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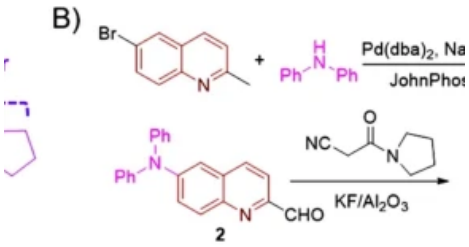

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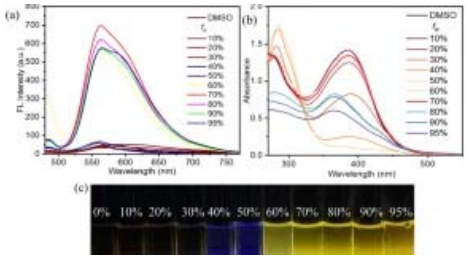

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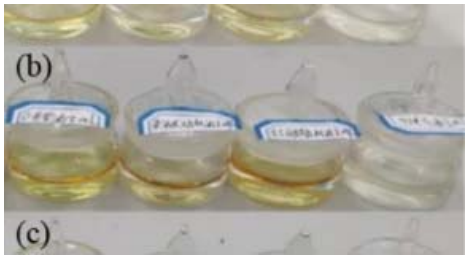

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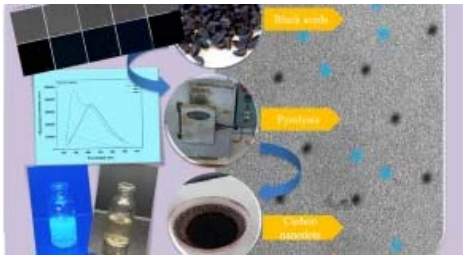

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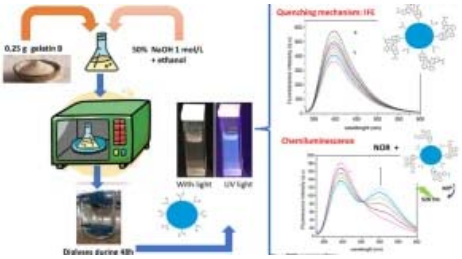

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RESEARCH

Hydrazide Schiff base Compound Containing Triphenylphosphonium Units for Fluorescence Sensing of Al^{3+} and its real Sample Applications

Ozge Gungor[1] · Muhammet Köse[1]

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Abstract

Al^{3+} excess in the body can cause many diseases. The development of chemosensors for the detection of Al^{3+} is therefore highly desirable. A hydrazide Schiff base compound containing triphenylphosphonium units (ER) was prepared and used as fluorescence turn-on sensor for the sensing of Al^{3+}. Detection of Al^{3+} among various metals has been achieved successfully through the formation of Al^{3+}-ligand coordination complexes. To detect Al^{3+}, the "turn on" property of the fluorogenic chemosensor was investigated. Fluorescence sensing studies were carried out in CH_3OH-Water (v/v, 9/1, pH 7.0) at $\lambda_{em} = 528$ nm. The LOD for sensing of Al^{3+} was found to be 0.129 μM. Using Job's graph, the stoichiometric ratio of **ER**- Al^{3+} was determined to be 1:1. The binding constant was determined to be 1.7×10^7 M^{-1} between Al3 + and the chemosensor ER. Finally, the determination of Al^{3+} in real herbal teas was carried out by using the sensing function of the chemosensor ER.

Keywords Hydrazide Schiff base · Chemosensor · Al^{3+} · Herbal tea

Introduction

In recent years, fluorescent chemosensors have become increasingly important for the detection of environmentally and biologically important metal ions, especially metal cations [1, 2]. Both in the environment and in biology, metal ions play an indispensable role [3–8]. The sensor provides direct results with fast response based on fluorescence changes caused by metal ions. Developing such sensors is therefore important. Aluminium is the third most common metal ion of biological importance. It makes up about 8% of the mass of the Earth's crust [9]. It is widely used in our everyday lives, from food additives and packaging to building and water treatment [10, 11]. Too much aluminium can be harmful to the human body, although it is not an essential element. It can damage the nervous system, tissues and cells and cause diseases like Alzheimer's, Parkinson's and kidney failure [12, 13]. Furthermore, excessive levels of Al^{3+} may stunt plant growth and threaten the existence of fish [14, 15]. The maximum concentration of Al^{3+} in drinking water should be limited to 7.4 μM according to the World Health Organization [16, 17]. For this reason, it is essential that effective analytical methods be developed for the detection of Al3 + in the environment and in biological systems.

