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RESEARCH ARTICLE

Novel luminescent Schiff's base derivative with an azo moiety for ultrasensitive and sensitive chemosensor of Fe³⁺ ions

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Abstract

Chemosensors with ultrasensing capabilities for detection of metal ions have received particular attention when using luminescent organic compounds. Even though hundreds of chemosensor agents have been reported for Fe³⁺ ion sensing, the designs of those molecules have been complicated and time consuming, in addition to having limited application for aquatic samples due to their poor hydrophilicity. Here, we synthesized a novel azo-imine derivative (L2) that showed ultrasensitive and selective sensing for Fe³⁺ ions. L2 exhibited ultrasensitive detection of Fe³⁺ ions with a turn-off of its emission intensity at 341 nm in H₂O:MeOH (4:1 v/v) aqueous medium. This quenching phenomenon was in good agreement with its colour change from orange-yellowish to colourless. Its capability was shown due to its very low limit of detection and limit of quantification values of 0.31 and 1.04 μM, respectively. The interference study showed that L2 is ultrasensitive for the detection of Fe³⁺ ions without a significant reduction in its sensing capability even in competitive metal mixtures. Furthermore, direct Fe³⁺ quantification of tap and drinking water showed that L2 gave good recovery percentages. These findings demonstrated that the Schiff's base with an azo fluorophore derivative is a potential chemosensor agent for Fe³⁺ ions sensing applications in aqueous media.

KEYWORDS

azo-imine, chemosensor, Fe³⁺ ions, fluorescence, ultrasensitive

1 | INTRODUCTION

Metallurgical industries of iron and steel manufacturing tend to generate large amounts of waste, especially in liquid form containing metallic irons that are released into the aquatic environment.^[1] Among the other transition metals, iron is a relatively abundant essential ion that has a pivotal role in the area of biological and environmental chemistry.^[2-5] However, excess intake of iron may cause some health issues such as cancers and dysfunction of several vital organs.^[6] Fe³⁺ ions, as the most stable oxidation state of the iron species, are mainly found in water systems with dissolved oxygen.^[7] Therefore, the development

of the selective and sensitive detection methods for Fe³⁺ ions is critical. In recent years, current research has focused on the design and development of chemosensor agents with high selectivity for detection of Fe³⁺ ions.^[8-11]

Chemosensors with fluorescence properties have received significant attention due to their great advantages such as highly sensitive and selective, as well as the possibility to be used for real-time monitoring.^[12] Many types of research into chemosensors have been reported based on organic fluorescence receptors for the detection of Fe³⁺ ions. However, their synthesis routes are often complicated, and they have had poor limits of detection (LOD) as well as limits of

quantification (LOQ).^[2,8,13–22] Additionally, several reports have shown low solubility in water, limiting their application for environmental samples. For example, a polyphenyl derivative¹ was synthesized from 2,3,4,5-tetraphenyl-cyclopentenone, which could be used for the fluorescence detection of Fe³⁺ ions in ethanol:water (EtOH:H₂O) at a 10:1 v/v ratio with 4 μM as the LOD value.^[23] The carbazole-based Schiff's base compound gave better LOD values (3.62 μM) towards Fe³⁺ detection in acetonitrile (CH₃CN), however other metal ions such as Cu²⁺ produced significant interference.^[24] Additionally, a fluoranthene-based pyridine ligand, synthesized from acenaphthenedione under inert conditions, was used as a fluorescent chemosensor for Fe³⁺ ions in EtOH. However, selectivity was inhibited by the presence of other counteranions such as sulfate, which made its use unfavourable.^[25]

Chemosensor agents based on Schiff's base derivatives with imine or azomethine groups could be simply prepared through condensation reactions between benzaldehyde and amine compounds in a high yield. They also exhibited excellent performance to form complexes by binding to certain metal ions,^[26] as well as good LOD and LOQ values.^[27,28] To enhance their capabilities as chemosensors for Fe³⁺ ions, many fluorescent chemosensors based on Schiff's bases have focused on their modification with a chromophore group,^[2,8,13–15,18,29,30] such as rhodamine,^[30] naphthalimide,^[15] and coumarin^[14,19] moieties. Qin *et al.* prepared a fluorescent chemosensor based on a rhodamine–quinoline conjugate in methanol (MeOH) with LOD values as low as 0.83 μM for Fe³⁺ detection.^[30] Other researchers have reported that coupled naphthol containing an azo group (–N=N) could detect Fe³⁺ ions in a 1:1 ratio of CH₃CN:H₂O with LOD values as low as 0.937 μM.^[31]

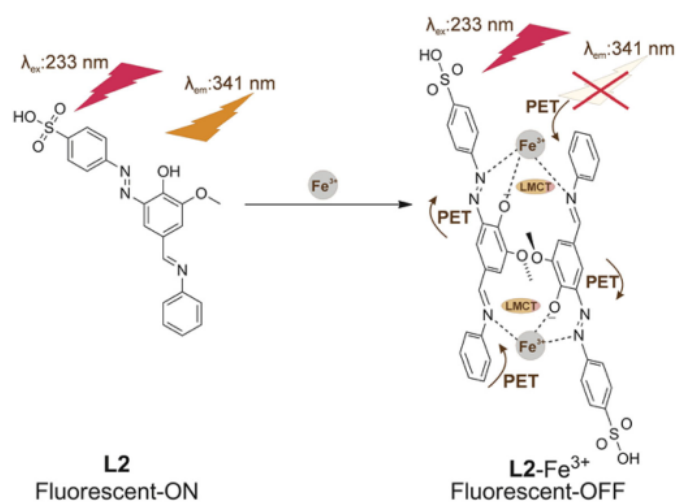
Conversely, azo derivatives have been extensively investigated because of their excellent fluorophore functionalization in dyes and molecular recognition applications. However, a systematic study on fluorescence detection of Fe³⁺ ions using modified Schiff's base derivatives consisting of azo fluorophores (–N=N) has rarely been reported. As an azo moiety is considered as an excellent fluorophore group,^[32]

combination of azo-imine moieties on chemosensor agents may serve as an excellent platform for the fluorescence detection and quantification with high selectivity and sensitivity of Fe³⁺ ions. Moreover, chemosensors with azo-imine moieties could increase the possibility for real application for environmental samples with the presence of a sulfonic acid functional group.^[33,34] Therefore, in the present work, we report the first successful synthesis of a Schiff's base azo-imine as a fluorescent chemosensor for Fe³⁺ ions in an almost aqueous system. The azo-imine derivative, named as 4-((E)-(2-hydroxy-3-methoxy-5-((E)(phenylimino)methyl)phenyl)diazenyl)benzene sulfonic acid (L2) was successfully prepared through simple two-step reactions, diazotization and nucleophilic addition reactions. In particular, the novel chemosensor L2 showed ultrasensitive and ultrasensitive properties for detection of Fe³⁺ ions through turn-off of its fluorescence properties with quenching of emission intensity at 341 nm (Scheme 1) and with an LOD of 0.31 μM in H₂O:MeOH (4:1 v/v). Indeed, this chemosensor L2 could be applied for the quantification of Fe³⁺ ions from tap and drinking water as the representations of real samples and with good recovery.

