

A Simple Spectrofluorimetric Method for Iron Determination with a Chalcone-Based Schiff Base

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Abstract A chalcone-based Schiff base (**5**), capable of detecting iron (III) in partially aqueous media, has been designed, then synthesized by the condensation of 3-formyl-2-hydroxyquinoline and acetophenone. To determine iron (III) ion, a simple spectrofluorimetric method was developed by using the synthesized Schiff base. The developed method was validated by analyzing the certified reference material (CRM-SA-C Sandy Soil C). During the process of the determination of iron in food samples, satisfactory accuracy was obtained for spinach and rocket. Nitric acid and hydrogen fluoride were used for the digestion of the certified reference material whereas only nitric acid was used for food samples, in a closed microwave system. Measurements were carried out by using the modified standard addition method. The standard addition graph was linear until 5.0 mg/L. in determination of iron (III). Detection and quantification limits were 0.06 and 0.20 mg/L., respectively. The presented method is simple, time-saving, cost-effective and suitable for determination of iron content of soil and foods.

Keywords Food · Soil · Iron determination · Spectrofluorimetric analysis · Chalcone · Schiff base

Introduction

The importance of metal ions is well known for living systems [1]. In particular, iron is vital for human life [2]. While it is causing a disease known as iron deficiency anemia in excess in causing other diseases [3, 4]. Therefore, it is important to take the iron balanced nutrition. On the other hand, iron is also an important element in the earth and is effective for plant growth. For this reason, the determination of the iron content in food and soil, is an important issue.

Atomic methods such as AAS, AES and GF-AAS are usually used to determine the iron content of food and soil samples [5–8]. Generally, separation and preconcentration processes are applied in these expensive methods before the determination stage. Moreover, these require long analysis time and using of some chemicals such as adsorbent and stripping reagents [9–11]. Consequently, it is important to develop an alternative simple, reliable, fast and cheap method to determine the iron content of food and soil samples.

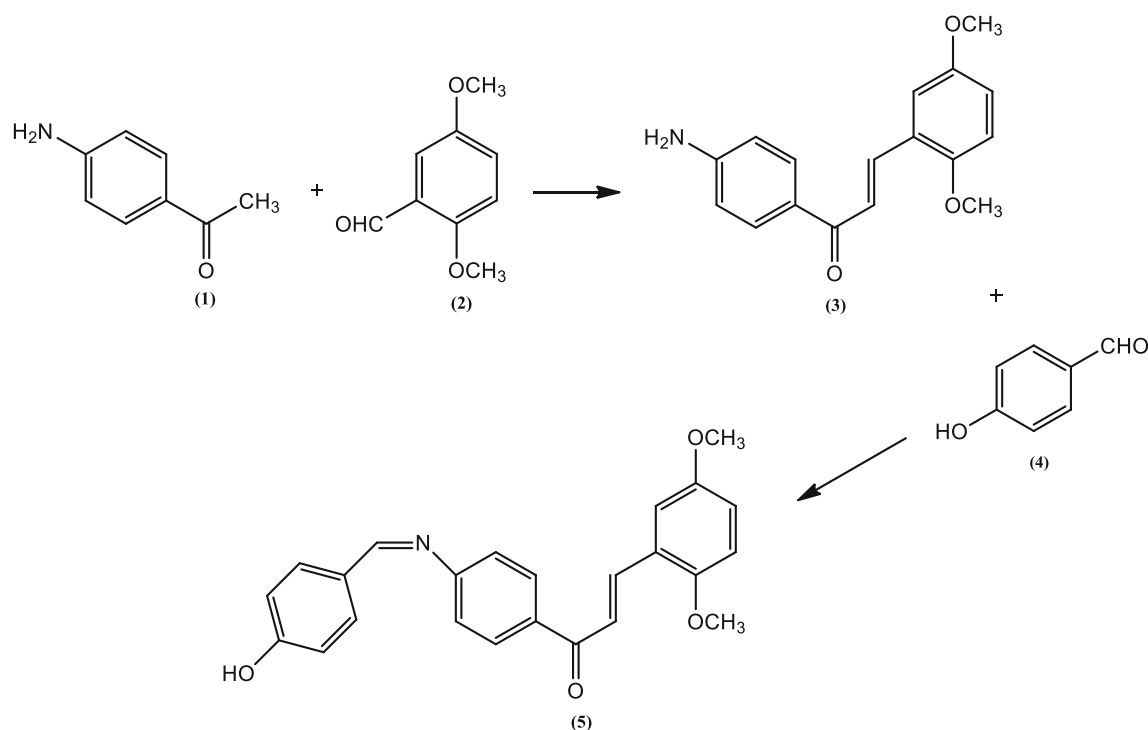
Many spectrofluorimetric methods to determine metal ions are reported in the literature [12–14]. However, there are limited spectrofluorimetric methods to determine iron in real samples [15–17]. Moreover, these methods require long time for sample treatment before the determination stage and the use of many chemicals and extra process [16, 17].