Among the various probes, chemical probes are attractive for fluorescence sensor development because of their cost and analytical configurations. Molecular computing is the principle on which chemical probes operate. Molecular changes and intramolecular interactions control them. Light, photons, different solvents, pH and metal ion complexes can induce these changes. They can also be easily tuned/adapted to the structure of molecules. These features provided rapid response, portability and ease of use, simplicity of operation, high selectivity, sensitivity and detectability by the naked eye for medical, biological and environmental applications. A number of important reports on fluorescence-based probes for the detection of Al3 + ions have been published, including [18].

Schiff-based compounds are well known for their relatively simple synthesis and easy coordination with metal ions [19]. Therefore, we herein prepared a novel Schiff-based *via* an easy one-step reaction by reacting pyridine-2,6-dicarbohydrazide and (3-formyl-4-hydroxybenzyl)triphenylphosphonium (Fig. 1). The properties of detecting Al^{3+} ions in the aquatic environment and in the tea samples were investigated.

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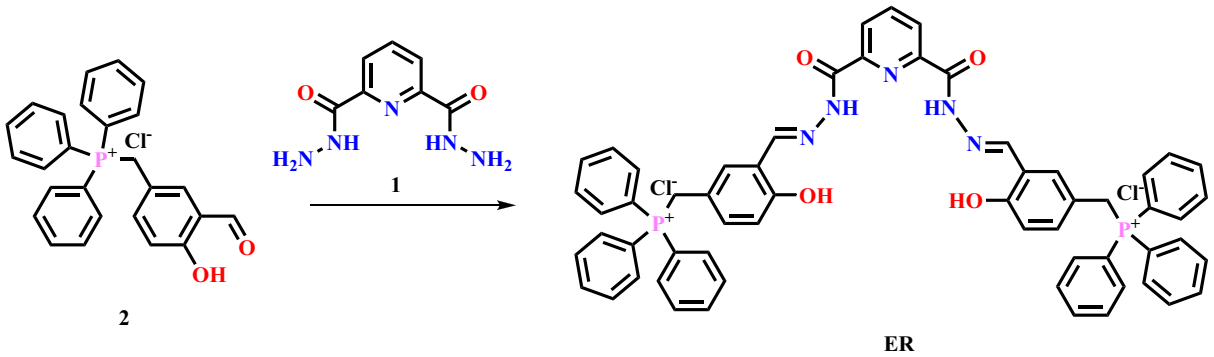

Fig. 1 Synthesis scheme of the compound

Experimental

General Methods

All starting materials (triphenylphosphine and hydrazine hydrate, salisilaldehit and HCl) and organic solvents were purchased from commercial sources and used as received, unless noted otherwise. A Perkin Elmer Paragon 1000 PC was used to measure FTIR spectra. Dimethyl pyridine-2,6-dicarboxylate, 5-chloromethyl salicylaldehyde and 3-formyl-4-hydroxybenzyl-triphenyl phosphonium chloride were prepared according to the reported procedures [20–22]. Experimental procedures and characterization data for these compounds were given in the supplementary documents.

Synthesis of Hydrazide Schiff base Compound (ER)

Compounds pyridine-2,6-dicarbohydrazide (0.19 g, 1 mmol) and (3-formyl-4-hydroxybenzyl)triphenylphosphonium (0.43 g, 2 mmol) were heated in ethanol (50 mL) solvent at reflux for 24 h. The mixture was allowed to cool to room temperature. Filtration was used to collect the resulting white solid product.

