MODESTUM

Synthesis a New Schiff Base as a Chelating Agent for Reliable Quantification of Zinc from Water and Biological Samples

Malihe Ebrahimpour [1], Ghodsieh Bagherzade [1*], Seyed Ali Akbar Sajadi [2], Rouhollah Khani [1]

[1] Department of Chemistry, Faculty of Science, University of Birjand, Birjand, IRAN
[2] Sharif University of Technology, Tehran, IRAN

ABSTRACT

A new Schiff base, 2-[(4-Chloro-2-oxo-2H-chromen-3-ylmethylene)-amino]-propionic acid was successfully synthesized and characterized by proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), Fourier transform infrared (FT-IR), elemental analysis (CHN) and UV–Vis spectroscopy. It was found out that the synthesized Schiff base could be used as a novel and reliable candidate to detect and quantify of Zn (II) ions in water and biological samples. Some effective parameters on quantification of Zn (II), such as pH, Schiff base and salt concentration were inspected by a (2^3) full factorial design using Design-Expert, software. Under optimum conditions, the calibration graph was linear in the range 0.04–10.0 µg mL^{-1} with a limit of detection of 0.01 µg mL^{-1}. The proposed method was successfully applied to the rapid determination of Zn (II) in water and biological samples. The validation was performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) with satisfactory results.

Keywords: synthesis, Schiff base, multivariate optimization, zinc, flame atomic absorption spectrometry, biological samples

INTRODUCTION

Zn (II), which is present in the variety of foods has an important role in the enzymes operations. It is an essential element for the proper functioning of the immune, cellular replication, digestive and nervous systems [1,2]. The low concentrations of this element can be harmful for plants, animals and human body due to its bioaccumulation, nonbiodegradable properties and its toxicity for the brain [3]. Zn (II) has the antioxidant properties at low concentrations and a potent prooxidant effect from moderate to high concentrations on the human body. The biological effects of Zn (II) exactly depend on its dose. A diet with excess Zn (II) can cause disorder of energy metabolism or enhance oxidative stress [4,5]. Growth retardation, altered immune response, premature birth, weight loss, and anorexia are some of the consequences of Zn (II) deficiency [6] can be caused by the lack of dietary intake of bioavailable Zn (II), or various illnesses, which impairs food intake [7,8]. Foods are the main intake source of Zn (II) as well as tap water; however, tap water should not have toxic levels of Zn (II). Toxicity could arise from corrosion processes in water pipes [9]. High levels of Zn (II) associated with other toxic metals have been recently found to be present both in the ground and tap water [10,11]. Therefore, it is necessary to develop a reliable and efficient method for monitoring and quantification of Zn (II) in water samples.

Several common methods have been proposed for the determination of zinc, such as UV–Vis spectrometry [12], flame atomic absorption spectrometry (FAAS) [13-15], electrothermal atomization (ETAAS) [16], inductively coupled plasma optical emission spectrometry (ICP-OES) [17-19], and inductively coupled plasma mass spectrometry (ICP-MS) [20]. Each kind of these techniques has advantages and disadvantages that make them suitable only for certain situations. Analytical methodologies based on inductively coupled plasma optical emission or mass spectrometry (ICP-OES or ICP-MS) have often been developed when a multi-elemental analysis is required. These techniques are time-consuming and expensive, although, they provide good sensitivity and excellent selectivity [21]. Moreover, to develop a proper inexpensive instrumental analytical method for determination of
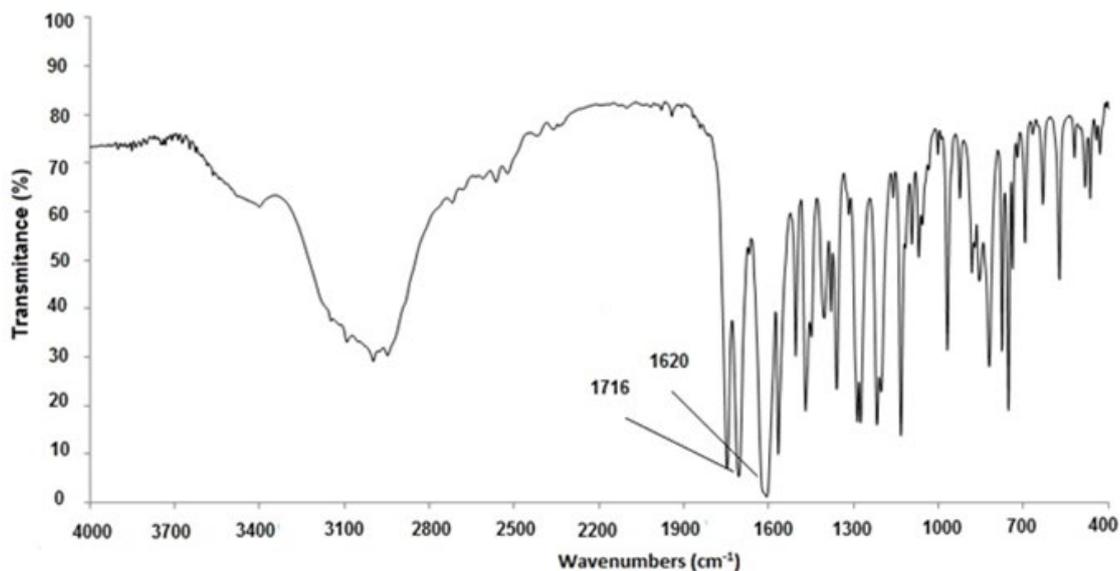
Figure 1. FT-IR spectra of Schiff base ligand derived from L-alanine