Selective fluorescent ligands can be used as complexation reagent to develop simple and fast metal determination methods [18–20]. In this context, we recently focused flavonoid-like compounds to evaluate them as analytical reagent. Conjugated π electron systems and heteroatoms in their structure make them potential fluorescent ligands to interact with metal ions. Moreover, flavonoids are natural products and known as harmless compounds.

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Scheme 1 Synthetic pathway to Schiff base derived from chalcone (5)

Therefore, flavonoid-like compounds may be considered as advantageous analytical reagents. The purpose of this study is to reveal the metal sensor properties of a flavonoid-class chalcone compound. Chalcones are the aromatic ketones belonging to 1,3-diaryl-2-propen-1-ones. Chemically they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α , β -unsaturated carbonyl system. The presence of reactive unsaturated ketone in chalcone may result in complexes with metal ions. [21, 22].

The new chalcone-based Schiff base was used as a selective fluorescent ligand to determine iron(III) ion. Fluorescence intensity of the system having many conjugated π electrons decrease increasing with iron(III) ion concentration. This property was used as an analytical response to develop a new spectrofluorimetric method to determine iron (III). The proposed method was applied in some food samples to determine iron amount. According to our knowledge, there is no report of a chalcone-based Schiff base used in metal determination in real samples in the literature. The present study is a first in this sense.

Fig. 1 Effects of Li^+ , Na^+ , K^+ , Be^{2+} , Mg^{2+} , Ca^{2+} , Sr^{2+} , Y^{3+} , Ti^{4+} , V^{5+} , Cr^{3+} , Mo^{6+} , W^{6+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Au^{3+} , Zn^{2+} , Cd^{2+} , B^{3+} , Al^{3+} , As^{5+} , Se^{2+} , NH_4^+ ions on fluorescence spectra of the ligand in ethanol-water (1:1). (Ligand concentration = 2.6×10^{-5} M. Metal concentrations = 2.6×10^{-4} M. Excitation at 360 nm)

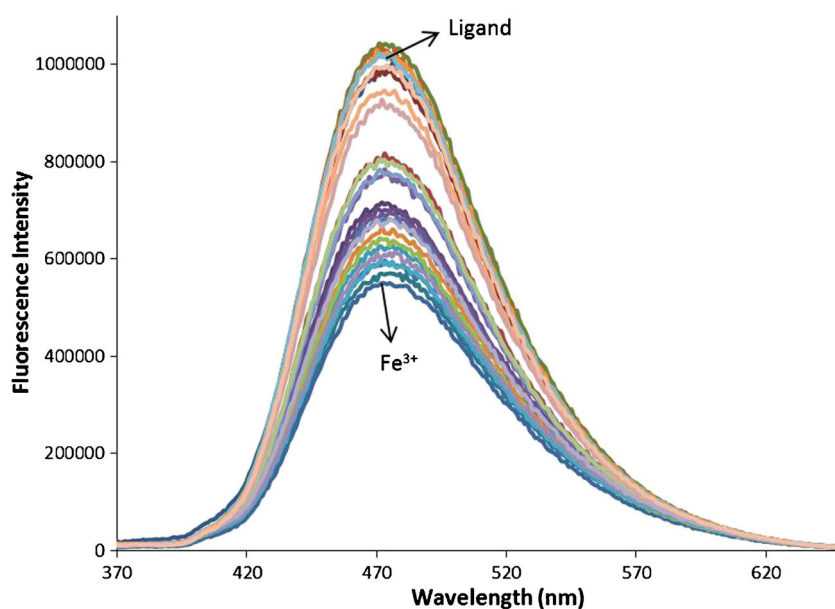
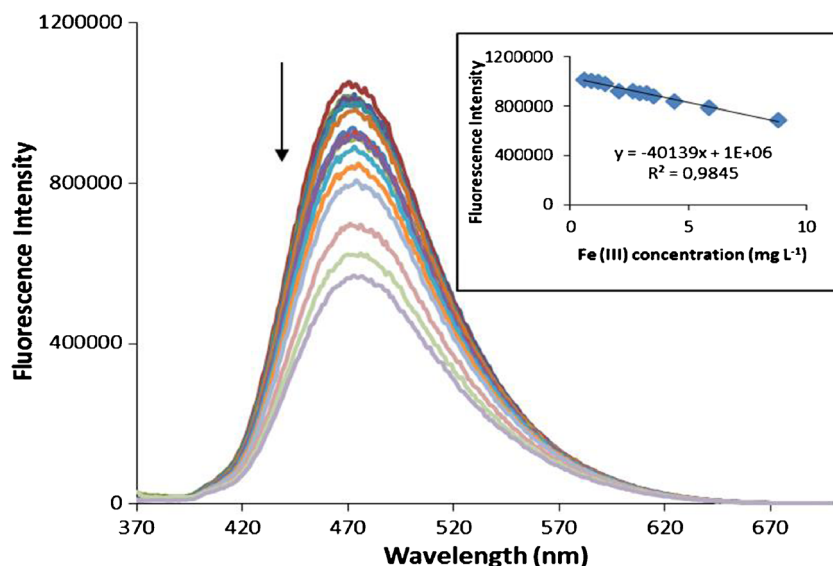