(ER) $C_{59}H_{49}N_5O_4Cl_2P_2$ MW: 1024.92 g/mol, Yield: 69%, Color: White, FT-IR (ATR, cm^{-1}): 3383, 1682, 1614, 1583, 1529, 1437, 1359, 1278, 1225, 1169, 1110, 995, 962, 839, 744, 687, 505 cm^{-1} (Fig. S1). ^{1} H-NMR (d_6-DMSO): 13.05 (2 H, s, OH), 11.29 (2 H, s, NH), 7.70 (2 H, d, Ar-H), 7.30 (2 H, s, Ar-H), 7.28 (2 H, d, Ar-H), 7.28 (2 H, d, Ar-H), 9.06 (2 H, s, CH = N), 8.36 (2 H, d, pry-CH), 8.31–8.29 (1 H, t, Pry-CH), 7.92 (7 H, s, Ar-CH), 7.94–7.90 (13 H, m, Ar-CH), 7.75–76.6 (13 H, m, Ar-CH), 7.33 (2 H, s, Ar-CH), 6.86 (4 H, d, -CH_2). ^{13} C NMR (101 MHz, DMSO) δ 160.2 (C-OH), 157.89 (C = O), 148.89 (PryAr-C), 148.72(PryAr-C), 140.22 (C = NH), 135.65 (Ar-C), 134.51 (Ar-C), 133.76 (Ar-C), 131.94 (Ar-C), 130.62 (Ar-C), 126.36 (Ar-C), 119.98 (Ar-C), 118.67 (Ar-C), 118.59 (Ar-C), 118.50 (Ar-C), 117.82 (Ar-C), 117.44 (Ar-C), 27.74 (-CH), 27,46 (-CH) (Fig. S2).

Fluorescence Sensing Studies

Fluorescence sensing experiments are given in the supplementary section.

Results and Discussion

The triphenylphosphonium based chemosensor ER was obtained from the condensation reaction of pyridine-2,6-dicarbohydrazide and (3-formyl-4-hydroxybenzyl)triphenyl phosphonium in high yield and purity. The structure of the compound was elucidated by FT-IR, ^{1} H/^{13} C NMR spectroscopies and elemental analysis.

Fluorescence Sensing of Al^{+3}

Fluorescence response of the chemosensor molecule ER towards Al^{+3}, Ba^{2+}, Mn^{2+}, Fe^{2+}, K^{+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+} and Fe^{3+} cations in CH_3OH-H_2O (1/9, v/v) media were investigated. The chemosensor molecule ER showed weak fluorescence at 450–600 nm range upon irradiation at 326 nm (Fig. S3). ESIPT (excited state intramolecular proton transfer) is often attributed to the weak fluorescence of Schiff bases. In the ESIPT phenomenon, the transfer of a hydroxy proton to the nitrogen atom of the imine group transforms the enol form of the molecule into the cis-keto tautomer at the excited state. The cis-keto tautomeric form may undergo relaxation to the trans-keto form (ground state, photochromic conversion). In the presence of Ba^{2+}, Mn^{2+}, K^{+}, Fe^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+} and Fe^{3+} cations, emission behaviour of the chemosensor ER did not show significant change. However, addition of Al^{3+} ion caused remarkable

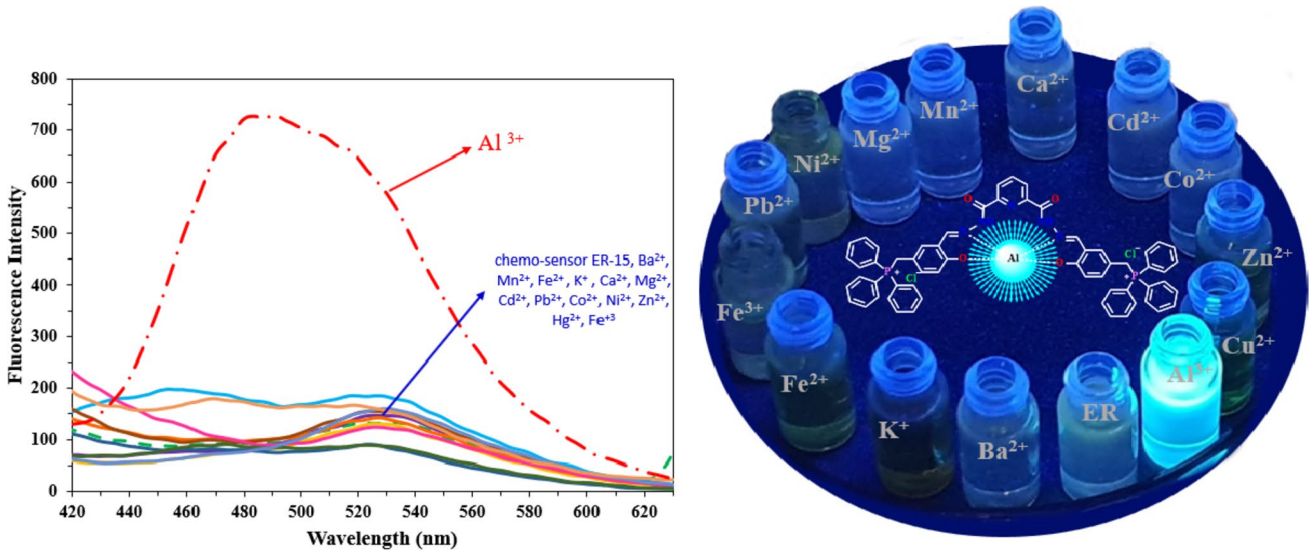

Fig. 2 The fluorescence spectra of the chemosensor ER (5.0 μM) and (**b**) fluorescence colors of the chemosensor ER in the presence of cations (10.0 eqv) in CH_3OH:H_2O, (v/v, 1/9) (λ_{ex} = 326 nm, λ_{em} = 528 nm)