heavy metals is still a challenging task in analytical chemistry. Electrochemical techniques such as anodic stripping voltammetry [22] offer very low detection limit, the small instruments and low cost, however, the reproducibility of the results is not as good as expected. Methods based on classical line source atomic absorption spectroscopy (AAS) are used for the single-element determination with adequate sensitivities, reasonable cost, and relatively simple operation. In fact, these approaches can be considered the most common spectrometric methods for routine quantitative tasks in the laboratory [23].

Schiff bases have been reported to form metal complexes of wide biological applications due to their stability in different oxidation states [24,25]. These compounds are bestowed by good anticancer, antibacterial, antifungal, and herbicidal activities [26,27]. Schiff base ligands have played an integral and important role in coordination chemistry since the late 19th century. They contain strong donor sites like phenoxo-oxygen atoms as well as imine nitrogen atoms and find extensive applications in different fields for their special coordination ability with transition metal ions.

In the present study, considering economic aspects and operation simplicity, a new Schiff base ligand derived from L-alanine was synthesized and used as a good and efficient chelating reagent for determination of Zn (II) in water and biological samples by FAAS spectrometry. Influence of important variables (pH, the concentration of Schiff base and $NaNO_3$) were investigated and optimized by central composite design (CCD) combined with response surface methodology (RSM). The results obtained by the proposed method were validated with ICP-AES.

RESULTS AND DISCUSSION

Characteristics of Schiff Base Ligand derived from L-Alanine

In the FT-IR spectrum of the ligand, a sharp band appeared at 1620 cm^{-1}, which is attributed to the vibrations of the imine group [28,29] showing the successful preparation of the Schiff base ligand and accompanying a strong band at 1716 cm^{-1} assigned to the carbonyl stretching vibration (**Figure 1**). The broad band in the region 2800–3300 cm^{-1} shows the presence of the intermolecular hydrogen bond due to carboxylic OH groups of Schiff bases.

The electronic absorption spectrum of the Schiff base ligand in ethanol depicted in **Figure 2** shows three bands at 216, 248 and 336 nm, the first two intense bands exhibit maximum absorbance, which is thought to be due to aromatic rings present in the ligand structure involving π-π* transitions. The less intense band at 336 nm are probably due to π-π* and n–π* transitions.
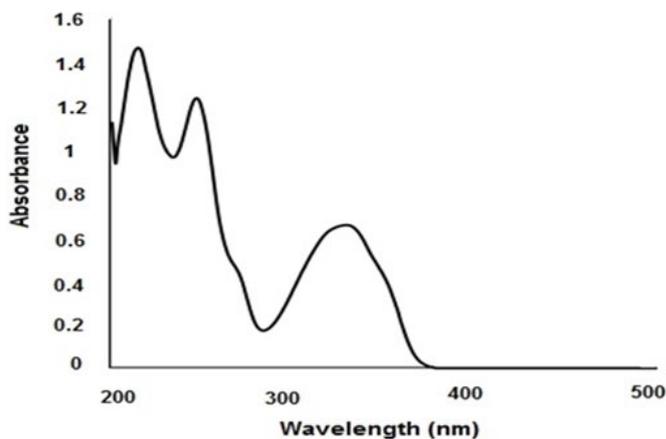
Figure 2. UV–Vis spectra of Schiff base ligand derived from L-alanine in ethanol

Table 1. Uncoded and coded levels of independent variables for determination of Zn (II)

Variables	Symbols	Range and levels				
		Low (-2)	-1	0	+1	High (+2)
pH	A	3.6	5.0	7.0	9.0	10.4
Conc. of Schiff base (M)	B	5.00×10^{-7}	8.00×10^{-6}	1.90×10^{-5}	3.00×10^{-5}	3.75×10^{-5}
Conc. of NaNO$_3$ (M)	C	0.03	0.10	0.20	0.30	0.37

The chemical structure was confirmed by the elemental analysis regarding the carbon (C), hydrogen (H) and nitrogen (N) contents in the ligand. The resulted data of the elemental analysis of the synthesized Schiff base ligand are in good agreement with the calculated values. Anal. Calc. for ligand ($C_{13}H_{10}NO_4Cl$): C 56.3%, H 3.26%, N 5.03%, Found: C 57.1%, H 3.69%, N 5.1%.

^{1}H NMR chemical shift data, expanded ^{1}H NMR spectra, ^{13}C NMR chemical shift data and expanded ^{13}C NMR spectra of the synthesized Schiff base ligand have been shown in **Figure 1** and **Figure 2**, respectively.