2 | EXPERIMENTAL

2.1 | Materials

The chemicals used in the present work, such as sulfanilic acid (4-aminobenzene sulfonic acid), anhydrous potassium carbonate (K₂CO₃), sodium nitrite (NaNO₂), vanillin (4-hydroxy-3-methoxybenzaldehyde), aniline, concentrated hydrochloric acid (HCl 37% w/v), sodium hydroxide (NaOH), acetone, MeOH, ethyl acetate (EtOAc), EtOH, metal chloride salts (i.e. KCl, NaCl, ZnCl₂, MnCl₂, FeCl₃, CoCl₂, NiCl₂, CuCl₂, MgCl₂, CaCl₂, BaCl₂, and LaCl₃) and thin-layer chromatography plate (silica gel 60 F₂₅₄) were purchased from Merck in proanalysis grade and used without further purification.



SCHEME 1 Schematic sensing mechanism of Fe³⁺ ions using Schiff's base azo-imine compound (L2) with 1:1 ratio of molecular recognition

2.2 | Instrumentation

The instrumentation used in this work was a melting point measurement apparatus (Electrothermal 9100), a Fourier transform infrared (FTIR) spectrometer (Jasco 6800), and a UV-vis spectrophotometer (Jasco V-760), as well as spectrofluorometer (Jasco F-8500ST). Other instrumentations were a liquid chromatography-high resolution mass spectrometer for liquid chromatography-high resolution mass spectrometry (LC-HRMS) consisting of a high performance liquid chromatography (Thermo Scientific Dionex Ultimate 3000 RSLCnano with microflow meter) and an HRMS system (Thermo Scientific Q Exactive parallel-reaction monitoring with MS2 at 17500 Resolution) with electrospray ionization (ESI), and an $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrometer (Jeol 400 MHz with 5 mm probe) which were used for characterization of molecular structure.

2.3 | Synthesis of compound L1

Sulfanilic acid (1.93 g, 11 mmol) and anhydrous K_2CO_3 (0.77 g, 5.5 mmol, 0.5 eq.) were dissolved in distilled H_2O (30 ml) and cooled at $0-5^\circ\text{C}$. Then, dissolved NaNO_2 (0.65 g, 11 mmol, 1.0 eq.) and concentrated HCl (3.13 ml) in cold distilled H_2O (20 ml) were slowly added (dropwise) to give mixture 1.

Conversely, mixture 2 was prepared by dissolving vanillin (1.70 g, 11 mmol, 1.0 eq.) in NaOH 10% w/v (60 ml) at $0-5^\circ\text{C}$. Mixture 1 was added slowly into mixture 2. The new mixture was stirred for 4 h and the formed precipitation was filtered and recrystallized with MeOH : EtOAc in 7:3 v/v to give (E)-4-([5-formyl-2-hydroxy-3-methoxyphenyl] diazenyl)benzene sulfonic acid (L1) as a reddish-brown solid at 31% yield (1.13 g). m.p. $>293^\circ\text{C}$ (decomposed). $\epsilon_{\text{L1}} = 1.05 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. $\phi_{\text{L1}} = 0.0019$ in $\text{H}_2\text{O}:\text{MeOH}$ (4:1 v/v). FTIR (KBr, ν/cm^{-1}): 3474 (-OH), 3073 (C-H sp^3), 2928 (CH sp^3), 2849 and 2753 (H-C=O), 1692 (C=O), 1601 (C=C aromatic), 1427 (N=N), 1201 (C-O), as well as 1034 and 671 (SO_3H). $^1\text{H-NMR}$ (DMSO- d_6 , δ/ppm): 9.88 (s, 1H, -CHO), 9.73 (s, 1H, - SO_3H), 8.73 (s, 1H, C-OH), 7.99 (d, $J = 7.16 \text{ Hz}$, 1H, H aromatic), 7.88 (s, 1H, H aromatic), 7.79 (d, $J = 7.16 \text{ Hz}$, 1H, H aromatic), 7.62 (m, $J = 7.52 \text{ Hz}$, 1H, H aromatic), 7.50 (s, 1H, H aromatic), 7.37 (m, $J = 8.28 \text{ Hz}$, 1H, H aromatic), and 3.17 (s, 3H, - OCH_3). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ/ppm): 192.11 (C=O), 151.97, 151.43, 150.59, 128.20, 127.56, 127.23, 123.16, 120.82, 116.59, 115.94 and 111.83 (C aromatics), 139.16 (C-OH), and 56.703 (- OCH_3). HRMS: m/z found 335.03445 Da ([M-H] $^-$) for $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_6\text{S}$ with a theoretical calculation of m/z at 335.03433 Da.