fluorescence enhancement (about 7-fold) (Fig. 2). Colour changes of chemosensor ER under UV lamp (365 nm) in the presence of cations are shown in Fig. 2. Free chemosensor showed pale green colour under 365 nm lamp and introduction of cations (Ba^{2+}, Mn^{2+}, K^{+}, Fe^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Cd^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+} and Fe^{3+}) did not cause significant colour change. In the presence Al^{3+} ion, a colour of the chemosensor solution turned to bright turquoise. The significant fluorescence enhancement can be attributed to Al^{3+} coordination to **ER** via lone pairs of imine nitrogen and phenolic oxygen (N_2O_2 donor site). The formation of a stable chelated complex system (**ER**/Al^{3+}) blocks ESIPT phenomenon and caused a strong chelation-enhanced fluorescence (Fig. 3). The initial results revealed that the chemosensor ER can be used as fluorescence probe for the determination of Al^{+3} in solution.

Since electron donor/acceptor based fluorescent sensors are often perturbed by protons in the detection of metal ions, it is necessary to investigate the pH effect on the **ER** in the absence of Al^{3+} and to find the optimal buffer system. The measurements were carried out at pH values (phosphate buffer) of the solution ranging from 0 to 14.

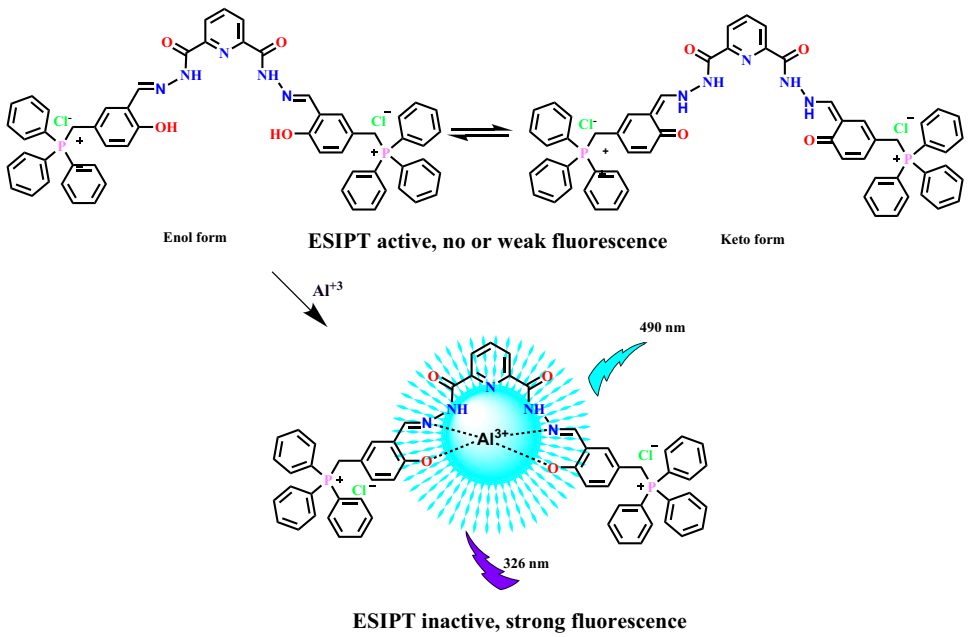

Fig. 3 The proposed sensing mechanism of ER and Al^{3+} ions

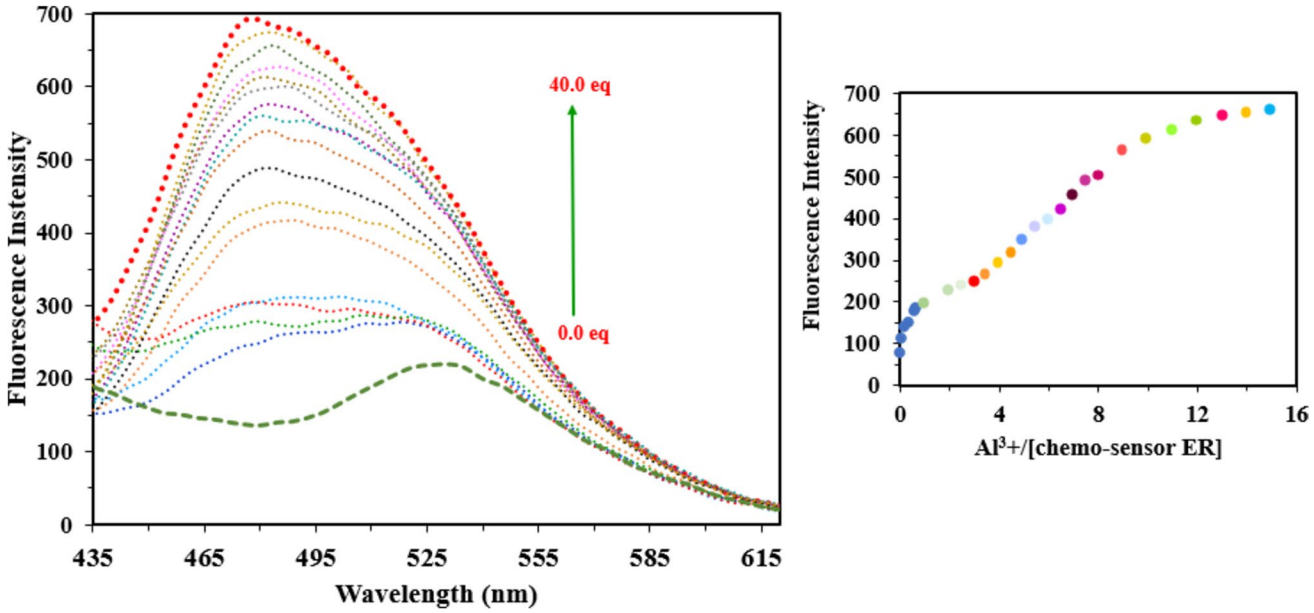

Fig. 4 Fluorescence spectral changes of the chemosensor ER solution (5.0 μM) with the various concentrations of Al^{3+} (0–10.0 eqv.) in CH_3OH-H_2O (1/9, v/v) (λ_{ex} = 326 nm)