Response Surface Modeling

Response surface modeling (RSM) is a statistical method that uses quantitative data from appropriate experiments to determine regression model and operating conditions [30]. RSM helps to evaluate the relative significance of the variables that affect the process. The main objective of RSM is to determine the optimum set of operational variables for the pertinent process [31]. In the present study, the linear, quadratic and interactive influences of the three process variables [pH (A), the concentration of Schiff base (B) and the concentration of NaNO$_3$ (C)] on the determination of zinc were investigated. The five different levels of the three selected variables are presented in **Table 1**.

## Experimental Design and Quadratic Model

Central composite design (CCD), a standard RSM design has been widely used for fitting a second-order model which requires a minimum number of experiments to be performed. The total number of experiments to be conducted in this type of design is 20 according to a 2 level study with three variables (as per 2^3 full factorial designs). A quadratic model was selected for developing the mathematical relationship between the response and the three operating process variables. A polynomial regression modeling was implemented between the response variable, absorbance and the corresponding coded values (A, B, and C) of the three different process variables ending up with the best fitted model equation as follows:

$$\text{Absorbance Zn (II)} = 0.95 + 0.00076 * A + 0.047 * B + 0.18 * C + 0.013 * AB + 0.0075 * AC - 0.017 * BC + 0.009 * A^2 + 0.0072 * B^2 - 0.037 * C^2 \tag{1}$$

Eq. (1) was used to estimate the influence of the process variables on the response factor (Absorbance), from aqueous solution by the synthesized Schiff base ligand derived from L-alanine. The statistical significance of the model equation was examined by the F-test for analysis of variance (ANOVA). The ANOVA statistics for the response absorbance is shown in **Table 2**. The regression coefficients and the ANOVA results (**Table 2**) further suggested that the concentration of Schiff base (B) and concentration of NaNO$_3$ (C) are among the parameters exhibiting the most significant effect on the absorbance intensity. As could be seen in **Table 2** the P-value for the Zn^{2+} absorbance (p-value <0.0001) is lower than 0.05 illustrating that quadratic model was significant. The low

Table 2. Tolerance limits of interfering species in determination of Zn (II) (3.0 µg mL^{-1})

Interferent species	Tolerance limit (µg mL^{-1})
Na$^+$,K$^+$, Cl$^-$	1000
F$^-$, SO$_4^{2-}$, SCN$^-$, NO$_3^-$, CO$_3^{2-}$, CH$_3$COO$^-$	500
Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Hg^{2+}, Fe^{3+}	100
Cu^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+}	30
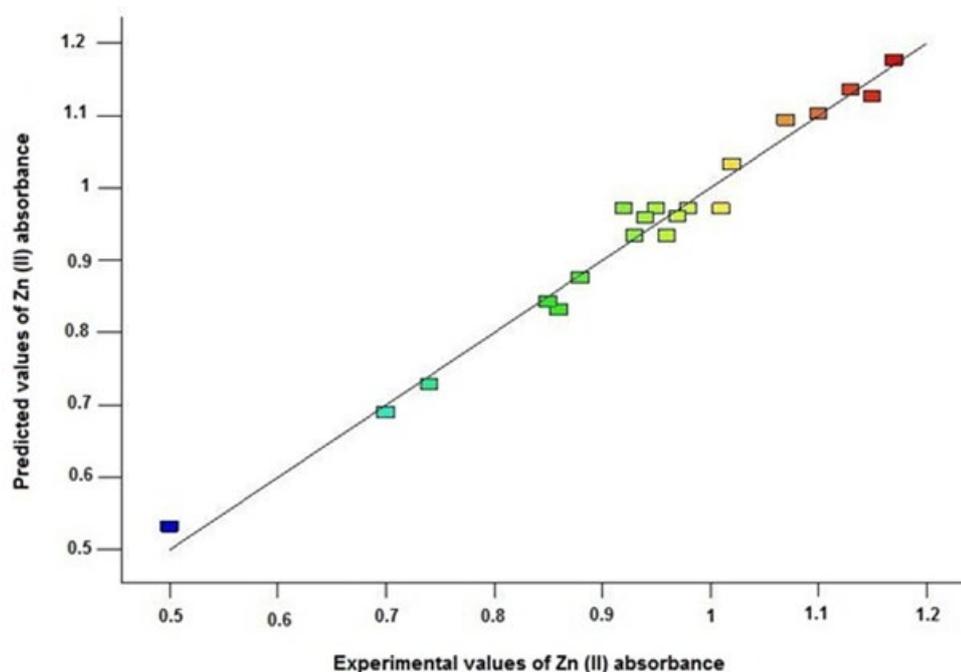
Figure 3. The experimental data vs. the predicted data of absorbance of Zn(II)

value of standard deviation (0.032) between the experimental and predicted results shows that Eq. (1) adequately represents actual relationship between the response and significant variables. High value of R² (98.2%) and R² (adjusted) (96.3%) exhibits a high dependence and correlation between the observed and the predicted values of response. This is also evident from the fact that the parity plot depicted in **Figure 3**, which shows the predicted values, obtained from the respective model of Eq. (1), versus the experimental results, contains the Zn^{2+} absorbance as response. The experimental values are the measured response data for a particular run, and the predicted values were evaluated from the model and generated by using the approximating functions. The predicted values are quite close to the experimental, imparting the model developed is successful in creating the correlation between the experimental and predicted values. The "lack of fit test" compares the residual error to the "Pure Error" from replicated experimental design points. The p-value, greater than 0.05, for the response indicated that lack of fit for the model was insignificant (p-value = 0.659). Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. The adequate precision for Zn^{2+} absorbance was 27.17, demonstrating that the model is significant for the process.