Fig. 2 Variation of the emission spectra of compound (**5**) with wavelength during the addition of iron (III). Ligand concentration: 2.6×10^{-5} M. Inset: Variation of fluorescence intensity with increasing iron (III) concentration in the range of 0.2 to 8.8 mg L⁻¹ at 470 nm



Materials And Methods

Instrumentation

An Analytik Jena Specord 210 spectrophotometer and a PTI spectrofluorimeter (QM 2006 model) were used to measure absorbance and fluorescence intensity, respectively. The excitation wavelength was 360 nm and fluorescence emission spectra were recorded in the range of 370–650 nm with a slit width of 1.0 nm. CEM Mars 6 Microwave system was used to digest the samples.

Reagents

In all analytical measurements, pipettes and vessels were kept in dilute nitric acid at least overnight and were subsequently washed three times with distilled water. Analytical grade chemicals and solvents were purchased from Merck (Darmstadt/Germany) and Fluka (Buch/Switzerland).

1000 mg/L. of stock solutions of metal cations were prepared by dissolving an appropriate amount of nitrate salts in deionized water. Working standard solutions were obtained by appropriate dilution of these stock standard solutions. Sandy soil standard reference material (CRM-SA-C) was supplied from High-Purity Standards, Inc. A 1.0×10^{-3} mol/L. solution of the ligand was prepared by dissolving appropriate amount of compound (**5**) in 100 ml ethanol and was kept in refrigerator at 4 °C for one week.

Samples

Rocket and spinach samples were purchased from local supermarkets in Trabzon, Turkey. The vegetable samples were washed thoroughly with tap and deionized water. Then, the samples were dried at 105 °C for 24 h and ground. The digestion of the dried samples (0.5 g) was carried out by using 10.0 mL of HNO₃ and the digestion of the standard reference material (0.5 g) was carried out by using 2.0 mL of HCl,

