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Spectrofluorimetric Determination of Zinc in Human Hair by Using Ortho Hydroxy Acetophenone Hydrazone Doped in Sol-Gel Matrix

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A B S T R A C T

A novel Schiff base (o- hydroxy acetophenone hydrazone) was synthesized via condensation of 2-OH acetophenone and hydrazine monohydrate. Photoluminescence characteristics of the ligand were investigated in different solvents including acetonitrile (AC), ethanol (ETOH), dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO). The ligand was found to have higher emission intensity in DMSO solution. The Chemosensor was synthesized for detecting Zinc ion in human hair for males and females with age range (15y – 31y). The assessment of zinc ion from the remarkable enhancement of the luminescence intensity at 425 nm of the ligand (o-hydroxyacetophenone hydrazone) doped in sol-gel matrix by various concentrations of the zinc ion. The calibration plot was achieved over the concentration range $1.0 \times 10^{-8} – 2.0 \times 10^{-4}$ mol L^{-1} of zinc ion with a correlation coefficient of 0.993 and a detection limit of 3.72×10^{-9} mol L^{-1}. The sensor showed excellent selectivity toward Zn^{2+} ion with respect to common coexisting cations. The proposed fluorescence sensor was successfully applied to detect Zn^{2+} ion in human hair for both females and males.

Introduction

Schiff's bases are important class of compounds, due to their variety of applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis [1]. Hydrazones, RR'C=N─NR''R", were used as intermediates in synthesis, as functional groups in metal carbonyls and in organic compounds [2]. Furthermore, hydrazones exhibit physiological activities in the treatment of several diseases such as tuberculosis. Zinc ion plays many roles in the body which has a special and practical importance in the growing organism: helping build cells, keeping hormones in check, assisting the absorption of various other vitamins and giving the body a helping hand with protein use. All of these actions are essential for hair growth and maintenance. Zn^{2+} ion is an essential metal cation of exceptional biologic and public health importance [3], where it has multiple roles in both extra-and intra-cellular functions, for example Zn^{2+} ion is known to be indispensable to growth, development and transmission of the genetic message [4]. The negative effect of Zn^{2+} ion appeared when its abnormal concentration existed in the natural and biological systems. For example, in the environment an excessive concentration of zinc ion may reduce the soil microbial activity resulting in the phytotoxic effect [5]. Moreover, it occurs commonly as an agriculture and food waste product in the environment. In the biological system, it is also known that disorders of zinc metabolism are closely associated with many severe neurological diseases such as Alzheimar's disease (AD). Hair is composed of proteins, lipids, water, and small amounts of trace elements. All proteins in animal and human bodies are built from permutations of amino acid molecules in a polypeptide string. The polypeptide chains of protein keratin are organized into filaments in hair cells. Hair is one of the most difficult proteins to digest or solubilize. Among the most common dissolving procedures for hair is acidic, alkaline, and enzymatic hydrolysis. For the analysis of hair, the solid samples are transferred by solubilization via digestion into a liquid phase [6]. Research during the past three decades suggests that the relationship between hair element concentrations and human health is a complex process related to exposure, absorption, and tissue distribution of essential and toxic elements. Follow-up blood testing or provocative urine testing is useful to confirm hair element findings. Blood provides information about what the body has recently

absorbed (hours to days in some cases weeks).

Blood levels are largely independent of tissue deposition. Blood levels vary according to the actual component analyzed (plasma, serum, RBC). They can be transient in nature, and are subject to the body's homeostatic mechanisms to maintain levels within narrow range [7]. In the present work a new synthesized *o*-hydroxy acetophenone hydrazone was used as a nano optical sensor for determination of zinc levels in human hair for women and men by the enhancement of the luminescence intensity of *o*-hydroxy acetophenone hydrazone by different zinc ion concentrations in dimethyl sulphoxide (DMSO).