2.4 | Synthesis of compound L2

A mixture of L1 (0.64 g, 2 mmol), K_2CO_3 (0.30 g, 2 mmol, 1 eq.) and aniline (2 ml, 2 mmol, 1 eq.) was dissolved in EtOH (25 ml) and the mixture was treated under reflux for 8 h. The mixture was evaporated and then neutralized using HCl 10% v/v. The formed precipitation

was washed with distilled water and recrystallized with acetone to obtain the desired 4-((E)-(2-hydroxy-3-methoxy-5-((E)-(phenylimino) methyl) phenyl) diazenyl)benzene sulfonic acid (L2) product as a brown solid at 71% yield (0.46 g). m.p. $>293^\circ\text{C}$ (decomposed). $\epsilon_{\text{L2}} = 2.24 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$. $\phi_{\text{L2}} = 0.0062$ in $\text{H}_2\text{O}:\text{MeOH}$ (4:1 v/v). FTIR (KBr, cm^{-1}): 3420 (-OH), 3065 (CH sp^2), 2928 and 2854 (CH sp^3), 1661 (C=N), 1586 (C=C), 1428 (N=N), 1305 (C-O), 1031 (C-O), 1010 and 671 (SO_3H). $^1\text{H-NMR}$ (DMSO- d_6 , δ/ppm): 9.90 (s, 1H, H-C=N), 9.74 (s, 1H, - SO_3H), 8.83 (s, 1H, C-OH), 7.99 (d, $J = 8.24 \text{ Hz}$, 2H, H aromatic), 7.89 (s, 1H, H aromatic), 7.76 (d, $J = 8.44 \text{ Hz}$, 2H, H aromatic), 7.49 (m, $J = 7.68 \text{ Hz}$, 1H, H aromatic), 7.47 (s, 1H, H aromatic), 7.45 (m, $J = 7.76 \text{ Hz}$, 1H, H aromatic), 7.40 (m, $J = 6.92 \text{ Hz}$, 1H, H aromatic), 7.36 (m, $J = 6.92 \text{ Hz}$, 1H, H aromatic), 7.30 (d, $J = 7.36 \text{ Hz}$, 1H, H aromatic), and 3.17 (s, 3H, - OCH_3). $^{13}\text{C-NMR}$ (DMSO- d_6 , δ/ppm): 192.10 (C=N), 151.99, 151.40, 150.59, 132.51, 130.01, 130.01, 128.55, 128.26, 127.31, 127.31, 123.70, 123.70, 123.17, 123.17, 121.57, 116.63 and 111.70 (C aromatics), 139.15 (C-OH), and 56.694 (- OCH_3). HRMS: m/z found 410.08163 Da ([M-H] $^-$) for $\text{C}_{20}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$ with a theoretical calculation of m/z at 410.08161 Da.

2.5 | Sensitivity test for L2 as a chemosensor of Fe^{3+} ions

The chemosensor L2 was dissolved in $\text{H}_2\text{O}:\text{MeOH}$ solvent in a 4:1 v/v ratio at 0.20 mM and called the ligand solution. The ligand solution was arbitrary mixed with Fe^{3+} ion solution at 3.0 mM in $\text{H}_2\text{O}:\text{MeOH}$ (4:1 v/v), giving the final concentration of ligand in the mixture of 0.03 mM. The final concentrations of Fe^{3+} ions were prepared in the series of 0, 0.003, 0.006, 0.0075, 0.015, 0.023, 0.024, 0.030, 0.060, 0.075, 0.150, 0.225, 0.240 and 0.30 mM to evaluate sensitivity. To calculate the LOD and LOQ values, Equations 1 and 2 were used as follows:

$$\text{LOD} = 3 \times \text{standard error/slope} \quad (1)$$

$$\text{LOQ} = 10 \times \text{standard error/slope} \quad (2)$$

2.6 | Complexation study between chemosensor L2 and Fe^{3+} ions

The stock solution of chemosensor L2 at 0.20 mM and the stock solution of FeCl_3 at 3.00 mM were mixed and diluted at various volume ratios to maintain a total mole fraction of 3.0 ml of L2 and Fe^{3+} mixture at 0.03 mM. The fluorescence spectrum of each mixture was measured using a spectrofluorometer to determine the stoichiometric ratio of the formed complex between L2 and Fe^{3+} ions.

The chemosensor L2 solution at 0.03 mM was mixed with 3.00 mM of Fe^{3+} ion solution. The final concentration of ligand in the mixture was 0.03 mM and the final concentrations of Fe^{3+} ions were 0, 0.003, 0.006, 0.0075, 0.015, 0.023, 0.024, 0.030, 0.060, 0.075,

0.150, 0.225, 0.240 and 0.30 mM. The fluorescence spectrum of each mixture was also measured using a spectrofluorometer to construct a Benesi-Hildebrand plot to calculate the association constant between chemosensor L2 and Fe³⁺ ions.

2.7 | Selectivity and interference test of chemosensor L2 for detection of Fe³⁺ ions

The chemosensor L2 solution at 0.20 mM was mixed with 15 μl stock solution of metal chloride solutions (FeCl₃, NaCl, KCl, MgCl₂, CaCl₂, BaCl₂, MnCl₂, CoCl₂, CuCl₂, NiCl₂, ZnCl₂, and LaCl₃) at 30.0 mM to give a mixture with a total volume of 3 ml in H₂O:MeOH (4:1 v/v). The fluorescence spectrum of each mixture was measured using a spectrofluorometer to evaluate the selectivity. Furthermore, the interference of the other metal ions on Fe³⁺ detection was investigated by mixing Fe³⁺ ions at 0.03 mM and other metal ions at 0.15 mM. Interference percentage was calculated using Equation 3 as follows:

$$\text{Interference (\%)} = \frac{\left(\frac{[\text{Fe}^{3+}]_{\text{observed}} - [\text{Fe}^{3+}]_{\text{theoretical}}}{[\text{Fe}^{3+}]_{\text{theoretical}}} \right) \times 100\% \quad (3)$$

Fe³⁺ quantification of the real samples, i.e. tap and drinking water was also carried out similarly as mentioned above.

3 | RESULTS AND DISCUSSION

3.1 | Synthesis of L1 and L2 compounds

Synthesis of L1 that contains an azo group was carried out through a diazotization reaction between vanillin and sulfanilic acid as precursors. In addition, the L2 compound was synthesized from a nucleophilic addition (condensation) reaction between L1 and aniline. First, the diazonium salt of sulfanilic acid was reacted with vanillin under alkaline conditions of K₂CO₃ to form L1. Next, L1 was reacted with aniline to produce L2 (Figure 1). The structure of the products was

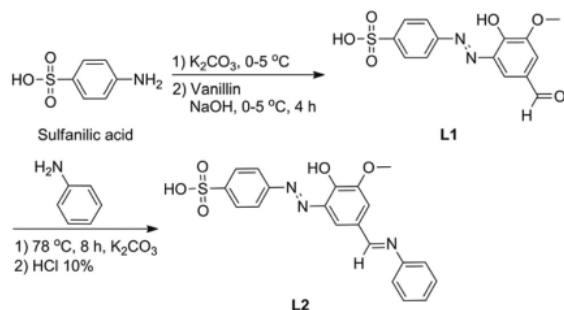


FIGURE 1 Synthesis scheme of L1 and L2 compounds from sulfanilic acid and vanillin

elucidated using FTIR, LC-HRMS, and ¹H-NMR and ¹³C-NMR spectrometry.