Fig. 3 Job's Plot of the iron (III) complex

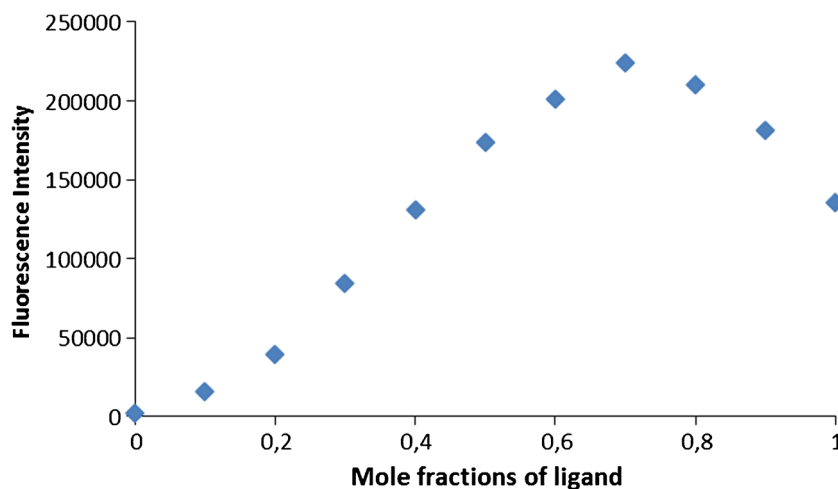
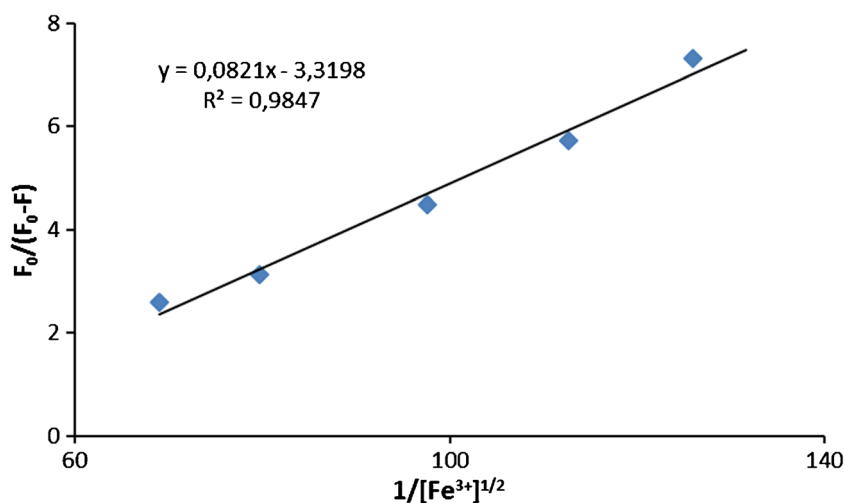


Fig. 4 Plot of $F_0/(F_0-F)$ versus $1/[\text{Fe}^{3+}]^{1/2}$ for the spectrofluorimetric titration of the ligand (2.6×10^{-5} M) with Fe (III)



3.0 mL of HNO_3 , and 5.0 mL of HF in a closed microwave digestion system. After the microwave digestion process, the solutions were filtered through a 0.45 mm cellulose nitrate membrane and evaporated. Then, the volume was completed to 10 mL with deionized water and used directly in the modified standard addition method as the sample solution.

Synthesis of the Ligand

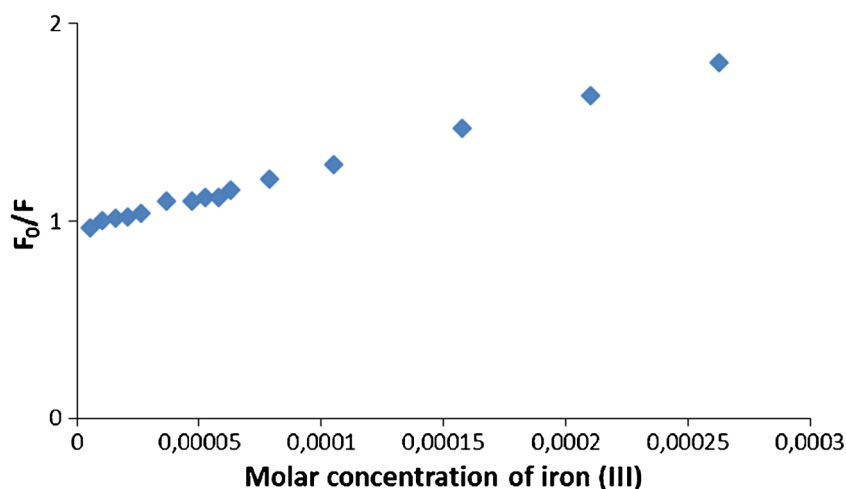
The synthetic pathway to new Schiff base ligand, (2E)-3-(2,5-dimethoxyphenyl)-1-((4-hydroxybenzylidene)amino)phenyl}prop-2-en-1-one (**5**), is summarized in Scheme 1. 4-Aminoacetophenone (10 mmol) (**1**) was dissolved in ethanol (25 mL) and an aqueous solution of NaOH (20%, 15 mL) was added dropwise to this solution. The reaction mixture was stirred for 30 min. at room temperature. Then, 2,5-diamino-methoxybenzaldehyde (10 mmol) (**2**) was added to the reaction mixture. The solid formed was filtered off and dried in vacuo. The yellow solid product (yield 80.0%) was obtained. To obtain the final compound (**5**), 4-hydroxybenzaldehyde (10 mmol) was added to the ethanolic solution