Interpretation of Normal Probability and Response Surface Plots

The normal (percentage) probability plot (NPP) is a graphical technique to assess whether or not a data set and its normal distribution. The NPP of the residuals is an important diagnostic tool to detect and explain the systematic departures from the assumptions. The errors are normally distributed and independent of each other, while the error variances are homogeneous [30]. Therefore, a NPP of the residuals is depicted in **Figure 4**. The residual is the difference between the observed and the predicted value (or the fitted value) from the regression. As it is obvious from **Figure 4**, the normal probability of residuals tells almost no serious violation of the assumptions underlying the analyses. Satisfactory normal distribution, confirms the normality assumptions made earlier and the independency of the residuals.
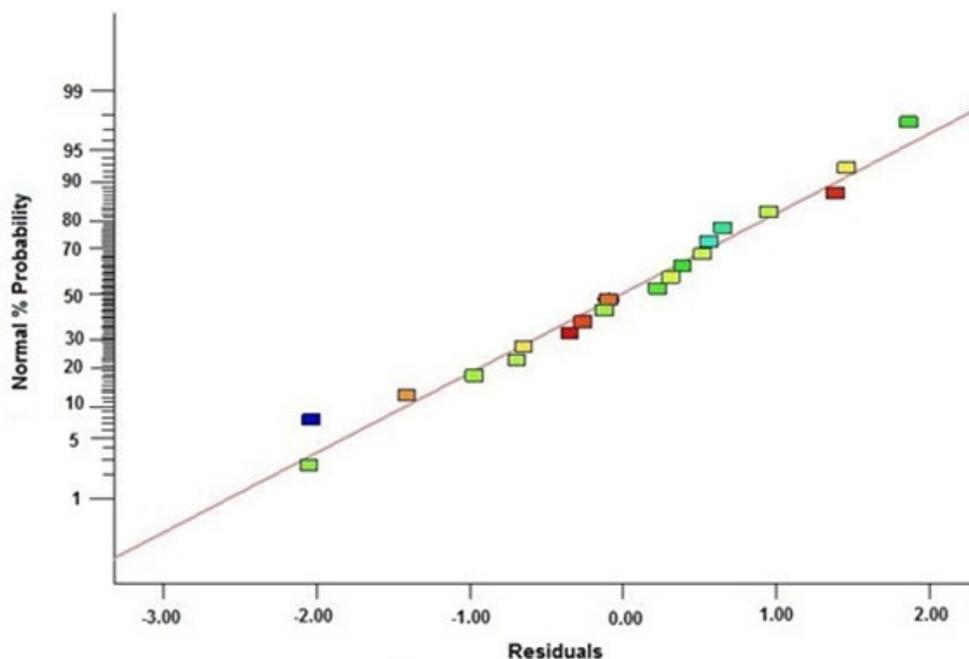
Figure 4. Normal probability plot for residuals