The FTIR spectra of L1 and L2 are shown in Figure S1. Differing from the FTIR standard of sulfanilic acid as the precursor, the FTIR spectrum of L1 showed the appearance of a C-H aldehyde functional group at 2849 and 2753 cm⁻¹ and also the existence of C=O aldehyde and N=N azo functional groups at 1692 and 1427 cm⁻¹. In addition, the C-H aldehyde peaks disappeared on the FTIR spectrum for L2, indicating successful synthesis through a condensation reaction. Furthermore, the vibration peak at 1692 cm⁻¹ was shifted to a lower wavenumber (1661 cm⁻¹) due to the transformation of C=O aldehyde to C=N Schiff's base. Based on the ¹H-NMR and ¹³C-NMR elucidation (Figures S2-S5), it was confirmed that both L1 and L2 compounds had been successfully synthesized. The presence of methoxy and C-H aldehyde protons on L1, corresponding to the vanillin moiety, was found at 3.17 and 9.88 ppm as singlet signals. The addition of aromatic protons at 7.30-7.49 ppm corresponded to the aniline moiety of L2. ¹³C-NMR spectra of L1 and L2 confirmed the total number of carbon atoms of each compound, i.e. 14 and 20 carbon atoms, respectively. Further characterization using HRMS spectrometry showed that the [M-H]⁻ of L1 and L2 were found at 335.03445 Da and 410.08163 Da, respectively, which was very close to the calculated [M-H]⁻ values for both compounds (335.03433 and 410.08161 Da) with accuracy less than 1 ppm, while the monoisotopic patterns were similar to the predicted ones. From FTIR, ¹H-NMR, ¹³C-NMR, and HRMS analyses, it was confirmed that the molecular structures of L1 and L2 were correctly synthesized and, therefore, had been isolated as pure compounds.

Figure 2 shows the fluorescence spectra of compounds L1 and L2 in H₂O:MeOH (4:1 v/v). Interestingly, the L1 (black line) gave two excitation peaks (black with dashed line) at 203 and 251 nm corresponding to aromatic and azo (-N=N) functional groups, while emission peaks were found at 341 and 427 nm (black with solid line).

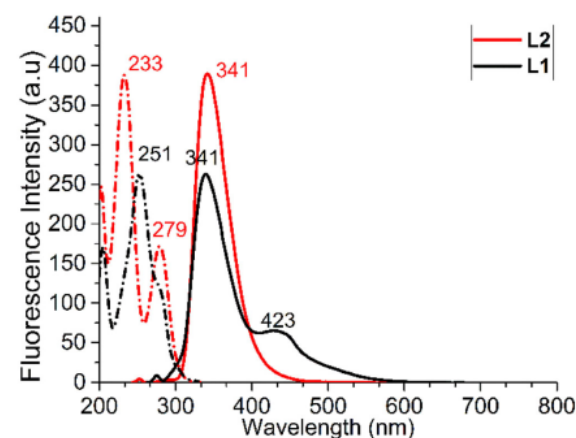


FIGURE 2 Fluorescence spectrum of L1 (black line) and L2 (red line). Dashed and solid lines correspond to excitation and emission spectra, respectively

Conversely, L2 (red line) showed three excitation peaks at 203, 233, and 279 nm (red with dash line), corresponding to the aromatic, azo ($-N=N$), and imine ($-C=N$) functional groups, respectively. The $-N=N$ group had a lower excitation wavelength, probably as it was adjacent to a hydroxyl group in the aromatic ring to form a tautomeric azoenol^[35] from the inductive effect. Upon monitoring at 233 and 279 nm (red with solid line), it was found that both excitation wavelengths gave an emission peak at 341 nm, whereas a higher intensity was given using 233 nm. Therefore, further studies have focused on monitoring emission spectral changes with an excitation wavelength at 233 nm. The molar extinction coefficient and fluorescence quantum yield values of L2 ($\epsilon_{L2} = 2.24 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\phi_{L2} = 0.0062$) were higher than that of L1 ($\epsilon_{L1} = 1.05 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, $\phi_{L1} = 0.0019$). Fluorescence quantum yield was measured using rhodamine B solution in water as the standard ($\phi = 0.3100$). This means that L2 had better optical and photoluminescence properties than the L1 compound. Low fluorescence quantum yield values for Schiff's base derivatives have also been reported in previous studies for the pyrene-Schiff's base and triphenylamine-Schiff's base ($\phi = 0.0900$).^[14,22]

3.2 | Sensing sensitivity of L2 toward Fe^{3+} detection and quantification

L2 was evaluated as a chemosensing agent towards the detection and quantification of Fe^{3+} ions. After addition of Fe^{3+} ions to L2 as shown from the fluorescence titration study (Figure 3a,b), it was found that both excitation and emission signals (Figure 3a,b) were gradually quenched by increasing the equivalence of Fe^{3+} ions. Moreover, quenching percentage was gradually increased up to almost 100%. Such sensing capability was well supported by decreasing ϕ_{L2} from 0.0062 to 0.000096 (almost 100%).^[33] Indeed, these emission and excitation spectral changes were also in good agreement with the colour of the ligand solution from orange-yellowish (before sensing) to colourless upon addition of Fe^{3+} ions (Figure 3c), indicating a rapid sensing property of L2 toward Fe^{3+} ions. These colour changes were clearly visualized by the addition of Fe^{3+} ions with a concentration of 0.75 eq.. From the contact time study with the equilibrium conditions even within a minute, such sensing capability^[29] for the rapid detection of Fe^{3+} ions (Figure S6) is really important in real-time sample analysis. Of interest, the emission spectral changes showed the same