Materials and methods

Chemicals and reagents

All chemicals used are analytical reagent of higher grade. Tetraethyoxy silane (TEOS) was supplied from Aldrich and also all solvents as Absolute ethanol (EtOH), Dimethyl sulphoxide (DMSO) were purchased from Aldrich and they were used as received. A stock solution of Zn^{2+} ion (1.0×10^{-2} M) was prepared by dissolving 0.2974 g Zn $(NO_3)_2$ - $6H_2O$ in exactly100 mL of deionized water and standardizing with EDTA [8].

Physical Methods of Measurements

All luminescence measurements were recorded with a Meslo- PN (222-263000) z Thermo Scientific Lumina fluorescence Spectrometer in the range (220– 750 nm). The absorption spectra of the samples were measured in the range of 190 –900 nm with Thremo UV-Visible double-beam spectrophotometer. The pH was measured using JAN-WAY 3330 PH meter. TEM image was taken on a JEOL JEM-1230 available at Ain Shams University. JEOL JEM-1230 is a high performance, high contrast, 40-120 kV Transmission Electron Microscope with excellent imaging capabilities. The FTIR spectra were measured as KBr pellets on the Mattson 1000 FTIR spectrophotometer.

General procedures

1. Synthesis of *o*-OH acetophenone Hydrazone as a Ligand

Orthohydroxy acetophenone hydrazone (ligand) was prepared by the slow dropwise of (0.05 mol, 6.05g) *o*-hydroxy acetophenone dissolved in 50 mL of absolute ethanol to hydrazine monohydrate (0.025 mol, 2.5g) dissolved in 50 mL of absolute ethanol. The reaction mixture was heated under reflux for 5 h, and then the mixture was left at room temperature to coagulate until the solid product was formed. The formed product was filtered off under suction and recrystallized from ethanol, yield 2 g [9].

2. Synthesis of *o*-OH acetophenone Hydrazone doped in Sol–gel Matrix

A mixture consisting of Tetraethoxysilane (TEOS), C_2H_5OH and H_2O in a molar ratio of 5:5:1 was refluxed for 1 h to give precursor sol solution using a few drops of diluted HCl solution as a catalyst. Subsequently, appropriate amount of the *o*-hydroxy acetophenone hydrazone (0.02g) dissolved in 10 mL DMSO and the

precursor solution were mixed and stirred together for 15 min until the mixture became homogeneous. The obtained ligand dispersed sol solution was dried into oven at 240^0 C for 10 min to give solidified and transparent composite sample [10].

3. Preparation of Hair Sample

Samples of hair (10-20 mm in length and weighing about 500 mg) were taken close to the skin from the same region of the nape of the neck for each male and female in the age range (15y – 31y); these were then cut into lengths of 1-2 mm with scissors. After exhaustive washing with acetone, detergent solution (sodium lauryl sulphate, 100 g/l), and distilled water in sequence, followed by a final rinsing in acetone and carbon tetrachloride in order to remove lipids and dust particles [11]. The hair samples were air dried between filter paper. In the procedure about 0.1 g of the hair sample was digested in a 15 mL test tube with 0.2 mL 10 M NaOH over a hot bath at 90 °C for 15 min. After cooling, the digest was made up to 25 ml with glass-distilled water and analyzed for zinc ion [12].

Results and discussion

Characterization of the *o*-OH acetophenone Hydrazone

The structure of the synthesized *o*-hydroxy acetophenone hydrazone (Ligand) was investigated using FTIR and elemental analyses. The morphology of ligand doped in sol-gel matrix was investigated by TEM **Scheme 1**.