As Fig. S4 shows, the fluorescence intensity (490nm) rose with increasing pH. In general, the complexes dissociate at low and high pH [23, 24]. The fluorescence intensity is similar to that of the sensor. In general, at low and high pH the complexes dissociate, similar to the fluorescence intensity of the sensor, because the -NH groups present in the structure have a potential 'buffering' effect, which only partly restores the fluorescence intensity of the complexes [23] The best pH under the experimental conditions was 7.0, within the biologically relevant pH range (5.5–7.5) (Fig. S4), indicating that the ER may serve as a sensor for Al^{3+} at neutral pH (around 7) for future investigations.

In order to evaluate binding properties chemosensor molecule with Al^{3+}, fluorescence titration experiments were conducted. When excited at 326 nm, chemosensor ER showed emission band at 480–600 nm range. Upon addition of particular concentrations of Al^{3+} (0.0–10.0 eqv), the emission band of ER showed blue shift accompanied by substantial fluorescence enhancement. Gradual fluorescence enhancement was observed upon incremental addition of Al^{3+} (0.0–10.0 eqv) (Fig. 4). The emission color change from pale green to bright turquoise under UV lamp (365 nm) was seen as result of the complex formation of ER with Al^{3+}. From the florescence titration experiment, the binding constant (K) of receptor ER with Al^{3+} was calculated by Benesi-Hildebrand plot (Fig. S5). The titration graph showed a linear regression curve {1/(I-I_0) versus 1/[Al^{3+}]} and binding constant of ER-Al^{3+} was calculated as 1.7×10^7 M^{-2}. From the fluorescence titration graph, limit of detection was calculated as 0.129 μM (Fig. S6).