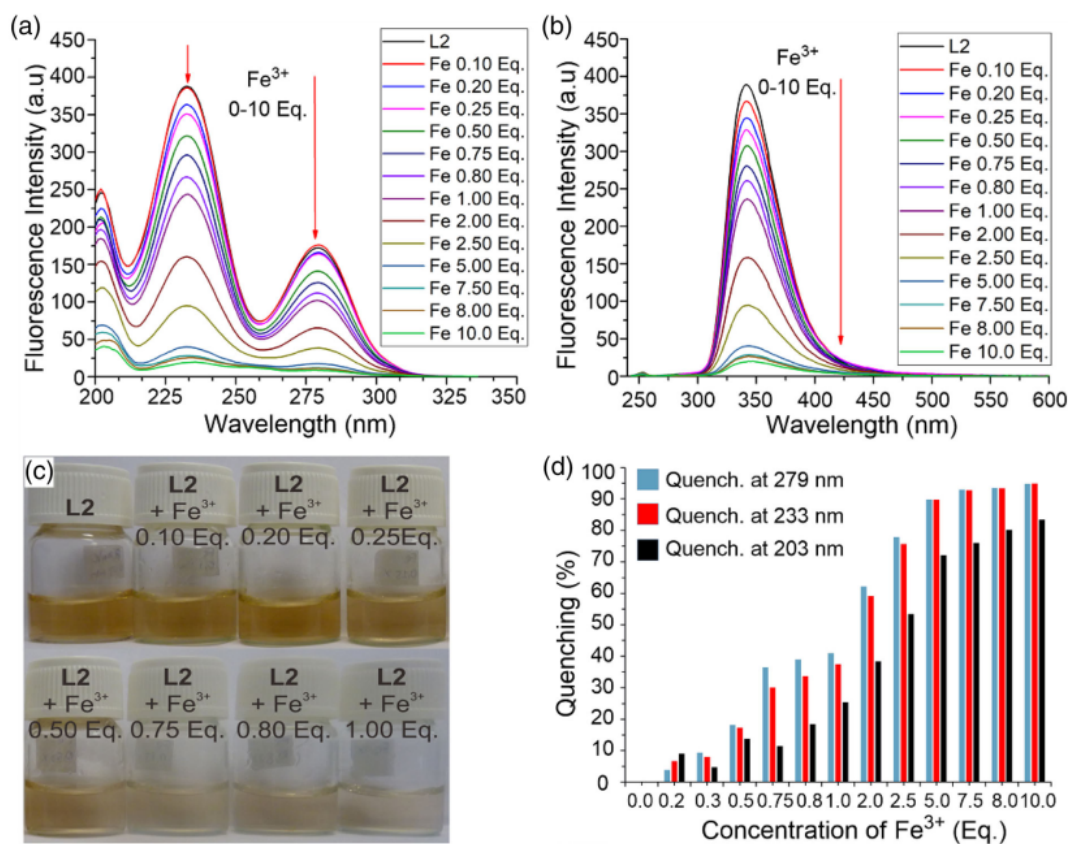


FIGURE 3 (a) Excitation ($\lambda_{em} = 341 \text{ nm}$); and (b) emission ($\lambda_{ex} = 233 \text{ nm}$) spectral changes of L2 in $\text{H}_2\text{O}:\text{MeOH}$ (4:1 v/v) with the addition of Fe^{3+} ions (0–10 eq.). (c) Photographs in daylight. (d) Per cent quenching (L2 = 0.03 mM)

quenching phenomena using different excitation wavelengths (203, 233, and 279 nm), as presented in (Figure 3d).

A Stern–Volmer plot was constructed from the study of fluorescence titration data as the plot of I/I_0 versus Fe^{3+} ions, where I_0 is

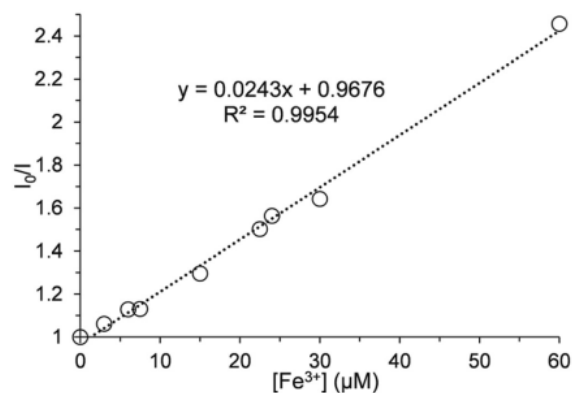


FIGURE 4 Stern–Volmer plot of L2 (0.03 mM) with the addition of Fe^{3+} ions (0–60 μ M) in $H_2O:MeOH$ (4:1 v/v)

initial emission intensity of L2, and I is the emission intensity of L2 with the addition of Fe^{3+} ions at various concentrations, with 341 nm as the emission signal. From the Stern–Volmer plot (Figure 4), the LOD and LOQ values were found to be 0.31 and 1.04 μ M with a linear dynamic range between 1.0 to 60 μ M, which indicated ultrasensitive detection. Moreover, L2 in fluorescence titration yielded 0–2.5% of % relative standard deviation (RSD), indicating good reproducibility for the fluorescent chemosensor of Fe^{3+} ions.

The calculated LOD value of L2 to detect Fe^{3+} ions was much lower compared with the permitted concentration of Fe^{3+} ions allowed in drinking water by the World Health Organization, that is, 0.3 $mg L^{-1}$ ($\sim 6 \mu$ M). This result indicated that L2 could possibly be applied for the detection and quantification of Fe^{3+} ions from drinking water samples.^[17] A comparison of LOD values for L2 with other reported chemosensor agents is listed in Table 1 and compared with previous reports.^[2,8,14–22,24,30,31,36–46] For comparison, the LOD value of L2 was even better than pyrene, oxadiazole, coumarin, naphthalimide, quercetin, acridine, crown ether, triphenylamine, carbazole, rhodamine, triazole, and isatin derivatives. More than that, the sensing capability of L2 was also better than hybrid inorganic/organic nanocomposite materials based on SBA-15 (see Table 1, entries

TABLE 1 Comparison of fluorescent chemosensors for Fe^{3+} ions from the present work and previously reported studies

