectrum quenching following the addition of $\text{Fe}^{2+/3+}$, while being unaltered by other tested metal ions (Figure 5a). The colour of the aqueous TPA–SNP suspension remained unchanged with the addition of several metal ions (200 μM), all of which failed to elicit any alterations in the spectral profile. Moreover, interference experiments demonstrated that the presence of competing analytes did not significantly affect $\text{Fe}^{2+/3+}$ detection, and the fluorescence response remained unchanged even presence of other metal ions (Figure 5b).

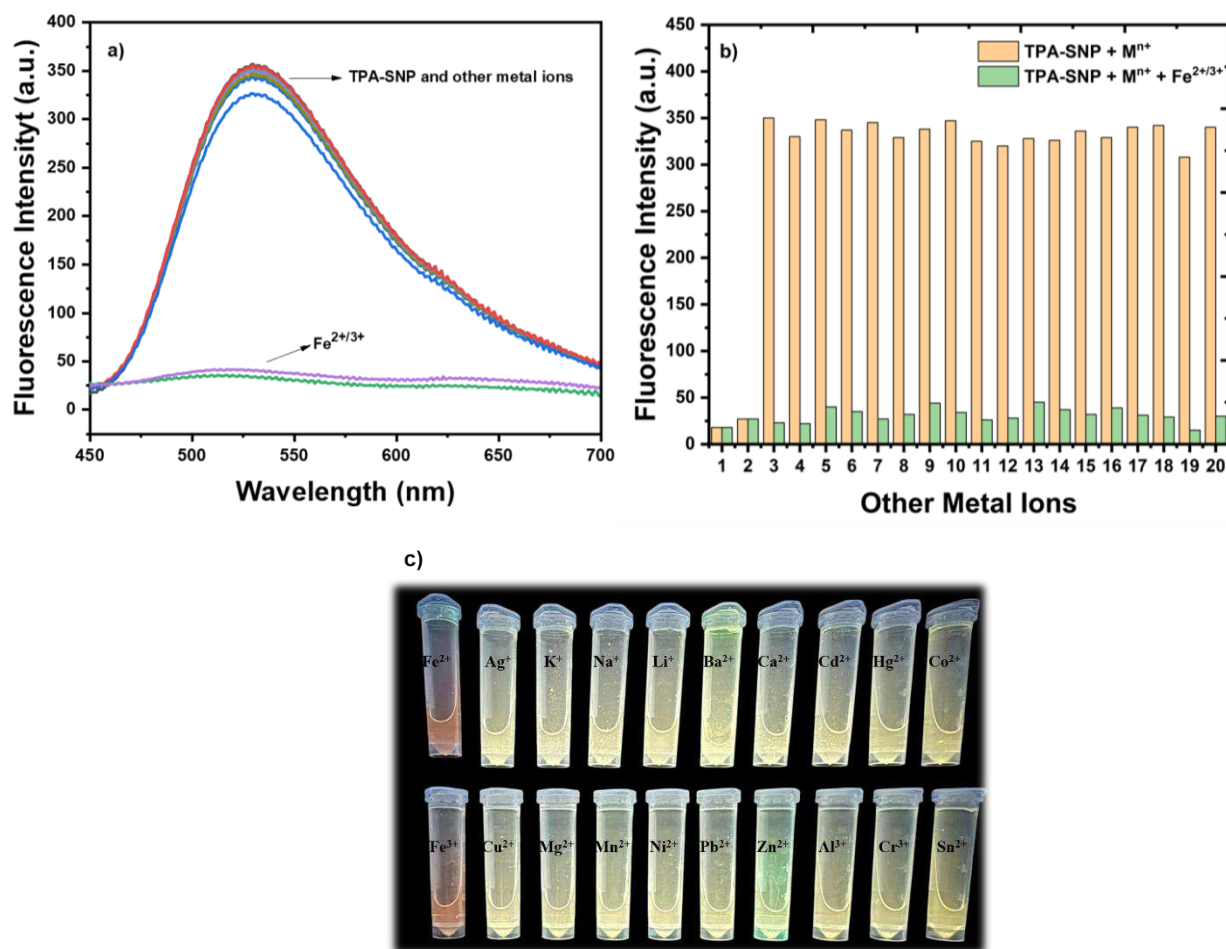


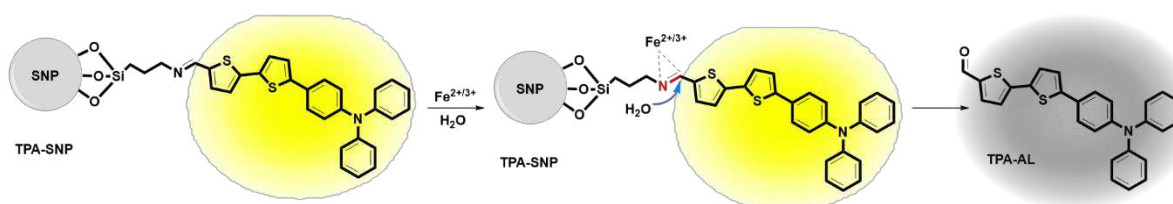
Figure 5. (a) Fluorescence intensity of TPA–SNP (25 ppm) in an environment of Fe^{2+/3+} (100 μM) and individual metal ions (200 μM) separately; (b) Fluorescence intensities of TPA–SNP (25 ppm) in the environment of Fe^{2+/3+} (100 μM) and (200 μM) of various metal ions collectively; 1, Fe³⁺; 2, Fe²⁺; 3, Ag⁺; 4, K⁺; 5, Na⁺; 6, Li⁺; 7, Ba²⁺; 8, Ca²⁺; 9, Cd²⁺; 10, Hg²⁺; 11, Co²⁺; 12, Cu²⁺; 13, Mg²⁺; 14, Mn²⁺; 15, Ni²⁺; 16, Pb²⁺; 17, Zn²⁺; 18, Al³⁺; 19, Cr³⁺ 20, Sn²⁺. (c) colorimetric alterations of the TPA–SNP solution recorded under 366 nm ultraviolet light in the presence of various metal ions.

The probe demonstrated good stability over a wide pH range (pH 4–10), with effective sensing performance observed throughout this interval (Fig. S4). Under physiological conditions, the pH of the reaction medium was adjusted to 7.4 using phosphate-buffered saline (PBS, 10 mM). TPA–SNP exhibited remarkable stability and sensing performance within the pH range of 4–10, indicating its suitability for applications in diverse environments.

3.3. Sensing Mechanism

Recently, some advancements have been made in the development of fluorescent probes for detecting Fe^{2+/3+} ions through the functionalization of a fluorophore with a specific imine moiety [34]. According to these reports, the proposed sensing mechanism indicates that Fe^{2+/3+} ions

facilitate the cleavage of the imine bond (C=N) on the nanoparticle surface resulting in the formation of corresponding aldehyde derivative, **TPA-AL**, having a donor- π -acceptor (D- π -A) structure. It is noteworthy that **TPA-AL** exhibited negligible fluorescence emission in aqueous environment due to the aggregation-caused quenching (ACQ) phenomenon [35]. The proposed sensing process and the possible interactions between the **TPA-SNP** and $\text{Fe}^{2+/3+}$ ions are illustrated in Scheme 2, consistent with previous general literature reports [34].



Scheme 2. Suggested mechanism illustrating the hydrolysis reaction involving **TPA-SNP** and $\text{Fe}^{2+/3+}$.

In order to validate this hypothesis, the UV-Vis absorption and fluorescence emission spectra of the pure **TPA-AL** molecule were compared with those obtained from **TPA-SNP** and $\text{Fe}^{2+/3+}$ system. As illustrated in Figure S5, the resulting spectra demonstrated strong similarity, which strongly suggests that $\text{Fe}^{2+/3+}$ ions hydrolysed the imine bond.