of the compound (**3**) (25 mL) and the reaction mixture was refluxed at 80 °C for 12 h. The yellow solid formed was filtered off and dried in vacuo. Yield: 70%; ^1H NMR ($\text{DMSO}-d_6$, TMS, 400 MHz, δ ppm): 10.62 (1H, bs, Ar-OH), 8.52 (1H, s, $-\text{CH}=\text{N}-$), 8.21 (2H, d, Ar-H, $J=8$), 8.05 (1H, d, $=\text{CH}$, $J=16$), 7.96 (1H, d, $=\text{CH}$, $J=16$), 7.81 (2H, d, Ar-H, $J=8$), 7.34 (2H, d, Ar-H, $J=8$), 7.04 (3H, bs, Ar-H), 6.91 (2H, d, Ar-H, $J=8$), 3.76 (3H, t, $-\text{OCH}_3$, $J=8$), 3.70 (3H, t, $-\text{OCH}_3$, $J=8$). ^{13}C NMR ($\text{DMSO}-d_6$, TMS, 100 MHz, δ ppm): 188.5, 162.1, 161.2, 156.6, 153.7, 153.1, 138.3, 134.9, 131.6, 130.4, 127.6, 124.0, 121.6, 118.5, 116.3, 116.2, 113.4, 113.1, 56.5, 56.1.

Optimum Conditions for Iron (III) Determination

The proposed method is based on the fluorescence quenching of the Schiff base compound (**5**) with iron (III) ion. Only a few minutes was enough before the fluorescence intensity of the solutions was measured. A modified standard addition method was used to determine iron content of the samples. A similar approach has already been used for iron (III) determination with other fluorescent ligands [23, 24]. Various constant iron

Fig. 5 Stern-Volmer plot for the quenching of the ligand by iron (III)



(III) concentrations (0.5–5.0 mg/L) were tested to obtain accurate results with the modified standard addition method. Optimum constant iron (III) concentrations were different for each sample matrix. 0.5 mg/L. was the optimum constant iron (III) concentration for the sandy-soil reference material while it was 1.5 mg/L. and 2.0 mg/L. for rocket and spinach, respectively.

Results And Discussion

Synthesis of the Ligand

There are some synthesis methods for chalcone-based Schiff bases in the literature [25–27]. In the presented study, the pathway is given in the Scheme 1. The chalcone compound (**3**) has been obtained by the reaction between aminoacetophenone and 2,5-diamino-methoxybenzaldehyde in NaOH media. Then, the chalcone based Schiff base (**5**) was obtained from the reaction between 4-hydroxy-benzaldehyde and the chalcone compound (**3**), in ethanolic solution. The characterization of the new compound (**5**) is given in the materials and methods part.

Spectrofluorimetric Measurements

Various excitation wavelengths between 400 and 300 nm were tested to obtain the highest fluorescence intensity for the ligand. The maximum emission band was obtained at 470 nm when the ligand is excited at 360 nm. The effect of many cations on the emission spectra of the ligand was investigated. Figure 1 shows the effects of Li^+ , Na^+ , K^+ , Be^{2+} , Mg^{2+} , Ca^{2+} , Sr^{2+} , Y^{3+} , Ti^{4+} , V^{5+} , Cr^{3+} , Mo^{6+} , W^{6+} , Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Au^{3+} , Zn^{2+} , Cd^{2+} , B^{3+} , Al^{3+} , As^{5+} , Se^{2+} , NH_4^+ ions (2.6×10^{-4} M) on the fluorescence spectra of the ligand (2.6×10^{-5} M). As

Table 1 Analytical performance data of the proposed standard addition method for total iron determination

Excitation wavelength (nm)	360
Emission wavelength (nm)	470
Limit of detection ($\mu\text{g mL}^{-1}$)	0.06
Limit of quantification ($\mu\text{g mL}^{-1}$)	0.20
Linear range ($\mu\text{g mL}^{-1}$)	0.2–5.0
Constant iron (III) conc. ($\mu\text{g mL}^{-1}$) for sandy-soil CRM	0.5
Constant iron (III) conc. ($\mu\text{g mL}^{-1}$) ($\mu\text{g mL}^{-1}$) for rocket	1.5
Constant iron (III) conc. ($\mu\text{g mL}^{-1}$) ($\mu\text{g mL}^{-1}$) for spinach	2.0
Ligand concentration (mol L^{-1})	2.6×10^{-5}
Ligand volume (mL)	2
Total volume (mL)	4
Solvent	ethanol:water (1:1)
Time before measurement	2 min.
Correlation coefficient (R^2) for spinach	0.9918