No	Ligand or compound	LOD (μ M)	Solvent system	Reference
1	Pyrene–Schiff's base	3.19	DMSO:H ₂ O (8:2)	[2]
2	Oxadiazole based	6.95	THF:Tris–HCl (4:1)	[8]
3	Pyrene–Schiff's base	1.37	DMSO:H ₂ O (7:3)	[14]
4	Coumarin based	50.0	HEPES–1% DMSO	[15]
5	Naphthalimide dyes	2.00	THF:H ₂ O (3:7)	[16]
6	Quercetin	20.5	DMSO:H ₂ O (9:1)	[17]
7	Aminopyrine	1.82	THF	[18]
8	Acridine	4.13	DMSO:H ₂ O (1:1)	[19]
9	Schiff's base–coumarin	4.30	MeOH	[20]
10	Schiff's base–crown ether	0.36	EtOH	[21]
11	Triphenylamine–Schiff's base	45.1	THF:H ₂ O	[22]
12	Carbazole-based Schiff's base	3.62	CH ₃ CN	[24]
13	Rhodamine–quinoline	0.83	MeOH	[30]
14	Naphthol–azo	0.94	CH ₃ CN:H ₂ O (1:1)	[31]
15	Rhodamine based	6.94	CH ₃ CN:H ₂ O (8:2)	[36]
16	Triazole derivative	3.21	Acetone:H ₂ O (1:1)	[37]
17	Bis–rhodamine based	4.10	Methanol	[38]
18	Rhodamine–thioxoquinazoline	4.11	CH ₃ CN:H ₂ O (8:2)	[39]
19	Triazole–coumarin derivative	3.81	DMSO:H ₂ O (n/a)	[40]
20	Naphthalimide–thiourea derivative	6.86	CH ₃ CN:H ₂ O (99:1)	[41]
21	Nanocomposite (SBA–15/isatin derivative)	0.60	H ₂ O	[42]
22	Nanocomposite (SBA–15/bis–Schiff's base benzenediamine)	1.98	EtOH:H ₂ O (9:1)	[43]
23	Diaza–18–crown–6 ether	0.31	DMF:H ₂ O (4:1)	[44]
24	Quinoline based	0.16	DMSO:H ₂ O (8:2)	[45]
25	Azo–Schiff's base anthranilic acid	6.44	DMF:HEPES (1:1)	[46]
26	Schiff's base–azo	0.31	H ₂ O:MeOH (4:1)	Present work

21 and 22). This marked result indicated that chemosensor **L2** formed a suitable complexation of Fe^{3+} ions with its Schiff's base, phenolic, and azo moieties. It was known that Schiff's base and phenolic compounds had high sensitivity for Fe^{3+} detection.^[21,31] By combining these functional groups with azo moieties, it was found that the LOD value of **L2** was lower than that of other hybrid compounds (see Table 1, entries 1, 9–12, and 25). In particular, compared with a quinoline-based chemosensor in DMSO:H₂O (4:1),^[45] our finding was almost that in aqueous system using MeOH:H₂O (1:4) and was close to the lowest reported LOD value. Conversely, while the other chemosensor agents based on organic compounds required DMSO or THF as the diluent (Table 1), in the present work a commercially available solvent from the mixture of H₂O:MeOH at 4:1 v/v volume ratio was used, which is relatively cheap and convenient.

This phenomenon has been also proved by investigating the sensitivity of **L1** for Fe^{3+} detection using fluorescence titration (Figure S7a,b) and Stern–Volmer plot for **L1** (Figure S8), yielding 2.49 and 8.29 μM as the LOD and LOQ values, respectively. Interestingly, the LOD and LOQ values for **L2** were almost 10 times better than that for **L1**, confirming that the presence of an imine ($-\text{C}=\text{N}$) functional group together with an azo ($-\text{N}=\text{N}$) moiety was pivotal for chemosensor sensitivity. Indeed, the Stern–Volmer constant (K_{SV}) was found to be $2.43 \times 10^4 \text{ M}^{-1}$ from the calculation using Figure 4, which was relatively high due to the higher sensing capability of **L2** to be used as the chemosensor agent for detection of Fe^{3+} ions. The K_{SV} value of **L2** was higher than that of **L1** ($7.50 \times 10^3 \text{ M}^{-1}$; Figure S8), indicating that the sensitivity of **L2** for Fe^{3+} detection was higher than that for **L1**.

3.3 | Investigation on the complex structure between **L2** and Fe^{3+} ions

The stoichiometric ratio between **L2** and Fe^{3+} ions was further studied for interaction in the formation of a complex structure using a

Job's plot experiment. Job's plot was obtained from the intensity of the excitation signal at 233 nm, as shown in Figure 5a. It was found that the complex between **L2** and Fe^{3+} ions was formed in a 1:1 stoichiometric ratio. To support this result, Benesi–Hildebrand plots for **L1** and **L2** were constructed to calculate the association constant, as shown in Figures S9 and S10. In this case, the Benesi–Hildebrand plot was found to be linear, with 0.9921 as the correlation factor (Figure S10) to give an association constant for the $[\text{L2-Fe}]^{3+}$ complex at $1.56 \times 10^4 \text{ M}^{-1}$. This result demonstrated a suitable 1:1 complexation interaction between **L2** and Fe^{3+} ions. The association constant for $[\text{L2-Fe}]^{3+}$ was higher, around 22 times that of $[\text{L1-Fe}]^{3+}$ in $6.98 \times 10^2 \text{ M}^{-1}$ (Figure S9), demonstrating that the complexation ability of **L2** for binding Fe^{3+} ions was stronger than **L1**.

Furthermore, to discover the role for each functional group in the complex interaction between **L2** and Fe^{3+} ions, FTIR spectra for **L2** before and after the addition of Fe^{3+} ions were recorded, and the percentage quenching of each signal on the fluorescence titration study was also calculated, as shown in Figure 3d. After the addition of Fe^{3+} ions at 10 eq. to the ligand **L2**, the vibration peak corresponding to the imine ($-\text{C}=\text{N}$) functional group was shifted from 1736 to 1715 cm^{-1} . In addition, the vibration peak for the azo ($-\text{N}=\text{N}$) functional group was shifted from 1401 to 1397 cm^{-1} , while the vibration peak for C–O decreased its intensity as shown in Figure 5b. These results suggested that both $-\text{C}=\text{N}$ and $-\text{N}=\text{N}$ groups in **L2** were affected by the addition of Fe^{3+} ions, as strengthened by the significant quenching of the fluorescence spectra of **L2** with the addition of Fe^{3+} ions at 233 and 279 nm. The decrease in the vibration band for C–O indicated that the phenolic group ($-\text{OH}$) adjacent to the azo group was also affected by the presence of Fe^{3+} ions.