3.4. Practical Application

To evaluate the practical applicability of the proposed probe, the determination of total iron was performed in an actual red wine sample. The wine samples were diluted with deionized water, and the diluted samples were analyzed under the optimized experimental conditions. The analysis revealed a total iron concentration of in the red wine samples, corresponding to 72.2 and 55.0 μM after accounting for the dilution factor. Then, 25.0 μM Fe^{3+} were added to the solution. The spiked recovery results of all actual samples are between 100.3% and 101.8%, with low RSD values (Table 2). These results demonstrate that the **TPA-SNP** probe can be successfully applied to the determination of total iron in red wine samples, highlighting its potential as a simple and practical fluorescence-based sensing platform.

Table 2. Determination of total iron content in red wine samples.

Samples	$\text{Fe}^{2+/3+}$ level found (μM)	Added (μM)	Found (μM)	Recovery (%)	RSD (%) (n=3)
Red wine 1	72.2	25	97.6 ± 1.2	101.8	1.2

Red wine 2	55.0	25	80.3 ± 2.0	100.3	2.5
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4. Conclusion

In conclusion, a novel triphenylamine-functionalized silica nanoparticle probe was successfully designed and synthesized for the selective and sensitive detection of Fe^{2+} and Fe^{3+} ions. Dual responsiveness toward Fe^{2+} and Fe^{3+} allows convenient total iron analysis. The developed sensing platform combines the advantageous optical properties of the TPA–thiophene fluorophore with the enhanced chemical and environmental stability provided by the silica nanoparticle matrix. **TPA–SNP** exhibited a rapid fluorescence quenching response toward iron ions with good selectivity, sensitivity, and stability over a broad pH range. The silica-based architecture effectively minimized the environmental limitations commonly associated with small-molecule fluorescent probes, including signal instability and susceptibility to external conditions. Furthermore, the probe was successfully applied to the determination of total iron in a real red wine sample, demonstrating its practical applicability for real-sample analysis. . This approach not only broadens the application of triphenylamine-based sensing materials but also highlights the advantages of silica-supported fluorescent probes, paving the way for the design of next-generation hybrid nanoprobe with enhanced stability and practical applicability.

Conflicts of interest

The authors declare no conflict of interest.

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Data Availability

The data supporting the findings of this study are available within the article and its Supporting Information.

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