Table 2 Determination of iron in two reference materials ($N = 3$)

Sample	Found value (mg/g) \pm % RSD	Certified value (mg/g) \pm % RSD
Sandy Soil C	16.5 ± 1.1	15.8 ± 1.2

seen from Fig. 1, there are moderate changes in fluorescence intensity of the ligand for the tested ions. Most of ions caused decrease of fluorescence intensity except for Ti^{4+} , Be^{2+} , B^{3+} and NH_4^+ ions. These ions caused a little fluorescence enhancement at 470 nm. Fe^{3+} causes the most fluorescence quenching. Spectrofluorimetric titrations showed that there is regular fluorescence quenching with increasing iron (III) concentrations at 470 nm. Therefore, this quenching was used as an analytical response for iron (III) determination.

Effect of Reagent Concentration

To determine optimum reagent concentration, spectrofluorimetric titrations were performed by using solutions of the ligand in the range of $5.0 \times 10^{-5} - 3.0 \times 10^{-6}$ M. The values of fluorescence intensity at 470 nm were plotted against the iron(III) concentration. A standard iron(III) solution was used in the experiments. When 2.6×10^{-5} M of ligand concentration was used, the highest R^2 value (0.9845) was obtained (Fig. 2). Therefore, further studies were carried out at this concentration.

Effect of Constant Iron Concentration

The constant iron (III) concentration used in the modified standard addition method was optimized by changing from 0.2 to 3.0 mg/L. for each sample matrix. The optimum constant iron (III) concentrations were 0.5 mg/L., 1.0 mg/L. and 1.5 mg/L. for Sandy Soil C, rocket and spinach, respectively. The relative errors below 5% were considered in the determination of the optimum constant iron (III) concentration.

Time before Measurement

Emission spectrum of the mixture containing an equivalent amount of iron (III) and the ligand (2.6×10^{-5} M) was obtained in the range of 1–10 min. It was seen that the fluorescence

Table 3 Determination of iron in the food samples ($N = 3$)

Sample	Proposed method Found value	Standard method (ICP-OES)	
		Found value	MU ^a
Spinach	230.1 ± 1.4^a	219.2	17.8
Rocket	615.7 ± 1.6^a	607.0	49.2

^a Measurement uncertainty

intensity had not changed after two minutes. There was very little change in the fluorescence intensity from 2.0 until 10 min, which means that a few minutes before the measurements was enough.

Composition and Stability of the Complex

The nature of the complex in ethanol/water (1:1) was investigated with Job method. The concentrations of ligand and iron (III) were 2.6×10^{-5} M and the total solution volume was 5 mL. As seen from Fig. 3, a plot of fluorescence intensity against mole fraction of the ligand shows a maximum at 0.7 that clearly indicates 2:1 (L:M) stoichiometric formation of iron (III) complex. The conditional formation constant ($\log K$), was calculated according to the known method [28] using the spectrofluorimetric titration data, was found to be 1.06 (Fig. 4).

Quenching Mechanism

The Stern-Volmer relationship [29] expresses the efficiency of quenching of a fluorophore by a quencher (Eq. 1).

$$F_0/F = 1 + K_{sv}[Q] \quad (1)$$

Where F_0 and F are the fluorescence intensity in the absence and the presence of the quencher, respectively. K_{sv} is the Stern-Volmer quenching constant and $[Q]$ is the concentration of the quencher. If a system obeys the Stern-Volmer equation, a plot of F_0/F versus $[Q]$ will give a straight line with a slope of K_{sv} and a y-axis intercept of 1.

Figure 5 shows the steady-state emission Stern-Volmer analysis for the complexation of iron (III) by the ligand. F_0/F term linearly increased with increasing iron (III) concentration until 2.6×10^{-4} M. These results are consistent with the static quenching.

Analytical Performance

An external calibration line based on fluorescence quenching of 2.6×10^{-5} M ligand showed linearity within the concentration range of 0.2 to 8.8 mg/L. (Fig. 2 inset). However, accuracy was not good in iron determination in samples using the external calibration graph. Because of this, a modified standard addition method was used in iron determination. The analytical performance data for the developed method under the optimized conditions are presented in Table 1.