The results were interpreted by using graphs obtained from the model. The three-dimensional (3-D) response surfaces plot of the response, using Eq. (1) when one of the variables is fixed at the central point and the other two are allowed to vary are shown in **Figure 5a** and **Figure 5b**. **Figure 5a** shows the 3-D response surfaces representing the combined effect of concentration of $NaNO_3$ and pH on the absorbance intensity of the Zn^{2+}. The complex formation reaction of Zn^{2+} ions with **L** depends on pH. Sodium acetate–acetic acid (pH 3.5–6.5), ammonium chloride–ammonia and phosphate buffers (pH 7.0–10.5) were used for pH studies. From the **Figure 5a**, it was observed that the complex exhibits maximum absorbance in the pH >7.0. Increase in pH of the solution above 9.0, leads to the formation of precipitation of Zn^{2+} ions and absorbance intensity reduction. Hence, further studies were carried out at pH 8.0. The influence of the ionic strength was also probed. The concentration of $NaNO_3$ varied from 0.1 to 0.3 mol L^{-1}. By increasing $NaNO_3$ concentration, the extraction efficiency enhanced due to the salting-out effect. So, a concentration of 0.3 mol L^{-1} $NaNO_3$ was selected for subsequent experiments in order to increase the recovery (**Figure 5a**).
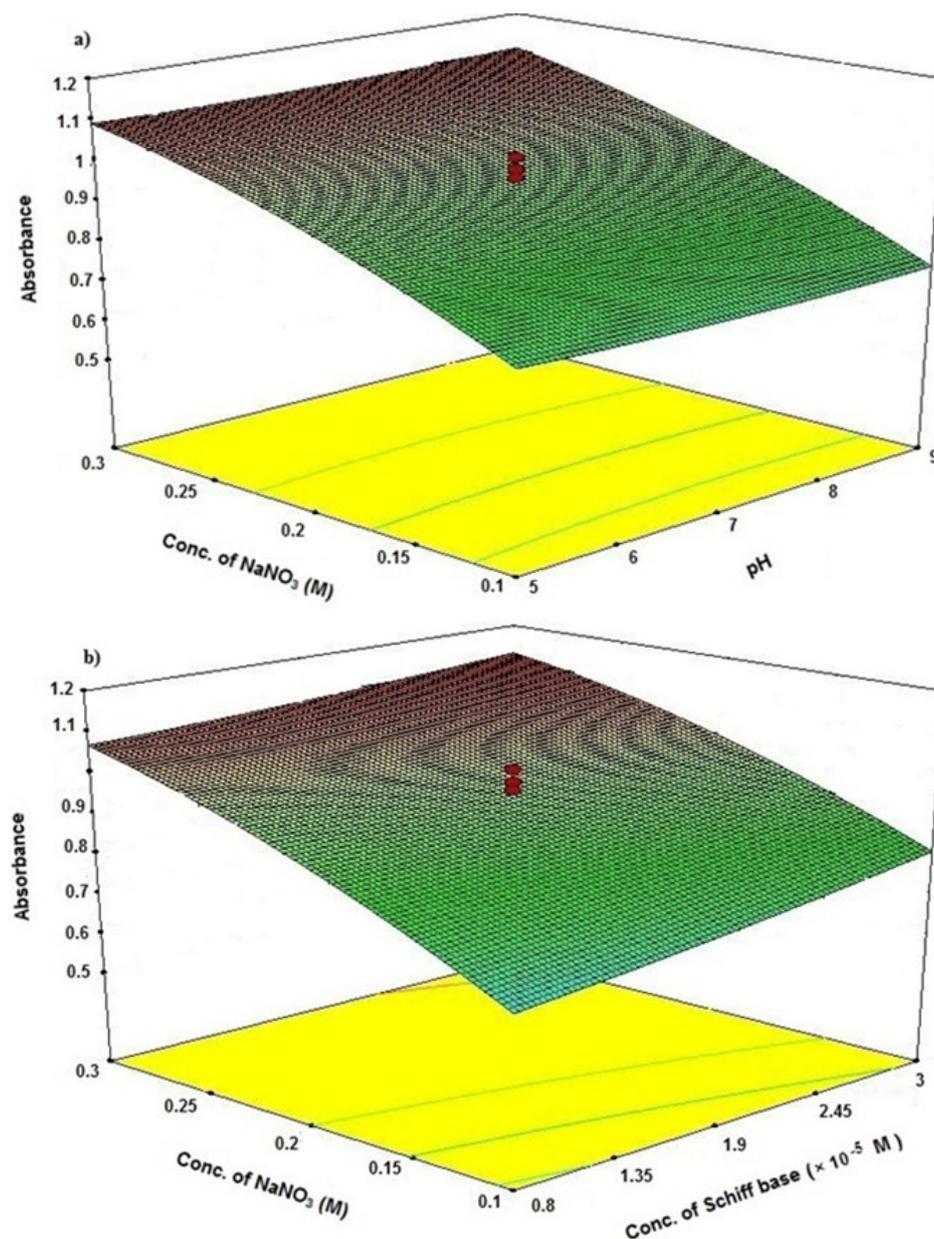
Figure 5. The 3-D plots for Zn(II) absorbance vs. (a) pH and concentration of NaNO$_3$ and (b) concentration of Schiff base and NaNO$_3$ concentration

The combined effect of the concentration of Schiff base and NaNO$_3$ concentration was analyzed and presented in **Figure 5b**. It indicates that the absorbance was elevated with the increase in concentration of Schiff base and raised with increase in NaNO$_3$ concentration. As **Figure 5b** shows, it is apparent that the lower concentrations of Schiff base leads to decrease in complex formation. Therefore from the absorbance values, it was observed that 3.0×10^{-5} mol L^{-1} Schiff base was sufficient to get maximum complex formation.

Optimization Values for the Determination of Zinc (II)

Optimization of the process variables to maximize the absorbance of the Zn^{2+} by the synthesized Schiff base ligand from the aqueous solution was performed using the quadratic model within the studied experimental range of various process variables. The process optimization modeling suggested the optimum values of different process variables (viz pH 8.0, Schiff base concentration 3.0×10^{-5} mol L^{-1}, and NaNO$_3$ concentration 0.3 mol L^{-1}) in order to obtain the maximum absorbance of the Zn^{2+}. The validity of triplicate assenting experiments at the optimized values of all parameters was also investigated. The results are closely related to the data obtained from desirability optimization analysis using CCD.