To investigate the selectivity of the chemosensor toward Al^{3+}, competitive experiments were performed in the presence of interfering ions [Ba^{2+}, Mn^{2+}, Fe^{2+}/Fe^{+3}, K^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Hg^{2+}]. First of all, chemosensor molecule ER was treated with Al^{3+} (10 eqv) and competitive cations (10 eqv) and changes in the emission intensity at 490 nm (l_{exc}=326 nm) were recorded (Fig. 5). The fluorescence competitive experiments revealed that Ba^{2+}, Fe^{+3}, Ca^{2+}, Mg^{2+}, Ni^{2+}, K^+, Cd^{2+}, $Co^{2+}$and Zn^{2+} ions did not show significant interference effect on the sensing of Al^{3+}. On the other hand, addition of Mn^{2+}, Fe^{2+}, Pb^{2+} and Hg^{2+} to the ER-Al^{3+} system resulted in slight decrease in the emission intensity at 490 nm. On the other hand, the most obvious interference was observed in the case of Cu^{2+} ion. Upon addition of Cu^{2+} to the Er-Al^{3+} system, the emission intensity was dramatically quenched. This indicates that the chemosensor ER has a higher binding affinity for Cu^{2+} than for Al^{3+}. In the presence of Cu^{2+}, the chemosensor ER cannot be used as a fluorescence sensor for the detection of Al^{3+}.

The reversibility of complexation between receptor ER and Al^{3+} were studied by addition of EDTA solution to the complex ER-Al^{3+}. As shown in Figs. 6 and 7, complexation of Al^{3+} with chemosensor ER is reversible. Addition of EDTA to the ER- Al^{3+} system cause a sharp decrease in the emission intensity at 490 nm and this was recovered upon addition of certain amount of Al^{3+}. The results showed that the change in emission intensity at 490 nm after

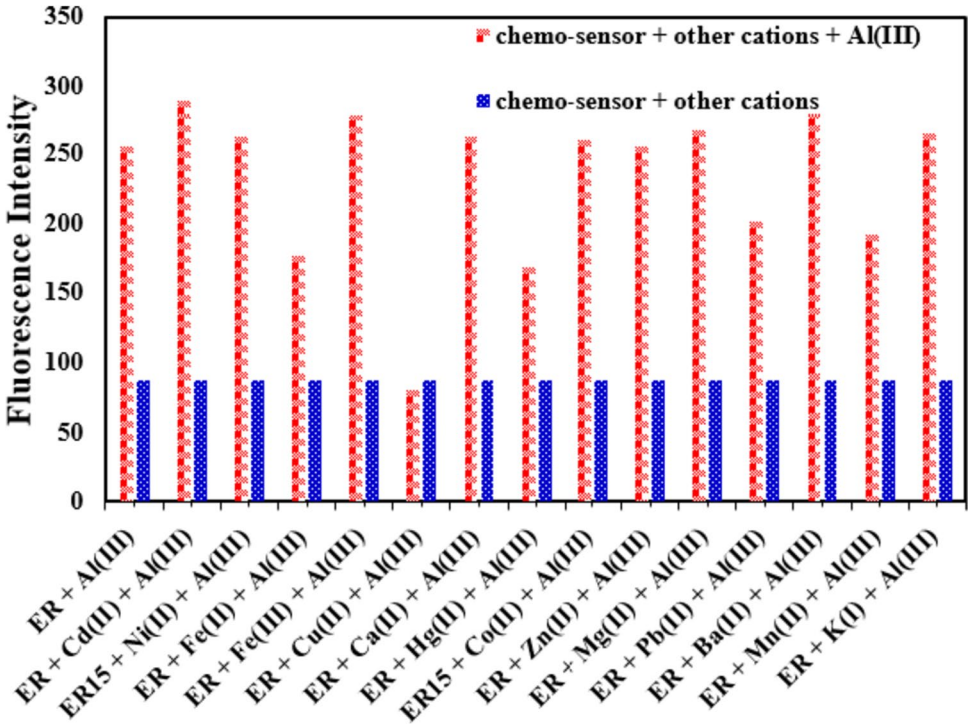

Fig. 5 Competitive experiments of chemosensors ER with Al3+ (10.0 eqv) in presence of competing cations (10 eqv) in CH3OH-H2O (1/9, v/v) (λex = 326 nm, λem = 490 nm)

alternating addition of EDTA follows a "fluorescence OFF-ON" process.