As mentioned previously, the quenching percentages of excitation signals at 203, 233 and 279 nm could be assigned to aromatic, azo, and imine functional groups. The quenching percentages of excitation signals from aromatic, azo, and imine groups were increased at a nearly similar amount by increasing the concentration of Fe^{3+} ions,

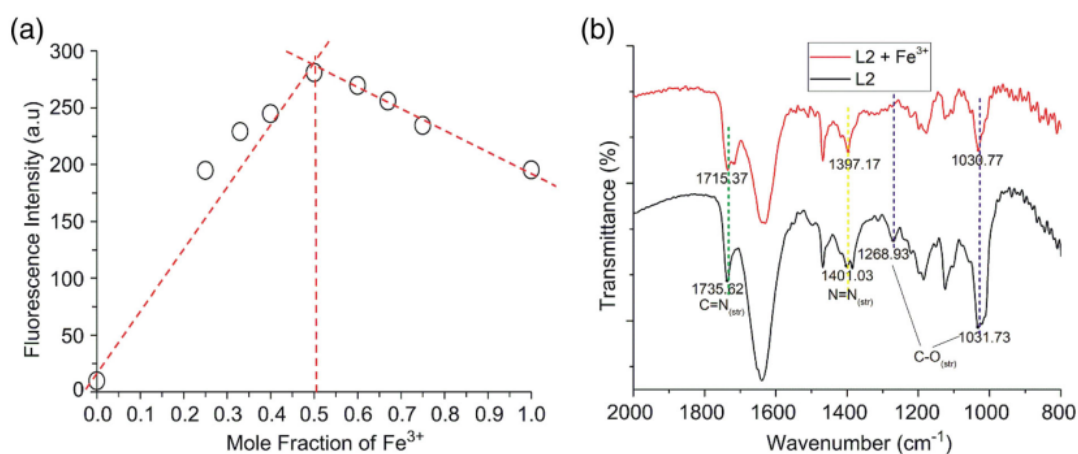


FIGURE 5 (a) Job's plot of **L2** and Fe^{3+} ions in 30 μM . (b) FTIR spectra of **L2** (300 μM) with and without the addition of Fe^{3+} ions at 10 eq. (300 μM)

indicating that the three functional groups were affected during Fe^{3+} ion sensing. Considering the Job's plot result, this could only have occurred if the complex between **L2** with Fe^{3+} ions reacted in a 1:1 stoichiometric ratio through head-to-tail and tail-to-head interactions. Moreover, **L2** contains an azo group (adjacent to a hydroxyl group) and is electron rich (N atom), and could chelate well with an Fe^{3+} ion. The imine group was also considered to induce good chelation as it was rich in electrons.^[26] As confirmed from the counteranion study, the hydroxyl group in **L2** was deprotonated due to the presence of Fe^{3+} . In addition, considering that the unfilled electrons in the d shell Fe^{3+} had strong paramagnetic properties, this could strongly affect the emission of a fluorophore unit through both electron or energy transfer to produce a quenching phenomenon.^[47] Therefore, the

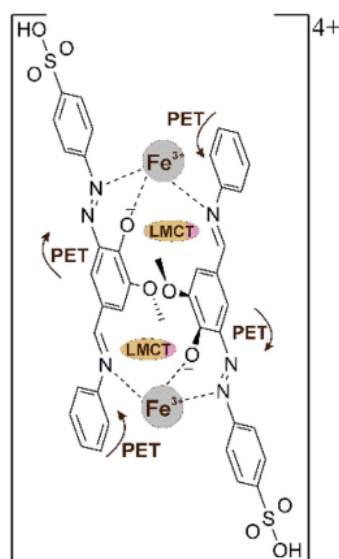


FIGURE 6 Proposed complex formation for the interaction of **L2** and Fe^{3+} ions

fluorescence intensity of **L2** was gradually quenched by the addition of Fe^{3+} ions, and ligand-metal charge transfer (LMCT) could be the most plausible mechanism for supporting the quenching phenomenon. The paramagnetic properties of Fe^{3+} could immediately trigger the $-\text{C}=\text{N}$ and $-\text{N}=\text{N}$ groups to open a deactivation channel with non-radioactive properties, and tended to facilitate electron/energy transfer, causing fluorescence quenching to occur. This interaction could lead to photoinduced electron transfer (PET) of the aromatic ring near the azo and imine groups. Therefore, binding between **L2** with Fe^{3+} ions during molecular recognition^[48] could induce the presence of both LMCT and PET (Figure 6). This formed complex could induce the quenching phenomenon with fluorescence properties from Turn-On to Turn-Off of its emission intensity, as shown in Scheme 1. Moreover, the interactions between Fe^{3+} ions and **L2** in this complex structure may attributed to the ultrasensitive and selective detection of Fe^{3+} ions in $\text{H}_2\text{O}:\text{MeOH}$ (4:1 v/v).

The effect of pH value on the $\log[\text{Fe}^{3+}]$ (detected amount Fe^{3+} ions) as calculated from the standard curve is shown in Figure 7a. The plot between ΔpH and $\log[\text{Fe}^{3+}]$ (detected amount Fe^{3+} ions) produced a linear plot with a slope of $y = 0.901x + 2.961$, demonstrating that one proton of **L2** was exchanged during sensing of Fe^{3+} ions. Considering that **L2** has a phenolic group that would allow chelation with an azo functional group, this phenolic group plays an important role in Fe^{3+} sensing. As expected and shown in Figure 7b, there was no effect when using either chloride or citrate or sulfate as counteranions due to the ion-exchange mechanism in Fe^{3+} sensing. These results were well supported for the plausible mechanism as explained above (Figure 6).

3.4 | Selectivity study on Fe^{3+} sensing in the presence of competitive metal ions

The selectivity study of Fe^{3+} sensing in the presence of other metal ions using **L2** was evaluated using other various metal ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Ba^{2+} , Mn^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , and La^{3+} .