As seen from the Table 1, a linear relationship was found between the fluorescence intensity at 470 nm and the concentration of the iron (III) in the range of 0.2–5.0 mg/L. For sandy-soil CRM, the correlation coefficient was 0.9918 indicating linearity. R^2 values were 0.9932 and 0.9898 for rocket and spinach, respectively. The LOD ($3 \times S_d/m$) and LOQ ($9 \times S_d/m$) were determined by using the standard deviation

of eleven measurements of the blank response (S_d) and the slope of the calibration line (m) according to the IUPAC recommendations.

Accuracy

The accuracy of the proposed method was verified by analyzing a sandy-soil standard reference material (CRM-SA-C Sandy Soil C). A good consistency was found between the obtained and certified values (Table 2). Statistical analysis of the results from the Student's t-test show no significant difference between the obtained and certified values.

The food samples were analyzed by the modified standard addition method. Analysis of the same food samples was carried out by a standard ICP-OES method. The results are given in Table 3. Statistical analysis of the results from the Student's t-test showed no significant difference between the results.

Advantages of the Proposed Method

The method hereby presented is effective in various food and sandy-soil samples. One of its many advantages is that before the determination stage, a closed microwave digestion of the sample is enough. The separation of iron (III) ion from the sample solution and sample treatment stage before measurement are both not needed in the usage of this method. It is also proved to have the ability to handle the matrix effect that might occur. It does not need more than a couple of minutes to stable fluorescence response before measurement. The reagent used in this report is a kind of flavonoid compound; being known as useful compounds for human body, making the reagent environment-friendly. Consequently, these advantages make the proposed method time-saving, simple and economic when compared to other several methods in the literature.

References

1. Sigel A, Sigel H, Sigel RKO (eds) (2006) Neurodegenerative diseases and metal ions: metal ions in life sciences. Wiley, London
2. Abbaspour N, Hurrell R, Kelishad R (2014) Review on iron and its importance for human health. *J Res Med Sci* 19:164–174
3. Aggett PJ (2012) Iron. In: Erdman JW, Macdonald IA, Zeisel SH (eds) Present knowledge in nutrition, 10th edn. Wiley-Blackwell, Washington, pp. 506–520
4. Murray-Kolbe LE, Beard J (2010) Iron. In: Coates PM, Betz JM, Blackman MR, et al. (eds) Encyclopedia of Dietary Supplements, 2nd edn. London and New York, pp 432–438
5. Ghaedi M, Niknam K, Shokrollahia A, Niknam E (2009) Determination of Cu, Fe, Pb and Zn by flame-AAS after preconcentration using sodium dodecyl sulfate coated alumina modified with complexing agent. *J Chin Chem Soc* 56:150–157
6. Dos Santos LMG, Araujo RGO, Jacob SDC, Vale MGR, Martens A, Martens IBG, Becker-Ross H (2009) Simultaneous determination of Cd and Fe in beans and soil of different regions of Brazil