Job's plot was used to investigate the stoichiometry and binding behaviour of **ER** with Al^{3+}. As seen in Fig. 7, in accordance with the Job's plot, a maximum absorbance value at 326 nm was obtained when mole ratio fraction ([ER]/([ER] + [Al^{3+}]) was 0.50. The results revealed the 1:1 complexation between ER and Al^{3+}. There are several fluorescence probes reported for the sensing of Al^{3+} in solution. Sensing performance of ER and previously reported chemosensors are tabulated in Table 1. Fluorescence sensing properties of ER towards Al^{3+} was comparable to the reported probes in literature. Limit of detection for the detection of Al^{3+} was found as 0.129 µM. Moreover, a remarkable increase in the quantum (Φ) yield of the chemosensor ER in the presence of Al^{3+} was observed (Φ:0.074 for free ER and Φ: 0.17 for ER- Al^{3+}) with a Stokes shift ($\Delta\lambda_{em-ex}$: 490 − 326 nm = 164 nm) (Fig. S7).

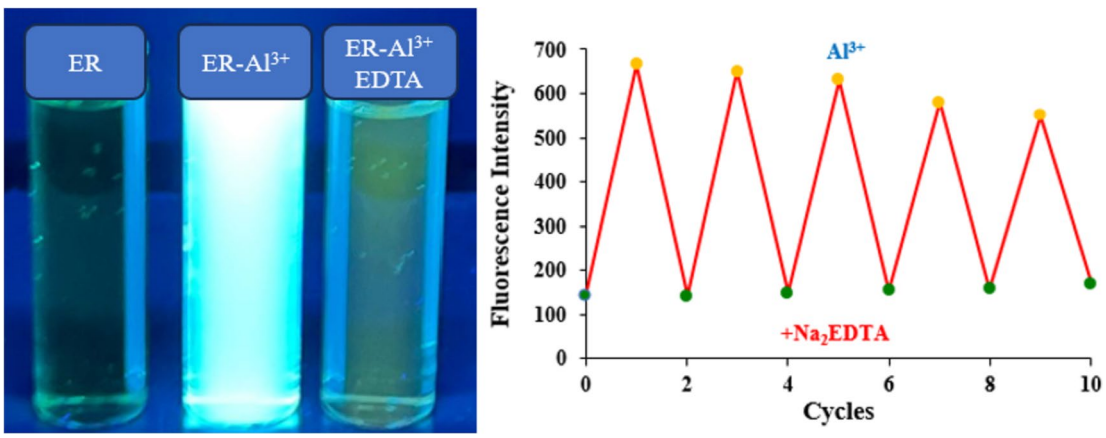

Fig. 6 **a** Color changes of Compound 1 solution in $CH_3OH:H_2O$ (9:1) by alternating addition of Al^{3+} and Na_2EDTA under UV light (λ_{ex} = 326 nm), **b** Fluorescence intensity of Compound ER at 526 nm by alternating addition of Al^{3+} and Na_2EDTA.

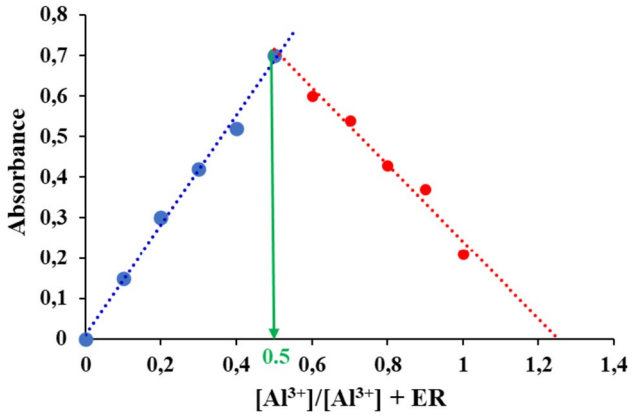

Fig. 7 Job's plot of ER and Al^{3+} ($[Al^{3+}] + [ER]$) at λ = 326 nm

Fluorescence Sensing Analysis of Al^{3+} in tea Samples

For the evaluation of the practical application of the fluorescent sensor ER, the detection of Al3 + using fluorometry was carried out on a number of herbal tea samples. The results were then compared with the values obtained from ICP-OES. According to the standard addition method, each tea sample was spiked with Al^{3+} (15 µL with 0.1 and 0.2 µM). Al^{3+} monitoring in herbal tea samples by fluorimetric and ICP-OES are given in Tables 2, S1 and S2. As can be seen in Table 2, chemosensor molecule (ER) can be employed as "fluorescence ON" sensor for the determination of Al^{3+} in foodstuffs with high sensitivity. The fluorescence data showed good recovery values (96.14-100.19%). In order to compare fluorescence

Table 1 Comparison of the fluorescence chemosensor ER with some reported studies for the recognition of Al^{3+}