Analysis of the IR spectra of o-Acetophenone hydrazone

The IR spectra in the range 4000-400 cm^{-1} of the hydrazone ligand is shown in **Fig. 1** The assignment of the ligand bands was reported in the **Table 1**.
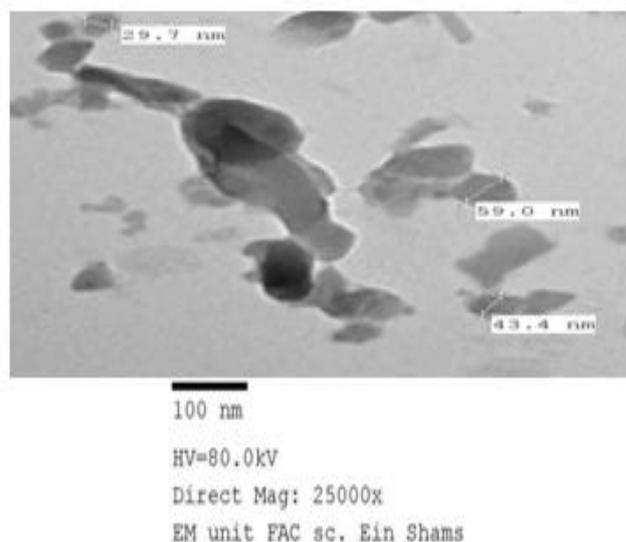
Scheme 1: TEM image of nano optical sensor o-OH acetophenone hydrazone (Average size of nano particle 44 ± 2)

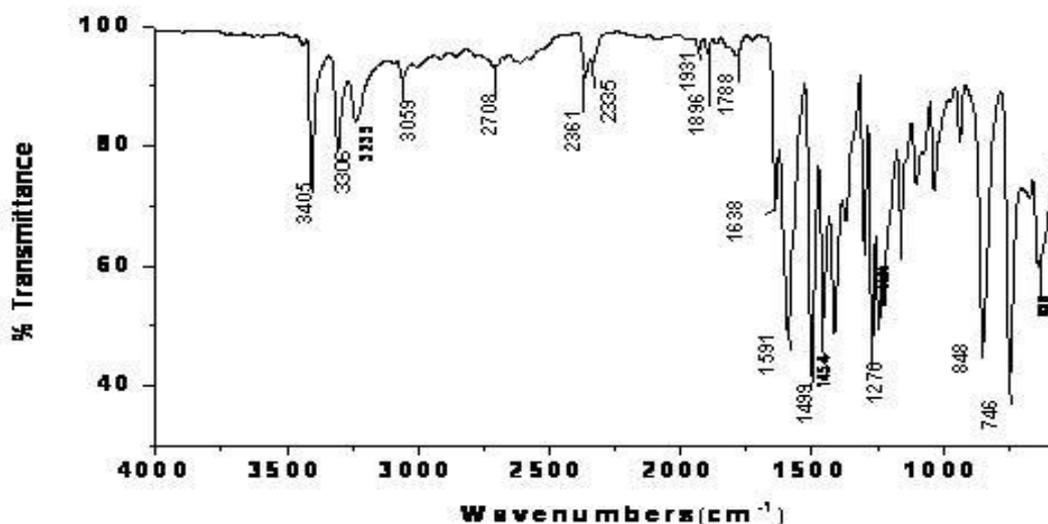**Fig. 1**: Infra-red spectra of o-hydroxy acetophenone hydrazone.

Table 1: FTIR spectra **o**f the Ligand o-OH acetophenone hydrazone.

Ligand bands (cm⁻¹)	Assignment
3405	$\nu_{(OH)}$
3239	$\nu_{a (NH2)}$
3059	ν_{C-H}
1638	$\nu_{(C=N)}$
1270	$\nu_{(C-O)}$

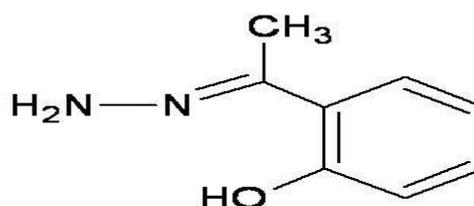**Assignment of *o*-OH Acetophenone hydrazone**