Table 3. Zinc contents in the water and biological samples determined by the proposed and ICP-AES methods

Sample	Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (%)	ICP-AES
Mineral water	-	0.20 (3.6)[a]	-	0.25 (3.4)
	0.5	0.67 (3.4)	94	0.73 (3.2)
Drinking water[b]	-	0.43 (3.7)	-	0.40 (3.3)
	0.5	0.88 (3.8)	90	0.92 (3.0)
Miami river water	-	1.80 (4.0)	-	2.00 (3.5)
	1.0	2.68 (3.6)	88	2.90 (3.7)
Blood serum 1	-	1.60 (3.5)	-	1.70 (3.3)
	0.2	1.78 (3.2)	90	1.92 (3.6)
Blood serum 2	-	1.45 (2.8)	-	1.50 (3.0)
	0.2	1.64 (3.3)	95	1.65 (3.2)

[a] Relative standard deviation (n= 3).
[b]Miami spring water.

Study of Interferences

The effect of other ions on the recovery of Zn^{2+} was tested using different amounts of ions commonly present in water, which were added to test solution containing 3 µg mL^{-1} Zn^{2+}. Any ion interferes when it causes a variation in the absorbance signal greater than ±5%. The tolerance limits for the various ions are given in Table 3. These results demonstrate that even large amounts of some common ions do not interfere with the determination of Zn^{2+}, and confirms the selectivity of the proposed method.

Analytical Performance

Under optimum conditions, the calibration curves were observed as linear in the concentration range of 0.04–10.0 µg mL^{-1} Zn^{2+} when using 5.0 mL of the solution. The correlation coefficient of the calibration curve equation was 0.9998 for Zn^{2+}, which indicates that a good linear regression has been established between the absorbance and the concentration. The limit of detection (LOD), based on a signal-to noise ratio (S/N) of 3, was 0.01 µg mL^{-1}. The relative standard deviation (RSD) of the method, determined by analyzing the standard solution at 1 µg mL^{-1} of Zn^{2+}, which was 3.5% (n = 5).

Application to Water and Biological Samples

The proposed method was applied to determine Zn^{2+} ions in various water (mineral, river and spring water) and biological samples to establish its applicability for real-world samples. The accuracy of the method was assessed by using the standard addition method and validated by ICP-AES. There is no statistical difference between them. The results are presented in **Table 3**. The percentage recovery (R) was calculated by using the equation: $R=100(C_s−C_0)/m$ where C_s is a value of the metal in a spiked sample, C_0 is a value of the metal in a sample and m is an amount of spiked metal. The results obtained indicate that the proposed method is suitable for Zn^{2+} determination in water and biological samples.

CONCLUSIONS

A new Schiff-based ligand derived from L-alanine was synthesized and investigated as a good candidate for detection and quantification of Zn^{2+} ion in real water and biological samples. The synthesized Schiff-based ligand structure was characterized by UV-Vis, FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The RSM based on full factorial CCD was used to determine the effect of three different process variables, such as pH, the concentration of Schiff base and NaNO$_3$ concentration on the zinc quantification. The optimization process for the maximum absorbance of the target metal gave the conditions process (pH 8.0, Schiff base concentration $3.0 × 10^{−5}$ mol L^{-1}, and NaNO$_3$ concentration 0.3 mol L^{-1}). The use of the response surface plots allow us to analyze the influence of the studied variables on the absorbance intensity yielding the combination of factors.

The proposed method represents a valuable contribution to green chemistry since the absence of the sample preparation step reduces the consumption of the various reagents. Additionally, the method is simple, economical, and rapid, which becomes a suitable alternative to costly ICP-OES or ICP-MS methods. Precision and accuracy were also tested; validation was performed by ICP-AES with satisfactory results. Finally, the proposed method was successfully applied to quantify of Zn^{2+} in mineral, Miami River, Miami spring water (as drinking water) and blood serum samples.

EXPERIMENTAL

Reagent and Standard Solutions

All the chemicals and solvents used for the synthesis without further purification were purchased from Merck (Darmstadt, Germany). 4-Chloro-3-formylcoumarin was synthesized by using literature procedures [32]. All solutions were prepared with analytical grade reagents. Stock solutions of Zn (II) (1000 mg L^{-1}) were prepared by dissolving Zn (NO$_3$)$_2$ (Merck, Darmstadt, Germany) in deionized water. Working standard solutions were freshly prepared by appropriate dilution of stock solutions with deionized water to the required concentrations. Deionized water was used throughout the experiments.

Apparatus and Software

A Shimadzu AA-6300 (Japan) flame atomic absorption spectrometer (air/acetylene flame) equipped with a deuterium lamp background and zinc hollow cathode lamp was used. A zinc hollow-cathode lamp (Hamamatsu Photonics, Shizuoka, Japan) was applied as the radiation source, operated at 5 mA with a monochromator spectral bandpass of 0.9 nm. For the zinc detection, the wavelength was set at 213.7 nm resonance line. The FT-IR spectra were recorded on a Shimadzu FT-IR spectrometer (4000–400 cm^{-1}) in KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were acquired with a Bruker Advance DPX-400. Spectrometer was operating at 400 MHz using CDCl$_3$ as the solvent. Chemical shifts are given in parts per million relative to tetramethylsilane (TMS) as the internal standard. The C, H, N contents were determined using Costech 4010 CHNS elemental analyzer. UV–Visible A-160 Shimadzu spectrophotometer having a photomultiplier tube (PMT) detector for absorption studies was utilized. A Varian 735-ES ICP-AES was used to validate the determination of Zn (II). A Metrohm (model 780) digital pH meter equipped with a combined glass electrode was used to adjust the pH.