- using high-resolution continuum source graphite furnace atomic absorption spectrometry and direct solid sampling. *J Agric Food Chem* 57:10089–10094
7. Da-Col JA, Domene SMA, Pereira-Filho ER (2009) Fast determination of Cd, Fe, Pb, and Zn in food using AAS. *Food Anal Methods* 2:110–115
 8. Jorhem L, Engman J (2000) Determination of lead, cadmium, zinc, copper, and iron in foods by atomic absorption spectrometry after microwave digestion: NMKL collaborative study. *J AOAC Int* 83: 1189–1203
 9. Baezzat MR, Maleki H, Moghadamhosseini E, Tabandeh M (2014) Determination of trace amounts of zinc, iron and copper by flame atomic absorption after their preconcentration using sodium dodecyl sulfate (sds) coated alumina nanoparticles modified with 3-mercapto-d-valin from environmental samples. *Am J Anal Chem* 5:1228–1238
 10. Khayatian G, Hassanpoor S, Nasiri F, Zolali A (2012) Preconcentration, determination and speciation of iron by solid-phase extraction using dimethyl (E)-2-[(Z)-1-(acetyl)-2-hydroxy-1-Propenyl]-2-Butenedioate. *Quim Nov* 35:535–540
 11. Roldan PS, Alcántara IL, Rocha JC, Padilha CCF, Padilha PM (2004) Determination of copper, iron, nickel and zinc in fuel kerosene by FAAS after adsorption and pre-concentration on 2-aminothiazole-modified silica gel. *Ecl Quím São Paulo* 29:33–40
 12. Kim SD, Lee DH, Kim JS (2008) Fluorescence spectrometric determination of aluminum(iii) ion in water using 4-chloro-2-phenyliminomethylphenol. *Bull Kor Chem Soc* 29:245–248
 13. Silvia C, Lopez P, Raimundo IM Jr, Moreno-Bondi MC, Orellana G (2010) Simultaneous determination of copper, mercury and zinc in water with a tailored fluorescent bipyridine ligand entrapped in silica sol-gel. *Anal Bioanal Chem* 398:3127–3138
 14. Al-Kindy SMZ, Al-Hinai KH, Suliman FEO, Al-Lawati HJ, Pillay A (2011) Development of a selective fluorimetric technique for rapid trace determination of zinc using 3-hydroxyflavone. *Arab J Chem* 4:147–152
 15. Zhou Q, Liu W, Chang L, Chen F (2012) Spectral study of the interaction between 2-pyridinecarbaldehyde-p-phenyldihydrazone and ferric iron and its analytical application. *Spect Chim Acta Part A: Mol Biomol Spect* 92:78–83
 16. Ragos CG, Demertzis MA, Issopoulos PB (1998) A high-sensitive spectrofluorimetric method for the determination of micromolar concentrations of iron(iii) in bovine liver with 4-hydroxyquinoline. *Il Farmaco* 53:611–616
 17. Shamsipur M, Sadeghi M, Garau A, Lippolis V (2013) An efficient and selective fluorescent chemical sensor based on 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophane as a new fluoroionophore for determination of iron(iii) ions. A novel probe for iron speciation. *Anal Chim Acta* 761:169–177
 18. Dutta M, Das D (2012) Recent developments in fluorescent sensors for trace-level determination of toxic-metal ions. *Trends Anal Chem* 32:113–132
 19. Liu SR, Wu SP (2011) An NBD-based sensitive and selective fluorescent sensor for copper(ii) ion. *J Fluoresc* 21:1599–1605
 20. Antony EJ, Raj M, Paulpandi RQ, Paulraj MS, Enoch ISMV (2015) A highly selective fluorescent sensor for Pb²⁺ based on a modified β -cyclodextrin. *J Fluoresc* 25:1031–1036
 21. Yue Y, Zhang Y, Song W, Zhang X, Liu J, Zhuo K (2014) Manganase dioxide-mediated oxidative coupling of 1,3-dicarbonyl compounds with α,β -unsaturated ketones: direct access to 3,4-dicarbonyl substituted furans. *Adv Synth Catal* 356:2459–2464
 22. Yue Y, Sun Y, Zhao S, Yan X, Li R, Shi Y, Zhuo K, Liu J (2016) Chloroacetate promotes and participates in the oxidative annulation of pyridines/isoquinoline by using oxygen and oxidant. *An Asian Journal*. doi:10.1002/asia.201601233
 23. Başoğlu A, Tosun G, Ocak M, Alp H, Yaylı N, Ocak Ü (2015) A simple and time-saving method for iron determination based on fluorescence quenching of an azaflavanon-3-ol compound. *J Agric Food Chem* 63:2654–2659
 24. Çağlar Y, Saka ET, Alp H, Kantekin H, Ocak U, Ocak M (2016) A simple spectrofluorimetric method based on quenching of a nickel(II)-Phthalocyanine complex to determine iron (III). *J Fluoresc* 26:1381–1389
 25. Mohsin HF (2013) Synthesis of some new pyrimidines from chalcone containing an imin group. *Asian J Research Chem* 6: 849–854
 26. Kudale SD, Deodhar MN (2012) Synthesis and evaluation of some coumarin containing potential antimicrobial agents. 9:2493–2500
 27. Kalanithi M, Rajarajan M, Tharmaraj P, Sheela CD (2012) Spectral, biological screening of metal chelates of chalcone based Schiff bases of N-(3-aminopropyl) imidazole. *Spectrochim Acta A* 87: 155–162
 28. Bourson J, Valeur B (1989) Ion-responsive fluorescent compounds. 2. Cation-steered intramolecular charge transfer in a crowned merocyanine. *J Phys Chem* 93:3871–3876
 29. Guilbault GG (1990) *Practical Fluorescence*, 2nd edn. Marcel Dekker, New York