The structure of *o*-hydroxy acetophenone hydrazone **Scheme 2** has been confirmed by FTIR technique. The most characteristic vibrational bands observed in the spectrum of the ligand are as follows: The broad band observed at 3405 cm⁻¹ was attributed to Ʋ (OH) stretching. The absorption band observed at 3239 cm⁻¹ was assigned to Ʋ (NH₂) stretching vibration. The absorption band observed at 3059 cm⁻¹ was due to the C–H stretching vibration of the phenyl ring. The sharp intense absorption peak located at 1638 cm⁻¹ was assigned to the Ʋ(C=N) stretching as has been reported [13]. The band observed at 1270 cm⁻¹ was attributed to Ʋ (C–O) stretching.

The U.V/visible spectra

The electronic spectrum of the free ligand (o-OH acetophenone hydrazone) exhibits two intense bands at 273 and 310 nm in DMSO as shown in **Fig. 2**.

Emission and excitation spectra

The fluorescence spectrum of the *o*-hydroxy acetophenone hydrazone sol-gel matrix showed fluorescence emission at λ_{em} 425 nm as shown in **Fig. 3**.

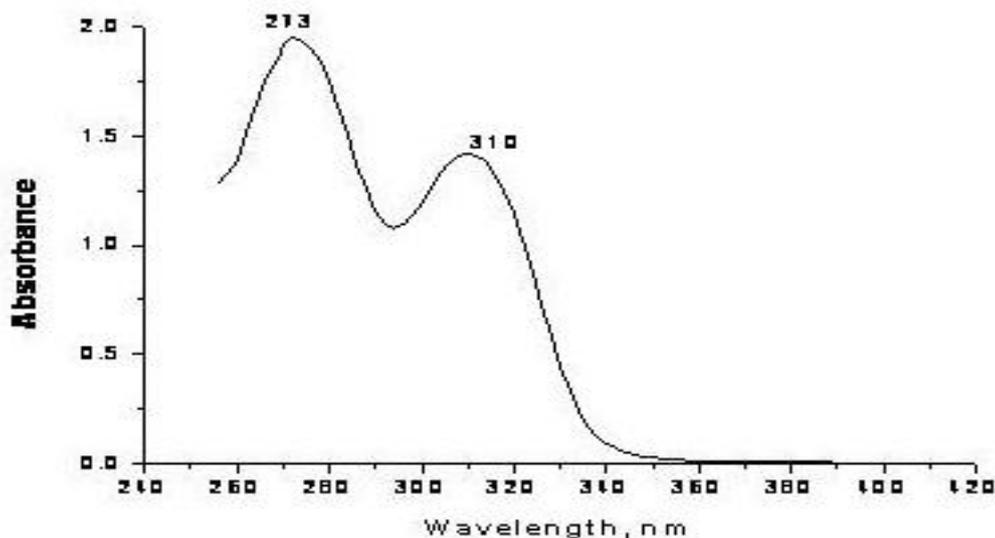**Scheme 2**: Structure of the ligand o-OH acetophenone hydrazone