The Design-Expert, a statistical package software version 9.0 (Stat-Ease Inc., Minneapolis, USA) was utilized for experimental design analysis and subsequent regression analysis of the experimental data.

Synthesis of 4-chloro-3- formylcoumarin

Phosphorus oxychloride (5.4 mL, 60 mmol) was added to 20 mL dry DMF at 0°C (cooled in ice) dropwise while stirring. In another vessel, containing 15 mL of dry DMF, 4-hydroxycoumarin solution (3.2 g, 20 mmol) was added dropwise. The solutions were mixed and the temperature of reaction mixture was raised to 55-65°C. Then it was cooled and poured into a beaker containing crushed ice. The yellow precipitate was filtered off, the solid materials were placed into a flask and recrystallized with 2:1 acetone-water to give yellow crystals (3 g, yield 72%), mp 125°C. IR spectrum, v, cm^{-1}: 1720 (O–C=O), 1690 (–C=O), 1603 and 1520. ^{1}H NMR chemical shift data, expanded ^{1}H NMR spectra, ^{13}C NMR chemical shift data and expanded ^{13}C NMR spectra of 4-chloro-3-formylcoumarin have been shown in **Figure 3** and **Figure 4**, respectively.

Synthesis of Schiff Base Ligand derived from L-Alanine

The Schiff base ligand (**L**) was synthesized as reported by literatures [33]. Alanine (0.01 mol) was added to a 30 mL of water in a beaker containing NaOH (0.002 mol). Another solution was prepared by dissolving 4-chloro-3-formylcoumarin (0.01 mol) in 40 mL ethanol and it was added to the first solution dropwise with constant stirring and heated for 2-3 h on a mantle at 50 °C. Then the reaction mixture was cooled to room temperature. The white precipitate, **L**, was formed, following filtering, it was washed with the ethanol-water mixture and stored in a vacuum desiccator over anhydrous calcium chloride (Yield: 78%), mp 231°C (**Scheme 1**).

Procedure for Zn (II) Determination

In the presence of optimum conditions, appropriate amount of the sample solution containing Zn (II) and Schiff base ligand **(L)** (3.0 ×10^{-5} mol L^{-1}) was placed in a 5.0 mL volumetric flask, and diluted to the mark. The solution pH was adjusted to 8.0 and allowed to stand for 20 min. Finally, a portion of the solution was introduced into the flame by conventional aspiration to determine of zinc.

Collection and Preparation of Real Samples

Water samples

In order to evaluate the capability of the proposed method, three various water samples including mineral water, river water (Miami's River) and drinking water (Miami's spring water) were selected and the developed

method was applied to determine the zinc content. The mineral water sample purchased from the local supermarket in Birjand (Birjand, Iran), the river water sample was collected from Miami's River (Shahroud, Iran), and the drinking water sample was obtained from the Miami's spring water (Shahroud, Iran). All the samples were collected in cleaned polyethylene bottles and only the river water was filtered through 0.45 µm pore size membrane filters immediately after sampling.

Biological samples (Blood serum)

Preparation of blood serum samples (two blood serum samples) was done according to the reported protocol by Harrington et al. [34]. Finally, solutions of prepared serum samples were measured according to recommended procedure. The good sensitivity of the atomic absorption method for zinc allowed of accurate measurements on blood serum samples.

ACKNOWLEDGEMENTS

SUPPLEMENTARY MATERIAL

It contains the ^{1}H NMR chemical shift data, expanded ^{1}H NMR spectra, ^{13}C NMR chemical shift data and expanded ^{13}C NMR spectra of synthesized Schiff base ligand and 4-chloro-3-formylcoumarin in **Figure 1**, **Figure 2**, **Figure 3** and **Figure 4**, respectively.