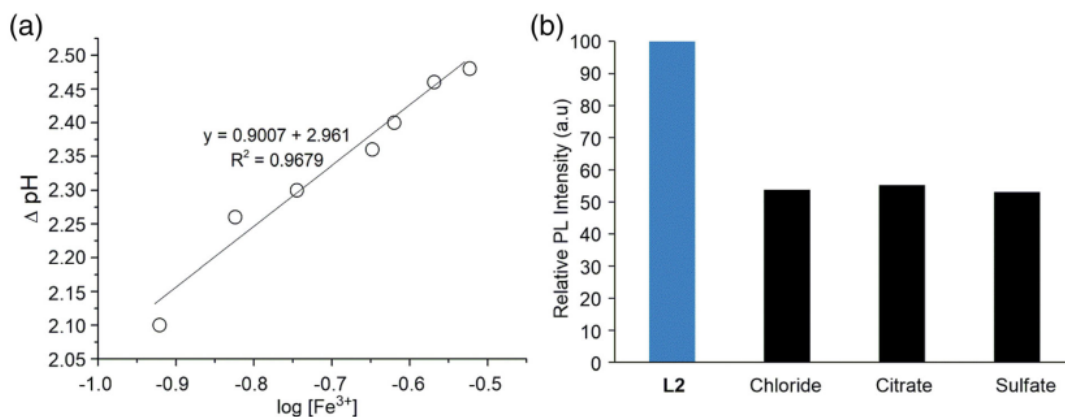


FIGURE 7 (a) Plot of ΔpH versus $\log[\text{Fe}^{3+}]$. (b) Counteranion effect of **L2** (30 μM) measured at emission 341 nm (Fe^{3+} ions in 1.0 eq.)

Figure 8 shows the fluorescence spectra of L2 after the addition of various metal ions at 10 eq. to the concentration of L2 (30 μM). Markedly, Fe^{3+} ions produced the quenching phenomenon only for the emission peak at 341 nm, while the other metal ions did not significantly change its initial emission intensity. Even though La^{3+} has the same positive charge as Fe^{3+} ions, L2 produced no significant response toward La^{3+} that was as high as that for Fe^{3+} ions. This result indicated that L2 is highly interactive only with Fe^{3+} ions to form the complex as mentioned above.

To evaluate the metal ion selectivity of L2 in the quantitative amount, the interference percentage for the detection of Fe^{3+} ions with the presence of other competitive metal ions is shown in Table 2. The presence of other metal ions together with Fe^{3+} ions did not produce any significant interference (less than 2.5%) on Fe^{3+} ion quantification at 30 μM concentration. This finding clearly demonstrated that L2 is a promising candidate to be

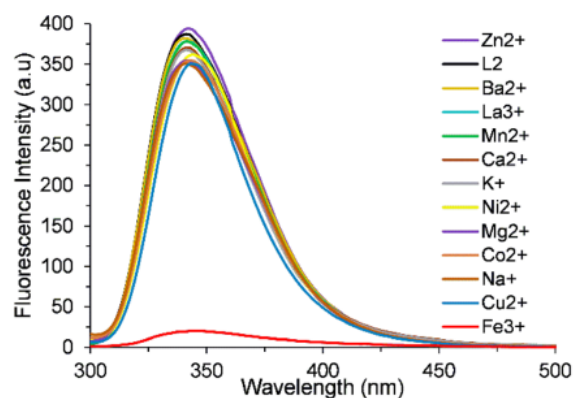


FIGURE 8 Emission spectral changes of L2 after the addition of various metal ions at the same concentrations

TABLE 2 Interference percentage of Fe^{3+} ion sensing in the presence of other metal ions at 1.0 eq. ($[\text{Fe}^{3+}] = 0.03 \text{ mM}$)

Metal ions	Interference (%)
Na^+	0.09
K^+	0.09
Mg^{2+}	0.16
Ca^{2+}	0.70
Ba^{2+}	0.81
Mn^{2+}	0.86
Co^{2+}	0.52
Cu^{2+}	2.26
Ni^{2+}	1.54
Zn^{2+}	2.33
La^{3+}	1.08
All metal ions mixture	1.84

TABLE 3 Fe^{3+} quantification from real water samples using L2 through a spiking technique

Sample	$[\text{Fe}^{3+}] (\mu\text{M})$		Recovery (%)
	Before spiking	After spiking	
Tap water	15.12	30.75	102
Drinking water A	13.04	30.19	107

used as a chemosensor agent with high selectivity for detection of Fe^{3+} ions in real sample analysis.

L2 was used to quantify Fe^{3+} ions from tap and commercially available drinking water and evaluate its potential for real applications. The recovery percentage was calculated based on spiking with 15 μM Fe^{3+} ions as a standard solution. As shown in Table 3, L2 gave 102–107% recovery percentages, which was really good enough for such simple chemosensors. Moreover, three replicate measurements for each real sample displayed an RSD of less than 3%, indicating good reproducibility of L2 to be used as a fluorescent chemosensor agent for quantification of Fe^{3+} ions in real aquatic samples. Because of that, this novel L2 probe is a promising chemosensor to be developed for fluorescence detection of toxic elements and dangerous pollutants such as lead ions with high selectivity, including with their simple portable analytical devices.^[49,50]

4 | CONCLUSION

From the results, it was found that L2 as a fluorescent chemosensor could selectively detect and quantify Fe^{3+} ions through a significant quenching phenomenon up to 100% at its emission signal at 341 nm. The LOD and LOQ values were 0.31 and 1.04 μM , which is much lower than the permitted maximum Fe^{3+} ion concentration in drinking water as stated by the recent government regulation and shows ultrasensitive detection so far in aqueous systems. Such an increment of L2 sensing capability is due to the presence of the imine group together with the azo functional groups. Moreover, the complexation study between Fe^{3+} ions and L2 was performed using Job's plot and Benesi-Hildebrand equation, and it was found that L2 and Fe^{3+} ions could form a 1:1 complex with $1.56 \times 10^4 \text{ M}^{-1}$ value as the association constant. From the interference test, it clearly showed that there was no significant interference from other metal ions towards sensing Fe^{3+} ion with L2. These findings may also shed light on the practical issues for the development of L2 as a water-soluble and a selective fluorescent chemosensor of Fe^{3+} ions, in which excellent chemosensors could be potentially applied for water monitoring in real time.

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