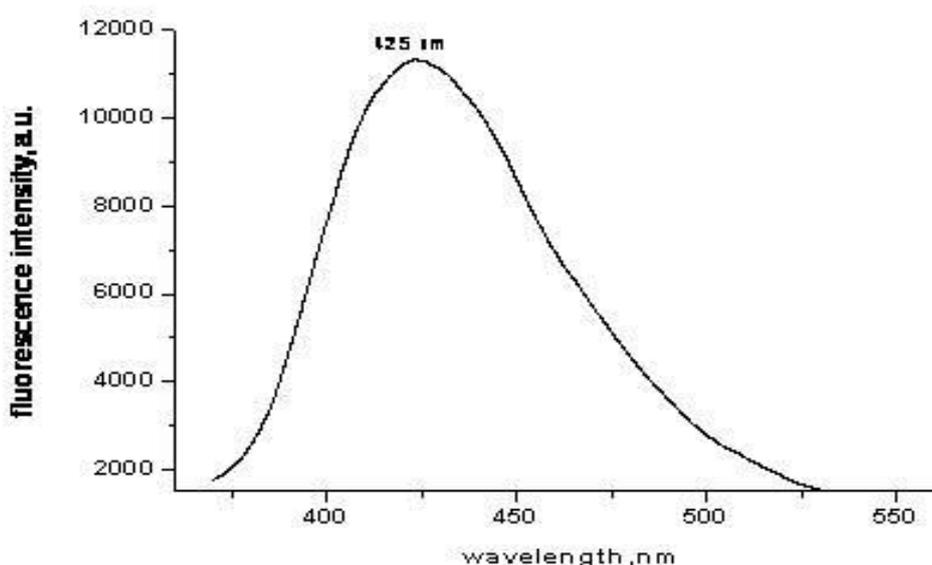**Fig. 2**: The UV/ Visible spectra of o-OH acetophenone hydrazone.

Fig. 3: The emission spectrum of the Ligand (1×10^{-3} mol/L) at $\lambda_{ex}/\lambda_{em} = 360/425$ nm.

The Effect of pH on the fluorescence spectrum

The pH of the medium has a large effect on the fluorescence intensity of the o-OH acetophenone hydrazone in the range (3–10). The pH has been adjusted using NH_4OH and HCl, The suitable pH value where the peak at 425 nm has the highest intensity was at pH 10.

The Effect of solvents on the fluorescence spectrum

The influence of the solvents on the fluorescence intensity of the ligand was studied; the results showed that the emission intensity was enhanced in the DMSO while a strong quenching effect was found in ethanol as shown in **Fig. 4**.

Response of sensor to Zn^{2+} ion

The chemical sensor exhibited remarkable fluorescence intensity enhancement upon exposure to Zn^{2+} ions. The nano optical sensor was placed in diagonal position in the quartz cuvette containing 2 mL buffer solution of pH 10. The sample cell was titrated with microliter amounts of a concentrated Zn^{2+} ion solution, using a precalibrated micro pipette and the fluorescence intensity of the system was measured at 425 nm (λ_{ex} 360 nm) after 5 min required to reach the equilibrium.

Analytical performance

1. Analytical parameters of the optical sensor

The six point's calibration curve as shown in **Fig. 5** was obtained by plotting the peak intensity of the sensor at

λ_{em} 425 nm versus the concentration of Zn^{2+} ion and the graph was described by the regression equation:

$$Y = a + bX$$

where Y = luminescence intensity of the optical sensor at λ_{em} 425 nm; a = intercept; b = slope and X = concentration in nmol mL^{-1}.

Regression analysis of luminescence intensity data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in **Table 2**. The limit of detection (LOD) and quantitation (LOQ) calculated according to IUPAC guidelines [14] using the formula:

$$\text{LOD= 3.3S/b and LOQ= 10S/b}$$

where S is the standard deviation of blank luminescence intensity values and b is the slope of the calibration plot, **Table 2**.

2. Selectivity

The selectivity behavior is obviously one of the most important characteristics of anion-selective optical sensor [15], which is the relative optode response for the primary ion over other ions present in solution. In order to assess the possible analytical application of the sensing method, the effects of various ions on the determination of Zn^{2+} ion were examined. To evaluate the metal bonding property and selectivity, the competition experiments were also conducted by adding the interfering ions to solutions containing 2.0 μM Zn^{2+}ion in Tris–HCl buffer, pH 8.0. The experiments were carried out by recording the change in the fluorescence intensity before (F_o) and after adding the interfering ion at 2.5×10^3 mol L^{-1} level (F) into Zn^{2+} ion solution. The resulting relative error (RE) is defined as RE (%) = [(F−F_o)/F_o] ×100.The results of these tests on potential interferences are summarized in **Fig. 6**.