Chemosensor	Fluorescence response	Solvent system	Detection limit (µM)	Binding constatnt (K_a M^{-1})	Ref.
Hydrazide-naphthlene	Turn-on	C_2H_5OH-H_2O (v/v, 1/1)	0.18	9.24×10^3	[25]
Naphthalen-Schiff base	Turn-on	C_2H_5OH-H_2O (v/v, 9/1)	3.30	1.03×10^4	[26]
Pyrene-based	Turn-on	Et-OH-HEPES (v/v, 1/1)	4.13	5.172×10^4	[27]
Schiff base	Turn-on	Et-OH-THF (v/v, 9.5/0.5)	0.38	16.2×10^3	[28]
Bis-Naphthale-Schiff base	Turn-on	CH_3CN	1	1.14×10^4	[29]
Fluorescein based	Turn-on	C_2H_5OH-H_2O (v/v, 2:3)	0.073	3.95×10^3	[30]
ER	Turn-on	CH_3OH-H_2O (v/v, 1:9)	0.113	1.7×10^7	**This work**

Table 2 Al^{3+} monitoring in herbal tea samples by ICP-OES and chemosensor **ER**

Al^{3+} (µmol L^{-1})					
	Al^{3+} added (µmol L^{-1})	Chemosensor ER	ICP-OES	Recovery (%)	difference of the means
herbal tea samples					
Fennel	0	4.157 ± 0.03	4.166 ± 0.03		-0.09
	0.1	4.165 ± 0.03	4.185 ± 0.03	97.65	-0.02
	0.2	4.204 ± 0.04	4.205 ± 0.04	98.78	-0.001
Green tea	0	9.703 ± 0.04	9.711 ± 0.04		-0.008
	0.1	9.758 ± 0.04	9.762 ± 0.04	99.54	-0.004
	0.2	9.783 ± 0.04	9.788 ± 0.02	98.78	-0.005
Chamomile tea	0	4.207 ± 0.04	4.207 ± 0.04		0
	0.1	4.251 ± 0.02	4.255 ± 0.02	98.69	-0.004
	0.2	4.278 ± 0.04	4.281 ± 0.04	97.07	-0.003
fennel tea (mixed with rose)	0	11.259 ± 0.03	11.27 ± 0.03		-0.011
	0.1	11.360 ± 0.04	11.362 ± 0.04	100.19	-0.002
	0.2	11.381 ± 0.04	11.396 ± 0.04	99.31	-0.015
Mint tea	0	3.718 ± 0.03	3.733 ± 0.03		-0.015
	0.1	3.725 ± 0.04	3.737 ± 0.04	97.56	-0.012
	0.2	3.767 ± 0.02	3.774 ± 0.02	96.14	-0.007
Hibiscus Tea	0	7.211 ± 0.02	7.233 ± 0.02		-0.022
	0.1	7.237 ± 0.02	7.259 ± 0.02	98.98	-0.022
	0.2	7.286 ± 0.04	7.288 ± 0.04	98.31	-0.002

sensing performance of ER, the amounts of Al^{3+} in herbal tea samples were also analysed by ICP-OES method. In a similar way, samples of tea were spiked with known concentrations of Al^{3+} (0.01–0.02 mg L^{-1}). The amounts of Al^{3+} was calculated from the calibrating graph. The recovery values for Al^{3+} were obtained in the range of 99.92–95.95%. There was a good correlation between the amount of Al^{3+} in the foundation and the amount of Al^{3+} in the addition. There was no significant difference between the fluorescence and ICP-OES results.

Conclusion

A novel hydrazide Schiff base compound containing triphenylphosphonium units was successfully prepared and used as fluorescence turn-on probe for the sensing of Al^{3+} in CH_3OH: H_2O, (v/v, 1/9). The fluorescence chemosensor ER revealed remarkable selectivity and sensitivity for the detection of Al^{3+} over the other common cations (except Cu^{2+}). The LOD for the detection of Al^{3+} was found as 0.129 μM and binding constant between chemosensor **ER** and Al^{3+} was found to be 1.7×10^7 M^{-1}. Finally, for the determination of Al^{3+} in herbal tea samples, the fluorescence switch-on chemosensor **ER** was used. For the determination of Al^{3+} in food, the fluorescence probe **ER** can be used.

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Declarations

Ethical Approval This article does not contain any studies involving animals performed by any authors.

Consent to Participate This article does not contain any studies involving animals performed by any authors.

Consent to Publish All authors mentioned in the manuscript have consented to submission and publication.

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