REFERENCES

1. Terres-Martos C, Navarro-Alarcón M, Martín-Lagos F, Giménez Martínez R, De La Serrana HLG, López-Martínez MC. Water Res. 2002;36:1912. https://doi.org/10.1016/S0043-1354(01)00373-6
2. Yılmaz S, Tokalıoglu S, Sahan S, Ulgen A, Sahan A, Soykan C. J. Trace Elem. Med. Biol. 2013;27:85. https://doi.org/10.1016/j.jtemb.2012.07.007
3. Trivedi P, Axe L. Environ. Sci. Technol. 2000;34:2215. https://doi.org/10.1021/es991110c
4. Tupe RS, Tupe SG, Tarwadi KV, Agte VV. Metab. Clin. Exp. 2010;59:1603. https://doi.org/10.1016/j.metabol.2010.02.020
5. Yanagisawa H, Miyakoshi Y, Kobayashi K, Sakae K, Kawasaki I, Suzuki Y, Tamura J. Toxicol. Lett. 2009;19:15. https://doi.org/10.1016/j.toxlet.2009.07.024
6. Calder PC, Yaqoob P. in Diet, Immunity and Inflammation. (Eds: K. Gruber, L. Rink) 2013:123-1567 (Woodhead Publishing: Cambridge).
7. Haase H, Overbeck S, Rink L. Perspect. Exp. Gerontol. 2008;43:394. https://doi.org/10.1016/j.exger.2007.12.002
8. Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Weill R, Boccio JR. Nutrition 2002;18:510. https://doi.org/10.1016/S0899-9007(01)00812-7
9. Alam IA, Sadiq M. Environ. Pollut. 1989;57:167. https://doi.org/10.1016/0269-7491(89)900080
10. Almeida MIGS, Chan C, Pettigrove VJ, Cattrall RW, Kolev SD. Environ. Pollut. 2014;193:233. https://doi.org/10.1016/j.envpol.2014.06.040
11. Patterson KY, Pehrsson PR, Perry CR. J. Food Compos. Anal. 2013;31:46. https://doi.org/10.1016/j.jfca.2013.03.004
12. Ghasemi JB, Hashemi B. Environ. Monit. Assess. 2011;183:57. https://doi.org/10.1007/s10661-011-1905-1
13. Abdolmohammad-Zadeh H, Sadeghi GH. Anal. Chim. Acta. 2009;649:211. https://doi.org/10.1016/j.aca.2009.07.040
14. Carletto JS, Roux KCDP, Maltez HF, Martendal E, Carasek E. J. Hazard. Mater. 2008;157:88. https://doi.org/10.1016/j.jhazmat.2007.12.083
15. Zhu X, Liang H, Zhao S, Yan H, Han D. Int. J. Environ. Anal. Chem. 2008;88:689. https://doi.org/10.1080/03067310801930758
16. Kiptoo JK, Ngila JC, Silavwe ND. Microchim. Acta. 2008;160:211. https://doi.org/10.1007/s00604-007-0831-y
17. Chang X, Luo H, Cui Y, Zhu X, Zheng HuY, He Q. J. Mol. Struct. Res. 2008;891:45. https://doi.org/10.1016/j.molstruc.2008.02.037
18. Suvardhan K, Suresh Kumar K, Rekha D, Kiran K, Jaya Raj B, Chiranjeevi P. J. Anal. Chem. 2007;62:336. https://doi.org/10.1134/S106193480
19. Khajeh M. J. Hazard. Mater. 2009;172:385. https://doi.org/10.1016/j.jhazmat.2009.07.025

20. Tormen L, Gil RA, Frescura VLA, Martinez LD, Curtius AJ. Spectrochim. Acta B 2010;65:959. https://doi.org/10.1016/j.sab.2010.09.007
21. Bersier PM, Howell J, Bruntlett C. Analyst, 1994;119:219. https://doi.org/10.1039/AN9941900219
22.
23. Pesavento M, Alberti G, Biesuz R. Anal. Chim. Acta. 2009;631:129. https://doi.org/10.1016/j.aca.2008.10.046
24. Welz B, Sperling M. Atomic absorption spectrometry 1999 (Wiley-VCH: Weinheim).
25. Veith M, Mathur S, Mathur C. Polyhedron 1998;17:1005. https://doi.org/10.1002/chin.199838302
26. Witkop WB, Ramachandran LK. Metabolism 1964;13:1016. https://doi.org/10.1016/S0026-0495(64)80021-4
27. Gerdemann C, Eicken C, Krebs B. Acc. Chem. Res. 2002;35:183. https://doi.org/10.1021/ar990019a
28. Kabeer AS, Baseer M, Mote N. Asian J. Chem. 2001;13:496.
29. Wang X, Wu G, Wang F, Wei W, Sun Y. Chin J. Catal. 2011;32:1812. https://doi.org/10.1016/S1872-2067(10)60272-0
30. Bellamy LJ. The infra- red spectra of complex molecules 1975 (Wiley; New York).
31. Khani R, Sobhani S, Hossein Beyki M. J. Colloid. Interface. Sci. 2016;466:198. https://doi.org/10.1016/j.jcis.2015.12.027
32. Myers RH, Montgomery DC. Response Surface Methodology 2001 (Wiley: New York).
33. Moorty S, Sundaram V, Rao NS. Indian J. Chem. 1973;11:854. https://doi.org/10.1002/chin.197406120
34. Singh HL, Singh J. Res. Chem. Intermed. 2013;39:1997. https://doi.org/10.1007/s11164-0120732-5
35. Harrington JM, Young DJ, Essader AS, Sumner SJ, Levine KE. Biol. Trace Elem. Res. 2014;160:132. https://doi.org/10.1007/s12011-014-0033-5

http://www.eurasianjournals.com