No significant interferences were observed if a less than ±5 % relative error was tolerated.

3. Analytical applications

To assess the applicability of the proposed sensor to real samples, an attempt was made to determine zinc ion in different human hair samples, collected under optimum conditions. Hair zinc levels of 9 healthy people between the 15 to 31 years age groups were examined with respect to age groups and their kind. As may be seen in **Table 3** it was found that hair zinc levels of female group were higher than male group. Deficiency of this element appears to be more common in male than female due to requirements for zinc increase at puberty, particularly in boys, presumably associated with the high concentration of zinc present in the testes and accessory male sex glands. Our findings are in agreement with hair zinc levels reported in the literature [13].

Conclusion

In conclusion, a novel sensor *o*-OH acetophenone hydrazone doped in sol-gel matrix for the determination of Zn^{2+} ions was developed, with good optical and mechanical properties. It has been shown that the response of this sensor in the presence of Zn^{2+} ions does not vary significantly in the pH range 8–10. The optimal emission and excitation wavelengths of the sensor are examined in different solvents and it was found that maximum quantum yield was achieved in DMSO. The sensor shows a high selectivity and quick response for Zn^{2+} ion over other common metal ions. The proposed sensor has a wide dynamic range from of $1.0 \times 10^{-8} – 2.0 \times 10^{-4}$ mol L^{-1} with a limit of detection (LOD) 3.72×10^{-9}mol L^{-1} and a regression coefficient of 0.993. As a result, the developed sensor was successfully used in quantitative determination of Zn^{2+} ion with high accuracy in human hair samples.
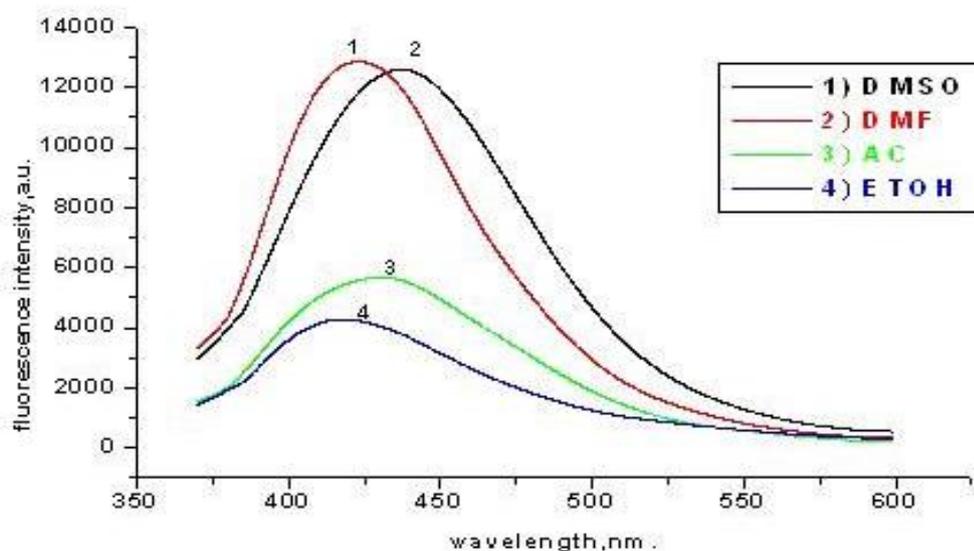
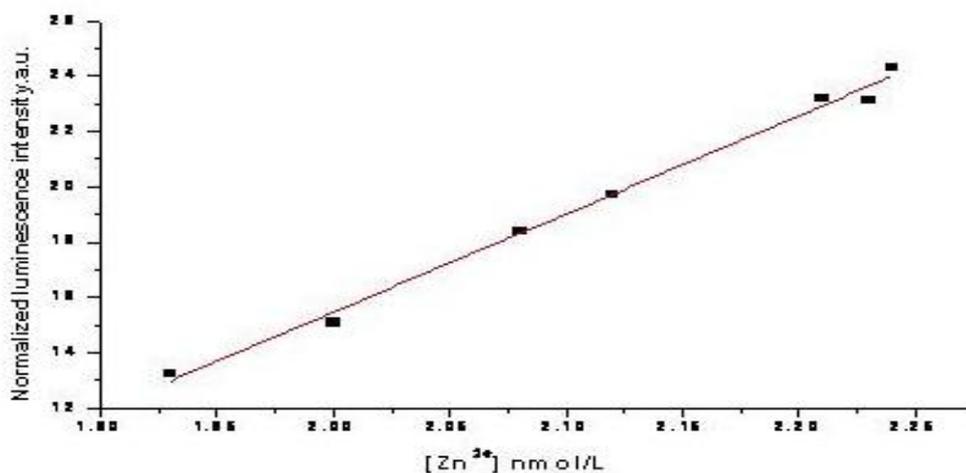**Fig. 4**: The fluorescence spectra of the ligand (1x10^{-3}M) in different solvents.

Fig. 5: Calibration curve of the luminescence intensity of the sensor (ligand) doped in sol gel matrix versus concentration of Zinc.
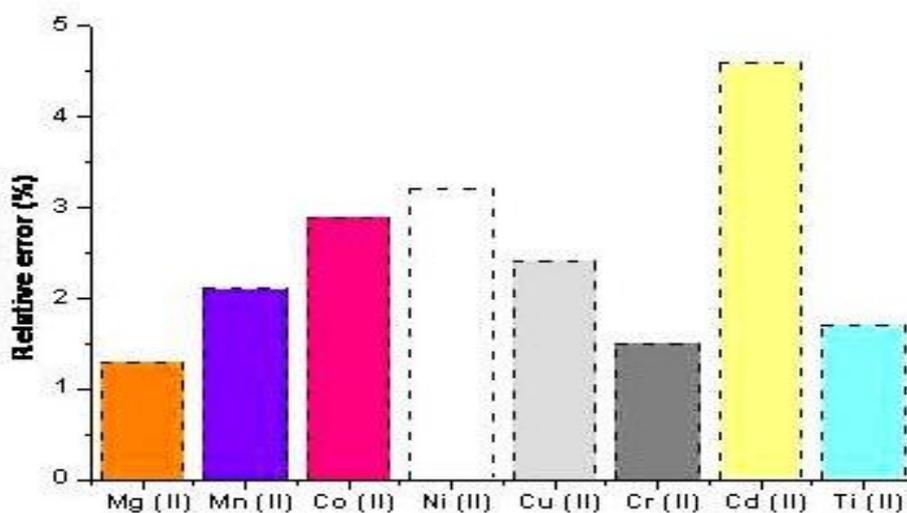

Fig. 6: Interferences of different metal ions (2.5×10^3 mol L^{-1}) onto the fluorescence determination of Zn^{2+} ion (2.0 μM) using the nano sensor at pH 7.0.

Table 2: Sensitivity and regression parameters for the optical sensor.

Parameter name	Parameter value
λ_{em}, nm	425
Linear range, mol L^{-1}	$1.0 \times 10^{-8} - 2.0 \times 10^{-4}$
Limit of detection (LOD), mol L^{-1}	3.72×10^{-9}
Limit of quantification (LOQ), mol L^{-1}	1.52×10^{-8}
Intercept (a)	2.9
Slope (b) x 10^9	1.4
Standard deviation x 10^{-3}	1.73
Regression coefficient (r)	0.993

Table 3: Comparison of hair zinc levels of male and female groups.

No. of participants	Age	Females	Males
		$\ddot{x} \pm SD$	$\ddot{x} \pm SD$
4	15y – 20y	**183 ± 1.7**	**171 ± 0.9**
5	21y – 31y	**143 ± 1.2**	**127 ± 1.1**

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